

# CLINAM

European Foundation for Clinical Nanomedicine

# 11/2018

Basel, September 2–5

# SUMMIT PROCEEDINGS

11TH EUROPEAN AND GLOBAL SUMMIT FOR CLINICAL  
NANOMEDICINE, TARGETED DELIVERY AND PRECISION MEDICINE  
THE BUILDING BLOCKS TO PERSONALIZED MEDICINE

Summit under the Auspices of the  
Swiss Confederation in Collaboration  
with 33 Organizations:



## 11TH EUROPEAN AND GLOBAL SUMMIT FOR CLINICAL NANOMEDICINE, TARGETED DELIVERY AND PRECISION MEDICINE THE BUILDING BLOCKS TO PERSONALIZED MEDICINE

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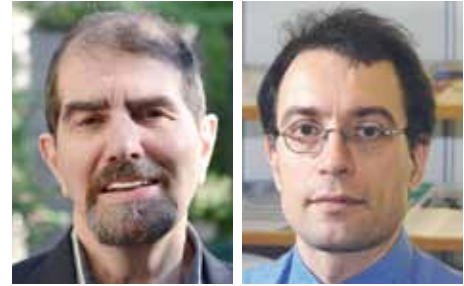
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# **EDITORIAL**

## **ON BEHALF OF THE CLINAM-FOUNDATION**



Welcome to Basel – Eleven years CLINAM FOUNDATION AND Eleven CLINAM SUMMITS.

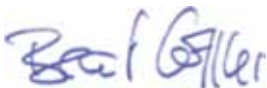
As you can see in the programme of this year we have further developed the concept of this Summit and in many cases the CLINAM Foundation has assisted as a service provider to bring together exponents of relevant fields in nanomedicine that use our neutral nonprofit platform as a place to create new international cooperation, discuss latest research results and to further nanomedicine, targeted delivery and precision medicine to the benefit of the patient.

Over the past eleven years the CLINAM Summit evolved to an exquisite and globally unique event. It builds on the principle that fundamental scientists, developers and professionals in clinical application and all other stakeholders in nanomedicine can mutually learn from each other to find better solutions for the medicine of the future.

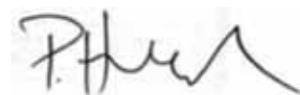
The CLINAM-Foundation's primary goal is to support the development and application from the stage of basic research all the way to the clinic.

This year the Nobel Laureates Prof. Jules Hoffmann, Prof. Ada Yonath and Prof. Harald zur Hausen will address the community and forty-one sessions will give room to 190 speakers and 100 poster presenters from the summit-community to highlight their work followed by lively discussions.

We look forward to host this summit in best way and thank you to be part in the event.



**Dr. med. h.c. Beat Löffler, MA**  
CEO of the CLINAM-Foundation



**Prof. Dr. med. Patrick Hunziker**  
CSO of the CLINAM-Foundation



# INTRODUCTION

## ON BEHALF OF SWITZERLAND



For the eleventh time the CLINAM Summit is held in Switzerland. CLINAM has become by now an international Service Platform with 32 supporting collaborating organizations to exchange the knowledge and research results in Nanomedicine, Targeted Medicine and Precision Medicine. CLINAM is in Europe **the** place to debate the unmet medical needs, hurdles and novel solutions that can be offered by the application of Nanomedicine, Targeted Delivery and Precision Medicine.

The CLINAM Foundation shall this year again host more than 450 members from the international community. The Health Regulatory Authorities being present at the meetings in the last years, have for this year decided to now and in the future hold their IPRP Meetings in Basel.

This International Pharmaceutical Regulators Programme Session holds its Nanomedicines Working Group meeting at the neutral CLINAM-Platform to discuss nanotechnology and specifically nanomedicine related issues relevant to regulated products. The meeting provides members a unique opportunity to leverage the expert scientific knowledge, regulatory and operational experience, on-going technical harmonization work, and information access of other participating regulators. We welcome you all, dear Regulatory Authority Members from Europe, from the United States, from China, from India, from Canada, from Japan and from Africa. We are proud that you have chosen Switzerland for your present important meeting.

The participants here are a mix of Clinicians, Biochemists, Chemists, Physicists, Pharmacologists, Engineers, Investors and Industrials. They meet face to face. This creates trust, understanding and will doubtlessly accelerate processes, create new cooperation and keen ideas, leading to new medications to the benefit of the patients and humankind.

Nanomedicine and Targeted Medicine are essential building blocks in the development towards Precision Medicine. Personalized medicine is certainly the future, which to some extent has already started and is at CLINAM a most important focus.

This Summit is under the auspices of the Swiss Confederation. We support it, because we believe that Switzerland has the political stability to actively promoting novel scientific fields as an Innovation leading country in Europe.

We wish all of you a fruitful meeting with new projects and worldwide cooperation that will accelerate the innovation process in medicine.

A handwritten signature in blue ink, appearing to read 'G. Haefliger'.

**Dr. Gregor Haefliger**, Vice Director, Head of National Research and Innovation Division,  
State Secretariat for Education Research and Innovation (SERI)





CLINAM

European Foundation for Clinical Nanomedicine

11/2018

Basel, September 2–5

# CURRICULA VITAE SPEAKERS



## Zahraa Al-Ahmady

Research Fellow in Advanced Drug Delivery

Zahraa obtained her BSc Degree in Pharmacy with a distinction from the College of Pharmacy, University of Baghdad in 2004. After training as a clinical pharmacist, she was awarded Chevining Scholarship to study the Masters in Drug Delivery at the

UCL School of Pharmacy. Zahraa completed her MSc studies with a distinction and was awarded the AstraZeneca Prize for the best overall performance. Zahraa completed her PhD studies with the Nanomedicine Lab at the UCL School of Pharmacy on the design, characterization and biological performance of temperature-sensitive vesicles for cancer therapy in 2013. She then joined the NANOSOLUTIONS (FP7-NMP) European project as a postdoctoral research associate at the University of Manchester. Her work was mainly focused on the structure – biological function relationship that determines the safety of engineered nanomaterials. Zahraa is currently a Research Fellow in advanced drug delivery working on the development of innovative therapeutics and *in vivo* imaging approaches for effective translocation through BBB in lesioned brain.

### RECENT PUBLICATIONS

- Zahraa S. Al-Ahmady. Selective Drug Delivery Approaches to Lesioned Brain through Blood Brain Barrier Disruption. *Expert Opinion on Drug Delivery* (2018).
- Zahraa S. Al-Ahmady, Marilena Hadjidemetriou, James Gabbins, Kostas Kostarellos., Formation of Protein Corona *in vivo* affects Drug Release from Temperature-Sensitive Liposomes. In press, *Journal of Controlled Release* (2018).
- Zahraa S. Al-Ahmady, Kostas Kostarellos, Chemical Components for the Design of Temperature-Responsive Vesicles as Cancer Therapeutics. *Chemical Reviews* (2016).
- Marilena Hadjidemetriou, Zahraa S. Al-Ahmady, Mariarosa Mazza, Kostas Kostarellos. In Vivo Biomolecule Corona around Blood-Circulating, Clinically-Used and Antibody-Targeted Lipid Bilayer Nanoscale Vesicles. *ACS Nano* (2015).
- Zahraa S. Al-Ahmady, Wafa' T. Al-Jamal, Tam T. Bui, Alex F. Drake, James Mason, Jeroen V. Bossche, Kostas Kostarellos, Lipid-Peptide Vesicle Nanoscale Hybrids for Triggered Drug Release by Mild Hyperthermia *In Vitro* and *In Vivo*. *ACS Nano* (2012).

ceutics (2012-2013) in the same university I designed and carried out pharmacodynamic investigations on artemether and lumefantrine-loaded solid lipid microparticles based on solidified reverse micellar solutions. These microparticles improved the aqueous solubility and bioavailability of these drugs and achieved significantly higher *in vitro* drug release and *in vivo* parasitaemia clearance compared to commercial drugs.

I am presently carrying out my PhD research on formulating and characterizing various nano-sized delivery systems of artesunate and quinine for intranasal administration. The success of this research work would provide a rapid, convenient and satisfactory alternative to parenteral route of administration for anti-malarial drugs for the treatment of these disease conditions. It would also prevent the neurological sequelae that arise from late initiation of therapy in some rural areas in sub-Saharan Africa with limited access to healthcare professionals trained to carryout intravenous and intramuscular administration of these drugs. It could also serve as an alternative to the parenteral route of delivery for patients suffering from uncomplicated malaria that can receive drugs orally. My desire to explore and gain experience from other fields of Pharmacy practice led to my working briefly in the hospital and community Pharmacies and with the Sales Department of GlaxoSmithKline Pharmaceutical Nigeria Limited (2014-2015). I am currently a lecturer in the Department of Pharmaceutics, University of Nigeria, Nsukka where I teach and supervise undergraduate research projects. The courses I teach include Dispensing, Physical Pharmaceutics, Design and Formulation of Dosage Forms and Drug Delivery Systems. I am part of the Nanomedicine research group in my department and member, Pharmaceutical Society of Nigeria.

I completed my Bachelor's degree in Pharmacy in 2010 with a total of three (3) distinctions in my final professional examinations, and a first class in my Master's degree program in 2014 with a cumulative Grade Point Average (cGPA) of 4.91 on a 5-point scale. In 2017, I was awarded a commonwealth split-site scholarship in the University of Birmingham, United Kingdom. I am presently in the School of Pharmacy characterizing my nano-structured lipid carriers of artesunate and quinine.

My masters's degree research project has been published in the journal of Drug Development and Industrial Pharmacy and is titled "Formulation design, *in vitro* characterizations and anti-malarial investigations of artemether and lumefantrine-entrapped solid lipid microparticles". I have also written a book chapter titled "In vivo fate of synthetic biomaterial-based nanoparticles for drug delivery" in J.N. Govil (Ed). *Nanotechnology* Vol. 11.

I have been involved in many voluntary and charitable activities undertaken in my immediate community aimed at improving the health and quality of life of rural dweller.



## Chinazom Precious Agbo

I am a first stage researcher involved in the design and investigation of novel delivery systems for drugs used in the treatment of malaria and other neglected tropical diseases with a goal of achieving safer, more convenient and more efficacious therapies. At the moment am focusing on applying nanotechnology and novel drug delivery systems in improving the side effect profiles of anti-malarial drugs and in delivering of anti-malarial drugs to the Central Nervous System through the intranasal route to improve the treatment outcomes in cerebral and severe malaria.

My passion to pursue a career in pharmaceutical nanotechnology started during my undergraduate studies in the University of Nigeria, Nsukka (2004-2010). My first opportunity came in my final year research project that entailed the development of polymeric microparticles of Ibuprofen using Eudragit RS and RL 100. The purpose was to achieve a controlled release of the drug and prevent its gastro-erosive side effect.

In my master's degree research work in the department of Pharma-



## Adriano Aguzzi

Professor, Director of the Institute of Neuropathology, University Hospital Zurich

Adriano Aguzzi is professor and director of the Institute of Neuropathology at the University of Zurich. He has devoted the past 25 years to studying the immunological and molecular basis of prion pathogenesis, combining transgenetics with molecular and immunological techniques to clarify the pathogenesis of the disease, and to identify cells and molecules involved in prion neuroinvasion. He is the Founder and Director of the Swiss National Reference Center for Prion Diseases and has developed diagnostic and therapeutic methods in the field of transmissible spongiform encephalopathies. He serves on the editorial board of *Science* and is the Editor in Chief of the *Swiss Medical Weekly*; he also serves on the scientific advisory board of philanthropic foundations and biomedical companies. Among other honors, Prof. Aguzzi has won Ernst-Jung

Prize, the Robert Koch Prize, and the medal of the European Molecular Biology Organization, and has held two ERC Advanced Grants.

## PERSONAL INFORMATION

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Nationality: Italian and Swiss Date of birth: 01 December 1960  
web site: <http://www.en.neuropathologie.usz.ch/Pages/default.aspx>

## RECENT PUBLICATIONS

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## Khuloud T Al-Jamal

Chair of Drug Delivery and Nanomedicine, Institute of Pharmaceutical Science, King's College London

Professor Khuloud T. Al-Jamal is a Chair of Drug Delivery & Nanomedicine, King's College London. She is also a registered pharmacist at the General Pharmaceutical Council.

She has completed her pre-registration pharmacy training at The University College London Hospital and was awarded the Overseas Research Award Scheme (ORSA) Scholarship from The University of London (2000-2004) to complete her PhD in Drug Delivery from The School of Pharmacy, University of London (currently known as UCL-School of Pharmacy).

She was awarded the prestigious CW Maplethorpe Research and Teaching Postdoctoral Fellowship from The University of London (2005-2007) to explore the use of cationic dendrimers as anti-angiogenic agents for growth inhibition of solid and metastatic tumours.

She has developed an extensive experience in designing and developing novel nanoscale delivery systems including dendrimers, liposomes, quantum Dots (QDs), polymers, viral vectors, chemically functionalised carbon nanotubes and graphene oxide. Her current work involves pre-clinical translation of novel nanomaterials designed specifically for drug, protein, nucleic acids and radionuclide delivery for therapeutic or diagnostic applications.

She was awarded and is managing a number of research projects funded by The Royal Society, Worldwide Cancer Research, EPSRC,

BBSRC, FP6, FP7 and ITN Marie Curie research programmes. In February 2012, she was awarded the BBSRC New Investigator award exploring the use of chemically functionalised carbon nano-needles as vectors for delivering therapeutics across the BBB. In 2012, she was awarded the prestigious Royal Pharmaceutical Society Science Award in recognition for her outstanding scientific achievements in the field of Nanomedicine. She is a three-time winner of the Wellcome Trust Image Award (2014-2016). She is a management and steering committee member of the London Centre for Nanotechnology, a consortium that builds around nanotechnology research conducted at King's College London, University College London and Imperial College London.

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## Wafa T. Al-Jamal

Queen's University Belfast, School of Pharmacy, UK

Dr Wafa Al-Jamal completed her PhD in Drug Delivery and Nanomedicine in 2008 from the School of Pharmacy, University of London (now known as UCL-School of Pharmacy). She is currently a Reader in

Nanomedicine and Drug Delivery at School of Pharmacy, Queen's University Belfast. She is also a Prostate Cancer Fellow (2014-2019). Dr Al-Jamal joined School of Pharmacy, University of East Anglia, Norwich, as a Lecturer in Drug Delivery (2013-2017), after working as a Senior Research Fellow at University College London, and King's College London (2008-2013).

Dr Al-Jamal's main research interest focuses on engineering novel nanomaterials for biomedical applications. Her current research aims to design smart vectors to deliver a wide range of therapeutic agents and targeting moieties, and to fabricate multifunctional nanoparticles for combinatory therapy and theranostic applications. Her long-term research career is to facilitate the translation of nanoparticle-based therapeutics from the lab to the clinic.

Dr Al-Jamal is the GSK Emerging Scientist Award winner for 2015, and Gro Brundtland Award winner for 2017. Her multidisciplinary research has been funded by the Royal Society, Prostate Cancer UK, The Engineering and Physical Sciences Research Council (EPSRC), and Rosetrees Trust. She has published over 50 papers in high impact factor journals. Currently, she is a member the Prostate Cancer UK (PCUK) Research Advisory Committee, and a Visiting Professor at Guizhou Medical School, China.

## PUBLICATIONS:

- Kostevšek N, Monaco I, Dehsorkhi A, Conyard J, Hall C, Meech S, Mayes A, Serša I, Comes Franchini M, and Al-Jamal WT. *Temper-*

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- Silva V, Al-Jamal WT. Exploiting the Cancer Niche: Tumour-Associated Macrophages and Hypoxia as Promising Synergistic Targets for Cancer Therapy. *J Control Release*, In press.
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## Christoph Alexiou

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Prof. Dr. Christoph Alexiou, received his Ph.D. in 1995 from the Technical University of Munich, Medical school. After finishing his internship in the Gastroenterology Department at the University-hospital of the Technical University he started as a physician and researcher at the Department of oto-rhino-laryngology, head and neck surgery and founded a research group working on the field of local chemotherapy with magnetic nanoparticles (Magnetic Drug Targeting). In the year 2000 he received his degree as an ENT-Physician and 2002 he changed to the ENT-Department in Erlangen, Germany, where he performed his postdoctoral lecture qualification (Habilitation). He is working there as an assistant medical director in the clinic and leads the Section for Experimental Oncology and Nanomedicine (SEON). Since 2009 he owns the Else Kröner-Fresenius-Foundation-Professorship for Nanomedicine at the University-hospital Erlangen. He receives grants from the European Union, German Research Community (DFG), Ministry of Education and Science (BMBF) and Bavarian State Ministry of the Environment and Consumer Protection and is a member of the Executive Board of the European Technology Platform for Nanomedicine (ETPN). His research is addressing the emerging fields of Diagnosis, Treatment and Regenerative Medicine using magnetic nanoparticles and the translation from basic research into clinical trials. He received for his research several national and international renowned awards.

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- Tietze R, Lyer S, Dürr S, Struffert T, Engelhorn T, Schwarz M, Eckert E, Göen T, Vasylyev S, Peukert W, Wiekhorst F, Trahms L, Dörfler A, Alexiou C: Efficient drug-delivery using magnetic nanoparticles — biodistribution and therapeutic effects in tumour bearing rabbits. *Nanomedicine: Nanotechnology, Biology, and Medicine* 9:961–971, 2013
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## Namrata Anand

Dr. Namrata Anand born on 9th April 1985 is a PhD from Postgraduate Institute of Medical Education and Research, India and is currently working as a postdoctoral fellow in Department of Zoology Panjab University, India. During my PhD, I have worked on milk protein 'Lactoferrin' (Lf) isolated from bovine and buffalo colostrum and examined its antiparasitic properties against intracellular parasites like; Plasmodium, Toxoplasma and Leishmania. I have also worked on the Lf protein interaction towards RBCs and macrophages of human origin to better understand the mechanism of action as they are the host cells for intracellular parasites. I have found that, Nanoformulation of bovine and buffalo Lf using Alginate Enclosed Chitosan nanoparticles showed better results compared to native Lf form in controlling the disease progression in mice model. The mode of mechanism of Lf protein was found to be due to production of free radicle ions and accumulation inside the macrophages of mice visceral organs which are site for parasite multiplication. My current research work is to examine the antiparasitic properties of these nanoparticles and its comparison with conventional drugs using pregnancy mouse models of disease like malaria and toxoplasmosis.

### PUBLICATIONS

- Antiparasitic and immunomodulatory potential of oral nanocapsules encapsulated with lactoferrin protein against Plasmodium berghei. Anand et al., 2016. *Nanomedicine*.11 (1): 47-62.
- Oral administration of encapsulated iron binding bovine lactoferrin protein Nanocapsules against intracellular parasite :Toxoplasma gondii. Anand et al. *International Journals of Nanomedicine*. 2015.10: 6355-6369.
- Effect of lactoferrin protein on red blood cells and macrophages-mechanism of parasite host interaction. *Drug Design Development and Therapy*. Anand et al., 2015 :9 3821–3835.
- Standardization of Plaque Assay of Japanese Encephalitis Virus (Nakayama NIH Strain) on BHK-21 (Cl-13) Cell Line 2010. Anand, N., Kumar,S., and Goyal,D. *Am. J. Biomed. Sci.* 2010, 2(1), 43-50.
- Lactoferrin nanocapsules prevent malaria infection via immunomodulation and regulate miRNAs involved in iron metabolism.





## Marianne Ashford

Advanced Drug Delivery, Pharmaceutical Sciences, AstraZeneca

Marianne is Senior Principal Scientist in Pharmaceutical Sciences at AstraZeneca. In this role, Marianne is responsible for applying drug delivery approaches to improve therapeutic index of medicines and is working to enable novel targets via successful intracellular delivery of new modalities such as nucleic acid and peptide drugs. Marianne has initiated a number of collaborations, which have resulted in the introduction of nanomedicines into our Oncology portfolio. She is a member of the Pharmaceutical Sciences and Oncology Science Leadership teams and is an Honorary Professor at the University of Nottingham.

Marianne's previous experience includes being a project manager leading the pharmaceutical development of a number of AstraZeneca's Oncology development drugs and influencing their global product strategies. Prior to that Marianne was Associate Director of a Preformulation and Biopharmaceutics Group whose role was to evaluate the product design characteristics of candidate drugs, supplying pre-clinical formulations as well as providing solid state science and biopharmaceutics support across the portfolio. Marianne has authored a number of book chapters, research papers and patents in the pharmaceutical science arena and more recently has given several invited talks and plenaries in the Nanomedicine/Advanced Drug Delivery field. Marianne is Honorary Professor at School of Pharmacy, University of Nottingham, UK

### RECENT PUBLICATIONS

- Ashford, M (2017) Development and Commercialization of Nano-carrier-Based Drug Products in Pharmaceutical Nanotechnology. Eds J Cornier, A Owen, A Kwade, M Van de Voorde. Wiley-VCH Verlag GmbH & Co. KGaA
- Lallana et al (2017) Chitosan/hyaluronic acid nanoparticles: rational design revisited for RNA delivery. *Molecular pharmaceutics* 14 (7), 2422-243.
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- Hare et al (2016) Challenges and strategies in anti-cancer nanomedicine development: An industry perspective. *Advanced drug delivery reviews* 108, 25-38.



## Anthony Amaechi Attama

Anthony Amaechi Attama is a Professor of Pharmaceutics and the Director, Education Innovation Unit (Vice Chancellor's Office), University of Nigeria, Nsukka. He is also the Coordinator of Drug Delivery and Nanomedicines Research Group at the University of Nigeria. Prof. Attama was at the Institut für Pharmazeutische Technologie,

Technische Universität Carolo-Wilhelmina zu Braunschweig, Germany for his postdoctoral research in pharmaceutical nanotechnology sponsored by Alexander von Humboldt Foundation, Germany. He was a Visiting Professor at the Laboratory of Membrane and Liposome Research, Department of Biochemistry and Molecu-

lar Biology, Institute for Medical Research Israel-Canada (IMRIC), Hadassah Medical School, Hebrew University of Jerusalem, Israel (February to April 2018) under the sponsorship of the Lady Davis Fellowship Trust of the Hebrew University of Jerusalem. The focus of the Drug Delivery and Nanomedicines Research Group, which he coordinates, is to improve the effectiveness of drugs used for the treatment of mostly neglected tropical and poverty-related diseases, through engineering of novel drug delivery strategies (nanoparticles, microparticles, etc.). He has researched extensively on lipid drug delivery systems- micro and nanomedicines using natural and biocompatible lipids. While at the Hebrew University, Prof. Attama worked under Prof. Chezy Barenholz for the development of novel nanoliposomes to combat malaria. He welcomes collaboration with drug development scientists within and outside Nigeria. Prof. Attama has won many research and travel grants, and has attended many scientific conferences and workshops. He supervises postgraduate students in pharmaceutical sciences and has many research articles published in peer-reviewed high impact journals. In a bid to translate research results to products, he has fostered the establishment of some pharmaceutical industries in Nigeria and also serves as a consultant to many pharmaceutical companies. Prof. Attama is a registered pharmacist and a Federal Government licenced drug analyst..



## Simon Baconnier

Simon Baconnier completed his scholar cursus at Université Joseph Fourier Grenoble 1, where he graduated in 2003 with a PhD in Biophysics.

Then, he chose to shift his carrier towards international project management and partnership building.

After a first mission in the Biotech Start-up environment in Genopole (Evry, France) where he managed the optimisation of biotech integration in European projects, Simon Baconnier integrated Canceropole Lyon Auvergne Rhone-Alpes (CLARA, Lyon, France) for which he was first hired to develop the Europe Funding strategy to support the regional and local oncology stakeholders participation in EU funding programs. CLARA then asked him to set-up and managed a Nanotechnology for Cancer network in the Rhône-Alpes region supported by regional and international experts from both fields, and allows a strong development of his network in both fields.

from 2006 to 2013, Simon Baconnier has been coordinator assistant in European projects supporting the development of clinical and translational research in the field of soft tissue tumours: "Con-ticanet" (2005-2011) and "EuroSarc" (2012-2016), Currently, Simon Baconnier is coordinating an international KOL network in a rare cancer area since 2010 (World Sarcoma Network).

Simon Baconnier, also has a good knowledge of the field of industry. In 2010, he took the lead of the French affiliate of a Japanese Biotech (OncoTherapy Science Inc.), as Chief Scientific Officer, to manage the early clinical development of an innovative treatment in a rare cancer. This mission reinforced his skills in management and optimization of international partnership and allowed him to acquire a huge experience in early clinical development and investigational drug development in the pharma industry environment. Simon joined his last position as TransNational Access manager of the European Nanomedicine Characterisation Laboratory (EU-NCL) infrastructure in CEA end 2015. With this position, Simon is in close contact with Translation Advisory Board (TAB) and the 3 pilot lines to propose and implement synergistic collaboration between the 3 operational groups of the Nanomedicine Translation Hub.

In parallel, Simon is involved in several EU Projects in the nanomedicine field (NOBEL 2017-2020, REFINE, 2017-2021) as well as in the Clinical Working Group of the ESTER initiative.





## Lajos (Lou) P. Balogh

Editor-in-Chief, Precision Nanomedicine

Dr. Lajos (Lou) P. Balogh, Ph.D. is the Editor-in-Chief of Precision Nanomedicine, the official journal of the European Foundation for Nanomedicine (CLINAM). Dr. Balogh published 228 scientific papers, gave >230 invited lectures, and was awarded 12 patents. Lou's publications have been cited over 7000 times (17 papers with more than 100 citations, and 7 with more than 200 citations) and is considered to be an international expert in Nanomedicine and scholarly publications. He is one of the five Founders of the American Society for Nanomedicine, serves on the US Technical Advisory Group to ISO TC 229 Nanotechnology, and on the Science Board of a number of international and US national organizations. Some recent awards include Visiting Professorship for Senior International Scientists at the Chinese Academy of Sciences, Beijing and KOFST Fellowship, Seoul National University, Seoul, Korea. Lou has held faculty positions at Kossuth University Debrecen, Hungary, then in the USA at the University of Massachusetts Lowell, MA, at the Michigan Molecular Institute, Midland, MI, the University of Michigan, Ann Arbor, MI, the Roswell Park Cancer Institute, Buffalo, NY, and at Northeastern University, Boston, MA. Lou was the Editor-in-Chief of the Nanomedicine: Nanotechnology, Biology and Medicine Elsevier journal for 7 years until 2016. Dr. Balogh is the owner and Chief Scientific Advisor of AA Nanomedicine & Nanotechnology (AANMNT), a science consulting firm registered in Essex County, Massachusetts, USA (balogh@aananomedicine.com) and also serves as the Executive Editor of Manuscript Clinic (www.manuscriptclinic.us).

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Google Scholar: <https://scholar.google.com/citations?user=jbDIqSwAAAAJ&hl=en>

### RECENT PUBLICATION

- Balogh, LP, Balancing Interests of Science, Scientists, and the Publishing Business. *Prec. Nanomed.* 2018 Apr; 1(1):5-14. DOI:10.29016/180418.1
- Parak W, Pelaz B, Alexiou C, Alvarez-Puebla RA, Alves F, Andrews AM, Ashraf, S, Balogh LP, et al, Diverse Applications of Nanomedicine, *ACS Nano*. *ACS Nano*, 2017, 11 (3), pp 2313–2381. DOI: 10.1021/acsnano.6b0604
- Siccardi, M., Löffler, B., Balogh, L., & Owen, A. (2017). Integrated pharmacokinetic modelling for accelerated nanomedicine translation. *European Journal of Nanomedicine*, 9(1), 1-3. doi:10.1515/ejnm-2016-0041
- Keri M, Nagy Z, Novak L, Szarvas E, Balogh LP, Istvan Banyai I. Beware of phosphate: evidence of specific dendrimer–phosphate interactions, *Phys.Chem.Chem.Phys*, 2017, 19, 11540
- Yuliang Zhao, Lajos Balogh, Caging Cancer, *Nanomedicine: Nanotechnology, Biology, and Medicine*, 11 (2015) 867–869



## Ruchi Bansal

Ruchi Bansal is currently an Assistant Professor in the Department of Biomaterials Science and Technology at the University of Twente. Ruchi obtained her doctoral degree in 2012 from the University of Groningen, Netherlands. She carried out her PhD project on targeting of interferon gamma and its signaling domain in chronic diseases. From 2011-2012, she received two prestigious grants Sheila Sherlock research fellowship (European association for the study of liver diseases, EASL) and The Ruth and Richard Julin's Swedish Foundation research grant and she was also appointed as a visiting researcher at Karolinska Institute, Stockholm, Sweden. She has independently established her research on nano-targeting of inflammation, fibrosis and metabolic disorders. Her expertise and interests are to study the disease mechanisms, cellular cross-talk, identify key targets for molecular and cellular targeting, and deliver therapeutic drugs using targeted nanoparticles. She has published in multiple high impact journals including *Gastroenterology*, *Hepatology*, *Nature Reviews Gastroenterology & Hepatology*, *Advanced drug delivery reviews* and *Journal of controlled release*. She has received a prestigious VENI (Innovational Research Incentives for academic excellence) grant from The Netherlands Organization for Health Research and Development, ZonMW, NWO followed by several research funding to support her research group. She has also received several young investigator awards, Junior Scholar Award 2015 and UEG National Scholar Award 2017. She has also secured patents and a NWO valorization grant to start-up a company Tar-Mac Innovations.



## Yechezkel Barenholz

Professor Emeritus Yechezkel (Chezy) Barenholz is head of the Liposome and Membrane Research Lab at the Hebrew University-Hadassah Medical School, Jerusalem, Israel and is also the Daniel G. Miller Professor in Cancer Research at Hebrew University of Jerusalem. He has been on the faculty at Hebrew University since 1968 and has been a visiting Professor at leading Universities around the world. His current research focuses on the development of drugs and nano-drugs based on drug delivery systems (DDS) best exemplified by the anticancer Doxil<sup>®</sup>, the first liposomal drug as well as the first nano-drug approved by the FDA (1995) and used world-wide. Professor Barenholz is an author of >400 scientific publications, cited as of November 14 32,259 times, with h-index of 87times. Prof Barenholz is a co-inventor in > 45 approved patent families. He is a founder of four 5 start-ups, NasVax (now Therapix) Moebious, PolyPid, LipoCureRX, and Ayana. He received many National and International awards including Honorary Doctorate degree from the Technical University of Denmark (2012) and the 2012 founders award of the International Controlled Release Society. In 2003, Professor Barenholz founded the Barenholz Prizes from Doxil<sup>®</sup> royalties to encourage excellence and innovation in the applied sciences of Israeli PhD students. Professor Barenholz is married to Dr Hanna Barenholz together they have 4 daughters and 12 grandchildren.



## Jack Barokas

Running and owner of private audio visual equipment maintenance Company over 20 year's. Computer and network maintenance services at TAU-CD, Head of Digital Media team and

Digital Media Coordinator of International Projects at TAU, BA degree in learning technologies and Instructional Design,

HIT 2010 Israel. Online courses development to replace Face2Face learning at School of Medicine, Higher Education Reform Expert (HERE) in the framework of Israel National Tempus Office (NTO).

Life webcasting and lecture/course recording services for European projects such as, Nano2Life, NanoEI, NanoSkills, Qnano, Edunano, Horizon 2020 Up2U, ERASMUS+ NanoEI-Asia.

### RECENT PUBLICATIONS

- Danilo Demarchi, Gianluca Piccinini, Mariagrazia Graziano, Jack Barokas, Silvia Schintke, Philippe Morey-Chaisemartin, and Slavka Tzanova, HANDS-ON LABORATORIES IN THE NANOEL PROJECT, 9th European Workshop on Microelectronics Education EWME'12, Grenoble, France, May 11, 2012.
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## Matthias Barz

Junior Research Group Leader

Dr. Matthias Barz studied chemistry at the Johannes Gutenberg-University Mainz (Germany) and Seoul National University (South Korea) received a diploma degree in chemistry in 2006 and a PhD in polymer chemistry from the Johannes Gutenberg-

University Mainz (Germany) working under the supervision of Prof. R. Zentel in 2009. Afterwards he joined the groups of Dr. Maria J. Vicent at the CIPF (2010) and Prof. T. Kirchhausen at Boston Children's Hospital, Harvard Medical School (2011-2012). In 2013 he became independent junior research group leader and started his habilitation at the Institute of Organic Chemistry at the Johannes Gutenberg-University Mainz (Germany), which he successfully finished in 2016. Dr. Matthias Barz is steering committee member of the SFB 1066. He received the research prize of the Emil und Paul-Müller-Gedächtnisstiftung, the GDCh Scholarship for junior faculty in makromolekular chemistry and the Roche pRED Award for Excellence in Drug Delivery at the Roche-Nature Biotechnology Symposium 2014. He has published more than 60 publications, editorials & reviews, book chapters, patents and patent applications. He has introduced polypept(o)ides as novel biomaterial to the field of polymer science. His research focuses on the synthesis of polypept(o)idic nanomedicines for therapy (chemotherapy and immunotherapy) and diagnosis (MRI and PET imaging) with the aim to achieve the required complex functionality by simple, versatile and scalable synthetic methods

### RECENT PUBLICATION

- Birke A, Ling J, Barz M, Polysarcosine-containing copolymers: Synthesis, characterization, self-assembly, and applications. Progress in Polymer Science, 2018, DOI:10.1016/j.progpolymsci.2018.01.002.
- Klinker K, Schäfer O, Huesmann D, Bauer T, Capelôa L, Braun L, Stergiou N, Schinnerer M, Dirisala A, Miyata K, Osada K, Cabral H, Kataoka K, Barz M. Secondary Structure-Driven Self-Assembly of Reactive Polypept(o)ides: Controlling Size, Shape and Function of Core Cross-Linked Nanostructures. Angew. Chem. Int. Ed. 2017, 56, 9608-9813.
- Heller P, Hobernik D, Lächelt U, Schinnerer M, Weber B, Schmidt M, Wagner E, Bros M, Barz M\*. Teaming Reactive Triblock Copolymers with Functional Cross-linkers: A Versatile Pathway to Disulfide Stabilized-Polyplex Libraries and Their Application as pDNA Vaccines. J. Control. Release 2017, 2017, 258, 146-160.
- Bartneck M, Schlößer CT, Barz M, Zentel R, Trautwein C, Lammers T, Tacke F, Immunomodulatory Therapy of Inflammatory Liver Disease Using Selectin-Binding Glycopolymers. ACS nano 2017, 11 (10), 9689-9700.
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## Ana Benito

Dr. Ana Benito, Senior Researcher at CIDE-TEC Nanomedicine, obtained her MChem degree in the University of the Basque Country in 1998. She graduated as PhD in Organic Chemistry in the University of the Basque Country in 2004. She worked 9 years in R&D Department of Lilly SA (Alcobendas, Spain) where she specialized in

High Throughput Screening (HTS) for hits and leads identification until 2006. Then, she moved to medicinal chemistry group where she specialized in the synthesis of New Chemical Entities (NCE) for lead optimization and candidate selection. Her work was fully dedicated to research transfer into the pharma market. In 2012, she joined CIDETEC where she worked in the synthesis of single chain polymeric nanoparticles for drug delivery and imaging until 2015, when she started working in NanoPilot project (European Union Framework Programme for Research and Innovation H2020 funded project). Since 2015, she has been fully dedicated to setting-up a pilot plant operating under Good Manufacturing Practice (GMP) for the production of polymer-based nanopharmaceuticals. During this time, she has increased her expertise in GMP guidelines and technology transfer from the laboratory scale to a GMP environment to enable new developments to enter clinical trials. As a result of her work she has been inventor of 3 patents and is coauthor of 10 scientific articles. Some of them:

### RECENT PUBLICATIONS

- "Synthesis and functionalization of dextran-based single-chain nanoparticles in aqueous media". J. Mater. Chem. B, 2017, 5, 1143-1147. DOI: 10.1039/C6TB02773C.
- "Functional Single-Chain Polymer Nanoparticles: Targeting and Imaging Pancreatic Tumors in Vivo". Biomacromolecules, 2016, 17 (10), pp 3213-3221. DOI: 10.1021/acs.biomac.6b00941.
- "Discovery of a Novel Series of Orally Active Nociceptin/Orphanin FQ (NOP) Receptor Antagonists Based on a Dihydrospiro(piperidine-4,7'-thieno[2,3-c]pyran) Scaffold". J. Med. Chem., 2014, 57, 3418-3429. DOI: dx.doi.org/10.1021/jm500117r.
- "Development of LC-MS/MS-Based Receptor Occupancy Tracers and Positron Emission Tomography Radioligands for the Nociceptin/Orphanin FQ (NOP) Receptor". J. Med. Chem., 2012, 55,



## Nadia Benkirane-Jessel

Dr. Nadia Benkirane is Research director and head of the “Regenerative Nanomedicine” laboratory, at INSERM (French National Institute for Health and Medical Research), UMR 1260 Inserm-Unistra, Strasbourg, France. She was leader of “Active Biomaterials and Tissue Engineering” team INSERM 977. She received her Ph.D.

from University Louis Pasteur, ULP, Strasbourg, France for the work on Development of pseudopeptides as synthetic vaccines. Dr. Jessel (Benkirane) then held a postdoctoral position in collaboration with the Institut Pasteur, Paris, France, working on Immunotherapy HIV, and another postdoctoral position on the application of modified peptides as vaccines against FMDV (Plum Island Animal Disease Center, ARS, USDA, Greenport, NY 11944-0848, USA). She joined the INSERM U595 in 2002 as a post-doc, and received the diploma to direct the research (HDR) in 2004. Dr. Jessel got the permanent position (CR1) in the INSERM 595 laboratory in 2004 and Research Director (DR2) position in the INSERM 977 and head of “active Biomaterials and Tissue Engineering team from 2009 until 2012). Currently Research Director (DR1) and head of the INSERM UMR 1260 (Regenerative Nanomedicine”. Dr. Jessel possesses expertise in diverse fields of molecular and cellular biology, immunochemistry, tissue engineering and biomedical engineering. In the last 10 years, she focused her research on the bio-functionalization of multilayered polyelectrolyte architectures with emphasis on the use of these architectures to induce specific cellular responses and gain control over cell proliferation and differentiation. Dr. Benkirane-Jessel have 138 publications (h index: 36) with peer-reviewed publications in high impact factor journals (Proc. Nat. Acad. Sci. USA; Adv. Mater.; Adv. Funct. Mater.; Small; Nanoletters, Biomaterials, ACS Nano), 5 chapters reviews and 5 international patents, she is a regular referee for a number of scientific journals (Nature nanotechnology, Nature Materials, ACS nano, Biomaterials, Nanoletters...). She is under the contract (Interface INSERM/Clinic 2008-2013) and she got also “Prime d’Excellence Scientifique” from the INSERM, 2010-2014 and the PEDR from the INSERM on 2016 for 4 years.

Website:

<http://www.regmed.fr/>

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<http://www.linkedin.com/pub/nadia-benkirane-jessel/24/39b/345>

### RECENT PUBLICATIONS

- Briand J.-P, Benkirane-Jessel N et al., Bioactive coatings based on a polyelectrolyte multilayer architecture functionalized by embedded proteins. Proc Natl Acad Sci USA. 1997, 94:12545-12550 (IF-9.809).
- Benkirane-Jessel N, et al., Bioactive coatings based on a polyelectrolyte multilayer architecture functionalized by embedded proteins. Adv. Mater. 2004, 15:692-695 (IF-15.409).
- Benkirane-Jessel N et al., Control of monocyte morphology on and response to model surfaces for implants equipped with anti-inflammatory agents. Adv. Materials, 2004, 16, 1507-1511 (IF-15.409).
- Benkirane-Jessel N et al., Pyridylamino-beta-cyclodextrin as a molecular chaperone for lipopolysaccharide embedded in a multilayered polyelectrolyte architecture. Adv. Materials, 2004, 14, 963-969 (IF-15.409).
- Benkirane-Jessel N et al., Build-up of polypeptide multilayer coatings with anti-inflammatory properties based on the embedding of piroxicam-cyclodextrin complexes. Adv. Funct. Mater. 2004, 14:174-182 (IF-10.439).



## Henning Blume

Henning Blume is Associate Professor of pharmaceutical sciences at the Goethe University, Frankfurt/Germany. After receiving his PhD he completed postdoctoral research as Assistant Professor at the School of Pharmacy, University of Frankfurt.

His fields of research include biopharmaceutical and pharmacokinetic investigations, with particular focus on the characterisation of bioavailability and bioequivalence of medicinal products, also with highly variable drugs, studies in special populations and drug-food interactions. He published more than 230 peer-reviewed research papers.

Henning Blume is member of a number of international scientific associations on the fields of biopharmaceutics and clinical pharmacology. He was appointed as member of Editorial Boards of several international Journals and is Editor of the Bioavailability Section of the International Journal of Clinical Pharmacology and Therapeutics.

From 1989 until 2001 he was appointed as expert of the Bioavailability Commission at the German Authorities and was member of the German Pharmacopeial Commission from 1994-1998. He was appointed Member of the Expert Network at the European Medicines Agency (EMA). From 1989 until 2000 he served as president of the Section of Official Laboratories and Medicines Control Services of the International Pharmaceutical Federation (FIP), and was chairman of the FIP Special Interest Group on Bioavailability and Bioequivalence. From 1993-2000 he has been the chairman of the Committee on Pharmaceutical Policies of the European Federation of Pharmaceutical Societies (EUFEPS). Since 2006 he is elected chairman of the EUFEPS European Network on Bioavailability and Biopharmaceutics. In this capacity he is chair of the Global Bioequivalence Harmonisation Initiative conferences.

Henning Blume received the EUFEPS Ouroboros Award in 2001, the German Generics Award in 2007 and in 2012 the Honorary Award of the German Pain Award. Moreover he received the Honorary Membership of the Argentinian Diabetes Association in 2008 and was awarded in 2016 with the title “Dr. honoris causa” by the Semmelweis Medical University, Budapest/Hungary.



## Patrick Boisseau

VP Healthcare

Mr Boisseau has 28 year experience in the world of nanomedicine and innovative health technologies, working at CEATech, a French non-for-profit Technology Research Institute.

Patrick Boisseau’s current position is VP Healthcare responsible for the Strategic Planning in Life Sciences and Healthcare Technologies, at CEATech, a public non-for-profit Research & Technology Organisation, based in Grenoble, France. He has acquired a large expertise of coordination of EU projects like the Network of Excellence Nano2Life, EuroNanoBio, BIBA, TARGET-PDT, and recently the EU-NCL infrastructure on nanocharacterisation or REFINE project on emerging analytical characterisation techniques for nanomedicines. His field of technical expertise is nanomedicine, drug delivery, medical imaging and innovative medical technologies.

Patrick Boisseau is solicited for numerous expertise and evaluations for the European Commission and other European and national funding agencies.

He is elected Chairman of the European Technology Platform on Nanomedicine since December 2012.



He is chairing the ESTHER Task Force designing and implementing this European Industry Driven Initiative on Emerging and Strategic Technologies for Healthcare since May 2015, in cooperation with Medtech-Europe.



## Tobias Bopp

I am a basic researcher and immunologist who specializes in the field of immunological tolerance to foster our knowledge about context- and tissue-specific regulation of immune responses in health and disease. After completing my training in molecular biology at Johannes-Gutenberg-University Mainz in 2003 I joined the Institute for Immunology (IFI) at University Medical Center (UMC) Mainz to conduct my doctoral thesis in 2006. As a faculty member and later on as a Professor for Molecular Immunology in the IFI at UMC Mainz, I focus on a better understanding of the molecular and transcriptional regulation of T cell- and macrophage-regulated immune responses in situ.

### AREA OF EXCELLENCE

My scientific interests and expertise revolve around the regulatory T cell-, T Helper cell- and macrophage-mediated immune regulatory mechanisms. Since my postdoc and subsequent appointment as professor for molecular Immunology at the IFI of UMC Mainz I have endeavored to provide excellent research in order to enhance our knowledge about the molecular mechanisms underlying the transcriptional regulation of T cells and macrophages in the regulation of context- and tissue-specific immune responses, resulting in more than 80 publications in internationally renowned immunological journals (e.g. Nature Immunology, Immunity, Cell Metabolism and the Journal of Experimental Medicine). Besides the honors of being a co-speaker of the DFG-funded comprehensive research Center (CRC) 1292, a project leader in the CRCs 1066, 1292, TR52, TR128, a member of the steering committee of the University Cancer Center Mainz, a faculty member of the German cancer Consortium, expert reviewer for national and international research agencies and renowned journals, I was elected in 2013 to become the head and coordinator of the largest research focus group within the German Society for Immunology (DGfI), T cells: Subsets and functions.

### SUPPORTING ACTIVITIES

In addition to providing excellent teaching and basic research, I am also involved in the development and pursuit of superior scientific and university's administrative structures. Here, I am not only a founding member of the "Research Center for Immunotherapy" (FZI) of UMC Mainz, but also the head and coordinator of the FZI's leadership council, a member of the educational and the scientific committee of UMC and a board member of the Mainz Research School of Translational Biomedicine (TransMed).

### HONORS AND AWARDS

On the basis of my work I had the honor to receive funding for excellent junior scientists by the Carl Zeiss Foundation from 2007-2009, the Fritz-and-Ursula-Melchers award in 2010 and the Boehringer Ingelheim award in 2008.



## Sven Even Borgos

Invited speaker

Sven Even Borgos (born 1976) earned both his undergraduate and PhD degrees at the Norwegian University of Science and Technology in Trondheim, which is the main technical university of Norway. His undergraduate was from the Faculty of Physics and Mathematics, specialization in Biophysics and Medical Technology. His PhD, however, was in molecular biology. More specifically, it was concerned with genetic engineering of the antibiotic-producing soil bacterium *Streptomyces noursei* in order to develop mutants producing derivatives of the clinically important antifungal antibiotic nystatin and related compounds, with improved pharmacological properties. His post doc was earned in systems biology, developing and validating a genome-scale metabolic model of the alginate-producing bacterium *Pseudomonas fluorescens*. Since 2006, he has been working in SINTEF (Norway), which is one of the largest independent research institutes in Europe with more than 2000 employees. Here, he has been working with advanced analytical chemistry, mainly based on mass spectrometry coupled to chromatography. The last years, he has been specializing in physicochemical characterisation of nanomaterials, with an emphasis on nanomedicines, using various modalities of mass spectrometry and separation methods such as field flow fractionation (FFF). He is working in the European Nanomedicine Characterisation Laboratory (EUNCL) H2020 project leading the chemical part of the characterisation cascade, as well as leading the work package that identifies novel nanomedicines characterisation technologies. Following on to this, he is WP leader in the REFINE H2020 project supporting the relevant authorities in ongoing development and optimisation of regulatory frameworks for nanomedicines. Since 2017, the SINTEF Mass Spectrometry group is also a main analytical partner in the B-SMART H2020 project developing RNA-based nanomedicines against neurodegenerative disorders. He also has a keen interest in the novel field of mass spectrometry imaging (MSI), e.g. for label-free, spatially resolved analysis of drug biodistribution in tissues in terms of targeted drug delivery by nanomedicines.

### RECENT PUBLICATIONS

- Bremer-Hoffmann, S., Halamoda-Kenzaoui, B. and Borgos, S.E. (2018) Identification of regulatory needs for nanomedicines. *Journal of Interdisciplinary Nanomedicine*, 3, 4-15.
- Ritsema, J.A.S., Herschberg, E.M.A., Borgos, S.E., Lovmo, C., Schmid, R., Te Welscher, Y.M., Storm, G. and van Nostrum, C.F. (2017) Relationship between polarities of antibiotic and polymer matrix on nanoparticle formulations based on aliphatic polyesters. *Int J Pharm.*
- Mehn, D., Iavicoli, P., Cabaleiro, N., Borgos, S.E., Caputo, F., Geiss, O., Calzolari, L., Rossi, F. and Gilliland, D. (2017) Analytical ultracentrifugation for analysis of doxorubicin loaded liposomes. *Int J Pharm*, 523, 320-326.
- Borgos, S.E.F. (2016) In Cornier, J., Owen, A., Kwade, A. and Van de Voorde, M. (eds.), *Pharmaceutical Nanotechnology: Innovation and Production*, 2 Volumes. John Wiley and Sons, Vol. 1, pp. 135-156.



## Susanne Bremer-Hoffmann

Susanne Bremer-Hoffmann, Dr. rer. nat., holds a PhD degree in Biology obtained from the Charité University Hospital Berlin in Germany for her work on the development of immunotherapies against leukemia. After post-doctoral research at the Federal Institute for Risk Assessment in Germany, Susanne moved to the Joint

Research Centre (JRC) of the European Commission and became a team member of the European Centre for the Validation of Alternative Methods (ECVAM) in 1995. She was involved in formal validation studies of toxicological *in vitro* tests detecting embryotoxicity and endocrine disruption as well as their regulatory acceptance at the OECD. Susanne collaborated in several FP6, FP7 projects including the public/privat partnership initiative "SEURAT-1" that focussed on the development of alternative methods for chronic toxicities. Currently Susanne is leading JRCs project "SciNano" which is providing scientific support to the European Commission on nano-related policies. In addition, she is involved in the European Nanomedicine Characterisation Laboratory and the "REFINE" project aiming to advance regulatory science in the field of nanomedicine and nano(bio)material. She contributed to more than 80 peer-reviewed scientific paper.

### RECENT PUBLICATION

- B. Halamoda-Kenzaoui, U. Holzwarth, G. Roebben, A. Bogni, S. Bremer-Hoffmann (2018), Mapping of available standards against the regulatory needs for nanomedicines; Wiley Interdisciplinary Reviews in press
- S. Bremer-Hoffmann, B. Halamoda-Kenzaoui and S. Even Borgos (2018), Identification of regulatory needs for nanomedicines, Journal of Interdisciplinary Nanomedicine 3, pp 4-15
- S. Gioria, F. Caputo, P. Urban, S. Bremer-Hoffmann, A. Prina-Mello, L. Calzulai, Mehn D. (2018), Are existing standard methods suitable for the evaluation of nanomedicines: some case studies. Nanomedicine 13 (4), pp 539-554
- S. Bremer-Hoffmann, Amenta V., and Rossi F. (2015) Nanomedicines in the European Translational Process. Eur. J. Nanomedicine 7, pp 135-268



## Donald Bruce

Managing Director, Edinethics Ltd.,  
11/6 Dundonald Street, Edinburgh EH3  
6RZ, Scotland, UK

Dr Donald Bruce holds doctorates in chemistry and in theology. He is managing director of the independent consultancy Edinethics Ltd., working on the ethics and public engagement of emerging technologies. After working 15 years as a chemist in nuclear energy research, risk regulation, and energy policy, he became Director of the Church of Scotland's Society, Religion and Technology Project (SRT) from 1992-2007. In this role he did pioneering ethical assessment of many emerging technologies including GM crops and animals, cloning and stem cells. He has worked extensively on nanomedicine and related technologies from 2003 to the present, in a series of EC projects Nano2Life, NanoBio-Raise, NanoMedRound, Ethentech (on human enhancement). He is a partner in the NanoAthero EC FP7 project on nanodevices to detect and treat atherosclerosis. An integral part of this work has been in developing and writing public engagement tools with Perry Walker formerly of the New Economics Foundation. He helped develop the Democs/Decide card games and Open-up argu-

ment map concepts, on such issues as GM crops, synthetic biology, human enhancement, and stem cells for therapy and for toxicity testing. He will describe and demonstrate a Democs game on nanomedicine written for the NanoAthero project. He has also worked on the implications of distributed healthcare for patients, carers, medical staff and the healthcare system, using the ethical matrix method. He is a member of the UK Animals in Science Committee. He was a former member of the Scottish Science Advisory Committee, the Societal Issues Panel of Engineering and Physical Sciences Research Council, the Public Affairs advisory group of Biotechnology Research Council, and of the Advisory Board of the Institute of Nanotechnology.



## Christoph Brutschin

Christoph Brutschin (60) is Head of the Department of Economic, Social and Environmental Affairs of the Canton of Basel-Stadt since 2009, with the specialist fields of economics, of social insurance and social support and of environmental and energy policy. He represents the interests of the Canton within a variety of affiliated or-

ganisations, including: Member of the Board of EuroAirport Basel-Mulhouse, of MCH Group AG (exhibition centre) and of Swiss Rhine Ports. Since June 2016 Christoph Brutschin presides the Conference of the Cantonal Councillors for the Economy.



## Sara Busatto

Sara Busatto is a Ph.D. student in Prof. Bergese's Colloidal Clinical Chemistry Laboratory at the University of Brescia in Italy. Currently, she is also a visiting pre-doctoral student in Prof. Wolfram's Nanomedicine and Extracellular Vesicles group at Mayo Clinic in Florida (United States). In 2015, Busatto graduated from the Univer-

sity of Brescia in Italy with a master's degree in medical biotechnology. Her research focuses on exploring the targeting and drug delivery capacity of extracellular vesicles (EVs) *in vitro* and *in vivo*. She has several publications in the field of extracellular vesicles. Busatto's current research project involves studying the interface between EVs and biological membranes, especially focusing on the blood brain barrier. Her goal is to foster and contribute to the progress of personalized and biocompatible nanomedicine, translatable into more effective therapies for the treatment of life-threatening diseases.

### RECENT PUBLICATIONS

- C. Montis\*, S. Busatto\*, F. Valle, A. Zandrini, A. Salvatore, Y. Gerelli, D. Berti, P. Bergese. Biogenic supported lipid bilayers from nanosized extracellular vesicles. *Advanced Biosystems*, 2018: In press. \*Equal contribution
- L. Paolini, F. Orizio, S. Busatto, A. Radeghieri, R. Bresciani, P. Bergese, and E. Monti. Exosomes Secreted by HeLa Cells Shuttle on Their Surface the Plasma Membrane-Associated Sialidase NEU3. *Biochemistry* 2017;56(48):6401-6408.
- M. Berardocco, A. Radeghieri, S. Busatto, M. Gallorini, C. Raggi, C. Gissi, I. D'Agano, P. Bergese, A. Felsani, A. C. Berardi. RNA-seq reveals distinctive RNA profiles of extracellular vesicles from different human liver cancer cell lines. *Oncotarget*, 2017;8(47):82920-82939.



- C. Montis, A. Zendrini, F. Valle, S. Busatto, L. Paolini, A. Radeghieri, A. Salvatore, D. Berti and P. Bergese. Size Distribution of Extracellular Vesicles by Optical Correlation Techniques. *Colloids and Surfaces B: Biointerfaces*, 2017;158:331–338.
- L. Paolini, A. Zendrini, G. Di Noto, S. Busatto, E. Lottini, A. Dossi, A. Caneschi, D. Ricotta and P. Bergese. Residual matrix from different separation techniques impacts exosomes biological activity. *Scientific Reports*, 2016;6:23550.



## Luigi Calzolai

Ph.D.  
Project Leader, Joint Research Center of the European Commission

Dr. Calzolai obtained his M.Sc. in chemistry and his Ph.D. in chemistry from the University of Florence and the University of Siena, respectively.

After a Postgraduate Research at the University of California, Davis, he joined, in 1998, the Swiss Institute of Technology in Zurich, in the laboratory of the Nobel laureate Kurth Wuthrich, where he determined the 3D structure of prion proteins responsible of neurological disorders, such as Mad Cow Disease and Creutzfeld-Jacob disease.

In 2007 he moved to the School of Pharmacy of the University of Kent (UK) as Senior Lecturer in biochemistry.

In 2009 he joined the Joint Research Center of the European Commission where his research focuses on the development of methods for the detection and characterization of nanoparticles in complex matrices and nanomedicines. He is a member of the Core Expert Team of the European Union Nanomedicine Characterization Laboratory.

### PUBLICATION LIST AVAILABLE AT:

- Google Scholar (Calzolai Luigi): <https://scholar.google.com/citations?user=jgiELM8AAAAJ&hl=en>



## Aoneng Cao

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Prof. Cao is a full professor and the executive director of the Institute of Nanochemistry and Nanobiology, Shanghai University. He received the B. S. degree in Chemistry from Zhejiang University, China, in 1989, the M. S. degree in Material Sciences from Zhejiang University, China, in 1995, and the Ph. D. degree in Chemistry from Peking University, China, in 1998. After two years of postdoc training with Prof. Harold A. Scheraga at Cornell University, USA, he joined the faculty of the College of Chemistry and Molecular Engineering at Peking University, China, in 2000. He was a visiting associate professor at the Institute for Protein Research at Osaka University, Japan, in 2004. He moved to current institute in 2008.

Prof. Cao's research focuses on Nanomedicine and Nanobiotechnology, with a special emphasis on rational design of bio-functions on nanoparticles, as demonstrated by the creation of gold nanoparticle-based artificial antibody (denoted as Goldbody) through

conformational engineering. He has published over 70 peer-reviewed papers, and holds 10 patents.

### SELECTED PUBLICATIONS:

- G.-H. Yan, K. Wang, Z. Shao, L. Luo, Z.-M. Song, J. Chen, R. Jin, X. Deng, H. Wang, Z. Cao, Y. Liu and A. Cao. Artificial Antibody Created by Conformational Reconstruction of the Complementary-Determining Region on Gold Nanoparticles. *Proc. Natl. Acad. Sci. USA* 2018, 115(1), E34-E43.
- Y. Cao, Y. Cui, Y. Yang, J. Hua, Z.-M. Song, H. Wang, Y. Liu and A. Cao. Silica nanoparticle with a single His-tag for addressable functionalization, reversible assembly, and recycling. *Nano Research* 2018, DOI: 10.1007/s12274-017-1876-9.
- S.-T. Yang, Y. Liu, Y. Wang and A. Cao. Biosafety and bioapplication of nanomaterials by designing protein–nanoparticle interactions. *Small* 2013, 9, 1635-53.



## Fanny Caputo

Research Engineer

Dr. Caputo graduated in Materials Science (B.Sc. and M. Sc. in Materials Science and Technology), prior to achieve a PhD in “Materials for Health, Environment and Energy” in March 2015.

Dr. Caputo joined the Micro-technologies for Biology and Healthcare Division (DTBS) of CEA-LETI in July 2015 to head the physical-chemical characterization of medical nanoparticles within the European Nanomedicine Characterisation Laboratory (EU-NCL). She is currently focusing on the physical characterization of Med-NPs including particle size distribution, morphology, stability, particle concentration and NP-proteins interactions by multiple techniques including asymmetric field flow fractionation (AF4), and size exclusion chromatography coupled to static and dynamic light scattering, analytical ultracentrifugation, tunable resistive pulse sensing by and on Med-NPs imaging by electron microscopy.



## José M Carballido

Executive Director, Translational Research Fellow

José M. Carballido is an Executive Director at the Novartis Institutes for Biomedical Research (NIBR) in Basel (CH). José obtained his M. Sc. degree in Biology at the University of Barcelona (ES) in 1987 and,

after a short training in Immunology at the Pharmaceuticals Research Division of Ciba-Geigy, Basel, he entered the Swiss Institutes for Allergy and Asthma Research (SIAF) in Davos (CH), to work towards his Ph. D. (Dr. sc. Nat. for the University of Zurich, 1992). José spent one additional year at SIAF, as leader of the Allergy group and then he reached to Palo Alto, CA (US), to perform a Postdoctoral training at the DNAX Research Institute of Molecular and Cellular Biology. In 1997, José joined the Novartis Research Institute in Vienna (AT), where he held positions of increasing importance until he became the Head (Executive Director) of the Fully Integrated Program of Psoriasis. Following the closure of the Vienna site (2008), he was appointed to his current position at the Autoimmunity, Transplantation and Inflammation Disease Area of NIBR, in Basel. José has a strong background in basic and clinical immunology, particularly in the areas of Allergy, Dermatology, Transplantation and

Autoimmunity. José serves as ad-hoc reviewer for various scientific journals and private or governmental funds and he is an active member of several academic societies. He is author of over 60 scientific publications, has published 11 international patents and holds more than 20 inventions. José is currently focused on bringing to the clinic immune tolerance approaches, particularly using nanomedicines to ameliorate and cure type 1 diabetes.



## Cristiane Carvalho Wodarz

Senior Post Doc

I am a cellular microbiologist with interest on host-pathogen interactions. After the Ph.D. and one postdoc position in Brazil, working with intracellular infections, I moved to Germany for another postdoc position at the Helmholtz Institute for Infection Research, in Braunschweig. There I have studied mycobacterial infections in very challenging and exciting systems such as epithelial enucleated cells and primary human lymphatic endothelial cells. Since 2013 I am a senior postdoc at the Department of Drug Delivery, at HIPS, Saarbrücken, in the group of Prof. Claus Michael Lehr, working on the establishment of a 3D advanced lung *in vitro* models for drug delivery and bacterial infections, suitable to test new anti-infectives

### RECENT PUBLICATION

- Kletting, S, Barthold S, Repnik U, Griffiths G, Loretz B, Schneider-Daum N, de Souza Carvalho-Wodarz C, Lehr, CM. Co-culture of human alveolar epithelial (hAELVi) and macrophage (THP-1) cell lines. *ALTEX*. 2017;34(4). doi: 10.14573/altex.1607191. Epub 2017 Nov 23.
- Giovanni Monteiro Ribeiro, Cristiane Kayoko Matsumoto, Fernando Real, Daniela Teixeira, Rafael Silva Duarte, Renato Aruda Mortara, Sylvia Cardoso Leão, Cristiane de Souza Carvalho-Wodarz. Increased survival and proliferation of the epidemic strain *Mycobacterium abscessus* subsp. *massiliense* CRM0019 in alveolar epithelial cells. December 2017 *BMC Microbiology* 17(1):195 DOI: 10.1186/s12866-017-1102-7
- Murgja X, Yasar H, Carvalho-Wodarz C, Loretz B, Gordon S, Schwarzkopf K, Schaefer U, Lehr CM. Modelling the bronchial barrier in pulmonary drug delivery: A human bronchial epithelial cell line supplemented with human tracheal mucus. *Eur J Pharm Biopharm*. 2017 Sep;118:79-88. doi: 10.1016/j.ejpb.2017.03.020.
- Lerner TR, De Souza Carvalho-Wodarz C, Repnik U, Russell MR, Borel S, Diedrich CR, Rohde M, Wainwright H, Collinson LM, Wilkinson RJ, Griffiths G, Gutierrez MG. Lymphatic endothelial cells are a replicative niche for *Mycobacterium tuberculosis*. *J Clin Invest*. 2016 Mar 1; 126(3):1093-108. DOI: 10.1172/JCI83379.



## Luca Casettari

Luca Casettari is an Associate Professor and Principal Investigator, heading his group in Pharmaceutical Technology at the Department of Biomolecular Sciences in the University of Urbino (Italy).

From 2015 he is also co-founder and business development manager of the spin-off "Pharma & Food Consulting", an Italian

company working in the formulation technology field. Luca Casettari obtained a specialization in Hospital Pharmacy (2015) and a PhD in Chemistry and Pharmaceutical Sciences (2010), while in

2013 received the Braconnot Prize from the European Chitin Society (EUCHIS). From 2014 to 2015 he was appointed as Associate Researcher at the School of Pharmacy of the University of Nottingham (UK). His current research aims to develop novel drug delivery systems to facilitate absorption of drugs in various disease states through the overcoming of physiological barriers, and thus providing practical solutions for current healthcare issues. Moreover, his group synthesizes and characterizes novel biocompatible and biodegradable materials ranging from surfactants to polymers. These novel materials are then employed to formulate innovative pharmaceutical dosage forms. Since 2008, he has authored over 50 scientific publications in high impact multidisciplinary peer reviewed international journals.

Personal website: [www.pharmatech.uniurb.it](http://www.pharmatech.uniurb.it)

Twitter account: @PharmTechFabLab

Spin off website: <http://www.pharmafoodconsulting.com>



## Werner Cautreels

President and CEO

Prior to joining Selecta Biosciences in 2010, Dr. Cautreels was Chief Executive Officer of Solvay Pharmaceuticals, the pharmaceutical division of the Solvay Group, in Brussels, Belgium, from 2005 until Solvay Pharmaceuticals was acquired by Abbott Laboratories in 2010. Before becoming the CEO of Solvay Pharmaceuticals, Dr. Cautreels was its Global Head of R&D from 1998.

Prior to joining Solvay, he was employed by Sanofi, Sterling Winthrop from 1979 to 1994, and Nycomed Amersham from 1994 to 1998 in a variety of R&D management positions in Europe and in the United States. Dr. Cautreels is a director of Galapagos NV in Mechelen, Belgium. He held director positions at Seres Therapeutics, Inc. in Cambridge Massachusetts from 2013 to 2016, at Inrogenetics NV in Gent, Belgium from 1999 to 2006 and at ArQule Inc. in Woburn, Massachusetts each from 1999 to 2006. He was the President of the Belgian Luxemburg Chamber of Commerce for Russia and Belarus until June 2010. Dr. Cautreels received his Ph.D. in Chemistry, specializing in Mass Spectrometry, from the University of Antwerp (Antwerp, Belgium), and his financial and business training from the Advanced Management Program at Harvard Business School.



## Mark Chiu

6/2017 – present Associate Director, Analytical Resource Team, Janssen Pharmaceutical Development and Manufacturing Sciences, lead a team of 28 for analytical support for structure-function characterization for pre-NME, clinical trial samples, and commercial products and reduced turn-around-time 25% in 6 months. Coordinate co-qualification and co-validation, method preparation and transfers, process improvement. Support IND and BLA submissions and prepare for regulatory inspections

7/2013 – 6/2017 Associate Director, Janssen BioTherapeutics, Head of Biologics Engineering & Function that guides lead optimization and target validation for multispecific biologics using antibody and alternative scaffold platforms with Fc engineering for oncology, immunology, neuroscience, metabolic, and infectious diseases. Bridge projects within Discovery, Development, Clinical Pharmacology, Toxicology, Translational Research, and Commercial Divisions. Actively worked on a team to guide concept technical POC to NME

in 1 y, to FIH in 2.5

y. Working in teams that integrated multispecific technology that resulted in 9 NMEs in 3 y. Contributed to FDA filings of IND and BLA documentation. Designed experiments for patent defense.

## EDUCATION

Post Doc Microbiology, Biocenter of the University of Basel, 1994 - 1998

Bacterial Multiple Drug Resistance Transcriptional Activators and Membrane Proteins with Charles Thompson & Jürg Rosenbusch Physical Chemistry, Eidgenössische Technische Hochschule, 1992 - 1994

Solution State NMR Dynamics of Proteins with Richard Ernst

Ph.D. Biochemistry, University of Illinois at Urbana-Champaign, 1992

Thesis: Solution Structure and Dynamics of Sperm Whale Myoglobin and its Mutants with Stephen Sligar

## SELECTED REFERENCES

- Hinke<sup>1</sup>, S.A., Cieniewicz, A.M., Kirchner, T., D'Aquino<sup>1</sup>, K., Nanjunda, R., Aligo, J., Perkinson, R., Cooper, P., Boayke, K., Chiu, M.L., Jarantow, S., Lacy, E., Liang, Y., Johnson, D.L., Whaley, J.M., Lingham, R.B. and Kihm, A.J. (2018) "Unique Pharmacology of a Novel Allosteric Agonist/Sensitizer Insulin Receptor Monoclonal Antibody" *Mol. Metabolism* 10(18) 87-99
- Zhang, D., Whitaker, B., Derebe, M. & Chiu, M. (2018) "FcγRII-binding Centyrins mediate agonism and antibody dependent cellular phagocytosis when fused to an anti-OX40 antibody" *mAbs* Jan 23:1-13. doi: 10.1080/19420862.2018.1424611
- Goulet, D.R., Orcutt, S.J., Zwolak, A., Rispens, T., Labrijn, A.F., de Jong, R.N., Atkins, W.M., and Chiu, M.L. (2018) "Kinetic Mechanism of Controlled Fab-Arm Exchange for the Formation of Bispecific Immunoglobulin G1 Antibodies", *J Biol Chem.* 293(2):651-661. doi: 10.1074/jbc.RA117.000303
- Zwolak, A., Leettola, C., Tam, S.H., Goulet, D.R., Derebe, M., Pardinas, J.R., Emmell, E., Chiu, M.L. (2017) "Rapid Purification of Human Bispecific Antibodies via Selective Modulation of Protein A Binding", *Sci Rep.* 2017 Nov 14;7(1):15521. doi: 10.1038/s41598-017-15748-0
- Zwolak, A., Armstrong, A.A., Tam, S.H., Pardinas, J.R., Goulet, D.R., Zheng, S., Brosnan, K., Emmell, E., Luo, J., Gilliland, G.L., & Chiu, M.L. (2017) "Modulation of protein A binding allows single-step purification of mouse bispecific antibodies that retain FcRn binding" *MAbs.* 2017 Nov/Dec;9(8):1306- 1316. doi: 10.1080/19420862.2017.1375639



## Jeffrey D. Clogston

Principal Scientist, Physicochemical Characterization

Dr. Clogston joined the Nanotechnology Characterization Laboratory (NCL) as a Scientist in March 2006. In his position, Dr. Clogston conducts physicochemical characterization and standardization of nano-

particles intended for cancer therapeutics and diagnostics.

Dr. Clogston received his B.S. in chemical engineering from Manhattan College and his Ph.D. in chemical engineering from The Ohio State University. His research dissertation was on the application of the lipidic cubic phase for drug delivery, wastewater remediation, and membrane protein crystallization. His areas of expertise include physicochemical characterization of and *in vitro* release from lipid-based drug delivery systems, analytical methodology, and protein and lipid biochemistry.

## RECENT PUBLICATION

- Smith MC, Clogston JD. PEG Quantitation Using Reversed-Phase High-Performance Liquid Chromatography and Charged Aerosol

Detection, in Characterization of nanoparticles intended for drug delivery, S. McNeil, Editor. *Methods in Molecular Biology.* Vol. 1628, 2018, Humana Press, New York, NY. p. 49-55.

- Hansen MN, Clogston JD. Elemental Analysis in Biological Matrices Using ICP-MS, in Characterization of nanoparticles intended for drug delivery, S. McNeil, Editor. *Methods in Molecular Biology.* Vol. 1628, 2018, Humana Press, New York, NY. p. 37-47.
- Smith MC, Crist RM, Clogston JD, McNeil SE. Zeta potential: a case study of cationic, anionic, and neutral liposomes. *Anal Bioanal Chem.* 2017, 409(24), 5779-5787.
- Grossman, J.H., Crist, R.M., and Clogston, J.D., Early Development Challenges for Drug Products Containing Nanomaterials. *AAPS J.* 2017, 19(1), 92-102.
- Clogston, J. D.; Crist, R. M.; McNeil, S. E., Physicochemical characterization of polymer nanoparticles: Challenges and present limitations. in *Polymer nanoparticles: A Guide for Design, Preparation and Development as Nanomedicines.* Eds. Vauthier, C. and Ponchel, G. Springer International Publishing, Switzerland, 2016, 187-203.



## Patrick Couvreur

Patrick Couvreur is Full Professor of Pharmacy at the Paris-Sud University, member of the Académie des Sciences and holder of the chair of "Innovations Technologiques" (2009-2010) at the prestigious «Collège de France». He is appointed as a Senior Member of the "Institut Universitaire de France" since 2009. He is also

the recipient of an "ERC Advanced Grant" (2010–2015) and of an "ERC Proof of Concept" (2015–2016). He has held many important national and international academic positions as Director of the UMR CNRS 8612 (a CNRS associated department gathering together more than 120 researchers in the drug delivery field), Director of the Doctoral School "Therapeutic Innovation" (over 300 PhD students at Paris-Sud University), founder member of the pole of competitiveness MEDICEN, Extraordinary Professor at the University of Louvain (Belgium), member of the board of governors of many international scientific organizations (ie. The International Pharmaceutical Federation FIP, the Controlled Release Society CRS, the European Federation of Pharmaceutical Scientists, APGI etc.). He is the chair of the LS-7 panel of the European Research Council (ERC consolidator grant) and has served in many scientific committees in France (Institut Pasteur, ENS Cachan, Academic Council of Paris-Saclay University, Scientific Committee of the Région Centre, Comité National of the CNRS, Conseil National des Universités CNU etc.). Prof Patrick Couvreur's contributions in the field of drug delivery, nanomedicine and drug targeting are highly recognized around the world with more than 520 peer review research publications (Google Scholar H-index 120 and Thomson Reuters H-index 89), some of them in prestigious journals (*Nature Nanotechnology*, *Nature Materials*, *Nature Communications*, *Proceedings of the National Academy of Sciences*, *Angewandte Chemie*, *Cancer Research*, *Journal of the American Chemical Society* etc.). His research is interdisciplinary, aiming at developing new nanomedicines for the treatment of severe diseases. This research is at the interface between Physico-Chemistry of Colloids, Polymer Chemistry, Material Science, Cellular and Molecular Biology and Experimental Pharmacology.

Patrick Couvreur's research has led to the funding of two start-up companies (Bioalliance and Medsqual). Bioalliance (now ONXEO) entered the stock market in 2005 and a nanomedicine invented in Couvreur's lab is currently finishing phase III clinical trial for the treatment of the hepatocarcinoma.

The major scientific contribution of Patrick Couvreur to the Pharmaceutical Sciences is also recognized by numerous international (the "2004 Pharmaceutical Sciences World Congress Award", the prestigious "Host Madsen Medal", the "European Pharmaceutical Scientist Award" of the European Federation of Pharmaceutical Sci-



ences, the European Inventor Award 2013 given by the European Patent Office, the Speiser's award from ETH and the Higuchi Award 2015, Japan) and national awards (The Grand Prix de l'Innovation of «L'USINE NOUVELLE» 2008, the "Prix Galien 2009" and the "Médaille de l'Innovation 2012 of the CNRS). His appointment as a member of eight academies (Académie des Sciences, Académie des Technologies, Académie Nationale de Médecine and Académie Nationale de Pharmacie in France, as well as the Académie Royale de Médecine in Belgium, the Royal Academy of Pharmacy in Spain, the United States National Academy of Medicine and the United States National Academy of Engineering) is another recognition of major scientific and scholarly contributions of Patrick Couvreur.



## Daan J.A. Crommelin

PhD

Prof. Daan Crommelin is professor emeritus from the Department of Pharmaceutics at Utrecht University. Until December 2011 he was scientific director of the Dutch Top Institute Pharma – a public private partnership - in Leiden. He is adjunct

professor at the Department of Pharmaceutics and Pharmaceutical Chemistry at the University of Utah. Crommelin is co-founder of OctoPlus, a Leiden based company specialized in the development of pharmaceutical (mainly protein based) product formulations and advanced drug delivery systems. He published extensively and edited a number of books. He was Editor-in-Chief of the AAPS book series 'Advances in the Pharmaceutical Sciences'. He advises venture capital groups and acts as a consultant for several big pharma companies and SME's. He chaired the Board of the UCAB Foundation: the Utrecht Center of Excellence for Affordable Biotherapeutics, a WHO supported initiative and the Board of Pharmaceutical Sciences of the International Pharmaceutical Federation (F.I.P.). He was chair of the organizing committee of the Pharmaceutical Sciences World Conference 2007 in Amsterdam. He is past president of the European Federation of Pharmaceutical Sciences (EUFEPS) and past vice-chair of the scientific advisory board of the European Innovative Medicines Initiative (IMI).



## Bruno De Geest

Prof. Dr. Ir. Bruno De Geest graduated as Chemical Engineer in 2003 from Ghent University where he obtained his PhD in pharmaceutical sciences in 2006 on 'Poly-electrolyte Multilayer Capsules for Pharmaceutical Applications'. For his PhD work he was awarded the graduate student award for pharmaceutical technology

from the AAPS and the Andreas Deleenheer award from Ghent University. After 2 years of postdoctoral research at Utrecht University (The Netherlands) he returned to Ghent University at the Laboratory of Pharmaceutical Technology. From October 2012 onwards he is appointed as professor in Biopharmaceutical Technology. His research focuses on strategies to engineer the immune system via a materials chemistry approach. His group has is active in applying polymer chemistry and nanotechnology to develop interactive materials that can guide and modulate the immune system. We are applying this approach mainly for anti-cancer therapy, but also have a strong interest in developing nanoparticle vaccines and drug delivery systems.



## Jon de Vlieger

PhD

Lygature – Non Biological Complex Drugs Working Group

Dr. Jon de Vlieger, obtained his doctoral degree in bio analytical chemistry from the VU University in Amsterdam. In 2011 he joined Lygature (former Top Institute Pharma), an independent not-for-profit

organization based in the Netherlands that catalyzes the development of new medical solutions for patients by driving public-private collaboration between academia, industry, and society. Dr. de Vlieger coordinates several international public private partnerships, including the Non Biological Complex Drugs Working Group, an international network of scientific and clinical experts from academia, industry and regulatory bodies, with expertise in many aspects of the development and evaluation of NBCDs. He is a co-editor of the book on NBCDs in the AAPS Advances in the Pharmaceutical Sciences Series and is co-author on a series of key-papers on the topic of regulatory challenges for NBCDs.



## Paolo Decuzzi

Senior Researcher and Professor of Biomedical Engineering

Paolo Decuzzi is a senior researcher and director of the Laboratory of Nanotechnology for Precision Medicine at the Italian Institute of Technology in Genova – Italy. Dr. Decuzzi earned his M.Sc. degree in Mechanical Engineering from the Politecnico di Bari (Italy) in 1997 and his Ph.D. degree from the University of Naples – Federico II (Italy) in 2000, with a thesis on friction and adhesion at the nanoscale.

In 2002, he was nominated Assistant Professor at the Politecnico di Bari and, in 2005, he became Associate Professor in the School of Medicine of the University 'Magna Graecia'. In October 2007, he joined The University of Texas Health Science Center in Houston as an Associate Professor of Biomedical Engineering. In October 2010, he moved to the Houston Methodist Hospital where he served as a Professor of Biomedical Engineering till July 2015. There, he founded the Center for the Rational Design of Multifunctional Nanoconstructs and served first as the co-chair of the Nanomedicine Dept and then as the interim chair of the Translational Imaging Dept. In July 2014, Dr. Decuzzi was awarded a 5-year European Research Council "Consolidator Grant" to design, synthesize and develop nanoconstructs for imaging and therapy in brain cancer. Dr. Decuzzi has been visiting scientist at the University of Michigan - Ann Arbor (1998-1999 and 2001); and visiting professor at the Princeton Material Institute – Princeton (2003); Ohio State University (2003 and 2004); University of Texas Health Science Center (2006). Dr. Decuzzi has published over 150 papers in international journals, conferences and books. He holds over 5 patents in the field of Nanomedicine. He is involved in multiple dissemination activities to foster the collaboration between medicine and engineering. He serves on multiple study sections for NIH, NSF, ESF, Italian Government, EuroNanoMed and ERC. His research activity is primarily supported by ERC, AIRC and MSCA in EU. Decuzzi's lab mission is to rationally design polymeric nanoconstructs for multimodal imaging and combination therapy in cancer, cardiovascular and neurological diseases; fabricate microfluidic chips for the rapid screening of novel molecular and nano-based therapeutic agents; develop multi-scale, hierarchical computational models; organize dissemination activities at the interface between engineering and medicine, and promote the professional development of lab members in a multidisciplinary environment.

- Di Francesco, M., ... D., Decuzzi, P. Hierarchical Microplates as Drug Depots with Controlled Geometry, Rigidity, and Therapeutic Efficacy (2018) *ACS Applied Materials and Interfaces*, 10 (11), pp. 9280-9289.
- Palomba, R., Palange, A.L., ... Decuzzi, P. Modulating Phagocytic Cell Sequestration by Tailoring Nanoconstruct Softness (2018) *ACS Nano*, 12 (2), pp. 1433-1444.
- Stigliano, C., ... Decuzzi, P. Methotrexate-Loaded Hybrid Nanoconstructs Target Vascular Lesions and Inhibit Atherosclerosis Progression in ApoE<sup>-/-</sup> Mice (2017) *Advanced Healthcare Materials*, 6 (13), art. no. 1601286.
- Aryal, S., ... Decuzzi, P. Paramagnetic Gd<sup>3+</sup> labeled red blood cells for magnetic resonance angiography (2016) *Biomaterials*, 98, pp. 163-170.
- Key, J., Palange, A.L., ... Decuzzi, P. Soft Discoidal Polymeric Nanoconstructs Resist Macrophage Uptake and Enhance Vascular Targeting in Tumors. (2015) *ACS Nano*, 9 (12), pp. 11628-11641.



## Heleen Dewitte

Post-doctoral Fellow

Heleen Dewitte (°1987) obtained her master's degree in pharmaceutical sciences – drug development at Ghent University (Belgium) in 2010. In 2015, she obtained her PhD in Pharmaceutical and Medical Sciences at both Ghent University (UGent)

and the Vrije Universiteit Brussels (VUB) under the joint mentorship of Prof. dr. Stefaan De Smedt and Prof. dr. Karine Breckpot.

Her thesis, entitled “design and evaluation of immunotherapeutic imaging for cancer vaccination”, was awarded the 2017 national thesis award of the Belgian Society for Pharmaceutical Sciences (BSPS). After her PhD, she obtained a scholarship to continue her research on the development of nanomaterial- and ultrasound-based systems to achieve immunization against cancer.

Her research was awarded several times, among which the “Therapeutic use of microbubbles” oral presentation award (Rotterdam, The Netherlands) and the Jan Feijen poster prize at the 13th European symposium on controlled drug delivery (Egmond aan Zee, The Netherlands).

In February 2018, Heleen was inaugurated as a member of the Flemish Young Academy for Science and Arts. Moreover, she is active in the field of science communication, bringing scientific themes such as cancer immunotherapy and ultrasound-based therapies to a broader audience (both adults and children) on many different occasions.

### RECENT PUBLICATION

- Rein Verbeke, Ine Lentacker, Laura Wayteck, Karine Breckpot, Mieke Van Bockstal, Benedicte Descamps, Christian Vanhove, Stefaan C. De Smedt, Heleen Dewitte, Co-delivery of nucleoside-modified mRNA and TLR agonists for cancer immunotherapy: Restoring the immunogenicity of immunosilent mRNA. *Journal of Controlled Release*, 266C (2017) p287-300.
- Laura Wayteck, Heleen Dewitte, Lynn De Backer, Karine Breckpot, Jo Demeester, Stefaan C. De Smedt, Koen Raemdonck, Hitchhiking Nanoparticles: Reversible Coupling of Lipid-Based Nanoparticles to Cytotoxic T Lymphocytes. *Biomaterials*, 77 (2016) 243-254.
- Heleen Dewitte, Rein Verbeke, Karine Breckpot, Stefaan C. De Smedt, Ine Lentacker, Nanoparticle design to induce tumor immunity and oppose the immunosuppressive tumor microenvironment, *Nano Today*, 9(6) (2014) p743-758.
- Heleen Dewitte, Katrien Vanderperren, Hendrik Haers, Emmelie Stock, Luc Duchateau, Jimmy H. Saunders, Stefaan C. De Smedt, Ine Lentacker, Theranostic mRNA-loaded microbubbles in the lymphatics of dogs: implications for drug delivery, *Theranostics*, 5 (2015) 97-109 .
- Heleen Dewitte, Sandra Van Lint, Carlo Heirman, Kris Thielemans,

Stefaan C. De Smedt, Karine Breckpot, Ine Lentacker, The potential of antigen and TriMix sonoporation using mRNA-loaded microbubbles for ultrasound-triggered cancer immunotherapy. *Journal of Controlled Release*. 194 (2014) 28-36.



## László Dézsi

PhD, DrHabil, Senior Research Associate, Nanomedicine Research and Education Center, Institute of Pathophysiology, Semmelweis University, Budapest, Hungary (*in vivo* animal laboratory).

He obtained his MSc degree in biology at Eötvös Loránd University and his PhD in physiology at Semmelweis University Medical School, Budapest, Hungary. He conducted teaching and research activities at Semmelweis University (1981-1999), and meanwhile he received fellowships at Albert Ludwigs Universität, Freiburg, Germany working in the field of local regulation of blood flow in skeletal and cardiac muscle studying nitric oxide; and at the University of Pennsylvania, Philadelphia, USA, Cerebrovascular Research Center working in the field of cerebral blood flow and metabolism as well as cerebral ischemia and reperfusion in animal stroke models. He had been head of laboratory, CRO monitor and research project manager in vascular and safety pharmacology at Gedeon Richter (GR) Pharmaceutical Plc. (1999-2012). He was manager of Analgesic Research Laboratory (2006-2012), a joint venture of GR and University of Pécs, Department of Pharmacology. He was involved in curriculum development and had been Secretary of Biomedical Engineering (BE) Course Committee (1994-2000), currently member of the MSc BE Committee at Technical University, Budapest. He made his habilitation at Semmelweis University in 2005 and became Adjunct Professor (PD) of physiology in 2006. He established his own teaching course in 2008 entitled “Cardiorespiratoric and neurophysiological measuring techniques” at the Department of Clinical Experimental Research. He participates in postgraduate education in nanomedicine. He is a physiology and pathophysiology teacher at Semmelweis University Medical and Health Faculties. Currently he is working at the Nanomedicine Research and Education Center (2012-) in the field of nanomedicine investigating cardiopulmonary and immunological effects of nanoparticles in various *in vivo* models of complement activation related pseudoallergy (CARPA) and participates in the development of new models. He has been working as a member of the EU FP7 “NanoAthero” Consortium (2013-2018).

### RECENT PUBLICATIONS

- Unterweger H , Dézsi L , Matuszak J , Janko C , Poettler M , Jordan J , Bäuerle T , Szebeni J , Fey T , Boccaccini A R , Alexiou C , Cicha I. Dextran-coated superparamagnetic iron oxide nanoparticles for magnetic resonance imaging: Evaluation of size-dependent imaging properties, storage stability and safety. *INTERNATIONAL JOURNAL OF NANOMEDICINE* 13: pp. 1899-1915. (2018)
- Raffai G , Csekő C , Nádasy GL , Kocsis L , Dézsi L , Hunyor S , Monos E. Environmental stress and vestibular inputs modulate cardiovascular responses to orthostasis in hypertensive rats. *HYPERTENSION RESEARCH* 41: pp. 18-26. (2018)





## Amit Kumar Dinda

Dr. Amit K. Dinda is a Professor in Department of Pathology, at All India Institute of Medical Sciences, New Delhi. He graduated (MBBS) from Calcutta Medical College, Calcutta. He did his post-graduation (MD) in Pathology at All India Institute of Medical Sciences, New Delhi followed by PhD in the same institute. He is officer in-charge

of Division of Renal & Immunopathology. He has keen interest in experimental pathology with interdisciplinary research in immunopathology, tissue engineering, cancer biology and Nanomedicine. For the past five years he is actively working in the area of application of Nanotechnology in Medicine and Nanotoxicology. His laboratory has developed nanoparticle based oral gene delivery system and nanoadjuvant for developing single shot booster free vaccine. The on-going projects include development of novel nanocarriers for Amphotericin B, technology for delivering 4 antitubercular drugs in one nanoparticle, nanoadjuvant for single shot booster free vaccine for Hepatitis B, macrophage targeted siRNA delivery system for reversal of Atherosclerosis plaque, combined chemo and photothermal therapy for superficial bladder cancer, study of long term fate and toxicity of gold nanoparticle, novel antioxidant collagen nanoparticle for cosmetics and wound healing. He is actively involved in collaborative groups for developing marketable dermal supplement, genetically engineered human serum albumin, extracellular matrix spray as a first aid device for superficial wound and burn.

His laboratory is interested for in-depth study in the area of Nanocell biology. Cellular interaction of nano particles starting from endocytosis, endosomal maturation, intracellular metabolism and fate are major areas of focus which will give the capability of sub-cellular targeting. This will also help to explore the area of Nanotoxicology. He has published more than 350 articles in indexed journal, edited 4 books, written 14 chapters in books and filed 6 patents. He is President of Indian Society of Renal & Transplant Pathology, Founder Secretary of Indian Society of Nanomedicine, Ex Vice President of Electron Microscopy Society of India and Ex-Vice President of Society of Tissue Engineering & Regenerative Medicine (India). He is an assessor for NABL (Laboratory Accreditation), GLP (Good Laboratory Practice) & GCP (Good Clinical Practice) systems. He is an active member of several scientific advisory and policy making committees of Government of India. He worked as a Visiting Professor in Molecular Nephrology at Long Island Jews Medical Center & Albert Einstein Medical College, New York, USA and Dept. of Biomedical Engineering at University of New South Wales, Sydney, Australia. Website: [www.akdinda.com](http://www.akdinda.com)

### RECENT PUBLICATION

1. A V T, Dinda AK, Koul V. Evaluation of nano hydrogel composite based on gelatin/HA/CS suffused with Asiatic acid/ZnO and CuO nanoparticles for second degree burns. *Mater Sci Eng C Mater Biol Appl*. 2018 Aug 1;89:378-386.
2. Mohanty S, Jain KG, Nandy SB, Kakkar A, Kumar M, Dinda AK, Singh H, Ray A. Iron oxide labeling does not affect differentiation potential of human bone marrow mesenchymal stem cells exhibited by their differentiation into cardiac and neuronal cells. *Mol Cell Biochem*. 2018 Feb 15. doi: 10.1007/s11010-018-3309-9
3. Quadri JA, Sarwar S, Sinha A, Kalaivani M, Dinda AK, Bagga A, Roy TS, Das TK, Shariff A. Fluoride-associated ultrastructural changes and apoptosis in human renal tubule: a pilot study. *Hum Exp Toxicol*. 2018 Jan 1;960327118755257. doi: 10.1177/0960327118755257.
4. Barwal I, Kumar R, Kateriya S, Dinda AK, Yadav SC. Targeted delivery system for cancer cells consist of multiple ligands conjugated genetically modified CCMV capsid on doxorubicin GNPs complex. *Sci Rep*. 2016 Nov 22;6:37096.
5. Dinda AK, Bhat M, Srivastava S, Kottarath SK, Prashant CK. Novel nanocarrier for oral Hepatitis B vaccine. *Vaccine*. 2016 Jun 8;34(27):3076-3081.



## Alexander Eggermont

Alexander M.M. Eggermont, Director General of Gustave Roussy Comprehensive Cancer Center, Cancer Campus Grand Paris, France

Full Professor of Oncology (classe exceptionnelle) (2012-20) at the Paris-Sud University in Paris, France. Full Professor of Surgical Oncology (2003-2016) as well as

Endowed Professor of International Networking in Cancer Research (2011-2018) at Erasmus University MC Rotterdam, Netherlands. Joseph Maisin Chair in Oncology at Catholic University of Louvain in Belgium. PhD in tumor immunology at Erasmus University Rotterdam (1987) and Fellow of the NCI Surgery Branch (head: S.A. Rosenberg), Bethesda, USA

Specialties: Clinical specialties include immunotherapy, melanoma, sarcoma, general drug development. Basic research: tumor immunology, advanced imaging models and tumor patho-physiology. Scientific Output: author or co-author of > 800 peer-reviewed publications; 36 PhD theses from research programs@Erasmus University MC. H-index: 90; > 36000 citations (Scopus)

### INTERNATIONAL FUNCTIONS

- President European Academy of Cancer Sciences
- President Cancer Core Europe (Gustave Roussy – Cambridge Cancer Center - NKI Amsterdam- Vall d'Hebron Institute of Oncology- Istituto Nazionale Tumori de Milano- Karolinska Institutet – DKFZ/ NCT Heidelberg)
- ECCO: Past President of ECCO (the European Cancer Organization)
- EORTC: Past President of EORTC (European Organisation for Research and Treatment of Cancer), past Chairman of the Melanoma Group
- ASCO: Past Member Board of Directors of ASCO (Surgery Chair) and JCO Editorial Board
- AACR: Past Deputy Editor of Clinical Cancer Research
- Deutsche Krebshilfe: Chair International Jury for Comprehensive Cancer Centres Program
- FNCLCC/Unicancer: Vice-President French Federation of Cancer Centers, Chair Research
- ESMO Executive Board since 2014
- Member Board of Trustees CRUK since 2016
- Member (oncology) Wissenschaftsrat (DFG), Germany since 2017
- European Journal of Cancer: Editor-in-Chief since 01-01-2011

### SOCIETAL AWARDS:

- Légion d'Honneur, France (2015)
- Presidential Medal, Kazakhstan (2014)



## Adrian Egli

Head of Department Clinical Microbiology, University Hospital Basel

After studying medicine at the University of Basel (MD 1998 - 2004, PhD thesis 2006 - 2008) at the University of Basel, I went abroad for a Clinical Fellowship "Transplant Infectious Disease" as well as a Post-doctoral fellowship, Li Ka Shing Institute for Virology (2010 - 2011), both

at the University of Alberta, Canada. Back in Switzerland, I became a fellow in Clinical Microbiology (FAMH) (2012 - 2015) at the University Hospital of Basel. During the same time (11/2014 - to date) I became Research Group Leader Applied Microbiology Research" Laboratory in the Department of Biomedicine of the University of Basel.

From September 2015 up to the present time, I am holding the position as Head of Department, Clinical Microbiology at the University Hospital of Basel. The following activities and topics focus my main interest and drive me forward constantly: The rapid detection of pathogens is an important first step in the work-up of a pathogen. Pathogen evolution is dependent on a series of complex factors such as host and bacterial factors. The recent technological advances allow rapid identification based on molecular and protein profiles e.g. via PCR or mass-spectrometry. In addition, sequencing of the whole genome of pathogens and metagenomic approaches at the highest resolution allows us to explore this fascinating complexity. My main aims are: (i) to develop new diagnostic for rapid detection of multidrug resistant and virulent pathogens; (ii) to explore novel typing technologies such as whole genome sequencing (including long reads e.g. Pacbio, Minion) and MALDI-TOF mass spectrometry for clinical applications; (iii) to combine phylogenetic tree structures on single bacterial colony levels from culture but also metagenomic approaches directly from patient samples using computational models; (iv) to understand pathogens evolution within the host (e.g. during antibiotic treatment); (v) to finally understand pathogen evolution in the broad context of the host/pathogen/environment interaction. This could lead to the identification of the most critical factors for pathogenicity and resistance development. Such information will allow the generation of novel intervention strategies to impact disease outcomes for a single patient but also the population burden of infections.

## RECENT PUBLICATION

- Evaluation of the rapid biochemical  $\beta$ -CARBA™ test for detection of carbapenemase-producing Gram-negative bacteria. Hinic V, Reist J, Egli A. *J Microbiol Methods*. 2018 Jan;144:44-46. doi: 10.1016/j.mimet.2017.10.008. Epub 2017 Oct 19.
- Burkholderia stabilis outbreak associated with contaminated commercially-available washing gloves, Switzerland, May 2015 to August 2016. Sommerstein R, Führer U, Lo Priore E, Casanova C, Meinel DM, Seth-Smith HM, Kronenberg A, On Behalf Of Anresis, Koch D, Senn L, Widmer AF, Egli A, Marschall J, On Behalf Of Swissnoso. *Euro Surveill*. 2017 Dec;22(49). doi: 10.2807/1560-7917.ES.2017.22.49.17-00213.
- Associations among Antibiotic and Phage Resistance Phenotypes in Natural and Clinical Escherichia coli Isolates. Allen RC, Pfrunder-Cardozo KR, Meinel D, Egli A, Hall AR. *MBio*. 2017 Oct 31;8(5). pii: e01341-17. doi: 10.1128/mBio.01341-17.
- Impact of MALDI-TOF-MS-based identification directly from positive blood cultures on patient management: a controlled clinical trial. Osthoff M, Gürtler N, Bassetti S, Balestra G, Marsch S, Pargger H, Weisser M, Egli A. *Clin Microbiol Infect*. 2016 Aug 26. pii: S1198-743X(16)30329-9. doi: 10.1016/j.cmi.2016.08.009. [Epub ahead of print]
- Outbreak investigation for toxigenic Corynebacterium diphtheriae wound infections in refugees from Northeast Africa and Syria in Switzerland and Germany by whole genome sequencing. Meinel DM, Kuehl R, Zbinden R, Boskova V, Garzoni C, Fadini D, Dolina M, Blümel B, Weibel T, Tschudin-Sutter S, Widmer AF, Bielicki JA, Dierig A, Heining U, Konrad R, Berger A, Hinic V, Goldenberger D, Blaich A, Stadler T, Battegay M, Sing A, Egli A. *Clin Microbiol Infect*. 2016 Dec;22(12):1003.e1-1003.e8. doi: 10.1016/j.cmi.2016.08.010.

Health in 2003-2005. Dr. Elnkave completed radiology training at Albert Einstein Medical Center and trained in Vascular and Interventional Radiology at Memorial Sloan-Kettering Cancer Center. His clinical and research focus is on the combination of anatomical and molecular targeting to treat disease in the most precise and minimally invasive method possible. Dr. Elnkave also serves as the founding Chief Medical Officer of Zebra Medical Vision, LTD.



## Alke Fink

Professor BioNanomaterials, Adolphe Merkle Institute, University of Fribourg

Prof. Alke Fink received her Ph.D. in Chemistry from the University of Ulm, Germany in 1999. After a post-doctoral stay at the University of Gainesville, Florida, she joined the Institute of Materials Science at the École Polytechnique Fédérale de Lausanne (EPFL), first as a post-doctoral researcher, then as a senior scientist. She became an Associate Swiss National Science Foundation Professor in the Department of Chemistry at the University of Fribourg in 2009, and Full Professor in 2011 at the Adolphe Merkle Institute, Switzerland. Her research focuses on inorganic nanoparticles, their synthesis, surfaces, and interactions with biological cells.

## RECENT PUBLICATIONS

- Characterizing nanoparticles in complex biological media and physiological fluids with depolarized dynamic light scattering, S. Balog, L. Rodriguez-Lorenzo, C. A. Monnier, M. Obiols-Rabasa, B. Rothen-Rutishauser, P. Schurtenberger, A. Petri-Fink, *Nanoscale* 7, 5991-5997 (2015)
- Nanoparticle colloidal stability in cell culture media and impact on cellular interactions, Thomas L. Moore, Laura Rodriguez-Lorenzo, Vera Hirsch, Sandor Balog, Dominic Urban, Corinne Jud, Barbara Rothen-Rutishauser, Marco Lattuada, Alke Petri-Fink, *Chemical Society Reviews* 44, 6287-6305 (2015)
- Surface charge of polymer coated SPIONs influences the serum protein adsorption, colloidal stability and subsequent cell interaction *in vitro*, Vera Hirsch, Calum Kinnear, Marc Moniatte, Barbara Rothen-Rutishauser, Martin J. D. Clift, Alke Fink, *Nanoscale* 5, 3723 (2013)
- Form Follows Function: Nanoparticle Shape and Its Implications for Nanomedicine, Calum Kinnear, Thomas L. Moore, Laura Rodriguez-Lorenzo, Barbara Rothen-Rutishauser, Alke Petri-Fink, *Chemical Reviews* 117, 11476-11521 (2017)



## Flavia Fontana

Flavia Fontana (MSc. Pharm.) is a graduate student in Associate Professor Santos' lab, under the Drug Research Program, at the Faculty of Pharmacy, University of Helsinki (Finland). She obtained her Master's Degree in Medicinal Chemistry and Pharmaceutical Technology at the University of Pavia (Italy) in 2014. Her main research

interests are the development of innovative nanovaccines, immunotherapy, biohybrid nanosystems, and microfluidics.

## RECENT PUBLICATIONS

- Immunostimulation and Immunosuppression Nanotechnology on the Brink; Fontana, F.; Figueiredo, P.I.; Correia, A.; Bauleth-Ramos, T.; Santos, H.A. *Small Methods*, in press



## Eldad Elnkave

Eldad Elnkave, MD serves as the director of the Clinic for Interventional Oncology at the Davidoff Cancer Institute, Rabin Medical Center, Israel. He obtained his medical degree from Tufts University in Boston, Massachusetts and spent two years as a Howard Hughes Medical Institute Research Scholar at the National Institutes of

- Tailoring Porous Silicon for Biomedical Applications: from Drug Delivery to Cancer Immunotherapy; Li, W.; Liu, Z.; Fontana, F.; Ding, Y.; Liu, D.; Hirvonen, J.T.; Santos H.A. *Advanced Materials*, in press
- Nutlin-3a and Cytokine Co-loaded Spermine-Modified Acetalated Dextran Nanoparticles for Cancer Chemo-Immunotherapy; Bauleth-Ramos, T.; Shahbazi, M.A.; Liu, D.; Fontana, F.; Correia, A.; Figueiredo, P.; Zhang, H.; Martins, J.P.; Hirvonen, J.T.; Granja, P.; Sarmiento, B.; Santos, H.A. *Advanced Functional Materials*, 2017, 27 (42)
- Multistage Nanovaccines based on Thermally Oxidized Porous Silicon@Acetalated Dextran@Cancer Cell Membrane for Cancer Immunotherapy; Fontana, F.; Shahbazi, M.A.; Liu, D.; Zhang, H.; Mäkilä, E.; Salonen, J.; Hirvonen, J.T.; Santos, H.A. *Advanced Materials*, 2017, 29, 1603239
- Biomimetic Engineering Using Cancer Cell Membranes for Designing Compartmentalized Nanoreactors with Organelle-like Functions; Balasubramanian, V.; Correia, A.; Zhang, H.; Fontana, F.; Mäkilä, E.; Salonen, J.; Hirvonen, J.; Santos, H.A. *Advanced Materials*, 2017, 29, 1605375



## Ronit Freeman

Associate Professor, Department of Applied Physical Sciences, University of North Carolina Chapel Hill

### EDUCATION/TRAINING

- Bar-Ilan University, Ramat-Gan, Israel **B.Sc. 2005 Chemistry**
- Bar-Ilan University, Ramat-Gan, Israel, **B.Sc. 2005 Computer Science**
- The Hebrew University of Jerusalem, Israel **M.Sc. 2008 Chemistry**
- The Hebrew University of Jerusalem, Israel **Ph.D. 2013 Chemistry**
- Northwestern University Postdoctoral **2017 Chemistry/Biomaterials Science**

### POSITIONS AND EMPLOYMENT

2018 - present: **Associate Professor, Department of Applied Physical Sciences, University of North Carolina**  
 2013 - 2017 **Postdoctoral researcher at Northwestern University, laboratory of Samuel I. Stupp**

### RECENT PUBLICATION

- *Nature Communications*, 8:15982 (2017)
- *MRS Bull.* 40:1089-1101 (2015)
- *Nano Letters*, 15, 603-609 (2015)
- *Chem. Soc. Rev.*, 2012, 41, 4067-4085
- *J. Am. Chem. Soc.*, 2011, 133, 11597-11604



## Lisa E. Friedersdorf

PhD  
 Dr. Lisa Friedersdorf is the Director of the National Nanotechnology Coordination Office. She has been involved in nanotechnology for over twenty-five years, with a particular interest in advancing technology commercialization through university-industry-government collaboration. She is

also a strong advocate for science, technology, engineering, and mathematics (STEM) education, and has over two decades of experience teaching at both the university and high school levels. While at the NNCO, Lisa has focused on building community and

enhancing communication in a variety of ways. With respect to coordinating research and development, her efforts have focused on the Nanotechnology Signature Initiatives in areas including nanoelectronics, nanomanufacturing, informatics, sensors, and water. A variety of mechanisms have been used to strengthen collaboration and communication among agency members, academic researchers, industry representatives, and other private sector entities, as appropriate, to advance the research goals in these important areas. Lisa also led the establishment of a suite of education and outreach activities reaching millions of students, teachers, and the broader public. She continues to expand the use of targeted networks to bring people together in specific areas of interest, including the Nano and Emerging Technologies Student Network and the U.S.-EU Communities of Research focused on the environmental, health, and safety aspects of nanotechnology. Nanotechnology entrepreneurship and nanomedicine are areas where new communities of interest are developing.

Prior to joining the NNCO, Lisa served in a number of roles at the intersection of academia, industry, and government. At Lehigh University, Lisa served as Associate Director of the Materials Research Center (now the Center for Advanced Materials and Nanotechnology) and Director of the industry liaison program where she oversaw dozens of membership programs and was responsible for developing and coordinating multi-investigator interdisciplinary research programs including a multimillion-dollar public-private partnership in microelectronics. As Director of the Virginia Nanotechnology Initiative, she led an alliance of academic institutions, industry, and government laboratories with an interest in nanotechnology across the Commonwealth of Virginia. At the University of Virginia, she served as Managing Director of the nanoSTAR Institute and led the development of pan-university initiatives as a Program Manager in the Office of the Vice President for Research. Additionally, Lisa has been active in the start-up ecosystem assisting small companies with business development and access to resources and as an advisor to vet emerging technologies for investors.

Lisa earned her PhD and MSE in Materials Science and Engineering from the Johns Hopkins University and BS in Mechanical Engineering from the University of Central Florida.



## Heico Frima

Heico Frima obtained his Masters Degree in Applied Physics from the Technical University of Delft in 1980 and then worked in various R&D and product management functions in the semiconductor equipment industry. Since 1990 he is Programme Officer in the Directorate-General for Research & Innovation of the European

Commission in Brussels in the field of micro-technology and then from 2002 as Programme Officer for nanoscience and nanotechnology. As Programme Officer he contributes to programme policy development, the organisation of research proposal evaluations, contract negotiations and follow-up of research projects that are funded by the European Commission. Presently he is responsible for research policy in the field of nanomedicine, working in the Unit 'Advanced Materials and Nanotechnology'. Heico has the Dutch nationality, is married and has two children.





## Gregor Fuhrmann

Head of Research Group Biogenic Nanotherapeutics at the Helmholtz-Institute for Pharmaceutical Research

Dr Gregor Fuhrmann received his PhD in 2012 from ETH Zurich in Pharmaceutical Sciences. His doctoral thesis was awarded the "ETH Medal" and the "Rottendorf Europe-Award" for excellent pharmaceutical research. From 2013 to 2016 he was a Marie Curie Intra-European Fellow and a German Academic Exchange Service postdoc at Imperial College London, Department of Bioengineering. Since 2016, Gregor is Head of the Research Group „Biogenic Nanotherapeutics“ (BION) at the Helmholtz-Institute for Pharmaceutical Research Saarland. His research is supported by the young investigators programme NanoMatFutur from the German Federal Ministry of Education and Research.

Gregor is a founder and a member of board of the German Society for Extracellular Vesicles (gsev.org). In August 2017 he received the prestigious "Technology Award" from the Galenus Foundation for his innovative work in the field of pharmaceutical technology.

Gregor's research is focussed on engineering biogenic drug carriers utilising principles established in nature, such as extracellular vesicles. He has authored several peer-reviewed publications in high-impact journals such Nature Chemistry, PNAS or Journal of Controlled Release and he has been invited speaker at international conferences such as the Annual Meeting of the Controlled Release Society. He was Guest-Editor for Pharmakon, the proceedings of the German Pharmaceutical Society.

### RECENT PUBLICATION

- Goes A & Fuhrmann G\* (2018) Biomimetic carriers as smart transporters for antibiotics. *ACS Infectious Diseases*, DOI: 10.1021/acscinfecdis.8b00030
- Fuhrmann G, Chandrawati R, Pamar PA, Keane TJ, Maynard SA, Bertazzo S & Stevens MM (2018) Engineering extracellular vesicles with the tools of enzyme prodrug therapy. *Advanced Materials*, 30: 1706616 (selected for *Advanced Materials* backcover)
- Fuhrmann K & Fuhrmann G\* (2017) Recent advances in oral delivery of macromolecular drugs and benefits of polymer conjugation. *Current Opinion in Colloid and Interface Science*, 31:67-74
- Fuhrmann G\*, Serio A, Mazo M, Nair R & Stevens MM (2015), Active loading into extracellular vesicles significantly improves the cellular uptake and photodynamic effect of porphyrins. *Journal of Controlled Release* 205: 35-44



## Audrey Gallud

I hold a PhD in biology and I am specialized in nanomedicine and nanosafety.

My doctoral research, lead at the Institute of Biomolecules Max Mouseron in Montpellier (France), focused on glyco- and nano-vectors for therapeutic targeting against rare pathologies. In collaboration

with chemists from national and international research centers, I contributed to the development of innovative nanosystems for personalized and non-invasive treatments of small solid cancers based on targeted drug delivery and photodynamic therapy.

I have extended my knowledge in nanosafety by working 3 years at the Karolinska Institutet in Stockholm (Sweden) where I have focused mainly on immunotoxicity of engineered nanomaterials. This work was conducted in the frame of the EU-funded project FP7-Nanosolutions which aimed to achieve a systems biology un-

derstanding of nanomaterial interactions with biological systems at the molecular, cellular, and organism levels.

Currently, my research is conducted at Chalmers University of Technology in Gothenburg (Sweden) and within the FoRmulaEx collaboration – a Swedish industrial research center for functional RNA delivery – and contributes to create fundamental knowledge and novel technologies that will allow successful translation of RNA modalities into the clinic.

### FIVE SELECTED PUBLICATIONS:

- Identification of MRC2 and CD209 receptors as targets for photodynamic therapy of retinoblastoma using mesoporous silica nanoparticles. Gallud A., Warther D., Maynadier M., Sefta M., Poyer F., Thomas C.D., Rouxel C., Mongin O., Blanchard-Desce M., Morere A., Raehm L., Maillard P., Durand J.O., Garcia M. and Gary-Bobo M. *Rsc Advances*, 2015, 5(92), 75167-75172.
- pH-operated hybrid silica nanoparticles with multiple H-bond stoppers for colon cancer therapy. Théron C.\*, Gallud A.\*, Giret S., Maynadier M., Grégoire D., Puche P., Jacquet E., Pop G., Sgarbura O., Bellet V., Hibner U., Zink J. I., Garcia M., Wong Chi Man M., Carcel C. and Gary-Bobo M., *Rsc Advances*, 2015, 5(80), 64932-64936.
- Investigation of cyano-bridged coordination nanoparticles Gd 3+ / [Fe (CN) 6] 3- / d-mannitol as T 1-weighted MRI contrast agents. Perrier M.\*, Gallud A.\*, Ayadi A., Kennouche S., Porredon C., Gary-Bobo M., Larionova J., Goze-Bac C., Zanca M., Garcia M., Basile I., Long J., De Lapuente J., Borrás M. and Guari Y., *Nanoscale*, 2015, 7(28), 11899-11903.
- Keeping it small: towards a molecular definition of nanotoxicology. Gallud A., and Fadeel B., *European Journal of Nanomedicine*, 2015, 7(3), 143-151.
- Macrophage activation status determines the internalization of mesoporous silica particles of different sizes: Exploring the role of different pattern recognition receptors. Gallud A., Bondarenko O., Felio N., Kupferschmidt N., Atluri R., Garcia-Bennett A. and Fadeel B., *Biomaterials*, 2017, 121:28–40.



## Jérôme Galon

Dr. Jérôme Galon is Director of Research at INSERM (French NIH), and Head of the laboratory of Integrative Cancer Immunology, in Paris, France. Dr. Galon was trained as an immunologist at the Pasteur Institute and at the Curie Institute (Paris, France). He holds a Ph.D. degree in Immunology (Jussieu University, Paris, France, 1996).

Between 1997 and 2001 he worked at the NIH (National Institute of Health, Bethesda, USA). Since his full-tenured position at INSERM in 2001, he directs interdisciplinary research programs on tumor-Immunology. He is associate Director and co-founder of European Academy of Tumor Immunology (EATI), board Director of the Society for Immunotherapy of Cancer (SITC). His work on the comprehensive analysis of the tumor-microenvironment and the role of T-cells in human cancer led to the demonstration of the importance of adaptive pre-existing immunity in human cancer, and the concept of cancer immune-contexture. He pioneered the Immunoscoper. He is the co-founder of HaliOx company and the Chairman of its scientific council. His contributions have been recognized with numerous awards, including the William B. Coley Award, an international prize which honors the best scientists in the fundamental and cancer immunology, and Award from the National Academy of Science, and Award from the National Academy of Medicine.



## Robert Geertsma

Senior Scientist, Centre for Health Protection, National Institute for Public Health and the Environment (RIVM), Robert.Geertsma@rivm.nl

Robert Geertsma has worked at the Dutch National Institute for Public Health and the Environment (RIVM) for more than twentyfour years. As a senior scientist and project leader he is responsible for the provision of scientific advice to regulators on quality and safety of medical technology and nanomedicine. He works on multiple research projects on opportunities as well as risks of nanotechnologies and nanomaterials in medical applications, performing both desk research and experimental research. He participated in FP7-projects ObservatoryNano and NanoMedRoundTable, and is currently one of the partners in the H2020 project REFINE (Regulatory Science Framework for Nano(bio)material-based Medicinal Products and Medical Devices). He is also one of the experts of the Risks of Nanotechnology Knowledge and Information Centre (KIR nano), a Dutch government-supported observation organisation based at RIVM. His areas of expertise include risk management, biological safety, nanotechnology and emerging medical technologies. He participates actively in international ISO/CEN Standards Committees on these subjects and he is chairman of the joint CEN/CENELEC/TC3 responsible for horizontal standards on topics like quality and risk management systems. He was a member of the SCENIHR WG that wrote the Scientific Opinion "Guidance on the Determination of Potential Health Effects of Nanomaterials Used in Medical Devices". He is co-chairing the ISO/TC194/WG17 on Biological Evaluation of Medical Devices – Nanomaterials, and he is a member of the Nanomedicines WG of the International Pharmaceutical Regulators Forum. Furthermore, he frequently represents the Dutch competent authority in European Commission's working groups such as the New & Emerging Technologies WG, of which he was appointed co-Chair in 2009. He is a member of the European Society for Nanomedicine and the European Technology Platform Nanomedicine.

### PUBLICATION LIST

- Brand W, Noorlander CW, Giannakou C, Kooi MW, Jong WH de, Vandebriel RJ, Park MVDZ, Bosselaers IEM, Scholl JHG, Geertsma RE. Nanomedicinal products - Survey on specific toxicity and side effects. *Int J Nanomedicine* 2017; 12:6107-29.
- Giannakou C, Geertsma RE, Jong WH de, Loveren H van, Vandebriel RJ, Park MVDZ. Immunotoxicity Testing of Nanomedicinal Products: Possible Pitfalls in Endotoxin Determination. *Current Bionanotechnology* 2016; 2:95-102.
- Giannakou C, Park MVDZ, Jong WH de, Loveren H van, Vandebriel RJ, Geertsma RE. A comparison of immunotoxic effects of nanomedicinal products with regulatory immunotoxicity testing requirements. *International Journal of Nanomedicine* 2016; 11: 2935–2952.
- Geertsma RE, Park MVDZ, Puts CF, Roszek B, Stijl R van der, Jong WH de. Nanotechnologies in medical devices. RIVM Report 2015-0149, 2015. [http://www.rivm.nl/en/Documents\\_and\\_publications/Scientific/Reports/2015/december/Nanotechnologies\\_in\\_medical\\_devices](http://www.rivm.nl/en/Documents_and_publications/Scientific/Reports/2015/december/Nanotechnologies_in_medical_devices)
- Bleeker EAJ, Evertz S, Geertsma RE, Peijnenburg WJGM, Westra J, Wijnhoven SWP. Assessing health & environmental risks of nanoparticles: Current state of affairs in policy, science and areas of application. RIVM Report 2014-0157, 2015. [http://www.rivm.nl/en/Documents\\_and\\_publications/Scientific/Reports/2015/april/Assessing\\_health\\_environmental\\_risks\\_of\\_nanoparticles\\_Current\\_state\\_of\\_affairs\\_in\\_policy\\_science\\_and\\_areas\\_of\\_application](http://www.rivm.nl/en/Documents_and_publications/Scientific/Reports/2015/april/Assessing_health_environmental_risks_of_nanoparticles_Current_state_of_affairs_in_policy_science_and_areas_of_application)
- Noorlander CW, Kooi MW, Oomen AG, Park MV, Vandebriel RJ, Geertsma RE. Horizon scan of nanomedicinal products. *Nanomedicine* 2015; 10:1599-1608.



## Matthieu Germain

Matthieu holds a doctorate in biotechnology. He has joined NBTX in 2004. He's in charge of the Curadigm business unit in Nanobiotix. Previously Matthieu managed the development of the intravenous formulation of the NanoXray platform (NBTX-IV) and was in charge of sonodrugs European project for NBTX. Matthieu leads the chemistry team of NBTX discovery department for several years before the creation of Curadigm business unit. He acquired extensive experience in the design and physico-chemical characterization of nanomedicines and in project management while he was working on the different projects developed by Nanobiotix.



## Christina Giannakou

Christina Giannakou was born in 1988, in Greece. In 2006, she started her Diploma in Biology at Aristotle University of Thessaloniki, Greece. During the diploma studies she studied as an ERASMUS exchange student at the University of Hamburg for 6 months in 2009. She conducted a 4 month diploma internship in LEITAT on Industrial Microbiology Laboratory in 2011. She started her master education on the MSc program of Toxicology and Environmental Health at Utrecht University in 2012. During the two years of her master studies she worked on food allergy at the Immunotoxicology group of IRAS under the supervision of and on Nanotoxicology at the Centre for Health Protection of the National Institute for Public Health and Environment (RIVM). From September 2014, she is started her PhD at Toxicogenomics department of Maastricht University on a project in collaboration with the National Institute for Public Health and the Environment-RIVM. Her project is focusing on the methods and criteria for evaluating potential immunotoxicity of nanomedicinal products.

### PUBLICATION LIST

- Giannakou C, Park MV, de Jong WH, van Loveren H, Vandebriel RJ, Geertsma RE. A comparison of immunotoxic effects of nanomedicinal products with regulatory immunotoxicity testing requirements. *Int J Nanomedicine*. 2016;11:2935-2952.
- Braakhuis HM, Giannakou C, Peijnenburg WJ, Vermeulen J, van Loveren H, Park MV. Simple *in vitro* models can predict pulmonary toxicity of silver nanoparticles. *Nanotoxicology*. 2016;1:10.
- Giannakou C, Geertsma RE, de Jong WH, van Loveren H, Vandebriel RJ, Park MVDZ. Immunotoxicity Testing of Nanomedicinal Products: Possible Pitfalls in Endotoxin Determination. *Current Bionanotechnology*. 2016;2(2):95-102.
- Brand W, Noorlander CW, Giannakou C, et al. Nanomedicinal products: a survey on specific toxicity and side effects. *Int J Nanomedicine*. 2017;12:6107-6129.



## Maria Gianneli

Dr Maria Gianneli holds a PhD in Materials Science and Technology from the University of Crete, Greece. Her PhD research was funded by a Marie – Curie Host Fellowship and the largest part of the project was completed at the Max Planck Institute for Polymer Research (MPI-P) in Mainz, Germany. Her previous postdoctoral research

was funded by the European Union project “Love wave fully integrated Lab-on-Chip platform for food pathogen detection”, focusing on the detection of whole bacteria using acoustic wave sensors. Since July 2015, she is employed by Attana AB, Sweden, in the where she employs QCM for rapid label-free bio-nano-interface evaluation. Currently she is designing and executing binding assays to characterise the bio-nano interface and cell binding partners in complex biological environments.

## RECENT PUBLICATIONS

- M. Gianneli, Y. Yan, E. Polo, D. Peiris, T. Aastrup, K. A. Dawson; “Novel QCM-based method to predict *in vivo* behaviour of nanoparticles”, *Procedia Technology* 2017, 27, 197-200
- M. Gianneli, E. Polo, H. Lopez, V. Castagnola, T. Aastrup, K.A. Dawson; “Label-free in-flow detection of receptor recognition motifs on the biomolecular corona of nanoparticles”, *Nanoscale* 2018, 10, 5474-5481



## Daniel Gonzalez-Carter

With the support of an ‘Early Career Scientist’ research grant (Kakenhi, Japan), Dr. Gonzalez-Carter’s research focuses on developing poly-ionic micellar nanoparticles as drug delivery vehicles to target individual brain cell populations to overcome the inadequately low drug delivery levels which currently prevent successful treatments of brain disorders, such as Alzheimer’s disease. This work is being carried out at the recently formed Innovation Center of NanoMedicine (iCONM), Kawasaki, Japan under the supervision of Prof. Kazunori Kataoka. Previous to this position, Dr. Gonzalez-Carter completed post-doctoral work under the supervision of Prof. Alex E. Porter at the Department of Materials of Imperial College, London. Here, he examined the potential of carbon nanotubes and silver/gold nanoparticles as drug-delivery vehicles with an emphasis on the cellular effects at the blood-brain barrier and brain inflammation. This position was preceded by an over-seas Ph.D. scholarship from the National Council of Science and Technology (CONACyT), Mexico, to carry out doctoral work at the Division of Brain Sciences, Imperial College, London, under the supervision of Prof. David T. Dexter. His research focused on examining the potential of metabotropic glutamate receptors to modulate brain inflammation as a neuroprotective therapeutic strategy against neurodegenerative diseases.

## RECENT PUBLICATIONS

- D. Gonzalez-Carter, B. Leo, P. Ruenraroengsak, S. Chen, A. Goode, I. Theodorou, K. Chung, R. Carzaniga, M. Shaffer, D. Dexter, M. Ryan, A. Porter. Silver nanoparticles reduce brain inflammation and related neurotoxicity through induction of H2S-synthesizing enzymes. *Nature Scientific Reports*, 7:42871, 2017.
- D. Gonzalez-Carter, A. Goode, I. Dunlop, A. Porter, D. Dexter. Inhibition of leptin-ObR interaction does not prevent leptin translocation across a human blood-brain barrier *in vitro* model. *Journal of Neuroendocrinology*, 28 (6),2016
- D. Duricki, T. Hutson, C. Kathe, S. Soleman, D. Gonzalez-Carter, J. Petruska, H. Shine, Q. Chen, T. Wood, et al. Delayed intramuscular human neurotrophin-3 improves recovery in adult and elderly rats after stroke. *Brain*, 139 (Pt1):259-275, 2016
- A. Goode, D. Gonzalez-Carter, M. Motskin, I. Pienaar, S. Chen, S. Hu, P. Ruenraroengsak, M. Ryan, M. Shaffer, D. Dexter, A. Porter. High resolution and dynamic imaging of biopersistence and bioreactivity of extra- and intra-cellular MWNT exposed to microglial cells. *Biomaterials*, 70: 57-70, 2015
- D. Gonzalez-Carter, Z. Ong, C. McGilvery, I. Dunlop, D. Dexter, A. Porter. L-DOPA functionalized, multi-branched gold nanoparticles as brain-targeted nano-vehicles. *Nanomedicine: Nanotechnology, Biology and Medicine*. Manuscript under revision



## Stephan Grabbe

Stephan Grabbe, MD (born 1961), is a Dermatologist and currently holds the position as Director and Chairman of the Department of Dermatology, University of Mainz Medical Center (UMMC), Germany. He received his medical and scientific education at the University of Münster, Germany (Department of Dermatology (1987-2003),

as well as at Harvard Medical School (Research fellowships at the Department of Dermatology, Massachusetts General Hospital, Harvard Medical School, Boston, USA (1989-1992) and at the Department of Dermatology, Brigham and Womens’ Hospital, Harvard Medical School, Boston, USA (1998)). Before being appointed to his current position, Stephan Grabbe was Director and Chairman, Dept. of Dermatology, University of Essen Medical center (2003-2007). His clinical focus is on skin oncology and immune-mediated skin diseases. Currently, he is also Head of the UMMC Skin Cancer Center, Member of the board of the University of Mainz Cancer Center (UCT), Head of the Biomedical Research Center of the Johannes Gutenberg University, and co-speaker of the Research Center Immunotherapy (FZI) of the University of Mainz.

Stephan Grabbe’s scientific focus is in the field of cellular immunology and immunotherapy, dendritic cells, as well as nanoparticle-mediated immunomodulation. In this respect, he is deputy speaker of the collaborative research center SFB 1066 of the German Research Council (“Nanoparticle-mediated immunotherapy”), and deputy speaker of the collaborative research center SFB TR156 (“Skin immunology”). Stephan Grabbe has published more than 150 original papers in peer-reviewed journals, has been cited more than 10.000 times and has an h-index of 50.



## Stephen Grobmyer

Professor of Surgery, Cleveland Clinic, Cleveland, Ohio

Dr. Stephen R. Grobmyer graduated from Rice University and University of Texas Southwestern Medical School at Dallas. He completed residency in general surgery and a research fellowship at New York Hospital-Cornell Medical Center in New York City. He completed the Surgical Oncology fellowship at Memorial Sloan Kettering Cancer Center. Currently, Dr. Grobmyer is The Lula Zapis Endowed Chair to Support Breast Cancer Research, and Co-Director of the Cleveland Clinic Comprehensive Breast Cancer Program in Cleveland, Ohio. He is Professor of Surgery at Cleveland Clinic Lerner College of Medicine of Case Western Reserve University. He has published over 150 peer reviewed manuscripts and 20 book chapters. He is a member of the American Surgical Association, Southern Surgical Association, Central Surgical Association, Surgical Biology Club II of the American College of Surgeons, and The Society of Surgical Oncology.

## RECENT PUBLICATIONS

- Wang, H., Altemus, J., Niazi, F., Green, H., Calhoun, B. C., Sturgis, C., Grobmyer, S. R., Eng, C. “Breast tissue, oral and urinary microbiomes in breast cancer” *Oncotarget* 9(5): 88122, 2017.
- Grobmyer, S.R., Krishna, V. “Minimally invasive cancer therapy using polyhydroxy fullerenes” *European Journal of Radiology* 81 Suppl 1: S51-3, 2012.
- Xi, L., Grobmyer, S.R., Zhou, G., Qian, W., Yang, L., Jiang, H. “Molecular photoacoustic tomography of breast cancer using receptor targeted magnetic iron oxide nanoparticles as contrast agents” *Journal of Biophotonics* 7(6): 401-409, 2014.



- Gutwein, L. G., Singh, A. K., Hahn, M. A., Rule, M. C., Knapik, J. A. Moudgil, B. M., Brown, S. C. Grobmyer, S. R. International Journal of Nanomedicine 7: 351-7, 2012.
- Krishna, V., Singh, A., Sharma, P., Iwakuma, N., Wang, Q., Zhang, Q., Knapik, J. Jiang, H. Grobmyer, S. R. Koopman, B. Moudgil, B. "Polyhydroxy fullerenes for non-invasive cancer imaging and therapy: Small 6(20): 2236-4



## Heinrich Haas

VP RNA Formulation & Drug Delivery

Heinrich Haas has more than 20 years of experience in academic research and industrial pharmaceutical development. After he received his Ph.D. in physical chemistry, Dr. Haas researched lipid membranes and organized biomolecular systems. His professional focus is on colloidal/nanoparticulate formulations for targeted drug delivery with therapeutic and diagnostic applications. Before joining BioNTech RNA Pharmaceuticals GmbH (former Ribological GmbH), he was responsible for a variety of projects in biopharmaceutical research and development, ranging from the exploration of novel colloidal therapeutic and diagnostic carriers to up-scaling and development of market-compliant manufacturing methods for liposome products. After joining BioNTech RNA Pharmaceuticals GmbH in 2010, he helped build the formulation development and analytics unit, which develops formulations for delivery of RNA and small molecules. He has an active record of publications in peer-reviewed journals and patent applications in the field of drug delivery.

### RECENT PUBLICATIONS

- L. M. Kranz et al., "Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy," Nature, pp. 1–16, 2016.
- S. Grabbe et al., "Translating nanoparticulate-personalized cancer vaccines into clinical applications: case study with RNA-lipoplexes for the treatment of melanoma." Nanomedicine (Lond). 2016 Oct;11(20):2723-2734
- A. Ziller et al., "Incorporation of mRNA in Lamellar Lipid Matrices for Parenteral Administration," Mol. Pharm., vol. 15, pp. 642–651, 2018
- A.B. Vogel et al., "Self-Amplifying RNA Vaccines Give Equivalent Protection against Influenza to mRNA Vaccines but at Much Lower Doses" Mol Ther. 2018 Feb 7;26(2):446-455.



## Stefan Halbherr

Ph.D.  
Manager Research and Development  
InnoMedica

Studied Biochemistry at the University of Bern/Switzerland. At the University Institute for Immunology in the Insel hospital in Bern he investigated disease-specific antibody signatures in Hemophilia patients using Designed Ankyrin Repeat Protein (DARPin) technology. During his PhD, he developed genetically engineered RNA vaccines for against influenza A viruses including highly pathogenic H5N1 strains. During his doctoral studies already, he joined in 2013 the biomedical research team of InnoMedica and contributed to the initiation of the lead project "Talidox", a novel targeted liposomal formulation of the widely used cytostatic drug doxorubicin. In his role as Manager Research and

Development he brought the research concepts of the acquired Yamazaki DDS, Ltd. to a marketable product, introducing many innovations in the processes of industry-scale liposome assembly, drug loading, and especially design of biologically functional liposome surfaces. At the same time, he was involved in the creation of the SwissMedic approved liposome manufacturing facility of InnoMedica in Marly/Switzerland. With his research and development team, Stefan Halbherr is now leading InnoMedica to create and clinically translate new types of targeted liposome-nanodrugs. This offers new ways to approach key medical applications like chemotherapeutic treatment and diagnosis of cancer, but also management of bacterial infections, and control of diseases of the central nervous system.



## Clemens Helmbrecht

Director of Research and Development  
Particle Matrix GmbH

Clemens Helmbrecht studied Physics and Nanotechnology in Munich, Germany. After his graduation in physics, he received his PhD in Chemistry at Technische Universität München (TUM) under Prof. Dr. Reinhard Nießner working on next generation particle characterization and separation techniques. He continued his work as group leader of the Particle Separation Laboratory. During that time, he taught analytical chemistry and higher statistics (TUM, faculty of chemistry) and instrumental analysis (Ludwigs Maximilians Universität, faculty of pharmacy).

Clemens Helmbrecht develops and releases new applications of particle technology to medicine, chemistry and biology. Over more than a decade he has been working in the areas of nanoparticle characterization and automatization of measurement equipment. His is interested in the combination of bioanalytical technology with light scattering, laser spectroscopy, zeta potential, fluorescence and nanoparticle tracking analysis for research and industry applications.



## Wim Hennink

Professor at Utrecht University

Professor Wim Hennink obtained his Ph.D. degree in 1985 at the Twente University of Technology on a thesis of a biomaterials research topic. From 1985 until 1992 he had different positions in the industry. In 1992 he was appointed as professor at the Faculty of Pharmacy of the University of Utrecht. From 1996 on he is head of the Department of Pharmaceutics. From 1997 on he is European Editor of the Journal Controlled Release. From 2012-2015 he was the scientific director of the Utrecht Institute for Pharmaceutical Sciences and since September 2015 he is head of the Department of Pharmaceutical Sciences. His main research interests are in the field of polymeric drug delivery systems. He published over 520 papers and book chapters and is the inventor of 20 patents.

### RECENT PUBLICATIONS

- Shi Y, van der Meel R, Theek B, Oude Blenke E, Pieters EH, Fens MH, Ehling J, Schiffelers RM, Storm G, van Nostrum CF, Lammers T, Hennink WE. Complete regression of xenograft tumors upon targeted delivery of paclitaxel via  $\Pi$ - $\Pi$  stacking stabilized polymeric micelles. ACS Nano 9, 3740-3752, 2015.

- Rahimian S, Fransen MF, Kleinovink JW, Amidi M, Ossendorp F, and Hennink WE. Polymeric microparticles for sustained and local delivery of antiCD40 and antiCTLA-4 in immunotherapy of cancer. *Biomaterials* 61, 33-40, 2015.
- Li D, Kordalivand N, Fransen MF, Ossendorp F, Raemdonck K, Vermonden T, Hennink WE, and van Nostrum CF. Reduction-sensitive dextran nanogels aimed for intracellular delivery of antigens. *Advanced Functional Materials* 25, 2993-3003, 2015.
- Li D, Sun F, Bourajjaj M, Chen Y, Pieters EH, Chen J, van den Dikkenberg JB, Lou B, Camps MG, Ossendorp F, Hennink WE, Vermonden T, and van Nostrum CF. Strong *in vivo* antitumor responses induced by an antigen immobilized in nanogels via reducible bonds. *Nanoscale* 8, 19592-19604, 2016.
- Buwalda SJ, Vermonden T, and Hennink WE. Hydrogels for therapeutic delivery: current developments and future directions. *Biomacromolecules* 18, 316-330, 2017.



## Jules Hoffmann

Jules Hoffmann is the Chair for Developmental Biology at the University of Strasbourg Institute for Advanced Study and Emeritus Research Director at CNRS. He dedicated much of his work to the study of the cellular, genetic and molecular mechanisms responsible for innate immunity in insects. The work of Hoffmann and associates has provided new insights into the defense mechanisms that organisms, from the most primitive up to humans, employ against infectious agents. By demonstrating the marked conservation of innate defense mechanisms between insects and humans, the work initiated by Hoffmann and his collaborators has led to a re-evaluation of the role of innate immunity in mammals. More generally, the *Drosophila* model has enabled biologists throughout the world to make considerable progress, not only in developmental genetics and innate immunity but also in the study of certain human pathologies and in the understanding of memory, behavior, sleep and nutrition phenomena. With Bruce A. Beutler and Ralph M. Steinman, Hoffmann was awarded the Nobel Prize for Medicine in 2011. Hoffmann set up and headed the CNRS laboratory "Endocrinology and Immunology of Insects" within the CNRS Institut de Biologie Moléculaire et Cellulaire in Strasbourg, which he also directed from 1994 to 2006 and where he still works with some of his collaborators. He was President of the French Académie des Sciences in 2007 and 2008, and is a Foreign Associate member of the Academy of Sciences of the United States of America, Germany and Russia and of the American Academy of Arts and Sciences. For his contributions to immunity, Hoffmann was awarded numerous prizes, including, in recent years, the Robert Koch Prize (2004, with Bruce Beutler and Shizuo Akira) the Balzan Prize (2007, with Bruce Beutler), the Rosenstiel Award (2010, with Ruslan Medzhitov), the Keio Medical Science Prize (with Shizuo Akira, 2010), the International Gairdner Award (2011, with Shizuo Akira) and the Shaw Prize in Life Science and Medicine (2011, with Bruce Beutler and Ruslan Medzhitov). He also received in 2011 the CNRS Gold Medal. Hoffmann is Officier de la Légion d'Honneur in France and is an Immortel at the Académie Française (2012).



## Krisztian Homicsko

Dr. Homicsko finished undergraduate medical education and received his MD at the Semmelweis University, Faculty of Medicine, in Budapest, Hungary in 2001. After undergraduate studies, first Dr Homicsko completed a Masters (DEA) in Medical Biology at the University of Geneva and then started an MD-PhD doctoral thesis at the Swiss Institute of Experimental Cancer Research (ISREC) in Lausanne in the Laboratory of Prof Richard Iggo. His doctoral thesis focused on gene therapy of colorectal cancer with adenoviral vectors. After the end of his MD-PhD thesis in 2007, he continued in the laboratory of Prof Richard Iggo for a short post-doctoral work at the University of St Andrews in Scotland. After a short passage in the University Hospital of Dundee, Scotland, he started a medical training in the Curie Institute in Paris, France from 2007, where he also worked on clinical trials within the clinical trial unit. In 2009, Dr Homicsko joined the University Hospital (CHUV) in Lausanne to complete medical oncology training. In parallel and since 2012, Dr. Homicsko also works in the laboratory of Prof Douglas Hanahan at EPFL as a clinician scientist on translational oncology. Dr. Homicsko works as chief resident (chef de clinique) in the Department of Oncology and is responsible for the molecular tumor board of the Department of Oncology.



## Alexander Huber

Dr. Alexander Huber started his career in the pharmaceutical industry at the research department of F. Hoffmann- La Roche Ltd in Basel, Switzerland in 2002. He held several positions with increasing responsibility. 2009 he joined Novartis Pharma technical research and development (TRD) as director of the global centre of excellence for parenteral clinical supply. In 2013 he changed to the Cell & Gene-Therapy group as global CMC head. He is responsible of managing a global team for clinical and commercial manufacturing of CTL019 / Kymriah in Japan and Luxturna in Europe. He also manages several CMOs to ensure supply of critical materials for this therapy. Alexander received his PhD from the Federal Institute of Technology (ETH) Zürich in the field of molecular neuropharmacology, developing a first ex-vivo gene therapy approach against focal epilepsy in an animal model. He also holds a MBA from the same university.



## Patrick Hunziker

Patrick Hunziker has studied Medicine the University of Zurich, Switzerland. He received a doctoral decree based on thesis work in experimental immunology from the University of Zurich and did further research in experimental haematology at University Hospital in Zurich, Switzerland. He earned specialist degrees in Internal Medicine, Cardiology and Intensive Care Medicine. As a fellow the Massachusetts General Hospital, Harvard Medical School, worked on cardiac imaging in a joint project with the Massachusetts Institute of Technology, Cambridge. 2008 Patrick Hunziker became professor for Cardiology and Intensive Care Medicine at the University of Basel. His professional activities in Europe, the U.S.,



Africa and China gave him a broad insight into the needs for the medicine of the future in a variety of settings. Hunziker became involved in medical applications of Nanoscience in the late nineties and has been the pioneer physician in Nanomedicine in Switzerland since then. With improved prevention, diagnosis and cure of cardiovascular disease as his main research topic, he worked in the nanoscience fields of atomic force microscopy, nano-optics, micro/nanofluidics, nanomechanical sensors and polymer nanocarriers for targeting. He is the co-founding president of the European Society of Nanomedicine, co-founder of the European Foundation for Clinical Nanomedicine and co-initiator of the European Conference for Clinical Nanomedicine and is clinically active as deputy head of the Clinic for Intensive Care Medicine at the University Hospital Basel, Switzerland. In November, he is President of the International Society for Nanomedicine, which is uniting members from all continents in the world.



## Darrell Irvine

Professor  
Dept. of Biological Engineering and  
Dept. of Materials Science and  
Engineering, Massachusetts Institute of  
Technology  
Howard Hughes Medical Institute  
Investigator

- University of Pittsburgh, Pittsburgh, PA; B.S./B.Phil: 1991-1995; Engineering Physics
- MIT, Cambridge, MA; Ph.D: 1995-2000; Polymer Science
- Stanford University, Stanford, CA; Postdoc: 2000-2002; Immunology

### PERSONAL STATEMENT

Our laboratory is focused on developing approaches to engineer the immune system, by fusing immunology with biotechnology and materials science. Our first area of focus is in the development of technologies for enhanced vaccines against infectious disease. We are developing nanoparticles and microparticles that can be used to deliver vaccine antigens and immunostimulatory adjuvant molecules, and testing these in collaboration with colleagues in the Boston area in relevant preclinical models. We are also applying model systems developed in our lab for studying T-cell activation to understanding the properties of immune cells that may be important to control HIV infection. Much of this work is in collaboration with investigators of the Ragon Institute of MGH, MIT, and Harvard. Our second focus is in cancer immunotherapy. Using our lab's expertise in drug delivery particles and gels, we are exploring strategies to promote, amplify, and maintain anti-tumor immune responses by controlling where and when cells of the immune system are triggered to respond to tumor cells, and seeking to overcome the immunosuppressive milieu developed in solid tumors.

### RECENT PUBLICATION

- Zhang Y, Li N, Suh H, and Irvine DJ, "Nanoparticle anchoring targets immune agonists to tumors enabling anti-cancer immunity without systemic toxicity," *Nature Communications* 9(1) 6 (2018).
- Huang B, Abraham W, Zheng Y, Bustamante Lopez SC, Luo SS, and Irvine DJ, "Active targeting of chemotherapy to disseminated tumors using nanoparticle-carrying T cells," *Science Translational Medicine*, 7(291) 291ra94 (2015). PMID:PMC4687972
- Hanson MC, Crespo MP, Abraham W, Moynihan KD, Szeto GL, Chen SH, Melo MB, Mueller S, and Irvine DJ, "Nanoparticulate STING agonists are potent lymph node-targeted vaccine adjuvants," *Journal of Clinical Investigation*, 125(6) 2532-2546 (2015). PMID:PMC4497758
- Liu H, Moynihan KD, Zheng Y, Szeto GL, Li AV, Huang B, Van Egeren DS, Park C, and Irvine DJ, "Structure-based programming of

lymph node targeting in molecular vaccines," *Nature* 507 519-522 (2014). PMID: PMC4069155

- Li AV, Moon JJ, Abraham W, Suh H, Elkhader J, Seidman MA, Yen M, Im EJ, Foley MH, Barouch DH, and Irvine DJ, "Generation of effector memory T-cell-based mucosal and systemic immunity with pulmonary nanoparticle vaccination," *Science Translational Medicine* 5 204ra130 (2013). PMID: PMC3934930 (cover article)



## Tatsuhiro Ishida

Professor of Department of  
Pharmacokinetics and Biopharmaceutics,  
Institute of Biomedical Sciences,  
Tokushima University, Japan

Tatsuhiro Ishida has studied Pharmaceutical Sciences, the University of Tokushima, Japan. He received a doctoral degree based on thesis work in liposomal drug delivery system from the University of Tokushima in 1998. He has worked as postdoctoral fellow under supervision of Dr. Theresa Allen, University of Alberta, until 2000. He became Associate Professor in the University of Tokushima 2000 and became full Professor 2014. He published more than 130 papers. His major is immunological responses against PEGylated materials.

### RECENT PUBLICATION

- Mészáros, T., Kozma, G., Shimizu, T., Miyahara, K., Turjeman, K., Ishida, T., Barenholz, Y., Urbanics1, R., Szebeni, J., Involvement of complement activation in the pulmonary vasoactivity of polystyrene nanoparticles in pigs: Unique surface properties underlying alternative pathway activation and instant opsonization. *Int. J. Nanomed.*, in press
- Shimizu, T., Abu Lila, A.S., Fujita, R., Awata, M., Kawanishi, M., Hashimoto, Y., Okuhira, K., Ishida, T., A hydroxyl PEG version of PEGylated liposomes and its impact on anti-PEG IgM induction and on the accelerated clearance of PEGylated liposomes. *Eur. J. Pharm. Biopharm.*, 127, 142-149 (2018)
- Ando, H., Abu Lila, A.S., Kawanishi, M., Shimizu, T., Okuhira, K., Ishida, T., Reactivity of IgM antibodies elicited by PEGylated liposomes or PEGylated lipoplexes against auto and foreign antigens. *J. Control. Release*, 270, 114-119 (2018)
- Mima, Y., Abu Lila, A.S., Shimizu, T., Ukawa, M., Ando, H., Kurata, Y., Ishida, T., Ganglioside inserted into PEGylated liposome attenuates anti-PEG immunity. *J. Control Release*, 250, 20-26 (2017)
- Mima, Y., Hashimoto, Y., Shimizu, T., Kiwada, H., Ishida, T., Anti-PEG IgM is a major contributor to the accelerated blood clearance of polyethylene glycol-conjugated protein. *Mol. Pharmaceutics*, 12, 2429-2435 (2015)



## David Jacob

Technical Director

David Jacob (52) holds a doctorate in lasers physics from the University of Rennes (1995) and a diploma in aeronautics (ENSAE 1991). David Jacob is Technical Director of Cordouan Technologies (Pessac, 33). He has over 20 years of industry experience in the design and industrialization of optoelectronic systems and R&D management; Co founder and technical director of Cordouan technologies since 2007, he is in charge of collaborative R&D project management and of the development and industrialization of innovative instruments dedicated to the physico-chemical

characterization (size, charge, shape) of nanoparticles. David Jacob represents Cordouan Technologies on two AFNOR standardization committees at the national level (AFNOR / X457 Nanotechnologies commission) and internationally (ISO / TC 24 / SC 4 & ISO / TC 229). He is also a member of the French Nanometrology association

## RECENT PUBLICATION

- Thermosensitive polymer-grafted iron oxide nanoparticles studied by in situ dynamic light backscattering under magnetic hyperthermia ; Gauvin Hemery · Elisabeth Garanger · Sébastien Lecommandoux · [...] · Olivier Sandre ; Dec 2015 · Journal of Physics D Applied Physics
- Nonideal effects in electroacoustics of solutions of charged particles: combined experimental and theoretical analysis from simple electrolytes to small nanoparticles ; R Pusset · S Gourdin-Bertin · E Dubois · [...] · D Jacob Article · Apr 2015 · Physical Chemistry Chemical Physics
- Combining SAXS and DLS for simultaneous measurements and time-resolved monitoring of nanoparticle synthesis ; A. Schwamberger · B. De Roo · D. Jacob · [...] · J.P. Locquet ; Jan 2015 · Nuclear Instruments and Methods in Physics Research Section B Beam Interactions with Materials and Atoms



## Michael Johnston

Research Scientist

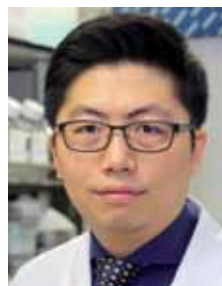
Dr. Michael Johnston completed his graduate studies at the University of British Columbia in 2006 which focused on regulated drug release from liposomal based nanoscale drug delivery systems. Since 2007, Dr. Johnston has been with Health Canada where he heads the nanomedicine laboratory continuing his research on nanoscale drug delivery systems, nanoscale vaccine adjuvants and therapeutic protein aggregates. Dr. Johnston also heads the Health Products and Food Branches nanotechnology working group within Health Canada, Chairs the International Pharmaceutical Regulators Forum Nanomedicines working group and is a member of the Government of Canada's Interdepartmental Nanotechnology working group.

## RECENT PUBLICATION

- 2017 Luebbert, C.C., Clarke, T.M., Pointet, R., Frahm, G.E., Tam, S., Lorbetskie, B., Sauvé, S., Johnston, M.J.W.; Nanoparticle size and production efficiency are affected by the presence of fatty acids during albumin nanoparticle fabrication. PLoS ONE, 12(12)
- 2017 Luebbert, C.C., Clarke, T.M., Pointet, R., Frahm, G.E., Tam, S., Lorbetskie, B., Sauvé, S., Johnston, M.J.W.; Nanoparticle size and production efficiency are affected by the presence of fatty acids during albumin nanoparticle fabrication. PLoS ONE, 12(12)
- 2017 Rahman, L., Wu, D., Johnston, M., William, A. and Halappanavar, S.; Toxicogenomics analysis of mouse lung responses following exposure to titanium dioxide nanomaterials reveal their disease potential at high doses. Mutagenesis, 32(1): 59-76
- 2016 McConnell, E.M., Bolzon, R., Mezin, P., Frahm, G., Johnston, M. and DeRosa, M.C.; PHAST (pH-Driven Aptamer Switch for Thrombin) Catch-and-Release of Target Protein. Bioconjugate Chem., 27 (6): 1493–1499
- 2016 Frahm, G.E., Pochopsky, A.W.T., Clarke, T.M. and Johnston, M.J.W.; Evaluation of microflow digital imaging particle analysis for sub-visible particles formulated with an opaque vaccine adjuvant. PLoS ONE, 11(2)
- 2016 Decan, N., Wu, D., Williams, A., Bernatchez, S., Johnston M., Hill, M. and Halappanavar, S.; Characterization of *in vitro* genotoxic, cytotoxic and transcriptomic responses following exposures to amorphous silica of different sizes. Mutation Research, 796: 8-22. Rahman, L., Wu, D., Johnston, M., William, A. and Halappanavar, S.; Toxicogenomics analysis of mouse lung responses following

exposure to titanium dioxide nanomaterials reveal their disease potential at high doses. Mutagenesis, 32(1): 59-76

- 2016 McConnell, E.M., Bolzon, R., Mezin, P., Frahm, G., Johnston, M. and DeRosa, M.C.; PHAST (pH-Driven Aptamer Switch for Thrombin) Catch-and-Release of Target Protein. Bioconjugate Chem., 27 (6): 1493–1499.
- 2016 Frahm, G.E., Pochopsky, A.W.T., Clarke, T.M. and Johnston, M.J.W.; Evaluation of microflow digital imaging particle analysis for sub-visible particles formulated with an opaque vaccine adjuvant. PLoS ONE, 11(2)
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## Jinmyoung Joo

Assistant Professor

Jinmyoung Joo is Assistant Professor of Convergence Medicine at the University of Ulsan College of Medicine, Seoul, Rep. of Korea. He holds affiliate appointment in the biomedical engineering research center at Asan Medical Center. He received B.S. (2007) and Ph.D. (2012) degrees in Chemical Engineering from Pohang University of Science and Technology (POSTECH) under the direction of Prof. Sangmin Jeon. He joined the faculty in the Department of Convergence Medicine at the University of Ulsan College of Medicine in 2016, after postdoctoral studies at University of California, San Diego under Prof. Michael J. Sailor in strong collaboration with the laboratory of Prof. Erkki Ruoslahti at Sanford Burnham Prebys Medical Discovery Institute and with the laboratory of Prof. Sangeeta Bhatia at MIT. His lab is interested in understanding the interaction of nanomaterials with complex biological systems, engineering novel nanostructures that can effectively target diseases such as cancer and infection, and developing theranostic nanoplatfoms for bioimaging and drug delivery. In order to fulfill these goals, Prof. Joo leads a multidisciplinary team dedicated to advancing human health by emerging engineering approaches with discovery in molecular biology.

## RECENT PUBLICATION

- Enhanced antibacterial efficacy of antibiotics-loaded nanoparticles targeted to the site of infection, Nature Biomedical Engineering, 2, 95-103 (2018).
- Tracking the fate of porous silicon nanoparticles delivering a peptide payload by intrinsic photoluminescence lifetime, Advanced Materials, DOI:10.1002/adma.201802878 (2018).
- Thermally induced silane dehydrocoupling on silicon nanostructures, Angewandte Chemie International Edition, 55, 6423-6427 (2016).
- Gated luminescence imaging of silicon nanoparticles, ACS Nano, 9, 6233-6241 (2015).
- A highly sensitive diagnostic assay for the detection of protein biomarkers using microresonators and multifunctional nanoparticles, ACS Nano, 6, 4375-4381 (2012).



## Dong-Wan Kang

I received an MD from Seoul national university college of medicine, Seoul, South Korea, in 2012. I completed resident training in neurology in Seoul national university hospital in 2017. After completing training, I am working in Gangjin public health center as an alternative military service, providing clinical care.

I have been actively involved in clinical work, providing treatment for patients with stroke, dementia, movement disorders, neuromuscular disorders, epilepsy, etc. I received the best resident award in my PGY2 for my good work attitude and sincerity. I also have been interested in studying neuroimaging. I reported a study on susceptibility vessel sign (SVS) visualized in magnetic resonance imaging (MRI) (Stroke, 2017, 48(6): 1554-1559). Besides working as a clinician, what has motivated me was that great researches can contribute a lot to give a breakthrough to treatment of diseases. To be specific, I have been interested in nanomedicine which may enables novel approaches to the various diseases that has not been conquered by current medical technology. In 2013, I met my mentor, Seung-Hoon Lee, MD, PhD, FAHA (Fellow of American Heart Association), professor in Seoul national university hospital. His published work on ceria nanoparticles for treating ischemic stroke (Angewandte Chemie Int Ed, 2012, 51(44): 11039-11043) and continuing effort fit my research interest very well. I joined his lab, Laboratory of Innovative Nanobiotechnology. I worked with collaborators and studied on the biomedical applications of inorganic nanomaterials. I, my colleagues, and Prof. Lee reported that ceria nanoparticles reduced inflammation after intracerebral hemorrhage via reactive oxygen species (ROS) scavenge, resulting reducing in perihematomal edema in the animal model (Nano Research, 2017, 10(8): 2743-2760). We also reported that ceria-zirconia nanoparticles, Zr4+-incorporated ceria nanoparticles with higher Ce3+/Ce4+ ratio and faster conversion from Ce4+ to Ce3+ than those exhibited by ceria nanoparticles, was effective in reducing mortality and systemic inflammation in sepsis animal models (Angewandte Chemie Int Ed, 2017, 11(56): 11399-11403). I presented my works in several domestic and international conferences as well. We are continuing our research to investigate pharmacokinetics and toxicity of ceria nanoparticles and determine whether it can be further developed into an investigational new drug.



## Keon Wook Kang

Dr. Keon W. Kang, a nuclear medicine physician, is a professor and the Chairman of the Department of Nuclear Medicine, Seoul National University College of Medicine. He received an M.D. degree from Seoul National University College of Medicine (1991). He was trained as an intern and a resident for Internal Medicine at Seoul National University Hospital (1991-1996). He received Ph.D. in Medical Science at Seoul National University College of Medicine (2001). He has worked as Chief, Department of Nuclear Medicine, National Cancer Center, Korea (2000-2007). He studied molecular imaging at Prof. Sam Gambhir's lab as a visiting scientist of Molecular Imaging Program at Stanford (2003-2004). His research areas are *in vivo* molecular imaging of cancer using PET & nanoparticles. He published more than 160 articles in peer reviewed journals. He is President of the Korean Society for Nanomedicine since 2017

### RECENT PUBLICATION

- Jang JT, Lee J, Seon J, Ju E, Kim M, Kim YI, Kim MG, Takemura Y, Arbab AS, Kang KW, Park KH, Paek SH, Bae S. Giant Magnetic Heat

Induction of Magnesium-Doped  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> Superparamagnetic Nanoparticles for Completely Killing Tumors. *Adv Mater.* 2018 Feb;30(6).

- Lee IJ, Park JY, Kim YI, Lee YS, Jeong JM, Kim J, Kim EE, Kang KW, Lee DS, Jeong S, Kim EJ, Kim YI, Chung JW. Image-Based Analysis of Tumor Localization After Intra-Arterial Delivery of Technetium-99m-Labeled SPIO Using SPECT/CT and MRI. *Mol Imaging.* 2017 Jan 1;16
- Kim YI, Jeong S, Jung KO, Song MG, Lee CH, Chung SJ, Park JY, Cha MG, Lee SG, Jun BH, Lee YS, Hwang DW, Youn H, Kang KW, Lee YS, Jeong DH, Lee DS. Simultaneous Detection of EGFR and VEGF in Colorectal Cancer using Fluorescence-Raman Endoscopy. *Sci Rep.* 2017 Apr 21;7(1):1035
- Bakht MK, Oh SW, Hwang DW, Lee YS, Youn H, Porter LA, Cheon GJ, Kwak C, Lee DS, Kang KW. The potential roles of radionanomedicine and radioexosomic in prostate cancer research and treatment. *Curr Pharm Des.* 2017;23(20):2976-2990.
- Jung KO, Youn H, Lee CH, Kang KW, Chung JK. Visualization of exosome-mediated miR-210 transfer from hypoxic tumor cells. *Oncotarget.* 2017 Feb 7;8(6):9899-9910



## Peter Kapitein

Patient Advocate

As a Patient Advocate of Inspire2Live Dr h.c. Peter Kapitein connects patients, researchers and clinicians to further research, treatments and care; in the Netherlands as well as international. He organizes congresses, lobbies the matrix of public authorities, health care organizations, insurance companies and health research institutes. Peter also gives lectures and talks to help patients and society to fight cancer where possible and live with cancer with a good quality of life. He writes blogs, articles and books that contribute to these topics. Peter has studied the Medical Industrial Complex, the complex in which the stakeholders in healthcare work together in a way that does not necessarily benefit the patient. Health care is (without bad intention) distracted from its essence: the patient.

Peter is the co-founder of Alpe d'HuZes, the foundation that is most famous for the annual cycling event on Mount Alpe d'Huez and that raised over 150 million euro for the fight against cancer. He works at the Central Bank of the Netherlands as a program manager and advisor for complex and politically difficult problems. His employer facilitates him in this job. Peter was honoured with a doctorate in October 2012 at the Free University in Amsterdam for connecting patients, researchers and clinicians all over the world.

### RECENT PUBLICATION

'Hoe heeft het zover kunnen komen? ... Een frisse blik op de gezondheidszorg'; Publisher Uitgeverij Water; Year of publication 2016; ISBN 978 94 92495 06 8; This one will be available in English at the beginning of 2018.



## Kazunori Kataoka

Director General, Innovation Center of NanoMedicine, Kawasaki Institute of Industrial Promotion  
Professor, Policy Alternatives Research Institute, the University of Tokyo

Dr. Kazunori Kataoka is a Professor at the Policy Alternatives Research Institute, The



University of Tokyo, and the Director General of the Innovation Center of NanoMedicine (iCONM) at the Kawasaki Institute of Industrial Promotion. He obtained his PhD degree in polymer chemistry in 1979 at The University of Tokyo. He was an Assistant Professor from 1979 to 1988, and an Associate Professor from 1988 to 1989, at The Institute of Biomedical Engineering, Tokyo Women's Medical College. In 1989, he became Associate Professor at the Department of Materials Engineering in the Tokyo University of Science until 1994, when he was promoted to Professor. From 1998 to 2016, Dr. Kataoka was Professor of Biomaterials at the Graduate School of Engineering, The University of Tokyo, and from 2004 to 2016, he was also appointed Professor of the Division of Clinical Biotechnology at the Center for Disease Biology and Integrative Medicine in the Graduate School of Medicine of The University of Tokyo. In 2016, he took mandatory retirement from the Graduate School of Engineering/Graduate School of Medicine, The University of Tokyo, and moved to the current position. He has been appointed as Adjunct Professor at Eshelman School of Pharmacy, the University of North Carolina Chapel Hill since 2015, and as the Director, Biomedical Institute for Convergence at SKKU (BICS) at Sungyunkwan University, Korea since 2016. Dr. Kataoka has been recipient of several awards, such as the Clemson Award from the Society for Biomaterials, USA (2005), the Founder's Award from the Controlled Release Society (2008), Humboldt Research Award from Alexander von Humboldt Foundation (2012), Leo Esaki Prize (2012) and Gutenberg Research Award from Johannes Gutenberg University Mainz, Germany (2015). In 2017, he has been elected to a Foreign Member of the National Academy of Engineering, U.S.A., and in 2018, a Fellow of the National Academy of Inventors, U.S.A.. Dr. Kataoka has published over 500 peer-reviewed papers (h-index 133). He has been on the board of over 15 international journals, including Editor of Journal of Biomaterials Science, Polymer Edition and Associate Editor of ACS Nano. His research aims are to develop functional polymeric nanosystems for controlling cellular functions in a desirable manner through the delivery of therapeutic agents, such as drugs and genes.

## RECENT PUBLICATION

- N. Yoshinaga, et al, Polyplex micelles with phenylboronate/glucanamide crosslinking in the core exerting promoted gene transfection through spatiotemporal responsivity to intracellular pH and ATP concentration. *J. Am. Chem. Soc.* 139(51) 18567-18575 (2017).
- Y. Anraku, et al, Glycaemic control boosts glucosylated nanocarrier crossing the BBB into the brain. *Nature Commun.* 8 1001 (2017).
- K. Katsushima, et al, Targeting the notch-regulated non-coding RNA TUG1 for glioma treatment. *Nature Commun.* 7 13616 (2016).
- P. Mi, et al, A pH-activatable nanoparticle with signal-amplification capabilities for non-invasive imaging of tumour malignancy. *Nature Nanotech.* 11(8) 724-730 (2016).
- Y. Matsumoto, et al, Vascular bursts enhance permeability of tumour blood vessels and improve nanoparticle delivery. *Nature Nanotech.* 11(6) 533-538 (2016).



## Daniela Katz

Dr. Daniela Katz, MD is a specialist in Internal Medicine and a Medical Oncologist. Dr. Katz is the head of breast and sarcoma services at Assaf Harofeh Medical Center in Israel.

Dr. Katz focuses on wide translational and clinical perspectives of breast cancer and sarcoma and studies specifically personalized targeted immunotherapy aspects in breast cancer and sarcoma.

Dr. Katz has graduated medical school, as MD, at the Hadassah Hebrew University Medical School, Jerusalem, Israel in 1996. In 2004

Dr. Katz has become an Internal Medicine specialist and in 2008 she has been certified as a Medical Oncologist.

Dr. Katz continued her education focusing on translational research in sarcoma at M.D. Anderson Sarcoma Center as a research fellow. Thereafter, she has established herself as a leading sarcoma specialist. She is an active member of the EORTC sarcoma group and serves as a reviewer of several journals amongst is *Annals of Oncology*. She has launched two investigator-initiated clinical trials in sarcoma one with ribociclib and the other with nivolumab and ipilimumab.



## Fabian Kiessling

Director of the Institute for Experimental Molecular Imaging (ExMI)

Since 2008 Professor Dr. Fabian Kiessling is leading the Institute of Experimental Molecular Imaging at the Helmholtz Institute for Biomedical Engineering at the RWTH-University in Aachen. Aim of his research

is the development of novel diagnostic, theranostic and therapeutic probes as well of advanced imaging technologies and image analysis tools. In this context, the main focus of his research is the investigation and diagnostic assessment of vascular and microenvironmental tissue properties and the exploration of its impact on disease progression and therapy response.

Fabian Kiessling studied Medicine and did his thesis at the University in Heidelberg. Until the end of 2002, he worked as resident in the Department of Radiology at the German Cancer Research Center (DKFZ) in Heidelberg. In 2003 he changed to the Department of Medical Physics in Radiology of the DKFZ as leader of the Molecular Imaging group. In parallel, he did his clinical training at different Departments of the University of Heidelberg and received the board certification as Radiologist in 2007. Fabian Kiessling did his habilitation in experimental radiology in 2006. In 2008 he founded the *in vivo* Contrast GmbH together with Matthias Braeutigam.

Fabian Kiessling is author of more than 250 scientific publications and book chapters, edited three books and received many research awards, among those the „Emil Salzer Price for Cancer Research“ and the „Richtzenhain Price“.

Professor Kiessling was in the Editorial board of several scientific journals including *Radiology*, *European Radiology*, *European Radiology Experimental*, and the *American Journal of Nuclear Medicine and Molecular Imaging*.

He is founding member of the European Society for Functional and Molecular Imaging in Radiology (ESMOFIR), currently treasurer of the European Society for Molecular Imaging (ESMI), founding member of the ESMI working group „Image Guided Therapy and Drug Delivery (IGTDD)“ and he was chairman of the „Molecular Imaging“ subcommittee of the European Society for Radiology (ESR). Furthermore, he was program chair of the European Molecular Imaging Meeting (EMIM) in Antwerp in 2014 and the World Molecular Imaging Conference (WMIS) in New York in 2016.

## RECENT PUBLICATIONS:

- Curaj A., Wu Z., Rix A., Sternkopf M., Alampour-Rajabi A., Gresch O., Lammers T., van Zandvoort M., Weber C., Koenen R.R., Liehn E.A., Kiessling F. (2018) Molecular Ultrasound Imaging of JAM-A Depicts Acute Alterations in Blood Flow and Early Endothelial Dysregulation. *Arterioscler Thromb Vasc Biol*, 38:40-48
- Tsvetkova Y., Beztsinna N., Baues M., Klein D., Rix A., Golombek S., Al Rawashdeh W., Gremse F., Barz M., Koynov K., Banala S., Lederle W., Lammers T., Kiessling F. (2017) Balancing Passive and Active Targeting to Different Tumor Compartments Using Riboflavin-functionalized Polymeric Nanocarriers. *Nano Lett*, 17:4665-4674
- Repenko T., Rix A., Ludwanowski S., Go D., Kiessling F., Lederle W., Kuehne A.J.C. (2017) Bio-degradable highly fluorescent con-



jugated polymer nanoparticles for bio-medical imaging applications. *Nat Commun*, 8:470

- Lammers T., Kiessling F., Ashford M., Hennink W., Crommelin D., Storm G. (2016) Cancer nanomedicine: is tumour targeting our target? *Nat Rev Mater*, 1(9). pii: 16069
- Lammers T., Koczera P., Fokong S., Gremse F., Ehling J., Vogt M., Pich A., Storm G., van Zandvoort M., Kiessling F. (2015) Theranostic USPIO-loaded microbubbles for mediating and monitoring blood-brain barrier permeation. *Adv Funct Mater*, 25:36-43



## Andreas Kjaer

Andreas Kjaer (MD, PhD, DMSc, MBA) is professor at the University of Copenhagen and chief physician and head of research at Dept. of Clinical Physiology, Nuclear Medicine & PET at Rigshospitalet, the National University Hospital of Denmark. He served 6 years as president of the Scandinavian Society for Clinical Physiology and Nuclear

Medicine (SSCPNM) and 6 years on the scientific board of the Danish Cancer Society. He is currently national representative of the European Society for Molecular Imaging (ESMI), member of the Oncology Committee of EANM, member of the ENETS advisory board, national director of the European Advanced Translational Research Infrastructure in Medicine and editor-in-chief of *Diagnostics* and on numerous editorial boards including *JNM* and *EJNMMI*. His research is focused on molecular imaging with PET and PET/MRI in cancer and his achievements include development of several new tracers that have reached first-in-human clinical use. He is holder of an ERC Advanced Grant and coordinator of an H2020 project. He has published more than 400 peer-review articles, supervised 30 PhD students, co-founded 3 biotech companies and has received numerous prestigious scientific awards over the years. He is a member of the Danish Academy of Technical Sciences.



## Andrey Klymchenko

Andrey Klymchenko was born in Ukraine, in 1976. He started his research with chemistry and photophysics of new fluorescent dyes, which was a subject of his PhD degree from Kiev National University in 2003. Then, he worked in the University of Strasbourg, where he could combine synthesis of new dyes with their bioimaging applica-

tions. In 2005, in order to extend his expertise towards supramolecular chemistry and nanotechnology, he moved to Catholic University of Leuven. Then, he joined CNRS in 2006, received CNRS Bronze Medal in 2010 and was promoted to Director of Research in 2014. In 2015, he obtained ERC consolidator grant BrightSens to work on fluorescent nanoparticles for ultrasensitive detection of cancer markers. He is a leader of "Nanochemistry and Bioimaging" group. His research interests include functional fluorescent molecules and nanomaterials for biosensing, imaging and theranostics. He has already developed a number of fluorescent probes for cellular imaging, notably a probe for apoptosis detection, which is currently on the market. Moreover, he recently introduced new concepts for development of ultrabright dye-loaded nanoparticles based on polymers and lipids for bioimaging applications. He is a co-author of over 160 peer-reviewed articles.

### SELECTED PUBLICATIONS

- Trofymchuk, K.; Reisch, A.; Didier, P.; Frasn, F.; Gilliot, P.; Mely, Y.; Klymchenko, A. S. Giant light-harvesting nanoantenna for single-

molecule detection at ambient light. *Nature Photonics* 2017, 11, 657.

- Reisch, A.; Klymchenko, A.S. Fluorescent Polymer Nanoparticles Based on Dyes: Seeking Brighter Tools for Bioimaging. *Small* 2016, 12, 1968.
- Reisch A, Runser A, Arntz Y, Mély Y, Klymchenko AS. Charge-controlled nanoprecipitation as a modular approach to ultrasmall polymer nanocarriers: making bright and stable nanoparticles. *ACS Nano* 2015, 9, 5104.
- Reisch A, Runser A, Arntz Y, Mély Y, Klymchenko AS. Charge-controlled nanoprecipitation as a modular approach to ultrasmall polymer nanocarriers: making bright and stable nanoparticles. *ACS Nano* 2015, 9, 5104.
- Reisch, A.; Didier, P.; Richert, L.; Oncul, S.; Arntz, Y.; Mély, Y.; Klymchenko, A. S. Collective fluorescence switching of counterion-assembled dyes in polymer nanoparticles. *Nature Commun.* 2014, 5, 4089.



## Netanel Korin

Assistat Professor

Netanel Korin is an Assistant Professor at the faculty of Biomedical Engineering at The Technion - Israel Institute of Technology and the head of the Cardiovascular NanoMed Engineering lab. Prior to joining the Technion, Dr. Korin was a Wyss Technology Development Fellow and a Research Associate at the Wyss Institute for Biologically Inspired Engineering at Harvard University. He received a Bachelor's degree in Mechanical Engineering, a Master's and a Ph.D. in Biomedical Engineering from the Technion. Netanel has authored over 20 papers in *Science*, *Nano letters*, *Lab on a Chip*, *Physical Review Letters*, *Journal of Biomechanics* and other major research journals. His work has been highlighted in other leading journals including *Nature*, *Nature Biotechnology*, *Nature Drug Discovery Reviews* and *New England Journal of Medicine*. Netanel has also won several honors and awards including a Wyss Technology Development Fellowship, Outstanding Paper Award ASME NEMB, IEEE MNM best poster award, the International Society for Clinical Hemorheology (ISCH) Travel Award, and the Aaron and Miriam Gutwirth Memorial Scholarship. Dr. Korin's research group focuses on engineering aspects of vascular biology with emphasis on the interplay between hemodynamics, vascular physiology, and transport phenomena in vascular diseases. The long-term objective of the group's research is to allow better understanding of the biophysical determinants of vascular disease and to leverage this knowledge to develop innovative therapeutic and diagnostic approaches.

### RECENT PUBLICATION

- Korin N, M. Kanapathipillai, B.D. Matthews, M. Crescente, A. Brill, T. Mammoto, K. Ghosh, S. Jurek, S.A. Bencherif, D. Bhatta, A.U. Coskun, C.L. Feldman, D.D. Wagner, D.E. Ingber "Shear-Activated Nanotherapeutics for Drug Targeting to Obstructed Blood Vessels", *Science*, 2012, 337(6095), 738-42.
- Marosfoi M.\*, N. Korin\*, M. Gounis\*, O. Uzun, S. Vedantham, E. Langan, A.L. Papa, O. Brooks, C. Johnson, A. Puri, D. Bhatta, M. Kanapathipillai, B. Bronstein, J. Chueh, D.E. Ingber, A. Wakhloo, "Shear-Activated Nanoparticle Aggregates Combined With Temporary Endovascular Bypass to Treat Large Vessel Occlusion", *Stoke*, 2015, 46(12), 3507-13.
- Papa A.L.\*, N. Korin\*, M. Kanapathipillai\*, A. Mammoto, T. Mammoto, A. Jiang, R. Mannix, O. Uzun, C. Johnson, D. Bhatta, G. Cuneo, D.E. Ingber, "Ultrasound-sensitive nanoparticle aggregates for targeted drug delivery", *Biomaterials*, 2017, 139, 187-194.
- Epshtein M. and N. Korin, "Shear Targeted Drug Delivery to Stenotic Blood Vessels", *Journal of Biomechanics*, 2017, 4 (50), 217-221.
- Korin N., Gounis MJ, Wakhloo AK, Ingber DE, Targeted Drug De-



## Sarah Kraus

Head of Biology Department,  
NewPhase Ltd., Israel

Sarah Kraus graduated at Tel Aviv University, Tel Aviv, Israel. She completed her Ph.D. studies in Immunology and Cell Biology on the involvement of second messengers and intracellular signaling molecules in the development of tumor cell resistance to the complement system. She did further research as a postdoctoral fellow at the Weizmann Institute of Science, Rehovot, Israel in the field of signal transduction and cancer research and investigated the activation of mitogen-activated protein kinase (MAPK) pathways and signaling pathways induced by G-protein coupled receptors, using the GNRH receptor as a prototype, in prostate and breast cancer models. She then continued her research as a Research Associate at the Department of Microbiology and Cancer Center, University of Virginia Health System, Charlottesville, Virginia, USA where she studied signaling pathways involved in the development of prostate cancer. After returning to Israel in 2006, Dr. Kraus joined the Integrated Cancer Prevention Center at the Tel Aviv Medical Center, Israel as a Senior Scientist and Head of the Molecular Biology research laboratory. She also joined the teaching staff at the Faculty of Medicine, Tel Aviv University and became an Assistant Prof. and Lecturer. On 2016, and after completing an M.B.A. degree in Biomedical management, she joined NewPhase Ltd., a company engaged in the development of a novel Nanotechnology-based therapeutic approach for the treatment of cancer.

### PUBLICATIONS (SELECTED AMONG 67 PUBLICATIONS):

- Kraus S, Shapira S, Kazanov D, Naumov I, et.al. 2015. Predictive levels of CD24 in peripheral blood leukocytes for the early detection of colorectal adenomas and adenocarcinomas. *Dis Markers*. 2015. doi: 10.1155/2015/916098.
- Naumov I, Zilberberg A, Shapira S, Avivi D, Kazanov D, Rosin-Arbesfeld R, Arber N, Kraus S. 2014. CD24 knockout prevents colorectal cancer in chemically induced colon carcinogenesis and in APCMin /CD24 double knockout transgenic mice. *Int J Cancer*. 135:1048-59.
- Kraus S, Nabiochtchikov I, Shapira, S, Arber N. 2014. Recent advances in personalized colorectal cancer research. *Cancer Lett*. 28; 347:15-21.
- Shapira S, Kazanov D, Weisblatt S, Starr A, Arber N, Kraus S. 2011. The CD24 protein inducible expression system is an ideal tool to explore its potential as an oncogene and a target for immunotherapy *in vitro* and *in vivo*. *J Biol Chem*. 286:40548-55.
- Kraus S, Gioeli D, Vomastek T, Gordon V, M J Weber. 2006. Receptor for activated C kinase 1 (RACK1) and Src regulate the tyrosine phosphorylation and function of the androgen receptor. *Can Res* 66:11047-54.



## Silke Krol

I.R.C.C.S Istituto tumori  
"Giovanni Paolo II". Bari, Italy &  
Fondazione I.R.C.C.S. Istituto Neurologico  
Carlo Besta, Milan, Italy  
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Since 2010 Silke Krol is with Fondazione I.R.C.C.S. Istituto Neurologico "Carlo Bes-

ta" in Milan, Italy and heads the laboratory for Nanomedicine. In 2016 she started to work in parallel for the Istituto tumori "Giovanni Paolo II" in Bari, Italy and leads the laboratory for translational Nanotechnology with focus on early diagnosis and advanced therapy of cancer.

At Besta, she is studying the transport mechanisms for differently functionalized gold nanoparticles across the blood brain barrier and how this is influenced by blood-derived proteins. Moreover, different novel metallic and non-metallic delivery systems for various other diseases (cardiovascular, prion disease, epilepsy, glioma, lymphomas, viral diseases) were designed for projects funded by Italian and European foundations. In collaboration with her Prof. Stellacci in Lausanne, Switzerland, they discovered the role of nanoparticles in vaccine stabilization, antiviral action and enhancer for viral infectivity for gene delivery. Additionally her group develops multifunctional polymer/nanogold based drug or drug delivery systems as well as diagnostic tool for medical applications such as a hand-held point-of-care device to measure chemotherapeutic drugs in real-time at the bedside of the patient. Moreover, the multilayer-nanocoating was used for encapsulation and immune protection of living cells like e.g. pancreatic islets. She has several pending patents for possible future drugs for prion disease and cancer treatment, viral diseases, and cancer diagnostics.

In Bari, her research aims at understanding the role of exosomes and the tumor secretome in cancer and predictive diagnosis as well as on the development of drug delivery systems for lung cancer and melanoma.

In 2009 she worked as an expert consultant for the United Nations and serves as external expert reviewer for National projects in France, Italy, Georgia and Greece. She worked as project technical advisor in 3 EU-FP7 projects and is external expert for the evaluation of EU project. She is member of the advisory board of "Euro-Nanotox-Letters" and the international advisory committee of the International scientific spring conference in Islamabad, Pakistan. She is member of the advisory board of the CLINAM-Foundation, in the editorial board of the journal "Precision Medicine", and associate editor of "Frontiers in Nanobiotechnology". Since 2013 she is adjunct faculty member at the Pakistan Institute of engineering and applied science (PIEAS).



## Reijo Laaksonen

Prof.

Reijo Laaksonen is a cardiovascular Clinical Pharmacologist with extensive scientific training at Clinical Research Institute of Montreal, Canada and Free University of Brussels (Erasmus hospital). He has over 150 original scientific publications and numerous patents in the cardiovascular field. Reijo was Coordinator of one of the largest EU funded cardiovascular FP7 project - "Atheroremo". Scientific manager of the 6 million euro AtheroFlux project and Steering Committee member of 6 million euro Risky-CAD project. He is also in charge of the medical operations at the Finnish Clinical Biobank, Tampere and Director of Finnish Cardiovascular Research Center Tampere. Prof Laaksonen is co-founder of Zora Biosciences Oy, a lipidomics technology based biomarker company.

### RECENT PUBLICATION

- Laaksonen R, Ekroos K, Sysi-Aho M, Hilvo M, Vihervaara T, Kauhane D, Suoniemi M, Hurme R, März W, Schrnagl H, Stojakovic T, Vlachopoulou E, Lokki ML, Nieminen MS, Klingenberg R, Matter CM, Hornemann T, Jüni P, Rodondi N, Räber L, Windecker S, Gencer B, Pedersen ER, Tell GS, Nygård O, Mach F, Sinisalo J, Lüscher TF. Plasma ceramides predict cardiovascular death in patients with stable coronary artery disease and acute coronary syndromes be-

yond LDL-cholesterol. *Eur Heart J.* 2016 Jul 1;37(25):1967-76. doi: 10.1093/eurheartj/ehw148. PubMed PMID: 27125947.

- Havulinna AS, Sysi-Aho M, Hilvo M, Kauhanen D, Hurme R, Ekroos K, Salomaa V, Laaksonen R. Circulating Ceramides Predict Cardiovascular Outcomes in the Population-Based FINRISK 2002 Cohort. *Arterioscler Thromb Vasc Biol.* 2016 Dec;36(12):2424-2430. PubMed PMID: 27765765.
- Tarasov K, Ekroos K, Suoniemi M, Kauhanen D, Sylvänne T, Hurme R, Gouni-Berthold I, Berthold HK, Kleber ME, Laaksonen R, März W. Molecular lipids identify cardiovascular risk and are efficiently lowered by simvastatin and PCSK9 deficiency. *J Clin Endocrinol Metab.* 2014 Jan;99(1):E45-52. doi: 10.1210/jc.2013-2559. PubMed PMID: 242436304.
- Cheng JM, Suoniemi M, Kardys I, Vihervaara T, de Boer SP, Akkerhuis KM, Sysi-Aho M, Ekroos K, Garcia-Garcia HM, Oemrawsingh RM, Regar E, Koenig W, Serruys PW, van Geuns RJ, Boersma E, Laaksonen R. Plasma concentrations of molecular lipid species in relation to coronary plaque characteristics and cardiovascular outcome: Results of the ATHEROREMO-IVUS study. *Atherosclerosis.* 2015 Dec;243(2):560-6. doi: 10.1016/j.atherosclerosis.2015.10.022. PubMed PMID: 26523994.
- Hilvo M, Salonurmi T, Havulinna AS, Kauhanen D, Pedersen ER, Tell GS, Meyer K, Teeriniemi AM, Laatikainen T, Jousilahti P, Savolainen MJ, Nygård O, Salomaa V, Laaksonen R. Ceramide stearic to palmitic acid ratio predicts incident diabetes. *Diabetologia.* 2018 Mar 15. doi: 10.1007/s00125-018-4590-6.



## Twan Lammers

Prof. Dr. Dr. Twan Lammers  
Dept. of Nanomedicine and Theranostics  
Institute for Experimental Molecular  
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Center for Biohybrid Medical Systems  
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52074 Aachen, Germany

Twan Lammers obtained a DSc degree in Radiation Oncology from Heidelberg University in 2008 and a PhD degree in Pharmaceutics from Utrecht University in 2009. In the same year, he started the Nanomedicine and Theranostics group at the Institute for Experimental Molecular Imaging at RWTH Aachen University Clinic. In 2014, he was promoted to full professor at the faculty of medicine at RWTH Aachen. He has published over 150 research articles and reviews (>8500 citations, h-index 47), and received several scholarships and awards, including a starting and two proof-of-concept grants from the European Research Council, and the young investigator award of the Controlled Release Society. He is associate editor for Europe for the *Journal of Controlled Release* and serves on the editorial board member of multiple other journals. His primary research interests include drug targeting to tumors and to the brain, image-guided drug delivery, and molecular imaging of liver and kidney fibrosis.



## Katharina Landfester

Director of the Max Planck Institute for  
Polymer Research

Katharina Landfester studied chemistry at the Technical University of Darmstadt. For her diploma thesis, she was at the Ecole d'Application des Hauts Polymères in Strasbourg (Prof. M. Lambla). In 1995, she re-

ceived her doctoral degree in physical chemistry from the Johannes Gutenberg University of Mainz after working with Prof. H.W. Spiess at the Max Planck Institute for Polymer Research on the synthesis and characterization of core-shell latexes by transmission electron microscopy and solid state NMR. After spending another year as a group leader at the institute, she moved for a postdoctoral stay at the Lehigh University (Prof. M. El-Aasser) where she first came in contact with the miniemulsion technique. She returned to Germany in 1998 joining the group of Prof. M. Antonietti at the Max Planck Institute of Colloids and Interfaces in Golm. There, she led the miniemulsion group working on new possibilities in the synthesis of complex nanoparticles. In 2002, she got her habilitation in physical chemistry at the University of Potsdam. In 2003, she accepted a chair (C4) of Macromolecular Chemistry at the University of Ulm. Here, she started her activities in the field of biomedical applications in cooperation with several medical groups working on the interaction of nanoparticles with different cell compartments, the labelling of cells and the delivery of substances to specific sides. Since 2008, she is director at the Max Planck Institute for Polymer Research. In 2001 she was awarded the Reimund Stadler price of the Gesellschaft Deutscher Chemiker (GdCh) and the price of the Dr. Hermann Schnell Stiftung. From 2002 to 2007, she was a member of the Young Academy (Junge Akademie) of the Berlin-Brandenburgischen Akademie der Wissenschaften und Deutschen Naturforscher Leopoldina; in 2003/2004 she acted as the spokesperson of the Young Academy. Since 2010, she is member of the Academy of Technical Science (Acatech). She has published more than 600 papers in international journals, 30 reviews and holds more than 50 patents.

## RECENT PUBLICATION

- B. Kang, P. Okwieka, S. Schöttler, S. Winzen, J. Langhanki, K. Mohr, T. Opatz, V. Mailänder, K. Landfester, F.R. Wurm, "Carbohydrate-Based Nanocarriers Exhibiting Specific Cell Targeting with Minimum Influence from the Protein Corona", *Angew. Chem. Internat. Ed.* 2015, 54, 7436-7440
- S. Schöttler, G. Becker, S. Winzen, T. Steinbach, K. Mohr, K. Landfester, V. Mailänder, F.R. Wurm, "Protein adsorption is required for stealth effect of poly(ethylene glycol)- and poly(phosphoester)-coated nanocarriers", *Nature Nanotechnol.* 2016, 11, 372-377.
- S.U. Frick, M.P. Domogalla, G. Baier, F.R. Wurm, V. Mailänder, K. Landfester, K. Steinbrink, "IL-2 functionalized nanocapsules for T cell-based immunotherapy", *ACS Nano* 2016, 10, 9216-9226
- M. Kokkinopoulou, J. Simon, K. Landfester, V. Mailänder, I. Lieberwirth, "Visualization of the protein corona: towards a biomolecular understanding of nanoparticle cell- interactions", *Nanoscale* 2017, 9, 8858-8870
- J. Müller, K.N. Bauer, D. Prozeller, J. Simon, V. Mailänder, F.R. Wurm, S. Winzen, K. Landfester; "Coating nanoparticles with tunable surfactants facilitates control over the protein corona", *Biomaterials* 2017, 115, 1-8.



## Philipp Langer

Philipp Langer was born in 1972 in Zurich, Switzerland.

He holds both the Swiss and the Austrian citizenships.

## EDUCATION:

Philipp Langer holds a BSc & MSc in Pharmaceutical Sciences (1992-1997) and a PhD in Biology/Behavioral Genetics from the University of Lausanne (1998-2003, in collaboration with the Flinders University of South Australia; main article in *Nature*, 2004). At Harvard University, USA, Philipp made a PostDoc in game theory and evolutionary dynamics in 2004-2006. Shorter research projects included work on host/virus interactions (University of Utah, Salt Lake City, USA), brain research (ETH/University of Zurich, Switzerland) and cardiovascular



research (University of British Columbia, Vancouver, Canada).

## PROFESSIONAL BACKGROUND:

After his research career, Philipp Langer was laureate of the 'Scientific Politics Scholarship' in Switzerland in 2008-2009 and worked as a scientific politics advisor ('Congress Fellow') in the Parliament of Switzerland. Following this, he participated in a law revision project on the Swiss Therapeutic Products Act for a public relations enterprise. He subsequently joined Switzerland's State Secretariat for Education and Research in 2009, first as Scientific Advisor, where he served as the Swiss Delegate in several committees of the EU (e.g. the FP7 Programme Committees 'Health', 'Research Infrastructures', 'Security', the European Research Infrastructures Committee ERIC, or the European Strategy Forum on Research Infrastructures ESFRI), of the EFTA or of the OECD (e.g. 'Global Science Forum' and 'Global Earthquake Model' GEM). A year later, Philipp became Deputy Head of the International Research Cooperation division; in this position, he took over the responsibilities for the EU Framework Programmes, where he notably developed the Swiss positions to the EC's Green Paper and to Horizon 2020. He was also project leader for the compensation payments made to Swiss researchers suffering from the negative effects of the strong Swiss franc in 2011.

## CURRENT RESPONSIBILITIES:

Since 2013, Philipp Langer is Head of the 'EU Framework Programmes' division in the then newly formed State Secretariat for Education, Research and Innovation (SERI). In 2018, he was appointed to the rank of Director of EU Framework Programmes and the European Research and Innovation Area (ERA) and as Swiss High-Level Negotiator for the EU Programmes. He is responsible for the international and national processes for Switzerland's association to the EU Framework Programmes in Research and Innovation (Horizon 2020, Horizon Europe and the Euratom Programme). As such, Philipp is in charge of negotiating the Association Agreements between the EU and Switzerland. He and his team are also responsible for the corresponding parliamentary dispatch in Switzerland, for funding directly Swiss researchers that were excluded from Horizon 2020 subsidies following Switzerland's partial association in 2014, and for the service providers related to the Framework Programmes (e.g. NCP Network Euresearch). Philipp Langer represents Switzerland in various European committees at strategic level (e.g. European Research Area and Innovation Committee ERAC, Research Policy Group RPG, JRC Board of Governors, Horizon 2020 Strategic Configuration of the Programme Committee, Joint Committee EU-CH in R&D, EFTA Working Group on S&T).



## Chuen-Neng Lee

Clinical Director

### CURRENT POSITION

1. Clinical Director, Biomedical Institute for Global Health Research & Technology (NUS)
2. The Abu Rauff Professor in Surgery, Department of Surgery, School of Medicine, National University of Singapore (NUS) Courtesy Professor in Engineering, Faculty of Engineering (NUS)
3. Senior Cardiothoracic Surgeon, Department of Cardiac, Thoracic & Vascular Surgery, National University Hospital, Singapore
5. Chairman, Medical Engineering Research & Commercialization Initiative (MERCi) (NUS)

### CURRENT RESEARCH AREAS

- MicroBiome in Surgical arenas
- Tissue bank / Clinical Analytics/ Machine learning/Artificial Intel-

ligence-Robotics

- Exosomes, Extra-cellular vesicles in clinical practices. Liposomes
- System wide approach for Elderly Surgical patients. (MILES Program). Management & Innovation
- Applied Engineering in Healthcare productivity. Home care Technologies, Hand Held Diagnostic Devices
- Pulsed Electromagnetic energy on ion channels for Sarcopenia, oncology, Aqua-cultures 2D Materials

### AWARDS

- College Overseas Medal, Royal College of Surgeons of Edinburgh, 2011
- National Outstanding Clinician mentor Award, Ministry of Health, Singapore, 2015

### RECENT PUBLICATION

- Peptide-Functionalized Polyurethane Coatings Prepared via Grafting-To Strategy to Selectively Promote Endothelialization. Ding X, Chin W, Lee CN, Hedrick JL, Yang YY. *Adv Healthc Mater.* 2017 Dec 4. doi: 10.1002/adhm.201700944. [Epub ahead of print] PMID: 29205938 (IF: 5.11)
- Myocardial Injury Is Distinguished from Stable Angina by a Set of Candidate Plasma Biomarkers Identified Using iTRAQ/MRM-Based Approach. Cheow ESH, Cheng WC, Yap T, Dutta B, Lee CN, Kleijn DPV, Sorokin V, Sze SK. *J Proteome Res.* 2017 Nov 7. doi: 10.1021/acs.jproteome.7b00651. [Epub ahead of print] PMID: 29068691 (IF: 4.27)
- Monocyte adhesion to atherosclerotic matrix proteins is enhanced by Asn-Gly-Arg deamidation. Dutta B, Park JE, Kumar S, Hao P, Gallart-Palau X, Serra A, Ren Y, Sorokin V, Lee CN, Ho HH, de Kleijn D, Sze SK. *Sci Rep.* 2017 Jul 18;7(1):5765. doi: 10.1038/s41598-017-06202-2. PMID: 28720870 (IF: 4.26)
- Plasma-derived Extracellular Vesicles Contain Predictive Biomarkers and Potential Therapeutic Targets for Myocardial Ischemic (MI) Injury. Cheow ES, Cheng WC, Lee CN, de Kleijn D, Sorokin V, Sze SK. *Mol Cell Proteomics.* 2016 Aug;15(8):2628-40. doi: 10.1074/mcp.M115.055731. Epub 2016 May 27. PMID: 27234505 (IF: 6.632)
- Identification of independent association signals and putative functional variants for breast cancer risk through fine-scale mapping of the 12p11 locus. Zeng C, ... Hartman M, ..., Lee CN, ... Zheng W et al. *Breast Cancer Res.* 2016 Jun 21;18(1):64. doi: 10.1186/s13058-016-0718-0. PMID: 27459855 (IF: 6.26)



## Dong Soo Lee

I am a nuclear medicine physician mostly interested in nuclear theranostics and brain itself. In nuclear theranostics, brain theranostics is the least explored mainly because of BBB for drugs (small molecules, peptides and antibodies) and also the concerns of possible toxicity if nanoparticles might cross the BBB. I use *in vitro* microfluidic chip model and *in vivo* (dementia) mouse models using microSPECT/CT or microPET/MRI.

However, as a physician doing clinical practice, I am mostly interested in clinical translation of nanomedicines (nanodrugs) use and thus believe radionanomedicine will pave the way of nanomedicine to have impact, I wish, on a variety of clinical disciplines.

As a nuclear medicine physician, in addition to small molecules or biomacromolecules, nanomaterials are to be another group of targeting molecules for *in vivo* use for therapy and diagnosis. Radiolabeled nanomaterials are going to enable us to follow up the bio-distribution and thus pharmacokinetics and radiation dosimetry. Extracellular vesicles are another candidate of delivery vehicle as radiolabeled form, which I think promising as theranostic agents.





## Hans Lehrach

Prof. Dr. Hans Lehrach, Director em., Max Planck Institute for Molecular Genetics, Ihnestr. 63-73, 14195 Berlin, Germany  
Founder and Scientific Director, Dahlem Centre for Genome Research and Medical Systems Biology Founder and Chairman of the Board, Alacris Theranostics GmbH, Max-Planck-Str. 3, 12489 Berlin

Hans Lehrach obtained his Ph.D. at the Max Planck Institute for Experimental Medicine and the Max Planck Institute for Biophysical Chemistry in 1974. Next he moved on to Harvard University, Boston (1974-1978) for a postdoc and then became group leader at EMBL, Heidelberg (1978-1987). He then moved to the Imperial Cancer Research Fund, London (1987-1994) to become head of the Genome Analysis Department. In 1994 he returned to Germany to become Director at the Max Planck Institute for Molecular Genetics (since 1994, em. 2014). He holds a position as a Visiting Professor at the Berlin Institute of Health (BIH), Berlin, Germany as well as at the Southern University of Science and Technology (SUSTech) in Shenzhen, China.

His expertise lies in genetics, genomics, systems biology and personalized medicine. Highlights include his key involvement in several large-scale genome sequencing projects, such as the human, rat, and *Schizosaccharomyces*. His group was part of the team which identified the Huntington's disease gene. Dr. Lehrach also performed key work on technologies such as protein microarrays, protein interactome analysis, yeast artificial chromosomes and RNAseq. He has been pioneer in the application of next generation sequencing techniques and systems medicine for the development of personalized therapies in cancer (Virtual Patient Model). He was partner in two German ICGC projects and partner in the European IHEC project BLUEPRINT, a steering committee member of the 1000 Genomes project, leader of the managing entity of the IMI OncoTrack project and co-ordinated the FET Flagship pilot initiative IT Future of Medicine (ITFoM, [www.itfom.eu](http://www.itfom.eu)), a finalist of the FET Flagship Call, which has established a strong technological roadmap and network of partners from 33 countries. In this current initiative, Hans Lehrach represents a growing network of research institutions, industry representatives, scientists and patient groups that share a long-term vision for sustainable health care ([www.healthcarecompactforeurope.eu](http://www.healthcarecompactforeurope.eu)) and the personalization of medicine in his new FET Flagship initiative Digital Twins for Better Health (DigiTwins).

Dr. Lehrach has founded several biotechnology companies such as Sequana Therapeutics, GPC Biotech, Scienion, Prot@gen, PSF Biotech, Atlas Biolabs. Dr. Lehrach is founder of the Berlin-based company Alacris Theranostics GmbH, specialising in the development of new approaches for personalised medicine for cancer patient diagnosis, treatment and drug stratification. He is chairman of the Supervisory Board and scientific advisor of the company since 2008. In 2010 he founded the non-for-profit research institute The Dahlem Centre for Genome Research and Medical Systems Biology (DCGMS).

Researcher ID - A. Lendlein:  
<http://www.researcherid.com/rid/K-9553-2013>

Andreas Lendlein received his doctoral degree in Materials Science from Swiss Federal Institute of Technology (ETH) in Zürich in 1996. He worked as a visiting scientist at the Massachusetts Institute of Technology in 1997-98 and completed his habilitation in Macromolecular Chemistry in 2002 at the RWTH Aachen University. Since 2002 he is institute director at the Helmholtz-Zentrum Geesthacht in Teltow and Professor for Materials in Life Sciences at the University of Potsdam, Germany. Furthermore, he is honorary professor in Chemistry at the Freie Universität Berlin and member of the medical faculty of Charité University Medicine Berlin. His research interests in material science & engineering are creation of material functions by design and implementation of multifunctionality in polymer-based materials with special emphasis given to stimuli-responsive polymers, especially shape-memory polymer actuators, biopolymer-based material systems and structured biomaterials. He also works on fabrication schemes for multifunctional materials including integrated processes and advanced manufacturing methods as well as on studies related to processes occurring at interfaces, e.g. biointerface or water/air interface. Biomaterial-based regenerative therapies, controlled drug delivery systems, health technologies and robotics recently are his interests in translational research. Andreas Lendlein published more than 500 peer-reviewed papers (H-factor: 54), is an inventor on about 300 issued patents and published patent applications, and received more than 20 awards for his scientific work and his achievements as an entrepreneur including the BioFUTURE Award, Hermann-Schnell Award, and the World Technology Network Award in the category Health & Medicine. He is founding Editor-in-Chief of the journal Multifunctional Materials (IOP Publishing) and serves on the Executive Advisory Board of VCH-Wiley's Macromolecular Journals.

### RECENT PUBLICATIONS:

- W. Wang, T. Naolou, N. Ma, Z. Deng, X. Xu, U. Mansfeld, C. Wischke, M. Gossen, A. T. Neffe, A. Lendlein, Polydepsipeptide Block Stabilized Polyplexes For Efficient Transfection of Primary Human Cells. *Biomacromolecules* 18 (11), 3819-3833 (2017)
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- Li, W. Wang, X. Xu, K. Kratz, J. Zou, L. Lysyakova, M. Heuchel, A. Kurtz, M. Gossen, N. Ma, A. Lendlein, Integrin  $\beta 1$  activation by micro-scale curvature promotes pro-angiogenic secretion of human mesenchymal stem cells. *J. Mater. Chem. B* 5, 7415-7425 (2017)
- W. Wang, M. Balk, Z. Deng, C. Wischke, M. Gossen, M. Behl, N. Ma, A. Lendlein, Engineering biodegradable micelles of polyethylenimine-based amphiphilic block copolymers for efficient DNA and siRNA delivery. *J. Contr. Release* 242, 71-79 (2016)
- A.T. Neffe, B. F. Pierce, G. Tronci, N. Ma, E. Pittermann, T. Gebauer, O. Frank, M. Schossig, X. Xu, B. M. Willie, M. Forner, A. Ellinghaus, J. Lienau, G. N. Duda, A. Lendlein, One Step Creation of Multifunctional 3D Architected Hydrogels Inducing Bone Regeneration. *Adv. Mater.* 27 (10), 1738-1744 (2015)



## Andreas Lendlein

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## Fransisca Leonard

Faculty Fellow  
Houston Methodist Research Institute

Dr. Leonard received Dipl.-Ing in Biotechnology from Berlin Institute of Technology and M.Eng from Dongseo University in South Korea in 2006. For her doctoral thesis, she joined Dr. Claus-Michael Lehr's lab

in Saarland University, where she was involved in Meditrans EU FP7 project and worked on developing 3D *in vitro* model for inflamed colonic mucosa for screening of nanoformulated drugs. During this time she obtained Euro-PhD Fellowship from Helmholtz Institute for Infectious Disease and went to Houston Methodist Research Institute in 2011 to conduct further studies on drug delivery utilizing silicon microparticles loaded with anti-inflammatory drugs. In 2013, she received her PhD degree from Saarland University, and then later joined Dr. Godin's team at Nanomedicine Department of Houston Methodist Research Institute as a Postdoctoral Fellow. She continued her research on various nanotechnology-based drug delivery approaches and development of the relevant *in vitro* testing systems. She has recently received her faculty appointment as Faculty Fellow since July 2018 at Nanomedicine Department of Houston Methodist Research Institute. During the course of her career, beside securing several scholarships, she also obtained various awards and recognitions, including awards from German Rhineland-Palatinate State and German Federal Ministry of Food, Agriculture and Consumer Protection Award for research on alternative and supplementary methods for animal testing in year 2010 and 2011, respectively. She was finalist for best work in 2016 NanoEngineering for Medicine and Biology (NEMB) annual meeting, and received March of Dimes award for best abstract in preterm labor at Society for Maternal-Fetal Medicine Annual meeting in 2016. Recently, she received Department of Nanomedicine Innovative Grant Award, and won the second place in podium presentation in MAPTA Winter Symposium, as well as a grant from Kostas Family Foundation for Research of Nanomedicine in Cardiovascular Disease. Dr. Leonard's research mainly focuses on nanomedicine targeting macrophages in inflammatory-related conditions such as cancer, cardiovascular, and infectious diseases. Her research includes development of liposomal & polymeric nanoparticles for drug delivery as well as development of disease-specific *in vitro* model for assessment of nanoformulations effects on cell uptake, cell-cell interaction, and the improvements in drug toxicity and efficacy.



## Didier Letourneur

Didier Letourneur, Engineer in Material Sciences, PhD in Chemistry from Paris North University, is Research Director at CNRS. Since 2014, he is the Director of the Laboratory for Vascular Translational Science (LVTS with about 220 persons) affiliated to Inserm and Universities Paris Diderot and Paris North. He also leads the team of Cardiovascular Bioengineering at Bichat Hospital in Paris. D Letourneur is actively involved in several national grants, in the regional Health Cluster Medicen, and since 2013 as European coordinator of NMP large scale project "NanoAthero" (16 partners, 10 countries). He was also involved in several FP7 European projects ("Fighting Aneurysmal Diseases"; "Nanoantenna"; "Prevention of Late Stent Thrombosis"). D Letourneur is the author of 170 international publications and inventor of 16 patents. He has more than 100 invited lectures and seminars and is the co-organizer of numerous national and international conferences (India, Tunisia, Canada) and two Inserm training workshops for Regenerative Medicine. He won several prizes: "Coup d'Élan for Research" Bettencourt Foundation 2001, Diderot Innovation Award 2009, Cardiovascular Innovation Award 2011 from French Medical Research Foundation, OSEO Emergence 2012, BPI Creation-Development 2013. In 2016, he obtained the G Winter Award the highest distinction from the European Society for Biomaterials, and in 2017 the Asian Polymer Association Jubilee Award. In 2016, he found the company SILTISS for the development of innovative implants from polysaccharide-based materials. He was from 2013 to 2016 at the French Council for Health technologies. He was from 2013 to 2015 vice-chairman for Regenerative Medicine at the European Technology Platform for Nanomedicine and is now General Secretaire. From 2016, he is the President of the Inserm committee on Health Technologies

& Social Sciences. From 2009, he is President of BIOMAT, French Society for Biomaterials.



## Lada Leyens

Lada Leyens works for the Swiss Therapeutics Agency (Swissmedic) as clinical study reviewer and GCP inspector in the clinical-trials division. She is also the international liaison for nanomedicine and represents the agency at national and international meetings. Lada worked at the European-Medicines Agency (EMA) as a National Expert on Secondment in the Scientific Support Team with a special focus on clinical pharmacology and pharmacogenomics. During her time at the EMA she also participated in several joint EMA-HTA Scientific Advice procedures. She has experience in the medical devices industry in the set up and management of clinical trials. Lada obtained her PhD on Personalised Medicine and Regulatory Science from Maastricht University, her MSc. in International Health Economics from London School of Economics and Political Sciences (LSE) and her BSc. Hons. in Human Genetics from University College London (UCL).



## Bei Li

PhD candidate

Bei Li, is studying in Australian Institute for Biomedical Engineering in The University of Queensland, Australia, with the supervision of Prof Gordon Xu. Her research interests in the novel, safe and efficient nanomaterials as multifunctional theranostic agents for cancer treatment. Her recent works mainly focus on designing novel inorganic layered nanoplatform for imaging-guided tumor-specific theranostics. She received a Master Degree based on thesis work in the preparation of inorganic layered catalyst and its photocatalytic performance from Beijing University of Chemical Technology, China, with the supervision of Prof Min Wei and Xue Duan (Academician of Chinese Academy of Sciences). She got a Bachelor degree in Applied Chemistry from Beijing University of Chemical Technology, China. During the PhD study, she got the Donald Tugby Prize in Nanotechnology in The University of Queensland for best paper in 2017, International Postgraduate Research Scholarship (IPRS) and UQ Centennial Scholarship (UQCent) in Australia and Chinese Government Award for Outstanding Self-financed Students Abroad.

### RECENT PUBLICATION

- Bei Li, Zi Gu,\* Nyoman Kurniawan, Weiyu Chen, Zhi Ping Xu,\* Manganese-Based Layered Double Hydroxide Nanoparticles as a T1-MRI Contrast Agent with Ultrasensitive pH Response and High Relaxivity. *Adv. Mater.* 2017, 29, 1700373.
- Bei Li, Jie Tang, Weiyu Chen, Guanyu Hao, Nyoman Kurniawan, Zi Gu,\* and Zhi Ping Xu,\* Novel Theranostic Nanoplatform for Complete Mice Tumor Elimination via MR Imaging-guided Acid-enhanced Photothermo-/Chemo-therapy. *Biomaterials* 2018, DOI: 10.1016/j.biomaterials.2018.05.055.
- Weiyu Chen, Huali Zuo, Bei Li, Chengcheng Duan, Barbara Rolfe, Bing Zhang, Timothy J. Mahony,\* Zhi Ping Xu,\* Clay Nanoparticles Elicit Long-Term Immune Responses by Forming Biodegradable Depots for Sustained Antigen Stimulation. *Small* 2018, 14, 1704465.
- Huali Zuo, Weiyu Chen, Bei Li, Kewei Xu, Helen Cooper, Zi Gu,\* Zhi Ping Xu,\* MnAl-Layered Double Hydroxide Nanoparticles as a Dual-functional Platform for Magnetic Resonance Imaging and siRNA Delivery. *Chem. Eur. J.* 2017, 23, 14299.



## Lars Lindner

Head Sarcoma Medical Oncology,  
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Lars Lindner is a medical oncologist having received his training as medical doctor at the University of Göttingen, Germany, and the Ludwig-Maximilians-Universität Munich, Germany. He spent his final year of medical school at the Cornell Medical Center, the Memorial Sloan Kettering Cancer Center and the Mount Sinai Medical College, all New York City, USA, and the Stadtspital Triemli, Zurich, Switzerland. He conducted his PhD studies with Prof. Eibl at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany, focusing on novel liposomal drug delivery technologies, and spent his Postdoctoral Fellowship at Erasmus Medical Center, Rotterdam, the Netherlands, at the Laboratory of Experimental Surgical Oncology with Prof. Dr. Alexander Eggermont. Today, he is an Professor of internal medicine, hematology and oncology at the University Hospital of the Ludwig-Maximilians-Universität Munich, Germany. He coordinates the Sarcoma Center SarkUM, one of Germany's largest and leading centers dedicated to the diagnosis and treatment of cancer patients with all types of sarcomas, and the hyperthermia treatment center, one of the world's largest and most renowned centers for regional deep hyperthermia in cancer patients. In addition, he supervises a research group dedicated to basic and applied research on novel, liposomal drug delivery technologies.

### RECENT PUBLICATION

- Issels RD, Lindner LH, Verweij J, Wessalowski R, Reichardt P, Wust P, Ghadjar P, Hohenberger P, Angele M, Salat C, Vujaskovic Z, Daugaard S, Mella O, Mansmann U, Dürr HR, Knösel T, Abdel-Rahman S, Schmidt M, Hiddemann W, Jauch KW, Belka C, Gronchi A; European Organization for the Research and Treatment of Cancer-Soft Tissue and Bone Sarcoma Group and the European Society for Hyperthermic Oncology. Effect of Neoadjuvant Chemotherapy Plus Regional Hyperthermia on Long-term Outcomes Among Patients With Localized High-Risk Soft Tissue Sarcoma: The EORTC 62961-ESHO 95 Randomized Clinical Trial. *JAMA Oncol.* 2018; 4:483-492
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## Neill Liptrott

Advanced Pharmacological Strategies  
(APS) Group, Materials Innovation  
Factory, Department of Molecular &  
Clinical Pharmacology, The University of  
Liverpool

Dr Liptrott has a background in pharmacology, immunology and molecular cell biology. His research is focused on investigating biocompatibility and immunological safety of conventional and nanotechnology-enabled medicines as well as cellular therapies. The knowledge generated is aimed at better understanding immune function, interindividual variability in immune response and identifying opportunities for immune modulation. Dr Liptrott also worked as a guest researcher at the National Institutes of Health (NIH) National Cancer Institute's (NCI) Nanotechnology Characterisation Laboratory (NCL) based in Frederick, Maryland, USA. During this time Dr Liptrott conducted research alongside global experts in nanomaterial toxicity and biocompatibility. In 2015 Dr Liptrott was awarded a tenure-track fellowship within the department of Molecular and Clinical Pharmacology and heads the nanotechnology biocompatibility research programme. Additionally Dr Liptrott is a member of the Executive Board, Core Expert Team (CET) and Assay Group leader (Immunotoxicity and Haematotoxicity) of the recently established European Nanomedicine Characterisation Laboratory (EU-NCL), funded by the European Commission (Horizon 2020). He leads the University of Liverpool work packages on nanoparticle biocompatibility and structure-activity relationships.

### RESEARCH

- 2015–present:** Tenure track fellow – Department of Molecular and Clinical Pharmacology, University of Liverpool, UK. Biocompatibility, Nanotoxicology and Immunopharmacology
- 2012–2015:** Senior Postdoctoral Research Fellow – Department of Molecular and Clinical Pharmacology, University of Liverpool. Towards nanomedicine interventions in HIV/AIDS.
- 2011–2012:** Postdoctoral Research Associate - Department of Molecular and Clinical Pharmacology, University of Liverpool. Determining the interaction between nanoformulated drug delivery systems and the human immune system.



## Beat Löffler

Born in Basel. After a study visit in the USA, he studied Philosophy, Communication Sciences and Politics at Free University in Berlin, (Master of Arts, magna cum laude.) He further developed his skills in Biology and Medicine absolving the training of the European Center of Pharmaceutical Medicine. 2014 he received an MD h.c. from the

University Basel in 1994 he co-founded an Agency for New Media From 1988 to 1994 he was Head of the International Hightech Forum Basel organizing congresses on new technologies in mobility, energy, CFD and medical technology. 1994 he founded his own company "L & A concept engineering" for translation of science-based visions and establishment of worldwide networks (mission and strategy for realizing visions). Six years Secretary General/coach of the tri-national BioValley Promotion Team, (Upper-Rhine Biotechnology network). 2003-06 mandated by NEC Hightech Performance Computing in charge of the Life Sciences Business Development in Biology/ Medicine. 2007 founded with Prof. Patrick Hunziker, MD the European Foundation for Clinical Nanomedicine. He launches the annual SUMMITS, the European Journal of Nanomedicine, (new open access Journal CLINAM Precision Medicine). He cofounded



the European Society and the International Society for Nanomedicine. He is head of dissemination in several framework programme projects and in the EU-flagship Application Team DigiTwins for the chapter Nanomedicine.



## Pasquale Maffia

I received my BSc (HONS) in Pharmacy and MPhil and PhD in Pharmacology, all from the University of Naples Federico II (Italy), where I became Aggregate Professor of Pharmacology in 2006. I then joined the University of Strathclyde as a Lecturer in Integrative Mammalian Biology, before moving to the University of Glasgow where I am currently Senior Lecturer at the Institute of Infection, Immunity and Inflammation and the BHF Centre of Excellence in Vascular Science and Medicine. I am the Senior Honours Immunology degree programme coordinator in Glasgow and serve on the Executive Committee of the International Union of Basic and Clinical Pharmacology (IUPHAR) Immunopharmacology Section and the British Pharmacological Society Policy & Public Engagement Committee. I am an Editorial Board Member of the British Journal of Pharmacology and *Frontiers in Immunology*, Associate Editor of *Pharmacological Research* and Executive Deputy Editor of *Cardiovascular Research*. I have a major interest in the immune response in cardiovascular disease. Current research activities address the study and imaging of cellular and molecular mechanisms involved in the pathophysiology of atherosclerosis and hypertension. I have authored 70 scientific papers in peer-reviewed journals including *Immunity*, *Circulation*, *Blood* and *PNAS*. I am an elected fellow of the Royal Society of Biology, the British Pharmacological Society and the European Society of Cardiology.



## Volker Mailänder

Univ.-Prof. Dr. med.

Volker Mailänder studied medicine at the University of Ulm supported by a stipend from the Studienstiftung des Deutschen Volkes and was in the graduate program "Molecular Biology". He worked in the Blume/Negrin lab in Stanford, California, on natural killer cells and was involved in patient care in the bone marrow transplantation unit. Afterwards he received training in internal medicine (haematology/oncology) in the Charité hospital in Berlin. After relocating to the Institute for Clinical Transfusion Medicine, University Clinic of Ulm, he worked on stem cell manipulation, the interaction of nanoparticles with cells and especially uptake mechanisms and the intracellular pathway. He was board certified in transfusion medicine. Further work focused on using polymeric nanoparticles for labelling or manipulation of stem cells and other cell types. Since 2008 he is leading a joint research group between the University Medical Clinic and the MPI for Polymer Science in Mainz. He has been appointed a professorship dealing with the translation of nanocarriers into medical applications. He is proficient in the procedures of manipulating, freezing and storing stem and immune cells for patients care as the head of production and qualified person. He is active in several cooperative projects (SFB1066 "Nanodimensional polymeric therapeutics for tumor therapy", BMBF projects) and is vice speaker of the center Biomaterials, Tissues and Cells in Science (BiomaTICS) of the University Medical Center. Since 1.1.2016 he is W2 professor at the University Medicine Mainz and associated to the Dermatology department and heads the Center for Translational Nanomedicine – CTN. He is

especially interested in understanding and overcoming the hurdles of applying nanocarriers for use in clinical applications. Therefore, protein corona, targeting and GMP-conform production of nanocarriers are the main focus of his research.

## RECENT PUBLICATIONS

- Schöttler S, Becker G, Winzen S, Steinbach T, Mohr K, Landfester K, Mailänder V, Wurm FR.: Protein adsorption is required for stealth effect of poly(ethylene glycol)- and poly(phosphoester)-coated nanocarriers. *Nat Nanotechnol.* 2016 Apr;11(4):372-7
- Schöttler S, Klein K, Landfester K, Mailänder V. Protein source and choice of anticoagulant decisively affect nanoparticle protein corona and cellular uptake. *Nanoscale.* 2016, 8: p. 5526-5536
- Hofmann, D., et al., Drug delivery without nanoparticle uptake: delivery by a kiss-and-run mechanism on the cell membrane. *Chemical Communications*, 2014. 50(11): p. 1369-71.
- Paven, M., et al., Super liquid-repellent gas membranes for carbon dioxide capture and heart-lung machines. *Nature Communications*, 2013. 4: p.2512
- Lerch, S., et al., Polymeric nanoparticles of different sizes overcome the cell membrane barrier. *European Journal of Pharmaceutics and Biopharmaceutics*, 2013. 84(2): p. 265-274.



## Harald Mangge

Deputy Head of the Clinical Institute for Medical and Chemical Laboratory Diagnostics (CIMCL), Medical University of Graz, Austria

Harald Mangge is a Medical Doctor and Professor at the Department of Laboratory Medicine of the Medical University of Graz, Austria. His research focuses on cardiovascular-, metabolic-, and oncologic diseases (pancreatic carcinoma) with emphasis on immune-mediated inflammation and Nanomedicine. In the framework of the STYJOBS/EDECTA cohort project, Harald Mangge conducts a large prospective, observational study to improve the understanding of metabolic and cardiovascular risk in obesity (<http://clinicaltrials.gov/ct2/show/NCT00482924>). Further, Harald Mangge holds the position of a Deputy Head of the Clinical Institute of Medical and Chemical Laboratory Diagnosis (CIMCL) and he is vice speaker of the Cardiovascular Research Field of the Medical University of Graz.

## RECENT PUBLICATION

- Adiponectin Predicts High-Density Lipoprotein Cholesterol Efflux Capacity in Adults Irrespective of Body Mass Index and Fat Distribution. Marsche G, Zelzer S, Meinitzer A, Kern S, Meissl S, Pregartner G, Weghuber D, Almer G, Mangge H. *J Clin Endocrinol Metab.* 2017 PMID:28938424
- New Diagnostic and Therapeutic Aspects of Pancreatic Ductal Adenocarcinoma. Mangge H, Niedrist T, Renner W, Lyer S, Alexiou C, Haybaeck J. *Curr Med Chem.* 2017;24: 3012-3024. PMID:28494747
- Work Intensity, Low-Grade Inflammation, and Oxidative Status: A Comparison between Office and Slaughterhouse Workers. Zelzer S, Tatzber F, Herrmann M, Wonisch W, Rinnerhofer S, Kundi M, Obermayer-Pietsch B, Niedrist T, Cvirn G, Wultsch G, Mangge H. *Oxid Med Cell Longev.* 2018:2737563, PMID:29849876
- Nanoparticles for intravascular applications: physicochemical characterization and cytotoxicity testing. Matuszak J, Baumgartner J, Zaloga J, Juenet M, da Silva AE, Franke D, Almer G, Texier I, Faivre D, Metselaar JM, Navarro FP, Chauvierre C, Prassl R, Dézsi L, Urbanics R, Alexiou C, Mangge H, Szebeni J, Letourneur D, Cicha I. *Nanomedicine* 2016 Mar;11:597-616. PMID:27003004
- Interleukin 10-coated nanoparticle systems compared for molecular imaging of atherosclerotic lesions. Almer G, Summers KL, Scheicher B, Kellner J, Stelzer I, Leitinger G, Gries A, Prassl R, Zimmer A, Mangge H. *Int J Nanomedicine.* 2014 Sep 3;9:4211-22, PMID:25214785





## Mira Marcus-Kalish

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Dr. Mira Marcus-Kalish is currently the Director of International Research Collaborations at the Tel Aviv University. Her main areas of interest are mathematical modelling, converging technologies and data mining.

Dr Kalish holds a Ph.D. in Operations Research from the Technion - Israeli Institute of Technology, where she developed one of the first computerized systems for electrocardiogram (ECG) diagnosis. She did her postdoctoral training at Harvard University, the MBCRR (Molecular Biology Computer Research and Resource) laboratory and at the Dana Farber Cancer Institute. Her B.Sc. is in Statistics and Biology from the Hebrew University of Jerusalem.

Upon her return to Israel, she joined the Tel Aviv University Business School establishing the Medical Management first program focusing on Medical Informatics. Then joined the Weizmann Institute of Science, working with Prof Ephraim Katzir, mainly on protein interactions, specificity and sensitivity. She moved with Prof Katzir back to Tel Aviv University, to the Biotechnology Department taking active part in NBIC, Converging Technologies and contributing to the recent EU-US Wtec-NBIC2 activities & publication.

She was involved in a private business enterprise and served as the scientific advisor and later as the head of the Enterprise Marketing Department of IBM Israel.

Dr. Kalish took an active part in many of the EU framework projects, such as the Nano2Life Network of Excellence, being the joint research WP leader, SkinTreat, ReNaChip, EpoCan, etc. Current active EU projects are NanoAthero, GLAM, ENATRANS and HBP- the Human Brain Flagship Project leading the Medical Informatics Sub Project. Her focus is Disease Signature identification based on targeted analysis of micro and macro environmental Knowledge & Data features. The newly developed approach & analytical tools are trying to meet the challenges of big versus small data analysis, missing values handling, various data sources combined analysis, etc. towards reliable, replicable, reproducible personalized medicine. Other area of research are rehabilitation of the discrete sensory motor, learning function, drug toxicity, machine learning systems, data mining and recently a broad band cross-disciplinary international initiative on Healthy Aging. Dr. Kalish is proud to be one of the establishers of the Dead Sea Research Institutes and the Porter University Institute for life under extreme conditions and a strong believer at the uniqueness of the Dead Sea region and its possible contribution to Life!



## Pascal Mäser

Pascal Mäser graduated from the University of Basel in 1998 with a Ph.D. in Microbiology on the mechanisms of drug resistance in African trypanosomes. As a postdoc at UC San Diego he studied ion transporters in plants. He returned to Switzerland, University of Bern, in 2002 as an assistant professor for Molecular Parasitology and Bioinformatics. In 2009 he joined the Swiss Tropical and Public Health Institute. Pascal Mäser is head of the Parasite Chemotherapy Unit and associate professor for Parasitology and Protozoology of the University of Basel. His research focus is on drug discovery for trypanosomatid parasites and malaria, mode of drug action, and mechanisms of drug resistance.

Pascal Mäser graduated from the University of Basel in 1998 with a Ph.D. in microbiology. He moved on to the University of California San Diego for postdoctoral research in molecular plant physiology,

and in 2002 returned to Switzerland as an assistant professor of the University of Bern. In 2009 he joined the Swiss TPH. Currently he is associate professor for parasitology and protozoology at the University of Basel and head of the parasite chemotherapy unit of the Swiss TPH. His research focuses on drug discovery for trypanosomatid parasites and malaria.



## Scott E. McNeil

Director of the Nanotechnology Characterization Laboratory (NCL)

Dr. McNeil serves as the Director of the Nanotechnology Characterization Laboratory (NCL) for Leidos Biomedical Research at the Frederick National Laboratory for Cancer Research, where he coordinates preclinical characterization of nanotech cancer therapeutics and diagnostics. At the NCL, Dr. McNeil leads a team of scientists responsible for testing candidate nanotech drugs and diagnostics, evaluating safety and efficacy, and assisting with product development - from discovery-level, through scale-up and into clinical trials. NCL has assisted in characterization and evaluation of nearly 400 nanotechnology products, several of which are now in human clinical trials.

### RECENT PUBLICATION

- McNeil, S., ed. Characterization of nanoparticles intended for drug delivery, 2nd Edition. Methods in Molecular Biology. Vol. 1628, 2018, Humana Press, New York, NY.
- McNeil, S.E., Evaluation of nanomedicines: stick to the basics. Nature Reviews Materials. 2016, 1, 16073.
- Skoczen, S., S.E. McNeil, and S.T. Stern, Stable isotope method to measure drug release from nanomedicines. J Control Release, 2015, 220(Pt A), 169-174.
- Crommelin, D. J., Shah, V. P., Klebovich, I., McNeil, S. E., Weinstein, V., Fluhmann, B., Muhlebach, S., and de Vlieger, J. S. The similarity question for biologicals and non-biological complex drugs. European journal of pharmaceutical sciences: official journal of the European Federation for Pharmaceutical Sciences, 2015, 76, 10-17.
- Fluhmann, B., Crommelin, D.J., de Vlieger, J.S., Borchard, G., Muhlebach, S., McNeil, S.E., Neervannan, S., Weinstein, V., and Shah, V.P., Non-Biological Complex Drugs (NBCDs) and their follow-on versions: time for an editorial section. Generics and Biosimilars Initiative Journal. 2015, 4(4).



## Peter J. Meier-Abt

Prof. PJ Meier-Abt received his MD from the University of Basel in 1974. After training in Internal Medicine and Clinical Pharmacology at the University Hospitals of Basel and Zurich, he completed a two year research fellowship in hepatology at Yale University School of Medicine, New Haven Ct USA. In 1984 he became chief of the Division of Clinical Pharmacology and Toxicology at the University Hospital Zurich. In 1992 he was promoted to full professor for Clinical Pharmacology and Toxicology. He served also as medical director of the Swiss Toxicological Information Center, Zurich (1989-2003) and as first director of the Center of Clinical Research of the Medical Faculty Zurich (2001-2004). His research interests focus around the molecular physiology of bile formation, hepatobiliary bile acid and drug transport, pathophysiology of cholestatic liver disease, drug and toxin induced liver damage, pharmacogenetics/-

genomics of adverse drug reactions and individualisation of drug therapy and drug safety. His research resulted in >250 publications and many honours and research awards. Prof. Meier-Abt served at numerous boards of national and international associations and research organisations including the Swiss National Science Foundation (SNSF; 1993-2004), the Swiss Academy of Medical Sciences (SAMS; 2004-now) and the Swiss Clinical Trial Organisation (SCTO; 2009-2012). He coordinated the national MD-PhD Programme (1998-2008) and is especially committed to the further development of translational/clinical research and personalized medicine in Switzerland. Between 2005 and 2011 Prof. Meier-Abt was vice-rector for research & talent promotion at the University of Basel, and between 2011 and 2016 he acted as president of the SAMS. Since 2017 he serves the SAMS council as vicepresident and chairs the National Steering Board of the Swiss Personalized Health Network, a SAMS led initiative to foster Personalized/Precision Health in Switzerland.



## Josbert M. Metselaar

Josbert M. "Bart" Metselaar (Rotterdam, July 6th 1971 ) obtained a MSc degree in Pharmaceutical Sciences in 1995 and a PharmD degree in 1998, both at Utrecht University. During his study he completed a research internship in pharmacology and PK/PD at the Dept of Pharmaceutics, University of Florida, US. In 1999 he started a

PhD at the Dept of Pharmaceutics and the Dept of Immunology in Utrecht where he studied novel targeted formulations of anti-inflammatory medicines. After completing his PhD and a Post Doc fellowship, he decided to translate part of his accomplishments into investigational medicinal products by starting his company Enceladus in 2005, with which he raised more than 6 million Euros funding over the years. With these investments and additional non-equity funding he successfully performed a series of preclinical and clinical development projects on three liposomal products. In 2012 he took a part-time academic position in the group of Targeted Therapeutics at the University of Twente, where he works on drug carrier design and formulation development in the field of advanced drug delivery for inflammation, atherosclerosis, and cancer. In 2015 he combines this with a position at the Dept. of Experimental Molecular Imaging at the RWTH Aachen University Clinic in Germany.



## Matthias Miederer

Deputy Director department of Nuclear Medicine, University Medical Center Mainz, Germany

After postgraduate training in the field of targeted alpha-radiation therapy at MSKCC, New York and a medical internship at the Charité in Berlin I trained in clinical Nuclear Medicine at the Technical University in Munich. Since 2009 I work as deputy director in the department of Nuclear Medicine at the Gutenberg University in Mainz, Germany. My research interests are to apply radiation for new diagnostic and therapeutic approaches. I have utilized small molecules as targeted carriers for longer lived therapeutic isotopes and work on imaging of processes of the immune system by positron emission tomography.

### RECENT PUBLICATION

- Wagener K, et al. Comparison of Linear and Hyperbranched Poly-ether Lipids for Liposome Shielding by <sup>18</sup>F-Radiolabeling and

- Positron Emission Tomography. *Biomacromolecules*. 2018 Apr 27
- Pektor S, et al. Toll like receptor mediated immune stimulation can be visualized *in vivo* by [<sup>18</sup>F]FDG-PET. *Nucl Med Biol*. 2016 Nov;43(11):651-660
- Graf F, et al. DNA double strand breaks as predictor of efficacy of the alpha-particle emitter Ac-225 and the electron emitter Lu-177 for somatostatin receptor targeted radiotherapy. *PLoS One*. 2014 Feb 7;9(2):e88239



## Moein Moghimi

Moein Moghimi is a Professor of Pharmaceutics and Nanomedicine (School of Pharmacy) and Research Professor (Institute of Cellular Medicine) at Newcastle University (UK) as well as an Adjoint Professor at the Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of

Colorado-Denver Medical Center (USA). His earlier appointments have included Professor and Chair in Pharmaceutics at the School of Medicine, Pharmacy and Health, Durham University, UK (2016-2017); Full Affiliate Member/Professor at the Methodist Research Institute, Houston Methodist Hospital Systems, Houston, Texas, USA (2013-2017); Visiting Professor at Università Degli Studi Di Padova, Padova, Italy (2015), where he designed and delivered the first integrated Nanomedicine-Business course in Europe with Dr. Farhangrazi (Denver University and S. M. Discovery Group LLC); Professor of Nanomedicine (at the Department of Pharmacy), Professor of Pharmaceutical Nanotechnology (at the NanoScience Center), and Founder/Director of the multi-million Dollar Center for Pharmaceutical Nanotechnology and Nanotoxicology, University of Copenhagen, Denmark (2008-2016); Honorary Professor of Nanomedicine at the Multidisciplinary Research Center, Shantou University, China (2008-2010); Senior Lecture in Biopharmacy and Molecular Pharmaceutics, School of Pharmacy, University of Brighton, UK (1998-2008); and University Research Fellow in Advanced Drug Delivery Systems, Department of Pharmaceutical Sciences, University of Nottingham, UK. Professor Moghimi is also co-founder of S. M. Discovery Group LLC (CO, USA) and S. M. Discovery Group Limited (UK) and further practices as an advisor/consultant to numerous multinationals, SMEs, governmental organizations and entrepreneurial enterprises worldwide. He also functions as Deputy Editor for *Molecular Therapy* (the flagship journal of the American Society for Gene and Cell Therapy, published by Cell Press), and editorial board member of several journals including *Advanced Drug Delivery Reviews* (Elsevier), *Nanomedicine-UK* (Future Medicine), *Journal of Controlled Release* (Elsevier), *Drug Delivery* (Taylor Francis) and *Scientific Reports* (Springer Nature).

Prof. Moghimi has made groundbreaking contributions to the field of advanced drug delivery, nanoparticle engineering, and nanopharmaceutical performance and safety. The work in Prof. Moghimi's laboratory is identifying realistic opportunities offered by understanding pathophysiological processes to the design and engineering of efficient and safe nanopharmaceuticals. This research has advanced fundamental understanding of biological barriers, and particularly the role of innate immune system in relation to nanoparticle performance and safety, and led to the development of new materials and approaches for treatment of various conditions to include cancer, cardiovascular diseases, immune disorders, and disease of the central nervous system, and many in translational stages. Prof. Moghimi has over 240 peer-reviewed publications/patents in the field of targeted drug delivery and nanomedicine to his credit, and has given over 400 invited talks, keynotes and plenaries world-wide.



## Stefan Mühlebach

Stefan Mühlebach, PhD, Prof. is a pharmacist by training. He chairs since 2010 the Non-Biological Complex Drugs (NBCDs) Working Group c/o Lygature, a non for profit, private-public partnership in the Netherlands (<http://lygature.org/nbcd>). He is professor for pharmacology and hospital pharmacy at the University of Basel in

Switzerland, a member of the Clinical Pharmacy & Epidemiology Unit in the Dept. of Pharmaceutical Sciences (<https://pharma.unibas.ch/home/>) and a member of the Medical Faculty. His research and teaching activities are within pharmacology, clinical nutrition, hospital pharmacy, and regulatory sciences. He authored more than 100 peer-reviewed papers, over 90 indexed in Pubmed/EMBASE, and several book chapters. He is a member of several (inter-)national professional associations and a board member of the Swiss Academy of Pharmaceutical Sciences (<http://www.saphw.ch/en>). From 1980-2005 he served as a Chief Hospital Pharmacist and head of department in Switzerland. From 2005 to 2008 he worked as Head of the Pharmacopoeia at Swissmedic, the Swiss Agency for Therapeutic Products, and of the Swiss Delegation at EDQM in Strasbourg. In 2008 he joined Vifor Pharma Switzerland as Chief Scientific Officer and holds actually a role as Regulatory Science Lead Non Biological Complex Drugs at the Vifor Pharma Headquarter in Switzerland (<http://www.viforpharma.ch/en/index.php>).



## Willem Mulder

Dr. Mulder is a Professor at Icahn School of Medicine at Mount Sinai and at the Academic Medical Center of the University of Amsterdam. His research – funded by several NIH grants and an NWO Vici – focuses on precision imaging<sup>1</sup> and targeted therapy<sup>2</sup> in cardiovascular disease<sup>3,4</sup> and cancer<sup>5,6</sup>. This involves library technology,

encompassing nanomaterials derived from natural lipoproteins (nanobiologics), that allows meticulously designing targeted immunotherapies<sup>7</sup>. When appropriately designed, such nanobiologics can be applied to empower the immune system's ability to fight disease, by promoting or inhibiting an immune response, by polarizing macrophage function, or by targeting myeloid cell dynamics. To facilitate translation, his team synchronously develops noninvasive imaging techniques to probe nanobiologics' *in vivo* behavior<sup>8,9</sup> and therapeutic function.

1. Mulder, W. J. M., Jaffer, F. A., Fayad, Z. A. & Nahrendorf, M. Imaging and Nanomedicine in Inflammatory Atherosclerosis. *Sci. Transl. Med.* 6, 239sr1 (2014).
2. Lobatto, M. E., Fuster, V., Fayad, Z. A. & Mulder, W. J. M. Perspectives and opportunities for nanomedicine in the management of atherosclerosis. *Nat. Rev. Drug Discov.* 10, 835–52 (2011).
3. Duivenvoorden, R. et al. A statin-loaded reconstituted high-density lipoprotein nanoparticle inhibits atherosclerotic plaque inflammation. *Nat. Commun.* 5, 3065 (2014).
4. Tang, J. et al. Inhibiting macrophage proliferation suppresses atherosclerotic plaque inflammation. *Sci. Adv.* 1, e1400223–e1400223 (2015).
5. Pérez-Medina, C. et al. Nanoreporter PET predicts the efficacy of anti-cancer nanotherapy. *Nat. Commun.* 7, 11838 (2016).
6. Zhao, Y. et al. Augmenting drug-carrier compatibility improves tumour nanotherapy efficacy. *Nat. Commun.* 7, 11221 (2016).
7. Tang, J. et al. Immune cell screening of a nanoparticle library improves atherosclerosis therapy. *Proc. Natl. Acad. Sci.* 113, E6731–E6740 (2016).

8. Pérez-Medina, C. et al. In Vivo PET Imaging of HDL in Multiple Atherosclerosis Models. *JACC Cardiovasc. Imaging* (2016). doi:10.1016/j.jcmg.2016.01.020
9. Perez-Medina, C. et al. PET Imaging of Tumor-Associated Macrophages with 89Zr-Labeled High-Density Lipoprotein Nanoparticles. *J. Nucl. Med.* 56, 1272–1277 (2015).



## Simona Mura

Pharmacist and Chemist, Dr MURA has been awarded her PhD in Chemistry and Technology of Drugs in 2009 (Univ. of Cagliari, Italy) working on the design and *in vitro* evaluation of novel vesicular systems for the topical delivery of drugs. Then, she has joined the Institut Galien Paris-Sud, UMR CNRS 8612 (group of Elias FATTAL) for a Post-Doctoral research stage during which she faced the issue of the potential pulmonary toxicity of biodegradable nanoparticles and the risk associated to their use as drug carriers for lung drug delivery. In 2011 she has been appointed Associate Professor in the same department within the framework of the “CNRS-Higher Education chairs” program (2011-2016) and she has integrated the team head by Pr Patrick COUVREUR. Since 2015 she is also Associate Visiting Professor at the University of Osaka (Japan). In 2017 she has been appointed Junior Member of the Institut Universitaire de France (IUF).

Her background is assigned to the design of colloidal systems for drug delivery and she holds a solid experience in their *in vitro/in vivo* preclinical evaluation. Recently, her research activity led to the discovery of the crucial role of endogenous lipoproteins as passive carriers for the delivery of the anticancer drug to the tumors. In parallel, the research interests of Dr MURA are oriented to the 3D culture methodologies aiming to construct relevant and easy to-handle tumor models, which are expected to allow a more predictive preclinical investigation compared to traditional flat monocultures on petri dishes.

Her research and contribution led to publications in leading journals of the field and she also authored several reviews in prestigious journals such as *Nature Mater*; *Chem Soc Rev*; *Adv Drug Deliv Rev*. Her scientific production includes 41 Peer-Reviewed Articles, 4 Book chapters and 1 Edited book, 15 international oral communications (5 invited) 4 national oral communications (2 invited); 9 invited lectures at Seminars/Workshops (3 international, 6 national) and 42 poster communications.

### SELECTED PUBLICATIONS

- Conjugation of squalene to gemcitabine as unique approach exploiting endogenous lipoproteins for drug delivery. D. Sobot, S. Mura, S. Yesylevskyy, L. Dalbin, F. Cayre, G. Bort, J. Mouglin, D. Desmaële, S. Lepetre-Mouelhi, G. Pieters, B. Andreiuk, A.S. Klymchenko, J.L. Paul, C. Ramseyer. P. Couvreur. *Nature Communications* 2017, 8, 15678 doi:10.1038/ncomms15678
- Multicellular tumor spheroids: a relevant 3D model for *in vitro* preclinical investigation of polymer nanomedicines. G. Lazzari P. Couvreur, S. Mura\*. *Polymer Chemistry* 2017, 8, 4947 4969
- Desmoplastic reaction in 3D-pancreatic cancer tissues suppresses molecular permeability. M. Matsusaki, M. Kameda, S. Mura, H. Tanaka, M. Kano, P. Couvreur, M. Akashi. *Advanced Healthcare Materials* 2017, doi 10.1002/adhm.201700057
- Peptide-functionalized nanoparticles for selective targeting of pancreatic tumor. S. Valetti, F. Maione, S. Mura, B. Stella, D. Desmaële, M. Noiray, J. Vergnaud, C. Vauthier, L. Cattel, E. Giraudo, P. Couvreur. *Journal of Controlled Release* 2014, 192, 29-39
- Stimuli-responsive nanocarriers for drug delivery. S. Mura, J. Nicolas, P. Couvreur. *Nature Materials* 2013, 12, 991–1003





## Maurice Mutro Nigo

My name is Maurice Mutro Nigo. I was born at Adi (DR Congo) in 1958. After my primary school at Adi, I joined Nyankunde Medical Centre in 1974 to be trained as paramedical worker. There, I soon recognized and appreciated the value of love in accompanying the sick people in their suffering time. Then, as joined the High School for Labora-

tory Technologies in the capital city of DR Congo, Kinshasa, where I graduated in 1984. Back to Nyankunde hospital, I served as laboratory technician for nine years. I was then chosen among other colleagues to continue my studies in the Master level in Belgium. This allowed me to become a teacher in our local High School for Medical Techniques since 1996 until 2014. On November 2014, I joined the Nanomedicine Group Laboratory (CLINAM), working together as a fellow in the former Discognosis Project. In the meanwhile, in 2015, I applied for PhD studies in Clinical Sciences at the University Hospital in Basel. I'm in my last year.



## André Nel

Dr. Nel is a Distinguished Professor of Medicine at UCLA, where he has successfully established one of the largest federally-funded nanotechnology research programs in the US. The UC Center for the Environmental Implications of Nanotechnology (UC CEIN) is the premier think tank for the safe and sustainable implementation of

nanotechnology in the US (<http://www.cein.ucla.edu/new/>), while the team-based science efforts he has put together as Research Director of the California Nanosystems Institute is spearheading nano medicine translation and commercialization on the UCLA campus. Professor Nel is a recipient of the Harry Truman Award and received the 2013 California Governor's Environmental Economic Leadership Award. He plays national leadership roles in science, biomedical research, nanotechnology and policy. He served as a chair of an NIH study section and was a NSF panel member for producing a comprehensive US Government blueprint to further develop the Nanotechnology Initiative (NNI) from 2010-2020 I. He was a member of the US Bilateral Presidential Commission for technology cooperation with Russia, and served as a panel member on Pres. Obama's PCAST panel for strategizing the NNI technological innovation and commercialization. Dr Nel has represented the US State Department and the NIH in cooperative research agreements with Japan and the Chinese Academy of Sciences, in which he was elected as Honorary Foreign Professor. In addition to groundbreaking work in nanotechnology, Dr Nel co-directed a leading EPA Particle Center studying the impact of air pollution on asthma. He was PI of the NIH-funded UCLA Asthma Center and received the John Salvaggio Award for outstanding service to the American Academy of Asthma Allergy and Immunology. He is a frequently invited plenary speaker at international multidisciplinary science conferences, a highly cited scientific scholar, and inventor contributing patents that spearheaded a startup company.

**SUMMARY OF PRESENT WORK:** Dr. Nel's current research focuses on nano-cancer and nanosafety. His interest in nano-cancer is focused around pancreatic cancer, with a view to develop an engineered approach by targeting the cancer stroma, accessing stromal vascular mechanisms and generating a systemic immune response to the primary and metastatic tumor. The nano-enabled immunotherapy platform is premised on the induction of immunogenic cell death plus interference in an immunomodulatory pathway through the use of synergistic, dual-delivery carriers. He is the co-inventor of the silicasome platform, comprised of lipid bilayer coated mesoporous

silica nanoparticles, which can be adapted for high drug loading of a large number of chemotherapeutics, including synergistic drug delivery, and the successful integration of chemo- with immunotherapy for the cancer treatment. His research in nanosafety is focused on developing high throughput screening approaches to assess nanomaterial behavior at the nano/bio interface for the purposes of toxicological profiling of broad and specific nanomaterial categories, tiered risk assessment approaches, safer by design strategies and utility of non-vertebrate, alternative test strategies for regulatory decision-making

## RECENT PUBLICATION

- 1. Lu, J.; Liu, X.; Liao, Y.-P. L.; Bergara, F.; Sun, B.; Jiang, W.; Chang, C. H.; Jiang, J.; Wang, X.; Wu, A. M.; Meng, H.; Nel, A. E.: Nano-enabled Pancreas Cancer Immunotherapy using Immunogenic Cell Death and Reversing Immunosuppression. *Nature Comm.* 8(1):1811-1825 (2017). doi:10.1038/s41467-017-01651-9
- Liong, M., Lu, J., Kovochich, M., Xia, T., Ruehm, S.G., Nel, A.E., Tamanoi, F., and Zink, JI., Multifunctional Inorganic Nanoparticles for Imaging, Targeting, and Drug Delivery. *ACS Nano.* 2:889-896 (2008).
- Liu X, Lin P, Perrett I, Lin J, Liao Y, Chang C, Jiang J, Wu N, Donahue T, Wainberg Z, Nel A, Meng H. Tumor-penetrating peptide enhances transcytosis of silicasome-based chemotherapy for pancreatic cancer. *The Journal of Clinical Investigation* 2017 127 (5): 2007-2018 DOI:10.1172/JCI92284
- Liu X, Situ A, Kang Y, Villabroza KR, Liao Y, Chang CH, Donahue T, Nel AE, Meng H. Irinotecan delivery by lipid-coated mesoporous silica nanoparticles shows improved efficacy and safety over liposomes for pancreatic cancer. *ACS Nano.* 2016, 10 (2), pp 2702–2715 DOI: 10.1021/acsnano.5b07781
- Meng H, Wang M, Liu H, Liu X, Situ A, Wu B, Ji Z, Chang CH, Nel AE. Use of a lipid-coated mesoporous silica nanoparticle platform for synergistic gemcitabine and Paclitaxel delivery to human pancreatic cancer in mice. *ACS Nano.* 2015 Apr 28;9(4):3540-57. doi: 10.1021/acsnano.5b00510. (2015)



## Bryant C. Nelson

Bryant is a staff research chemist at the National Institute of Standards and Technology (NIST) in the Material Measurement Laboratory (Gaithersburg, Maryland, USA). He was educated at the University of Texas at Austin, B.Sc., Chemistry, the University of Massachusetts at Amherst, Ph.D., Analytical Chemistry and completed his post-

doctoral training as an NRC research fellow at NIST in Analytical Chemistry (clinical mass spectrometry) in 1997. Bryant became a permanent staff member at NIST in 2003. Among other things, he has been responsible for the design and leadership of numerous large-scale research projects focused on using mass spectrometry to characterize the potential environmental health and human safety risks of engineered nanomaterials and nano-enabled materials. Current research programs are primarily focused on: (1) characterizing the potential causes and effects (and associated biases) of nanomaterial-induced artifacts in current and emerging analytical methods and bioassays used in nanomedicine and nanotoxicology and (2) the development of validated analytical methods, protocols and documentary standards for nano-enabled medical products. Bryant advises undergraduate/graduate students and postdocs in nanomedicine, analytical biochemistry and nanotoxicology. He is a member of the Analytical Chemistry Division of the American Chemical Society (ACS), a member of the American Society for Testing and Materials (ASTM International) and a member of Sigma Xi. He has authored over 80 peer-reviewed manuscripts and 2 book chapters. He has given over 60 oral presentations domestically and internationally and is a past Sigma Xi Distinguished Lecturer (2015



– 2017). He is Past President of the NIST Chapter of Sigma Xi (2008 – 2009), Past Associate Editor of the Journal of Environmental Quality (2011 – 2014) and is currently on the Editorial Review Board for the Journal of Nanotoxicology and Nanomedicine. He is currently the Chair of ASTM International E56.08 – Nano-Enabled Medical Products and a Councilor to the ACS representing the Analytical Chemistry Division.



## Guangjun Nie

Ph.D, Professor  
CAS Key Laboratory for Biomedical Effects of Nanomaterials & Nanosafety  
Direct of Project Management & International Collaboration, National Center for Nanoscience and Technology of China, 11 Beiyijie, Zhongguancun, Beijing 100190, China ;  
Tel:86-10-82545529; Niegj@nanoctr.cn

Guangjun Nie is a Professor at the National Center for Nanoscience and Technology, China. He received his B.S. degree from Northeast Normal University in 1996, followed by an M.S. degree from Jilin University in 1999. In 2000, he was a Visiting Scholar of Institute of Food Research, UK. In 2002, he obtained his Ph.D degree from Institute of Biophysics, Chinese Academy of Sciences. From 2002 to 2008, he worked as postdoc fellow at Jewish General Hospital, McGill University, Canada. Currently, he is a National Distinguished Youth Scientist. He was awarded the Hundred Talent Program Scholar of CAS in 2008 and was a Chief Scientist of a MoST National Basic Research Program. He has a long standing interest in cancer biology, blood physiology and pathophysiology of human disorders involving dysregulation of redox balance and metal metabolism. Currently, his main interests are design of bio-inspired materials to overcome the current barriers in tumor therapy and nanobiology. In particular, his group is working toward controlling the chemical properties of multi-functional nanoparticles in order to allow specific targeting and regulation of tumor cells and their microenvironment.

His research interests include: 1) Targeting and regulation of tumors and their microenvironment mediated by intelligent functional nanomaterials for diagnostic and therapeutic applications, especially pancreatic and liver cancers. 2) Novel biomaterials design and synthesis inspired by biological systems and design of functional molecular machinery. 3) Cellular membrane vesicle systems and the roles of exosomes in biological effects of nanomaterials and drug delivery. 4) Development of novel nanomedicines for treatment of iron and redox related human disorders.

His most recent research activities generated a group of interdisciplinary works in nanobiology, nanomedicine and blood physiology fields, including over 132 papers published in Nature Biotechnology, Nature Biomedical Engineering, Acc Chem Res, Adv Mater, Angew Chem, Adv Funct Mater, Blood, Biomaterials, Br J Haematol, JACS, JBC, Molecular Cancer Therapeutics, Nano Letters and Small. He has filled over 40 patents on novel nanomedicines and 22 of them have been granted, with two patents on antitumor drug development have been transferred to a biotechnology firm for pre-clinical investigation. He is an experienced supervisor of postgraduate students and collaborates widely both within China and internationally. Now he is leading a multidisciplinary team with over 40 people working toward better and safer antitumor nanomedicine for pancreatic cancer and liver cancer. He is also the Affiliated Professor of Northeast University, Shenyang, China and East China University of Science and Technology, Shanghai. He is also an Affiliated Senior Member of Houston Methodist Research Institute, Houston, US.



## Lutz Nuhn

Dr. Lutz Nuhn is a junior group leader in the department of Prof. Tanja Weil at the Max-Planck-Institute for Polymer Research (MPIP) in Mainz (Germany). He studied biomedical chemistry at the Johannes Gutenberg-University Mainz (Germany) and received his diploma degree in 2010. In 2008/09, he practiced first research experience in the laboratories of Prof. Robert Langer (MIT, USA).

For his doctoral degree he studied in the group of Prof. Rudolf Zentel, and during summer 2013 also in the group of Prof. Kazunori Kataoka (University of Tokyo, Japan). In 2014, he was awarded a PhD with distinction from Johannes Gutenberg-University Mainz. For his postdoctoral research, he moved to Belgium and worked together with Prof. Bruno De Geest and Prof. Richard Hoogenboom at Ghent University as a Feodor-Lynen fellow of the Alexander-von-Humboldt Foundation. Since summer 2017, Lutz Nuhn returned to Germany and joined the group of Tanja Weil at the MPIP as a Liebig fellow of the Fonds der Chemischen Industrie (FCI).

Lutz Nuhn was a member of the Max Planck Graduate School with the Johannes Gutenberg-University Mainz (MPGC) and received scholarships from the German National Academic Foundation, the Alexander-von-Humboldt-Foundation, the Research Foundation Flanders (“Fonds Wetenschappelijk Onderzoek Vlaanderen, FWO”) and the “Fonds der Chemischen Industrie” (FCI). He is also a project leader the Interdisciplinary Research Center “Nano-Sized Polymer Therapeutics for Tumor Immunotherapy” (SFB 1066) in Mainz and his research focuses on novel polymer-based cancer immunotherapeutics.

### REFERENCES:

- Lutz Nuhn, Markus Hirsch, Bettina Krieg, Kaloian Koynov, Karl Fischer, Manfred Schmidt, Mark Helm, Rudolf Zentel – “Cationic Nanohydrogel Particles as Potential siRNA Carriers for Cellular Delivery”, ACS Nano 2012, 6, 2198-2214.
- Lutz Nuhn and Sebastian Hartmann, Björn Palitzsch, Bastian Gerlitzki, Edgar Schmitt, Rudolf Zentel, Horst Kunz – “Water-Soluble Polymers Coupled with Glycopeptide Antigens and T-Cell Epitopes as Potential Antitumor Vaccines”, Angewandte Chemie International Edition 2013, 52, 10652-10656; Angewandte Chemie 2013, 125, 10846-10850.
- Lutz Nuhn, Nane Vanparijs, Ans De Beuckelaer, Lien Lybaert, Glenn Verstraete, Kim Deswarte, Stephan Lienenklaus, Nikunj M. Shukla, Alex C. D. Salyer, Bart N. Lambrecht, Johan Grooten, Sunil A. David, Stefaan De Koker, Bruno G. De Geest – “pH-Degradable Imidazoquinoline-Ligated Nanogels for Lymph Node Focused Immune Activation”, Proceedings of the National Academy of Sciences 2016, 113, 8098-8103.
- Nane Vanparijs, Lutz Nuhn, Bruno G. De Geest – “Transiently Thermoresponsive Polymers and Their Application in Biomedicine”, Chemical Society Reviews 2017, 46, 1193-1239.
- Lutz Nuhn, Lien Van Hoecke, Kim Deswarte, Bert Schepen, Yupeng Li, Bart Lambrecht, Stefaan De Koker, Sunil David, Xavier Saelens, Bruno G. De Geest – “Potent Anti-Viral Vaccine Adjuvant Based on pH-Degradable Nanogels with Covalently Linked Small Molecule Imidazoquinoline TLR7/8 Agonist”, Biomaterials 2018, accepted, DOI: 10.1016/j.biomaterials.2018.03.026



## Andrew Owen

Professor of Pharmacology

Andrew Owen is Professor of Molecular and Clinical Pharmacology at the University of Liverpool, UK. He is Chair of the British Society for Nanomedicine, a fellow of the Royal Society of Biology, and a fellow of the British Pharmacological Society. His clinical and basic research focuses on understanding mechanisms that underpin inter-patient variability in pharmacokinetics and pharmacodynamics. In recent years a major emphasis has been to employ knowledge of these mechanisms to accelerate the translation of nanomedicine candidates to clinical applications. He has published over 190 original publications, is co-inventor of patents relating to the application of nanotechnology to drug delivery and a co-founder of Tandem Nano Ltd ([www.tandemnano.com](http://www.tandemnano.com)) and PKTK ([www.PKTK.co.uk](http://www.PKTK.co.uk)).

### RECENT REFERENCES

- Long-acting injectable atovaquone nanomedicines for malaria prophylaxis. Bakshi RP, Tatham LM, Savage AC, Tripathi AK, Mlambo G, Ippolito MM, Nenortas E, Rannard SP, Owen A, Shapiro TA. *Nat Commun.* 2018 Jan 22;9(1):315. doi: 10.1038/s41467-017-02603-z.
- Towards a Maraviroc Long-Acting Injectable Nanoformulation. Tatham LM, Savage AC, Dwyer A, Siccardi M, Scott T, Vourvahis M, Clark A, Rannard SP, Owen A. *Eur J Pharm Biopharm.* 2018 Apr 20. pii: S0939-6411(18)30200-5. doi: 10.1016/j.ejpb.2018.04.009.
- Accelerated oral nanomedicine discovery from miniaturized screening to clinical production exemplified by paediatric HIV nanotherapies. Giardiello M, Liptrott NJ, McDonald TO, Moss D, Siccardi M, Martin P, Smith D, Gurjar R, Rannard SP, Owen A. *Nat Commun.* 2016 Oct 21;7:13184. doi: 10.1038/ncomms13184.
- Antiretroviral solid drug nanoparticles with enhanced oral bioavailability: production, characterization, and *in vitro-in vivo* correlation. McDonald TO, Giardiello M, Martin P, Siccardi M, Liptrott NJ, Smith D, Roberts P, Curley P, Schipani A, Khoo SH, Long J, Foster AJ, Rannard SP, Owen A. *Adv Healthc Mater.* 2014 Mar;3(3):400-11. doi: 10.1002/adhm.201300280.
- Strengths, weaknesses, opportunities and challenges for long acting injectable therapies: Insights for applications in HIV therapy. Owen A, Rannard S. *Adv Drug Deliv Rev.* 2016 Aug 1;103:144-56. doi: 10.1016/j.addr.2016.02.003.

with European and international partners and stakeholders at the forefront of Next Gen Medicines and Patient Centred Medicine innovation for the benefit of patients and society as a whole.

### BRIEF EMPLOYMENT HISTORY

MD, Internal Medicine specialists, Marisa joined the EMA in late 1994.

Appointed Deputy Head of Quality sector up to 2002, of the Efficacy and Safety Sectors up to 2009, Head of office until 2015, Marisa pioneered regulatory science work, at European and International level, to support emerging therapies and technologies with open discussions with innovators, involving of the best expertise available among partners and stakeholders and planning ahead for effective and efficient regulatory work.

### MAIN ACHIEVEMENTS INCLUDE

- 2016- to date:
  - Establishment and roll-out as co-chair of the EU Innovation offices Network (EU-IN) with its integration in an upcoming EU wide Horizon Scanning and participation to relevant European and international activities.
  - Pilot methodology for the EMA Regulatory Science Observatory pilot and contribution to the design of the EMA Regulatory Science Strategy to 2025.
- 2008-2013
  - The establishment and roll out of the initial EMA Scientific Support and Projects Office (now department) focussing on: Specialised Disciplines support to the Committees in multidisciplinary areas (statistical methodology, non-clinical drug development, environmental risk assessment, clinical pharmacology);
- 2000-2008
  - The initiation and establishment of innovative regulatory science platforms and processes to support innovation, regulatory preparedness and contributions to environmental analysis and strategic EMA Road Maps
  - Innovation Task Force and Business Pipeline Project (2001)
  - Establishing and chairing the EMA Biosimilars Task Force (2004)
  - Biomarkers and methods regulatory qualification (2007)
  - The establishment and full management of specialised expert groups and working parties covering emerging therapies and technologies (e.g. Gene therapy and Pharmacogenomics (2001), Cell-therapy (2004), Biosimilars (2005), Nanomedicines (2007), Biostatistics and methodology WP (2009)
- 1994-2000
  - Establishing and implementing foundation operational (centralised procedure) and scientific processes (European Public Assessment Reports - EPARs) of the European Medicines (evaluation) Agency
  - 1995-2000 Scientific secretariat of the Biotechnology Working Party



## Marisa Papaluca Amati

Senior Scientific Advisor  
Scientific Committees Regulatory Science Strategy (SciRS)

### CURRENT RESPONSIBILITIES

Marisa was appointed in March 2015 Agency as Senior Scientific Advisor in the Research and Development Division and since 1st September 2016 moved in to the advisory function of Scientific Committees Regulatory Science strategy.

In her new position Marisa co-chairs the EU Innovation offices Network with focus on the strengthening the overall EU regulatory support to innovation.

Marisa is also actively involved in the establishment of the EMA Regulatory Science Observatory, a matrix function aiming at informing strategies for the support of successful development of innovative medicines and modernisation of regulatory tools for the benefit of individuals, public health and society at large.

She is also responsible for a number of projects in collaboration



## Wolfgang Parak

Wolfgang Parak is Professor at the University of Hamburg. He has studied physics and obtained his PhD in Munich. After a postdoctoral fellowship at Berkeley he returned to Munich to start his own group. Before moving to the University of Hamburg he spent 10 years as professor at the Philipps University Marburg.

Wolfgang Parak is also Associate Editor of ACS Nano. The research of Wolfgang Parak is dedicated towards the development of new surface chemistries of inorganic nanoparticles and towards the characterization of their physicochemical properties. In particular, the development of an amphiphilic polymer coating is nowadays used by many different groups worldwide. Nanoparticles with such high colloidal stability are the bases of experimentally correlating their physicochemical properties with their interaction with cells (involving uptake and cytotoxicity), which has been the research topic of

the Parak group for the last 17 years. The group also uses polymeric polyelectrolyte capsules fabricated by layer-by-layer assembly for biological applications (*in vitro* sensing and delivery).

## RECENT PUBLICATIONS

- M. Colombo, L. Fiandra, G. Alessio, S. Mazzucchelli, M. Nebuloni, C. De Palma, K. Kantner, B. Pelaz, F. Corsi, W. J. Parak, D. Prosperi, "Tumour homing and therapeutic effect of colloidal nanoparticles depend on the number of attached antibodies", *Nature Communications* 7, 13818 (2016).
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- X. Ma, R. Hartmann, D. Jimenez de Aberasturi, F. Yang, S. J. H. Soenen, B. B. Manshian, J. Franz, D. Valdeperez, B. Pelaz, N. Feliu, N. Hampp, C. Riethmüller, H. Vieker, N. Freese, A. Götzhäuser, M. Simonich, R. Tanguay, X.-J. Liang, W. J. Parak, "Colloidal Gold Nanoparticles Induce Changes in Cellular and Subcellular Morphology", *ACS Nano* 11, 7807-7820 (2017).
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## Giorgia Pastorin

Associate professor

Giorgia Pastorin received her MSc degree in Pharmaceutical Chemistry and Technology in 2000 and her Ph.D. in 2004 from the University of Trieste (Italy), working on adenosine receptors' antagonists. She spent 2 years as a postDoc at CNRS in Strasbourg (France) where she acquired

some skills in drug delivery.

She joined the National University of Singapore in June 2006, as Assistant Professor in the Department of Pharmacy-Faculty of Science. She was promoted to Associate Professor in 2011, Assistant Head in 2014 and she is currently Deputy Head (Research) in the same Department.

She is the Editor of two books related to drug delivery and author in more than 100 articles on internationally peer-reviewed journals including *Nature Nanotechnology*, *Biomaterials*, *Nanoscale* etc.

For the work performed by her BioNano-Technology group in NUS, she recently received the Young Scientist Award (2015).

## RECENT PUBLICATIONS

- Wei Jiang Goh, Zou Shui, Wei Yi Ong, Federico Torta, Alvarez Fernandez Alexandra, Raymond M. Schifferers, Gert Storm, Jiong-Wei Wang, Bertrand Czarny\* and Giorgia Pastorin\*. Bioinspired Cell-Derived Nanovesicles versus Exosomes as Drug Delivery Systems: a Cost-Effective Alternative. *Scientific Reports*, 2017, 7, 14322 doi:10.1038/s41598-017-14725-x.
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## Anil Patri

Dr. Anil Patri serves as the Chair, Nanotechnology Task Force and Director of Nanocore, National Center for Toxicological Research, US Food and Drug Administration. His laboratory is very active in regulatory science research to understand material characteristics, safety and efficacy. Dr. Patri serves on the U.S. National

Nanotechnology Initiative (NNI) NSET Subcommittee and NEHI working group for inter-agency coordination. He is as member of ISO TC229 and serves on the executive committee of ASTM E56 to facilitate standards development.

Prior to joining FDA in 2014, Dr. Patri served as the Deputy Director of the Nanotechnology Characterization Laboratory (NCL) at the Frederick National Laboratory for Cancer Research. In a decade-long tenure at NCL, he assisted collaborators from industry and academia towards clinical translation of drug products utilizing nanotechnology, many currently in clinical trials. From 2006-2014, he served as a guest scientist at the National Institute of Standards and Technology (NIST) to co-develop nominal 10nm, 30nm, and 60 nm colloidal gold reference material standards. Dr. Patri developed nanotechnology-based targeted drug delivery and imaging agents until 2004 at the University of Michigan Medical School. He is a co-author of 65 publications, serves on editorial boards of *Molecular Pharmaceutics*, *Nanomedicine & Nanobiotechnology*. Dr. Patri obtained his B.Sc., from Osmania University, M.Sc., in Chemistry from Aligarh Muslim University in India. He moved to US in 1992 and earned his Ph.D. degree in Chemistry from the University of South Florida followed by a post-doctoral training at the University of Michigan. He worked at Astra Zeneca and as a lecturer in Chemistry prior to graduate school.



## Christiane Pauli-Magnus

Director department of Clinical Research

Prof. Christiane Pauli-Magnus is internist and professor of clinical pharmacology at the University of Basel. She is director of the Department of Clinical Research and director of the Clinical Trial Unit at the Medical Faculty of the University Basel. In this role, she was in charge for supervising the timely implementation of the general consent process with all involved stakeholders at the University Hospital Basel. As vpresident of the Swiss Clinical Trial organisation she is involved in the national consensus process for the development of a Swiss-wide harmonized content of the general consent information. She is also co-director of the Basel Personalized Health organisation. As such, she contributes to the strategic positioning and build-up of local data warehouse-, biobanking- and ELSI (Ethical Legal and Social Implication) -infrastructures as a prerequisite for data interoperability on a national level.





## Dan Peer

Manning Director, SPARK Tel Aviv, Center for Translational Medicine; Director, Laboratory of Precision NanoMedicine; Chair, Tel Aviv University Cancer Biology Research Center.

Dan Peer is a Professor and the Director of the Laboratory of Precision NanoMedicine at Tel Aviv University (TAU) funded by the US NIH and the European Union via ERC grant. Prof. Peer is also the Chair of Tel Aviv University Cancer Biology Research Center; the biggest Cancer Center in Israel that includes 17 affiliated hospitals. In addition, he heads the new SPARK program (Center for Translational Medicine) at TAU. Prof. Peer's work was among the first to demonstrate systemic delivery of RNA molecules using targeted nanocarriers to the immune system and he pioneered the use of RNA interference (RNAi) for *in vivo* validation of new drug targets within the immune system that has enormous implications in blood cancer and inflammation. Prof. Peer received more than 30 awards; among them, he was recognized by the Kenneth Rainin Foundation by their Innovator (2010) and Breakthrough (2011 – 2013) Awards for his pioneering work in inflammatory bowel diseases and by the AAAS and the Untold news breakthrough Award for his development of the Gagomers platform for cancer targeted drug delivery. In 2017, he received the 2017 Nanos Award for major contribution to the field of clinical nanomedicine in CLINAM 10th, Basal, Switzerland. Prof. Peer has more than 90 pending and granted patents. Some of them have been licensed to several pharmaceutical companies and one is currently under registration (as a new drug in inflammatory bowel disease). In addition, based on his work, five spin-off companies were generated aiming to bring innovative personalized medicine into clinical practice. Three of them are in clinical stage companies. Prof. Peer is a past Present of the Israeli Chapter of the Controlled Release Society, and a Member of the Israel Young Academy of Science.

### RECENT PUBLICATIONS

- Peer D.\*, Karp JM\*, Hong S\*, Farokhzad O, Margalit R, and Langer R (2007). Nanocarriers as emerging platforms for cancer therapy. *Nature Nanotechnology* 2,751-760. \* Highest cited paper in nanomedicine (cited > 5250).
- Peer D., Park EJ, Morishita Y, Carman CV, and Shimaoka M (2008). Systemic Leukocyte-Directed siRNA Delivery Revealing Cyclin D1 as an Anti-Inflammation Target. *Science*. 319, 627-630.
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## Beatriz Pelacho

Researcher

Beatriz Pelacho graduated in Biochemistry (1997) and Biology (2000) and obtained her PhD in Biochemistry (2002) in the University the Navarra. She performed a post-doctoral stay in the Stem Cell Institute in the University of Minnesota (USA) (2002-2005) and afterwards, joined to the Regenerative Medicine Area of the Center for Applied Medical Research (CIMA, Pamplona, Spain). Dr. Pelacho is also Associate Professor at the University of Navarra (Faculty of Medicine) since 2014. During all this time, she has developed diverse experimental and preclinical projects related to Regenerative Medicine, specifically focused in the study of the cellular and molecular mechanisms involved in the cardiovascular pathologies, as well as in the development of novel therapies based on Bioengineering, Nanotechnology and Cell and Gene Therapy strategies. Dr. Pelacho has authored more than 45 manuscripts and served as editorial board member in reputed journals, obtaining also several national and international grants as principal investigator. In addition, she has contributed to develop several studies linked to international companies, also for cardiac-related applications.

### RECENT PUBLICATIONS

- Ruiz-Villalba A, Simón AM, Pogontke C, Castillo MI, Abizanda G, Pelacho B, Segovia JC, Prósper F, Pérez-Pomares JM. "Embryonic epicardial and postnatal bone-marrow cellular derivatives dynamically interact to build up post-infarction cardiac fibrotic scar" (2015) *Journal of the American College of Cardiology* 65(19):2057-66.
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## Sara Pereira

My name is Sara Pereira and I have a BSc. in Biology from the University of Lisbon, Portugal (2008). After doing a 1-year research internship shared between the Gulbenkian Institute for Science (IGC) and the Molecular Medicine Institute (IMM), I then enrolled in a MSc. in Biotechnology in the Department of Bioengineering at the

Technical University of Lisbon (2010). After completing the MSc. dissertation entitled "Novel Aqueous Two-Phase Systems for the Purification of Antibodies", I was awarded a 2-year grant as a Research Assistant in the Nucleic Acid Bioengineering Lab to work in the field of DNA vaccination. During this period, I collaborated with the Nanomedicine Lab at King's College London, where I worked until 2014. In 2015, I started a PhD in the School of Pharmacy at the University of East Anglia, working in the development of a novel therapy for advanced prostate cancer based on the stimuli-responsive liposomes encapsulating doxorubicin-PSA cleavable peptides. In August 2017, my research group moved to Queen's University Belfast, where I will complete my PhD. During the PhD I have been able to collaborate in several different projects, which resulted in the following publications:

- Fluorinated tranlylcypromine analogues as inhibitors of lysine-specific demethylase 1 (LSD1, KDM1A). Borrello MT, Gerwien K, Benelkebir H, Pereira S, Al-Jamal WT, Douglas L, Duriez PJ, Packham G, Haufe G, Ganesan A. 2017 in: *Bioorganic & Medicinal Chemistry Letters*.
- Docetaxel-loaded liposomes: The effect of lipid composition and purification on drug encapsulation and *in vitro* toxicity. Pereira S, Egbu R, Jannati G, Al-Jamal WT. 2016 in: *International Journal of Pharmaceutics*.
- Synthesis of Diagnostic Silicon Nanoparticles for Targeted Delivery of Thiourea to Epidermal Growth Factor Receptor-Expressing Cancer Cells. Behray M, Webster CA, Pereira S, Ghosh P, Krishnamurthy S, Al-Jamal WT, Chao Y. 2016 in: *ACS Applied Materials and Interfaces*.
- Cationic Liposome- Multi-Walled Carbon Nanotubes Hybrids for Dual siPLK1 and Doxorubicin Delivery In Vitro. Pereira S, Lee J, Rubio N, Hassan HA, Suffian IB, Wang JT, Klippstein R, Ballesteros B, Al-Jamal WT, Al-Jamal KT. 2015 in: *Pharmaceutical Research*.



## Adriele Prina-Mello

Dr. Prina-Mello is an Ussher Assistant Professor in Translational Nanomedicine, at the Trinity Translational Medicine Institute (TTMI) at the University of Dublin, Trinity College Dublin, Ireland.

Dr Prina-Mello's multidisciplinary scientific interest and work falls, within and outside, TTMI, the AMBER (Advanced Materials

and BioEngineering Research as Science Foundation Ireland funded centre), CRANN nanoscience institute and the School of Medicine. Specialist interest are focused on the advanced translation research in NanoMedicine (*in vitro/ in vivo* diagnostic, imaging and therapeutics), microfluidic, biomedical devices and tissue engineering applications of nanotechnology and nanomaterials. Dr. Prina-Mello is part of the European Nanomedicine Characterization Laboratory (EUNCL) infrastructure, European Technology Platform of Nanomedicine (ETPN) association, COST action network RADIOMAG and UPCON, National Standard Authority of Ireland (NSAI) and several key H2020 projects (REFINE, BIORIMA). Most recently Dr. Prina-Mello, with collaborators, has published a series or book chapters focused on translational aspects and 3D models as alternative for *in vivo* testing.



## Signe Ratso

Signe Ratso is Deputy Director General of DG RTD of the European Commission since 1 March 2018. In this function her particular areas of responsibility include International Cooperation in Research and Innovation as well as R&I in Industrial Technologies and in the area of Transport. Before joining DG RTD she worked in dif-

ferent senior management positions in DG TRADE since 2006. From 2011 to 2018 she was Director for Trade Strategy, Analysis and Market Access in DG TRADE. She was also in charge of implementation issues as well as industrial sector aspects of the EU trade policy, including energy and access to raw materials. Previously (from 2007 to 2011) she was Director for WTO, legal matters and food-related sectors in DG TRADE. She also covered OECD issues, export credits and export controls of dual use goods. After joining the Commission at the beginning of 2006, she held the post of Principal Adviser in DG TRADE for the first year. Before joining the Commission Signe Ratso worked as Deputy Secretary General (from 1994 to 2005) at the Ministry of Economic Affairs and Communications of the Republic of Estonia. In this position she was responsible for all EU-related issues in the following policy areas: trade and industrial policy, energy, transport, telecommunications, information society, internal market affairs. During Estonia's accession negotiations she was responsible for negotiating 6 economic chapters. She has two University degrees. In 1983 she graduated from Tartu University in Estonia as an English philologist, in 1993 in International Trade and International Economics.



## Andreas Reisch

Maître de conférences (Assistant professor) in biophysics  
Université de Strasbourg – Faculté de Pharmacie  
Laboratoire de Bioimagerie et Pathologies (UMR 7021)  
Equipe Nanochimie et Bioimagerie – Nanochemistry and Bioimaging group

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My research focuses on the design of functional polymeric materials for biomedical applications. I am combining polymer synthesis and physical chemistry to assemble materials with precisely defined organization, functionality, and responsiveness. A major aim of my work is to control the interactions between polymeric materials and biological systems. Most recently I am working on ultrabright fluorescent polymer nanoparticles and their use in bioimaging.<sup>1-5</sup> In this context I coordinate the French young researcher grant (ANR JC/JC) "supertrack" since end of 2016.

After studies of chemistry with specialization in polymer chemistry and physical-chemistry at the universities of Stuttgart and Dresden and at the École Européenne de Chimie, Polymères et Matériaux (ECPM) in Strasbourg, I completed a Ph.D. thesis on biomimetic polymer surfaces under the supervision of Prof. Schaaf at the Institut Charles Sadron, Strasbourg in 2009. During my postdoctoral studies at the Florida State University, Tallahassee, in the group of Prof. Schlenoff I then developed saloplastic polyelectrolyte complexes.<sup>6</sup> A subject I continued working on during a postdoc at the University of Strasbourg alongside the development of mechano-responsive materials,<sup>7</sup> before joining the faculty in 2012.

### SOME PUBLICATIONS

- Reisch, A.; Klymchenko, A. S. Fluorescent Polymer Nanoparticles Based on Dyes: Seeking Brighter Tools for Bioimaging. *Small* 2016,

12 (15), 1968–1992.

- Reisch, A.; Trofymchuk, K.; Runser, A.; Fleith, G.; Rawiso, M.; Klymchenko, A. S. Tailoring Fluorescence Brightness and Switching of Nanoparticles through Dye Organization in the Polymer Matrix. *ACS Appl. Mater. Interfaces* 2017, 9 (49), 43030–43042.
- Andreiuk, B.; Reisch, A.; Lindecker, M.; Follain, G.; Peyri ras, N.; Goetz, J. G.; Klymchenko, A. S. Fluorescent Polymer Nanoparticles for Cell Barcoding In Vitro and In Vivo. *Small* 2017, 13 (38), 1701582.
- Reisch, A.; Runser, A.; Arntz, Y.; M ly, Y.; Klymchenko, A. S. Charge-Controlled Nanoprecipitation as a Modular Approach to Ultrasmall Polymer Nanocarriers: Making Bright and Stable Nanoparticles. *ACS Nano* 2015, 9, 5104–5116.
- Reisch, A.; Didier, P.; Richert, L.; Oncul, S.; Arntz, Y.; M ly, Y.; Klymchenko, A. S. Collective Fluorescence Switching of Counterion-Assembled Dyes in Polymer Nanoparticles. *Nat. Commun.* 2014, 5, 4089.



## Frances Richmond

Chair, Department of Regulatory and Clinical Sciences, University of Southern California

Dr. Frances Richmond is currently Chair of the Department of Regulatory and Quality Sciences at the University of Southern California in Los Angeles, California. She was educated as a neurophysiologist (BNSc, MSc, PhD) at Queen’s University, Kingston, Canada, then completed post-doctoral studies at the Universit  de Montr al and the National Institutes of Health. She returned to the faculty of Queen’s University, where she progressed to become professor and Associate Dean of Life Sciences (1989-1992). Research during this academic period focused on the neurological basis of normal and abnormal motor control. She was the first woman to be appointed Director of a research consortium, specifically the MRC Group in Sensory-Motor Research, funded by Canada’s Medical Research Council (1995-2000).

Dr. Richmond had several roles outside of her research and academic activities. She served for two years as a policy advisor on scientific labor needs to Industry Canada. After that, she participated in and graduated from a year-long executive program at Canada’s National Defense College. Dr. Richmond served as a clinical scientist at the Alfred E. Mann Foundation (1994-1995), and then as a consultant at Advanced Bionics Corporation in Sylmar, California. Dr. Richmond joined the faculty of the School of Pharmacy at USC in 1999 and was Director of Regulatory and Clinical Sciences at the Alfred E. Mann Institute. Research included seven preclinical/clinical projects related to medical product development, focused on electrical devices, microsensors and pharmaceutical products. Dr. Richmond is or has been a member of four large US research consortia (NIH Engineering Research partnership, NIH Bioengineering Research partnership, Clinical and Translational Science Institute, Tobacco Center of Regulatory Science). Dr. Richmond and her team have been responsible for the development and oversight of multiple undergraduate and graduate programs in the School of Pharmacy that provide certificate, MS and doctoral training in the regulatory and quality management of foods, dietary supplements, medical devices and drugs.

### RECENT PUBLICATION

- Jamieson, M and Richmond, FJ (2013) The role of universities in the commercialization of medical products: a survey of industry views. *Therapeutic Innovation and Regulatory Science*, 48:347-356
- Sinha, UK, Villegas, B, Kuo, C, Richmond, F, Masood, R, Nelson, NI and Loeb, GE. (2014); Safety of microstimulator during radiation therapy- A preliminary study on head and neck cancer patients, *Journal of Nuclear Medicine & Radiation Therapy*, 5(4), 1-5

- Storm, NE and Richmond, FJ (2015) Global regulatory dissonance: A case study of industry views on the development of drugs for postmenopausal osteoporosis. *Therapeutic Innovation and Regulatory Science* 49:269-278, On-line December, 2014,
- Medina, C and Richmond, FJ (2015) Incentivizing quality in the manufacture of pharmaceuticals: Industry views on quality metrics and ratings. *Therapeutic Innovation and Regulatory Science*, 49: 730-738 On-line, June, 2014,
- Yu, Z. and Richmond F.J.R. (2018) Regulatory Considerations for Genetically Manipulated Cell-Based Products. In: *Encyclopedia of Tissue Engineering and Regenerative Medicine*, in press.



## Bernd Riebesehl

Dr. Bernd Riebesehl is Senior Technical Project Leader in the Pharmaceutical Development Parenteral, Topical, Ophthalmic Dosage Forms of Novartis Pharma AG, Basel, Switzerland since 2008.

At Novartis he is leading the Global Pharmaceutical Innovation Committee and the early technical development of several parenteral drug products. Externally Dr. Riebesehl has been serving as Advisory Board Member of the European Society of Clinical Nanomedicine, and chaired drug delivery sessions for the Section Drug Delivery of International Association for Pharmaceutical Technology (APV). He completed his thesis in Pharmaceutical Technology at the Technical University of Braunschweig. 1992 he started his industrial career at Lilly Forschung GmbH in Hamburg leading several teams for preformulation, early phase development and formulation development. In his role as Research Advisor in Pharmaceutical R&D he led several initiatives enabling the formulation of poorly soluble drugs. In 2007 he became Director of Pharmaceutical Development at Speedel Experimenta AG, Basel.



## Cristianne Rijcken

Dr. Cristianne Rijcken is the founder of Cristal Therapeutics, and serves as Chief Scientific Officer of the company. Dr. Rijcken’s PhD thesis provided a strong basis for Cristal Therapeutics and she was awarded multiple grants and prizes including the Simon Stevin Gezel Award in 2008 and the Knowledge for Growth Inspiring Young Scientist Award in 2014. She is (co-) author of 28 scientific publications and co-inventor of all patents and patent applications of Cristal Therapeutics. Recently, Cristianne is selected as Limburg Businesswoman of the Year 2017 because of her innovative mind-set, the perseverance upon translational activities and her entrepreneurial attitude. Dr. Rijcken is pharmacist by training and holds a PhD degree in Pharmaceutics from Utrecht University.



## Matthias Roesslein

Empa

Dr. Matthias Roesslein - senior scientist - works at Empa since 1996. As a trained chemist with specialization in physical chemistry and statistics he thenceforward became one of the experts worldwide in 'measurement science in conjunction with

qualification, traceability, measurement uncertainty and quality management systems'. The main focus was first on applying the general principle of measurement science in analytical chemistry, where amongst other things he wrote major parts of a number of international guidelines as a member of the CITAC-Eurachem working group on 'measurement uncertainty and traceability'. In 2006 he obtained a position as 'Senior Scientist' and joined the laboratory for 'Materials-Biology Interaction' of Empa focusing on different subjects, such as the standardization of *in vitro* assays elucidating the effect of nanoparticles on different cell types and the quality of next generation sequencing results. For this purpose he established a close collaborations with two groups in the division 'bio-systems and biomaterials' at NIST and obtained in 2008 the status of a foreign guest researcher. This collaboration led to a number of novel approaches gauging the performance and quality of *in vitro* assays.

Beside that he contributed strongly to the progress of the Nanoparticle Tracking Analysis (NTA) measurement that are developed by NanoSight by participating in numerous round robins and consulting on the statistical analysis. Currently he is establishing different NGS methods, such as whole transcriptome or target exon sequencing, for answering specific questions related to the interaction of medical nanoparticles with biological system. Beside that he strongly focuses on different aspect of translational science.

### ROLE IN THE EU-NCL

Matthias Roesslein leads the effort to qualify all from the NCL transferred assays or methods that will be used in the characterisation cascade of the EU-NCL and heads the quality control management system. In this context he coordinates together with Simon Baconnier the scientific discussion of the core expert team (CET) of the EU-NCL.



## Barbara Rothen-Rutishauser

Co-Chair BioNanomaterials

Prof. Dr. Barbara Rothen-Rutishauser has received her Ph.D. in 1996 in cell biology at the Swiss Federal Institute of Technology (ETH) in Zurich. From 1996 to 2000 she held a post-doctoral position in Biopharmacy at the Institute of Pharmaceutical Sciences at the ETH where she developed and characterised cell culture models for drug transport studies. In 2000 she joined Prof. Peter Gehr's research group at the Institute of Anatomy, University of Bern, Switzerland. During the period of her research B. Rothen-Rutishauser has become an expert in the field of cell-nanoparticle interactions in the lung, with a special focus on the development of alternative lung models. Since 2011 she is the new chair in BioNanomaterials at the Adolphe Merkle Institute, University of Fribourg, Switzerland, the position is shared equally with Prof. Alke Fink. She has published more than 190 peer-reviewed papers and is an associate editor of the Particle and Fibre Toxicology. In 2013 the Swiss National Science Founda-

tion (SNSF) awarded a National Competence Centre of Research (NCCR) on Bio-Inspired Materials to the UNIFR, which includes Prof. Rothen-Rutishauser, as a module and project leader. Prof. Rothen-Rutishauser also serves as a Faculty Delegate for Women and Young Researchers in this NCCR

### RECENT PUBLICATIONS

- Milosevic A, Rodriguez-Lorenzo L, Balog S, Monnier CA, Petri-Fink A, Rothen B. Stability of fluorescently encoded nanoparticles in lysosomes. *Angew Chem Int Ed Engl.* 56(43): 13382-13386 (2017).
- Vanhecke D, Kuhn DA, Jimenez de Aberasturi D, Balog S, Milosevic A, Urban D, Peckys D, de Jonge N, Parak WJ, Petri-Fink A, Rothen-Rutishauser B. Involvement of two uptake mechanisms of gold and iron oxide nanoparticles in a co-exposure scenario using mouse macrophages. *Beilstein J Nanotechnol* 8:2396-2409 (2017).
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- Drasler B, Sayre P, Steinhäuser KG, Petri-Fink A, Rothen-Rutishauser B. In vitro approaches to assess the hazard of nanomaterials. *NanoImpact* 8, 99-116 (2017).



## Santi Sala

Dr. Santi Sala is CEO of Nanomol Technologies SL. With a strong background on applied research projects and technology transfer from academia to industry, he was founder on 2010 of this spin-off company, a science and innovation driven company providing cutting edge solutions to process and structure, at micro and nanoscale, active molecules of pharma, biotech and cosmetic companies. He is former Research Associate in the Spanish Biomedical Networking centre on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN). PhD in Chemistry at the Autonomous University of Barcelona in 2005 with a thesis on particle design using supercritical fluid technology, conducted at Materials Science Institute of Barcelona (ICMAB) CSIC. Master's Degree in Management and Business Administration - MBA of Barcelona School of Management - Pompeu Fabra University. Expert in particle design for drug delivery and Nanomedicine, and characterization of particles by optical and electron microscopy, laser diffraction and dynamic light scattering. Author of 18 publications in SCI journals and inventor of 6 international patents, 3 of them under operation. Solid experience in the preparation, management and implementation of collaborative research projects involving industry academia partnerships (national and international programs - H2020). Managing and promotion of applied research activities of Nanomol research group in ICMAB, as a member of TECNIO network of innovation centers (Catalonia Government) since 2006. Vast experience in management and commercialization of intellectual property arising from research results, including their valorisation.





## Kirsten Sandvig

Prof. Kirsten Sandvig is associated with Dept. of Biosciences, University of Oslo, Norway and she is heading a research group in Department of Molecular Cell Biology, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital. The Norwegian Radium Hospital is the main cancer hospital in Norway.

Sandvig's group, counting ~20 members from different countries, is interested in the mechanisms of endocytosis, intracellular transport and secretion. In some of our studies we are using protein toxins such as ricin and Shiga toxin, which are well established as markers for studies of membrane traffic, and which can be used as agents in cancer diagnosis and therapy. Our expertise is also applied to investigate uptake of nanoparticles, and we have obtained a large 5-year grant (Biodegradable nanoparticles in cancer diagnosis and therapy) from the Norwegian Research Council to build national competence in nanomedicine (running to the end of 2018). This project involves collaboration between 10 Norwegian research groups covering synthesis of nanoparticles, *in vitro* and *in vivo* biology studies, *in vivo* imaging and clinical studies. In addition, international collaboration is included. The Sandvig group is also involved in an INNO INDIGO granted project, which started April 2016. INNO INDIGO is an innovation-driven initiative for the development and integration of Indian and European research. We also characterize exosomes from prostate cancer cells and prostate cancer patients with the goal of detecting biomarkers. Our research spans all the way from basic to translational medicine, including innovation. We aim at providing a rational basis for diagnosis, treatment and prevention of disease. The group has extensive national and international collaboration.

**EDUCATION:** M.Sci. from The Technical University of Norway, Trondheim; Ph.D. from the Medical Faculty, University of Oslo, Norway. Research visits abroad at University of Michigan and at the biological laboratories, Harvard Cambridge, Mass. USA.

**SCIENTIFIC ACTIVITY:** Published more than 300 articles and supervised a large number of Ph.D. students and master students. Sandvig has been invited as plenary speaker at more than 100 international meetings, and the work is heavily cited, Hirsch index is 72.

**AWARDS AND HONOURS:** Anders Jahres Medical Prize for young researchers, 1989 (first woman to receive this prize); The Norwegian Research Councils research prize, 1990; Member of the Norwegian Academy of Science and Letters, 1993; Stiansens Biomedical Research Prize, 1995; King Olav V's Cancer Research Prize, 1998; Member of EMBO (European Molecular Biology Organization), 1998; Member of Academia Europea from 2002; Honorary Doctor at the University of Copenhagen, Denmark, 2007; Member of the American Academy of Microbiology, 2010; The Fridjof Nansen Award for outstanding research in science and medicine, 2014; Oslo University Hospital Prize for excellent research, 2017.



## Hélder A. Santos

Position: Associate Professor/Principal Investigator/Head Division

Research area: Nanomedicines and Biomedical Engineering

Affiliation: Faculty of Pharmacy and Helsinki Institute of Life Science, University of Helsinki

Dr. Santos (D.Sc. Tech., Chem. Eng.) received his doctorate degree (2007) in Chemical Engineering from Helsinki University of Technology, Finland. Currently, an Associate Professor (tenure track) in Pharmaceutical Nanotechnology, Head of Division of Pharmaceutical Chemistry and Technology, Head of the Preclinical Drug Formu-

lation and Analysis Group, and Director of the Doctoral Program in Drug Research at the Faculty of Pharmacy, University of Helsinki. He is also a Fellow Member of the recently established Helsinki Institute of Life Science (HiLIFE), leader of the nanomedicines and biomedical engineering group ([www.helsinki.fi/~hsantos](http://www.helsinki.fi/~hsantos)), and one of the World Portuguese Network Advisers for Science.

Dr. Santos research interests include the development of nanoparticles/nanomedicines for biomedical and healthcare applications. His current work makes the bridge between engineering, pharmaceutical and medical research. His main research focus is in the use of biodegradable and biocompatible nanoporous silicon nanomaterials, polymers, the application of microfluidics technology for nanoparticle production for simultaneous controlled drug delivery, diagnostic and treatment of cancer, diabetes, and cardiovascular diseases, and further translation of these nanotechnologies into the clinic.

Dr. Santos is author of more than 200 publications in prestigious high impact peer-reviewed journals, and author of more than 20 book chapters and more than 200 conference proceedings/abstracts. He has given over 110 invited talks at prestigious conferences, universities and summer schools around the world.

Dr. Santos has received a number of prestigious awards and grants, such as the "Talent Prize in Science" attributed by the Portuguese government in 2010, the European Research Council Starting Grant in 2013, the Young Researcher Award in 2013 and honour distinction for the exceptional scientific productivity in 2014, both attributed by the Faculty of Pharmacy at the University of Helsinki, the Academy of Finland Award for Social Impact in 2016, and honor nomination for the USERN Prize in Biological Sciences in 2017. Dr. Santos research career and development portfolio totals over 5 M€.

### HIGHLIGHTED PAPERS:

- Fontana F.\*, Shahbazia M.-A., Liu D., Zhang H., Mäkilä E., Salonen J., Hirvonen J. T., Santos H.A.\* Multistaged nanovaccines based on porous silicon@acetalated dextran@cancer cell membrane for cancer immunotherapy. *Adv. Mater.* 2017, 29(7), 1603239 (IF = 19.7).
- Balasubramanian V.\*, Correia A., Zhang H., Fontana F., Mäkilä E., Salonen J., Hirvonen J., Santos H.A.\* Biomimetic engineering using cancer cell membranes for designing compartmentalized nanoreactors with organelle-like functions. *Adv. Mater.* 2017, 29(11), 1605375 (IF = 19.7).
- Zehua Liu, Yunzhan Li, Wei Li, Chen Xiao, Dongfei Liu, Chao Dong, Ming Zhang, Ermei Mäkilä, Marianna Kemell, Jarno Salonen, Jouni T. Hirvonen, Hongbo Zhang\*, Dawang Zhou\*, Xianming Deng\*, Hélder A. Santos\*, "Multifunctional Nanohybrid Based on Porous Silicon Nanoparticles, Gold Nanoparticles and Acetalated Dextran for Liver Regeneration and Acute Liver Failure Theranostics", *Adv. Mater.* 2017 (IF = 19.7).
- Mónica P.A. Ferreira\*, Virpi Talman, Giulia Torrieri, Dongfei Liu, Gonçalo Marques, Karina Moslova, Zehua Liu, João F. Pinto, Jouni Hirvonen\*, Heikki Ruskoaho\*, Hélder A. Santos\*, "Dual-drug delivery using dextran-functionalized nanoparticles targeting cardiac fibroblasts for cellular reprogramming", *Adv. Funct. Mater.* 2018. (in press)
- Mónica P.A. Ferreira\*, Sanjeev Ranjan, Sini Kinnunen, Alexandra Correia, Virpi Talman, Ermei Mäkilä, Brianda Barrios-Lopez, Marianna Kemell, Vimalkumar Balasubramanian, Jarno Salonen, Jouni Hirvonen, Heikki Ruskoaho\*, Anu J. Airaksinen\*, Hélder A. Santos\*, "Drug-loaded Multifunctional Nanoparticles Targeted to the Endocardial Layer of the Injured Heart Modulate Hypertrophic Signaling", *Small* 2017, 13(33), 1701276.



## Rana Sanyal

CSO, RS Research Inc.;  
Director of Center for Life Sciences and  
Technologies, Bogazici University,  
Istanbul, (TR)

Dr Rana Sanyal graduated from Bogazici University in 1994 with a B.S. degree in Chemical Engineering. She continued her studies at Boston University on synthetic organic chemistry and received her Ph.D. degree in Chemistry. After her PhD, she worked as a research scientist in Amgen Inc., Thousand Oaks, California, where she gained extensive experience in protein therapeutics. Since 2004, she is a faculty member at the Department of Chemistry at Bogazici University. Her current research interests include targeted drug delivery agents including ADCs for cancer chemotherapy and preparation of novel materials for biomedical applications. She has received L'Oréal Turkey for Women in Science, Novartis Pharmaceutical and Medicinal Chemistry Drug Design and Turkish National Academy of Sciences Young Investigator awards. She is an inventor or co-inventor on 28 issued patents or pending applications. Since 2013, she is the director of the Center for Life Sciences and Technologies (<https://lifesci.boun.edu.tr/>), an international center of excellence, at Bogazici University. Rana Sanyal is also the co-founder and chief science officer of RS Research (<http://rsresearch.net/>), a pharmaceutical biotechnology start-up developing novel nanomedicines, established to enable preclinical research advance to the clinic. RS Research drug delivery platform provides targeted agents with improved efficacy and better safety profiles.

### RECENT PUBLICATION

- Sumer Bolu B, Golba B, Boke N, Sanyal A, Sanyal R "Designing Dendron-Polymer Conjugate Based Targeted Drug Delivery Platforms with a "Mix-and-Match" Modularity" *Bioconjugate Chemistry* (2017) 28(12), 2962-2975.
- Kaga S, Truong NP, Esser L, Senyschyn D, Sanyal A, Sanyal R, Quinn JF, Davis TP, Kaminskas LM, Whittaker MR "Influence of Size and Shape on the Biodistribution of Nanoparticles Prepared by Polymerization-Induced Self-Assembly" *Biomacromolecules* (2017) 18(12), 3963-3970.
- Oz Y, Barras A, Sanyal R, Boukherroub R, Szunerits S, Sanyal A "Functionalization of Reduced Graphene Oxide via Thiol-Maleimide "Click" Chemistry: Facile Fabrication of Targeted Drug Delivery Vehicles" *ACS Applied Materials & Interfaces* (2017) 9(39), 34194-34203.
- Karacivi M, Bolu BS, Sanyal R Targeting to the Bone: Alendronate-Directed Combretastatin A-4 Bearing Antiangiogenic Polymer-Drug Conjugates *Molecular Pharmaceutics* (2017), 14, 1373-1383.
- Aktan B, Chambre L, Sanyal R, Sanyal A "Clickable" Nanogels via Thermally Driven Self-Assembly of Polymers: Facile Access to Targeted Imaging Platforms using Thiol-Maleimide Conjugation" *Biomacromolecules* (2017), 18, 490-497.



## Raymond Schiffelers

Raymond Schiffelers studied Bio-Pharmaceutical Sciences at Leiden University (1990-1995). After an industrial traineeship at SmithKline Beecham Pharmaceuticals (UK) he did his PhD in medical microbiology at Erasmus University Rotterdam on liposomal targeting of antimicrobial agents (1996-2001). Subsequently he became post-doc at Utrecht University working on liposomes targeting tumor vasculature. In 2002-2003, at Intradigm Co (USA) he expanded his tumor vasculature-targeting work with polymers for delivery of siRNA. After his return to Utrecht University he became assistant

and then associate professor. In 2011, he moved to the Laboratory for Clinical Chemistry & Hematology of the University Medical Center Utrecht to become professor of nanomedicine. Next to work on synthetic drug delivery systems, he focuses on extracellular vesicles in the circulation as inspiration for new nanomedicine and diagnostic readouts. Regarding the extracellular vesicle field, he is founding member of the International Society for Extracellular Vesicles (ISEV), board member of the Netherland Society for Extracellular vesicles (NLSEV), Associate Editor of the Journal Extracellular Vesicles, and Founder of EXCYTEX-an extracellular vesicle-based company.

## Ruth Schmid

Vice President Marketing



Dr. Schmid is Vice President Marketing at SINTEF Industry in Trondheim, Norway with special responsibility for the area of medical technology, including nanomedicine. SINTEF is one of Europe's largest independent non-profit research institute.

Her present research activities include the preparation and characterisation of micro- and nanoparticles by various technologies and from a wide variety of materials (including biodegradable polymers and hybrid materials), as well as the surface modification of polymers and polymer particles by wet-chemistry, to introduce tailor-made properties. Lately, focus has been on the encapsulation and immobilisation of liquids and solids from emulsions, for protection and controlled release. Examples of encapsulated substances are liquid crystals, magnetic iron oxides, insect repellents and fragrances. Another focus has been on coating of biomaterials by self-assembling methods and covalent attachment with biocompatible, biomimetic and functional coatings, e.g. for introduction of antimicrobial properties, for increased osseo-integration or for immobilisation of biological molecules. There is special focus on applied research and product orientated solutions. Fields of special interest are the emerging fields of nanomedicine, targeted drug delivery and release, nanotechnology-based diagnostics and regenerative medicine, with special focus on applications based on particle technology and surface modification. Application of encapsulation technologies and controlled release in various industrial segments, e.g. medicine, animal health, cosmetics, house-hold and body care products, food and beverages, agriculture, etc. is another field of interest.

Dr. Schmid is a Swiss citizen living in Norway since 1979. She gained her Diploma (1975) and PhD (1979) in Natural Sciences (physical organic chemistry) at ETH Zürich, Switzerland. She is a member of ACS, CRS (member of the board of directors (2009-present), President 2016-2017), the European Technology Platform in Nanomedicine and the EARTO working group "Emerging Technologies for Healthcare" (2016-present). Dr. Schmid is author/co-author of 46 scientific publications, 21 patents and patent applications and 88 oral/poster presentations and 16 webinar, mass media and popular science publications.

### RECENT PUBLICATIONS

- K. Liefeth, M. Frant, U. Müller, P. Stenstad, H. Johnsen & R. Schmid, *Biointerphases* 13, 011004 (2018). "Archaeal tetraether lipid coatings – A strategy for the development of membrane analogue spacer systems for the site-specific functionalization of medical surfaces."
- E. Sulheim, T.G. Iversen, V.T. Nakstad, G. Klinkenberg, H. Sletta, R. Schmid, A.R. Hatletveit, A.M. Wågbo, A. Sundan, T. Skotland, K. Sandvig & Y. Mørch, *Int. J. Mol. Sci.* 18 (11), 2454 (2017); doi: 10.3390/ijms18112454. "Cytotoxicity of Poly(Alkyl Cyanoacrylate) Nanoparticles."
- J. Ritsma, E. Herschberg, SE. Borgtos, C. Løvmo, R. Schmid, YM

te Welscher, G. Storm & C van Nostrum, *Int J of Pharmaceutics* (2017), <http://dx.doi.org/10.1016/j.ijpharm.2017.11.017>. "Relationship between polarities of antibiotic and polymer matrix on nanoparticle formulations based on aliphatic polyesters."

- A.K.O Åslund, E. Sulheim, S. Snipstad, E. von Haartman, H. Baghirov, N. Starr, M. Kvåle Løvmo, S. Lelú, D. Scurr, C. de Lange Davies, R. Schmid & Y. Mørch, *Mol. Pharmaceutics*; doi: 10.1021/acs.olpharmaceut.6b01085. "Quantification and Qualitative Effects of Different PEGylations on Poly(butyl cyanoacrylate) Nanoparticles."
- Y. Mørch, R. Hansen, S. Berg, A.K.O. Åslund, W.R. Glomm, S. Eggén, R. Schmid, H. Johnsen, S. Kubowicz, S. Snipstad, E. Sulheim, S. Hak, G. Singh, B.H. McDonagh, H. Blom, C. de Lange Davies, P.M. Stenstad, *Contrast Media and Molecular Imaging* 10(5), 356-366 (2015); doi:10.1002/cmim.1639. "Nanoparticle-Stabilized Microbubbles for Multimodal Imaging and Drug Delivery."



## Avi Schroeder

PhD.

Avi Schroeder is an Assistant Professor of Chemical Engineering at the Technion – Israel Institute of Technology where he heads the Laboratory for Targeted Drug Delivery and Personalized Medicine Technologies (<https://www.schroederlab.com/>).

Dr. Schroeder conducted his Postdoctoral studies at the Massachusetts Institute of Technology, and his PhD jointly at the Hebrew and Ben Gurion Universities.

Avi is the recipient of more than 25 national and international awards, including named a KAVLI Fellow, a Horev Fellow – Leaders in Science and Technology, an Alon Fellow as well as the Intel Nanotechnology-, TEVA Pharmaceuticals-, and the Wolf Foundation Kill Award. Avi is the author of more than 45 research papers inventor of 17 patents and co-founder of several startup companies based on these discoveries.



## Ulrich S. Schubert

Professor and Chair, Laboratory of Organic and Macromolecular Chemistry, Friedrich Schiller University Jena, Jena, Germany

Education and appointments: Study of Chemistry in Frankfurt & Bayreuth (both Germany) and Richmond/Virginia (USA). PhD studies University of Bayreuth (Germany) and University of South Florida (USA) with Prof. G. Newkome and Prof. C.-D. Eisenbach. Post-doc Université Louis Pasteur Strasbourg (France) with Prof. J.-M. Lehn (Nobel laureate 1987). Habilitation (assistant professor) Technical University of Munich (Germany). Associate professor, Center for NanoScience, Ludwig Maximilians University Munich (Germany). 2000-2007 Full-Professor, Eindhoven University of Technology (The Netherlands). 2005-2014 Scientific Chairman Technology Area HTE Dutch Polymer Institute (DPI, The Netherlands). 2007 to date Full Professor and Chair, Friedrich Schiller University Jena (Germany).

Awards, fellowships and honors: Heisenberg-Fellowship (German Science Foundation), Habilitation award of the German Chemical Society (Macromolecular Division), Guest professorship Université Catholique de Louvain (Belgium), VICI award of the Netherlands Organization for Scientific Research (1.25 M€), Calls to Full Professorships at the Universities Hamburg and Stuttgart (both Germany, declined), Jan Pieter Lemstra Innovation Award (The Netherlands),

International Biannual BPG Award of the Belgian Polymer Group. Call Professor Materials Discovery and Director Materials Discovery Centre, University College London (UCL, declined 2012), Call Executive Director A\*STAR IMRE, Singapore (declined 2016), ACS Division of Polymer Chemistry, Polymer Division Fellow (USA), ISI "Highly cited researcher" in Materials Science (since 2014) & Chemistry (2016), Molecular Science Forum Professorship, Institute of Chemistry, Chinese Academy of Science, Beijing. Fellow Royal Society of Chemistry (FRSC, UK). Thüringer Forschungspreis (Kategorie Angewandte Forschung) 2017. Elected member of the German National Academy of Science and Engineering (acatech) and fellow of the National Academy of Inventors (NAI), USA. External scientific member of the Max-Planck-Gesellschaft (MPI for Colloid and Interfaces, Golm).

Administrative functions and memberships: 2013-2017 Dean of the Faculty of Chemistry and Earth Sciences, Friedrich Schiller University (FSU) Jena, Director of the Jena Center for Soft Matter and the Center for Energy and Environmental Chemistry Jena. Coordinator of the Priority Program (SPP 1568) "Design and Generic Principles of Self-healing Materials" of the German Science Foundation (since 2010). Vice chairman of the Board of the Division Macromolecular Chemistry of the German Chemical Society (since 2012). Deputy spokesman research board polymer research of the German Science Foundation (DFG). Spokesman DFG Center of Excellence (SFB 1278 "Polytarget"). >970 refereed publications in reviewed international journals, Web-of-Science ranking (July 2018): Sum of citations >40000, h-index: 93.

## RECENT PUBLICATION

- *Angew. Chem. Int. Ed.* 2018, 57, 2479; *Biomacromolecules* 2018, 19, 748; *Biomacromolecules* 2017, 18, 3280; *Polym. Chem.* 2017, 8, 6086; *Anal. Chem.* 2017, 89, 1185.



## Georg Schulz

Manager of the Core Facility Micro- and Nanotomography

**2008:** Diploma in theoretical physics at the University of Freiburg, Germany. Diploma thesis on Exciton dynamics in circular and elliptical aggregates.

**2008:** scientific collaborator at the group of Theoretical Quantum Dynamics (University of Freiburg, Germany).

**2012:** PhD at Biomaterials Science Center (University of Basel, Switzerland) on the topic of human brain imaging, in particular magnetic resonance imaging, phase contrast X-Ray computed tomography and small-angle X-ray scattering.

**2012-2015:** PostDoc at Biomaterials Science Center, University of Basel, Switzerland

**since 2015:** Group leader of the high-resolution X-ray imaging group, Biomaterials Science Center, Department of Biomedical Engineering, University of Basel, Switzerland

**since 2017:** Manager of the Core Facility 'Micro- and Nanotomography', Department of Biomedical Engineering, University of Basel, Switzerland

## RECENT PUBLICATION

- L. Costeur, C. Grohé, G. Aguirre-Fernández, E. Ekdale, G. Schulz, B. Müller, B. Mennecart, 'The bony labyrinth of toothed whales reflects both phylogeny and habitat preferences' *Scientific Reports* 8 (2018) 7841
- A. Khimchenko, C. Bikis, A. Pacureanu, S. E. Hieber, P. Thalmann, H. Deyhle, G. Schweighauser, J. Hench, S. Frank, M. Müller-Gerbl, G. Schulz, P. Cloetens, B. Müller, 'Hard X-ray nano-holotomography: Large-scale, label-free, three-dimensional neuroimaging be-



yond optical limit' *Advanced Science* 5(6) (2018) 1700694

- A. Khimchenko, G. Schulz, P. Thalmann, B. Müller, 'Implementation of a double-grating interferometer for phase-contrast computed tomography in a conventional system nanotom<sup>®</sup> m' *APL Bioengineering* 2 (2018) 016106
- C. Bikis, L. Degrugillier, P. Thalmann, G. Schulz, B. Müller, S. E. Hieber, D. F. Kalbermatten, S. Madduri, 'Three-dimensional imaging and analysis of entire peripheral nerves after repair and reconstruction' *Journal of Neuroscience Methods* 295 (2018) 37-44
- C. Bikis, P. Thalmann, L. Degrugillier, G. Schulz, B. Müller, D. F. Kalbermatten, S. Madduri, S. E. Hieber, 'Three-dimensional and non-destructive characterization of nerves inside conduits using laboratory-based micro computed tomography' *Journal of Neuroscience Methods* 294 (2018) 59-66



## Simó Schwartz

Director CIBBIM-Nanomedicine  
(Molecular Biology and Biochemistry  
Research Center for Nanomedicine).  
Barcelona Hospital Campus.  
Passeig de la Vall d'Hebron, 119-129 -  
08035 Barcelona, Spain

Dr Simó Schwartz Jr (1967th, Barcelona) is Director and Board member of CIBBIM-Nanomedicine, which fosters research on new biomedical advanced therapies and nanotechnology-based applications for the clinical practice. In particular, advanced cell therapies, new biomaterials and drug delivery systems, image based diagnostic systems and preclinical validation of therapeutic conjugates and bio-nanosensors, mainly in the areas of oncology and rare diseases. He is also member of the Science Advisory Board of the Vall d'Hebron Research Institute (VHIR) and member of the Science Advisory Board of the European Nanotechnology Characterization Laboratory (EU-NCL). He also leads the "drug delivery and targeting group" at the CIBBIM-Nanomedicine. He holds 12 patents, most transferred to leading companies of the biotech and pharma sectors and coauthors more than 85 papers in high impact factor journals.

Dr Schwartz Jr is coordinator and collaborator of several research projects directly related with the obtention and validation of therapeutic drug delivery systems. Among them are international and EU projects involving SME's in which animal models are being used for preclinical validation of new therapies directed against tumor cells. Dr Schwartz Jr is also member of the Nanomedicine Spanish Platform (NanomedSpain) and of the "European Platform for Nanomedicine". His research group is also a group member of the "CIBER de Bioingeniería, Biomateriales y Nanomedicina" (CIBER-BBN) of the Spanish Health Institute CarlosIII (ISCIII) which gathers a total of 45 research groups of national excellence in the field of nanotechnology and nanomedicine. Dr Schwartz Jr was the Nanomedicine Coordinador of CIBER-BBN at the national level and later appointed as Deputy Director and technology transfer coordinator. Dr Schwartz was also Co-founder and Science Advisor of ARGON Pharma SL (2008-2015), a Spin-Off company established at the Barcelona Science Park with the mission to develop new innovative therapies to provide solutions to unmet medical needs in the oncology field, and also to develop new technologies for drug delivery and diagnosis to improve current therapies. Dr Schwartz Jr is also member of the editorial Board of the *Eur. J. Nanomedicine* and until 2016 of *Nanomedicine-NBM*. He is currently Science Advisor of SOM BIOTECH and CELGENE, member of the Advisory Board of The Lundbeck Foundation Center of Excellence NanoCAN (Nanomedicine Research Center for Cancer Stem Cell Targeting Therapeutics), Southern Denmark University, and has been recently appointed as President of the European Society of Nanomedicine and Executive Board member of the International Society of Nanomedicine.

## RECENT PUBLICATIONS

- Diana Rafael, Francesc Martínez, Fernanda Andrade, Joaquim Seras-Franzoso, Natalia Garcia-Aranda, Petra Gener, Joan Sayós, Diego Arango, Ibane Abasolo, Simó Schwartz Jr. Efficient EFGR mediated siRNA delivery to Breast Cancer Cells by Cetuximab Functionalized Pluronic<sup>®</sup> F127/Gelatin *Chemical Engineering Journal*; In Press.
- Schwartz S Jr Unmet needs in developing nanoparticles for precision medicine. *Nanomedicine (Lond)*. 2017 Feb;12(4):271-274
- Giannotti, M; Abasolo, I; Oliva, M; Andrade, F; García-Aranda, N; Melgarejo, M; Pulido, D; Corchero, JL; Fernández, Y; Villaverde, A; Royo, M; Garcia-Parajo, M; Sanz, F; Schwartz Jr, S. Highly versatile polyelectrolyte complexes for improving the enzyme replacement therapy of lysosomal storage disorders. *ACS Applied Materials and Interfaces*, 2016 Oct 5;8(39):25741-25752
- I Cabrera, I Abasolo, JL Corchero, E Elizondo, P Rivera Gil, E Moreno, J Farauo, S Sala, D Bueno, E González-Mira, M Rivas, M Melgarejo, D Pulido, F Albericio, M Royo, A Villaverde, M F. García-Parajo, S Schwartz Jr., N Ventosa, J Veciana. Alpha-Galactosidase A Loaded Nanoliposomes with Enhanced Enzymatic Activity and Intracellular Penetration. *Advanced Healthcare Materials*. 2016 Apr 6;5(7):829-40
- Gener P, Rafael DF, Fernandez Y, Ortega JS, Arango D, Abasolo I, Videira M Schwartz S Jr. Cancer stem cells and personalized cancer nanomedicine. *Nanomedicine (Lond)* 2016 Feb; 11(3):307-20
- Gener P, Gouveia LP, Sabat GR, de Sousa Rafael DF, Fort NB, Aranja A, Fernández Y, Prieto RM, Ortega JS, Arango D, Abasolo I, Videira M, Schwartz S Jr. Fluorescent CSC models evidence that targeted nanomedicines improve treatment sensitivity of breast and colon cancer stem cells. *Nanomedicine-NBM*. 2015 Nov;11(8):1883-92.



## Torsten Schwede

Torsten Schwede studied Chemistry in Bayreuth and completed his PhD in Structural Biology in Freiburg, Germany. He then worked as a Bioinformatics Scientist at GlaxoWellcome in Geneva (later GSK) before he was appointed tenure-track assistant professor of Bioinformatics at the Biozentrum in Basel in 2001.

Since 2002, Torsten has been a group leader at the SIB Swiss Institute of Bioinformatics. In 2007, he was promoted to Associate Professor and, in 2014 he became Scientific Director of sciCORE, where he is responsible for the central infrastructure for scientific computing at the University of Basel. As a chairman of the Scientific Expert Board and a director of the Data Coordination Centre of the Swiss Personalized Health Network (SPHN), Torsten is committed to the development of research in the field of personalized health and medicine in Switzerland.



## Giacinto Scoles

Adjunct professor –  
University of Udine (Fac. of Medicine),  
Distinguished scientist at ELETTRA  
Synchrotron Light Source Laboratory in  
Trieste P.I. of an Advanced Grant from the  
ERC Donner  
Professor of Science, Emeritus, Princeton  
University Distinguished Adj. Prof. of Physics  
and Biology, Temple Univ. Philadelphia

## PERSONAL STATEMENT

GIACINTO SCOLES' scientific career has spanned an unusually long length of time and an equally unusually broad range of subjects. The general philosophy was to exploit new physical ideas and novel instrumentation to solve outstanding problems in chemistry and materials science before, and biology and medicine now. Much before the coming of age of Nanotechnology and Nanoscience, particular emphasis was given to the behavior of nano systems and materials

## POSITIONS AND LEADERSHIP

**2011 to date:** Adjunct Professor, University of Udine, Faculty of Medicine, Department of Biological and Medical Sciences, Ospedale Universitario Santa Maria della Misericordia Building #13, Udine, Italy.

**2011 to date:** Distinguished scientist at ELETTRA Synchrotron Light Source Laboratory in Trieste (It).

**2011 to date:** Holder of an ERC Advanced Grant within the Program IDEAS at the Univ. of Udine

**2008 to date:** Donner Professor of Science, Emeritus, Princeton University, Princeton, NJ 08544, USA and Distinguished Adjunct Prof. of Biology, Temple University, Philadelphia, PA, (USA).

**2003–2010:** Professor of Biophysics at SISSA Miramare (Trieste) Italy;

**2009:** Senior Consultant to the Inter. Center for Science and High Technology of the United Nations Industrial Development Organization (ICS-UNIDO) responsible for Nanotechnology & Nano Drug Delivery

**2005–2009:** Scientific Coordinator of LANADA the Laboratory for Nano Diagnostic, Drug Delivery and Analysis of CBM The Consortium for Biomolecular Medicine in Trieste (Italy).

**2003–2009:** Collaborator of ELETTRA, Sincrotrone Trieste S.C.p.A. Basovizza (Trieste), Italy;

**1987–2008:** Donner Professor of Science at Princeton University and Princeton Materials Institute;

**1971–1986:** Prof. of Chemistry and Physics Univ. of Waterloo, Waterloo, Canada;

**1982–1985:** Director of the Center for Mol. Beams and Laser Chemistry, University of Waterloo (Ca)

**1977–1979:** Professor of Solid State Physics, University of Trento, Italy;

**1974–1975:** Acting Director, of the Guelph- Waterloo Centre for Graduate Work in Chemistry.

**1968–1971:** Assoc. Prof., Physics Dept., University of Genova, Genova, Italy;

**1964–1968:** Assist. Prof., Physics Dept., University of Genova, Genova, Italy;

**1961–1964:** Research Associate, Kamerlingh-Onnes Lab., University of Leiden, The Netherlands

**1960–1961:** Assist. Prof., Physics Dept., University of Genova, Genova, Italy.

## HONORS AND AWARDS

**2013:** Herschbach Medal for Chemical Dynamics; **2006:** Benjamin Franklin Medal in Physics (with J.P.Toennies) from the Franklin Institute; **2003:** Creativity Award from the NSF 2003-5 and Earle K. Plyler Prize for Molecular Spectroscopy from the American Physical Society (with Kevin K. Lehmann). **2002:** Peter Debye Award in Physical Chemistry from the American Chemical Soc.; **2000:** Elected Foreign Member of The Royal Netherlands Academy of Arts and Sciences and Honorary Science Doctorate from the University of Waterloo; **1996:** Recipient of an Honorary Doctorate in Physics from the University of Genova; **1995:** Recipient of a Senior Fellowship of the Alexander von Humboldt Foundation and Recipient of the 1995 Lippincott Award of the Optical Society of America, the Coblenz Society, and the Society for Applied Spectroscopy; **1986:** Senior Killam Fellowship.

## RESEARCH SUPPORT

Giacinto Scoles has been recently granted an advanced ERC grant (2011, MONALISA QUIDPROQUO, MOlecular NANotechnology for Life Science Applications: QUAntitative Interactomics for Diagnos-

tics, PROteomics and QUantitative Oncology) of nearly 3M€ over 5 years for an ambitious collaborative research project. The focus and the goal of his research is to introduce innovative devices and protocols (based on micro/nano-fluidics and on the nano-mechanical response of bio-molecular nano-structures) to carry out precise, quantitative and low cost measurements on large, predetermined diagnostically relevant, subsets of the proteome obtained from very small samples in samples produced by a very small number of cells or within single cells with potential capability of measuring its interactions (Interactomics). By means of these measurements he hopes to make new inroads into quantitative diagnostics and disease monitoring.



## Sandro Sieber

Sandro Sieber is a PostDoc in the pharmaceutical technology laboratory of Prof. Jörg Huwyler at University of Basel. In 2014, he successfully achieved his Master Degree in Pharmaceutical Sciences with his master thesis entitled: "Hepatocyte Specific Drug Targeting using Liposomes and Preparation of Gold Nanoparticle Loaded Nanocarriers for the Investigation of Cellular Uptake Mechanisms". After an internship at Roche Basel, he continued his research in Prof. Huwyler's group focusing on the validation, and application of the zebrafish model for the pre-clinical characterization of nanomedicines. During his PhD, he spent three months at university of Leiden in the group of Prof. Alexander Kros as a visiting scientist. In 2018, he obtained his PhD degree with the thesis entitled: "The Zebrafish: A Preclinical Screening Model for the Optimization of Nanomedicine Formulations".



## Dmitri Simberg

Dr. Simberg received his Ph.D. in Biochemistry from the Hebrew University of Jerusalem, Israel. His thesis in the laboratory of Professor Chezy Barenholz was on biochemical and biophysical mechanisms of lipofection using cationic lipids *in vitro* and *in vivo*. After receiving the PhD he did a 2-year postdoctoral study on amplified tumor targeting of iron oxide nanoparticles in the laboratory of Prof. Erkki Ruoslahti at the Burnham Institute, La Jolla. This was followed by a 1-year postdoctoral research at the Department of Radiology, University of California San Diego. Dr. Simberg was a project scientist at the Center for Cancer Nanotechnology Excellence at UCSD, where he developed his research program in nano-bio interface and immune recognition of nanomaterials. In 2013, Dr. Simberg joined the faculty of the Skaggs School of Pharmacy, University of Colorado. Dr. Simberg is the corresponding author or coauthor of over 50 research papers, reviews, perspectives and book chapters, and a recipient of funding totaling over \$6M. His current research interests are focused on the development of iron oxide nanoparticles and red blood cells for drug delivery and imaging, on mechanisms of complement activation by nanomedicines, and isolation of cancer biomarkers from blood using nanotechnology.



## Tore Skotland

Tore Skotland is a biochemist by training and received his PhD from the University of Bergen, Norway in 1980. After 11 years at the university studying protein chemistry and enzymology, he moved to pharmaceutical R&D (Nycomed AS, Oslo, Norway) in 1983. He stayed within the same field of research for 26 years in one of the world

leading companies developing contrast agents for medical imaging; Nycomed was bought by Amersham in 1997 and Amersham was bought by GE Healthcare in 2003. During the last 20 years in pharmaceutical R&D he was heading work to describe the biodistribution, metabolism and excretion of all types of contrast agents (water soluble as well as particle based) for CT, MRI, ultrasound, SPECT, PET and optical imaging. He has been involved in bringing 5 products to the market (including 2 particle-based) and another 5 products into clinical trials (also including 2 particle-based). Skotland is the first or last author of publications related to all these 10 products. He is co-author of approx.115 publications and is used as referee for many journals in the field of bioanalysis, metabolism, biochemistry, nanomedicine and contrast agents for medical imaging. Skotland is since 2009 a senior researcher at the Institute for Cancer Research at The Norwegian Radium Hospital, the main cancer hospital in Norway, being part of Oslo University Hospital. He is there a member of a group studying exosomes, endocytosis and intracellular transport of protein toxins and nanoparticles. This group is heading a 5-year national competence building project in Norway going to the end of 2018. The project title is "Biodegradable nanoparticles for cancer diagnosis and therapy". Skotland is co-ordinating the in vivo studies in this project, which has members from academia, university hospitals, research institutes and pharmaceutical industry. The 10 groups involved have expertise in nanoparticle syntheses and characterization, in vitro studies of cellular uptake and intracellular transport, immunology studies, and studies using small animals with xenograft models, including use of different in vivo imaging modalities such as MRI, PET/CT and fluorescence. Clinicians are also involved. Our group is also partner in an INNO INDIGO granted project which started summer 2016. INNO INDIGO is an innovation-driven initiative for the development and integration of Indian and European research.

### Most important publications in the field of nanoparticle research:

- Skotland T, Iversen TG, Sandvig K: New metal-based nanoparticles for intravenous use: requirements for clinical success with focus on medical imaging. *Nanomedicine: NBM* 6 (2010) 730-737.
- Iversen TG, Skotland T, Sandvig K: Endocytosis and intracellular transport of nanoparticles: Present knowledge and need for future studies. *Nano Today* 6 (2011) 176-185.
- Skotland T, Iversen TG, Sandvig K: Development of nanoparticles for clinical use. *Nanomedicine (Future Medicine)* 9 (2014) 1295-1299.
- Skotland T: Injection of nanoparticles into cloven-hoof animals: Asking for trouble. *Theranostics* 7 (2017) 4877-4878.



## Scott Steele

Director, Regulatory Science Programs, Associate Professor, Public Health Sciences University of Rochester, NY (USA)

Scott Steele, PhD, serves as the Director of Regulatory Science Programs, in the Clinical and Translational Science Institute at the University of Rochester Medi-

cal Center (NY). He is actively involved in developing and leading regulatory science educational programs and mentoring trainees, serving as Program Director for a Certificate in Regulatory Science and Core Director of the Regulatory Science to Advance Precision Medicine initiative. Dr. Steele also coordinates U.S. NIH Clinical and Translational Science Award (CTSA) affiliated initiatives, including the Regulatory Science to Advance Precision Medicine Forum and the development of Regulatory Science competencies to guide training and education. The goal of the Forum is to bring together federal agencies, industry, foundations and academic institutions to help identify and address some of the key topics and opportunities for regulatory science to advance precision medicine. Topics have focused on the integration of omics approaches (genomics, proteomics and metabolomics), 3D printing of medical products and digital health. Dr. Steele serves as a member of the U.S. FDA Science Board and as a Senior Editor for the *Journal of Clinical and Translational Science*. He is an associate professor in the Department of Public Health Sciences, where his academic interests are focused on a range of science and technology policy issues including translational research and regulatory science, precision medicine, public health preparedness, and national security. Prior to joining the University of Rochester, Dr. Steele served in the U.S. White House Office of Science and Technology Policy (OSTP), initially as a policy analyst and later as the Executive Director of the President's Council of Advisors on Science and Technology (PCAST). Dr. Steele coordinated PCAST studies addressing issues in personalized medicine, nanotechnology, information technology, energy technologies, and approaches to enhance university-private sector research partnerships. At OSTP, he also led several programs related to medical countermeasures development, biotechnology, and science education. Dr. Steele received his BS with Honors in Biology from Union College in Schenectady, NY. Following this, he performed research at the General Electric Center for Research and Development (NY), was a fellow at the National Institutes of Health (Bethesda, MD) and performed research at the University of Geneva (Switzerland). Dr. Steele completed his MA and PhD in Molecular Biology at Princeton University.

### RECENT PUBLICATIONS

- Bahinski A, Freire MC, McLellan MR, Psaty BM, Roden DM, Steele SJ. 2016. "Scientific Engagement at FDA." FDA Science Board Subcommittee report.
- Adamo JE, Wilhelm EE, Steele SJ. 2015. "Advancing a Vision for Regulatory Science Training." *Clin Transl Sci.* Oct;8(5):615-8. doi: 10.1111/cts.12298. Epub 2015 Jun 17 PMID: 26083660.



## Nicole Steinmetz

Dr. Steinmetz is a Professor of NanoEngineering at the University of California, San Diego (07/2018-present). She started her independent career at Case Western Reserve University School of Medicine in the Department of Biomedical Engineering (in 2010), where she was promoted through the ranks of Assistant, Associate, Full

Professor. In 2017 she was named the George J. Picha Designated Professor in Biomaterials and Director for the Center for Bio-Nanotechnology. Dr. Steinmetz trained at The Scripps Research Institute, La Jolla, CA where she was a NIH K99/R00 awardee and AHA postdoctoral fellow (2007-2010); she obtained her PhD in Bionanotechnology from the University of East Anglia where she prepared her dissertation as a Marie Curie Early Stage Training Fellow at the John Innes Centre, Norwich, UK (2004-2007). Her early training was at the RWTH-Aachen University in Germany. Dr. Steinmetz is a standing member of the NIH Nanotechnology study section. She serves on the Editorial Board of *Wiley Interdisciplinary Reviews (WIREs)* on Nanomedicine and Nanobiotechnology; she serves on the Advisory Editorial Board for the ACS journal *Molecular Pharmaceutics* and



RSC Journal of Materials Chemistry B. Dr. Steinmetz has chaired various symposia; and she served as Chair of the Gordon Conference of Physical Virology (2015). Dr. Steinmetz is a Fellow of the American Institute of Medical and Biological Engineering (AIMBE, since 2017); she has won many awards, including an American Cancer Society (ACS) Research Scholar Award (2016), and a Young Innovator of Cellular and Molecular Bioengineering Award from the Biomedical Engineering Society (2015). Dr. Steinmetz has authored more than 120 peer-reviewed journal articles as well as numerous reviews, book chapters, and patents; she has authored and edited books on Virus-based nanotechnology. Research in the Steinmetz Lab is and has been funded through grants from federal agencies, including National Institute of Health, National Science Foundation (e.g. NSF CAREER award), as well as private foundations, including Susan G. Komen Foundation, American Cancer Society, and American Heart Association. Over the past 8 years, Dr. Steinmetz has been awarded grants as PI and Co-PI totaling \$18+ million in total costs.



## Francesco Stellacci

Prof. Francesco Stellacci got his degree in Materials Engineering at the Politecnico di Milano in 1998 with Prof. Zerbi. He then moved as a post-doc with Prof. J.W. Perry in the Department of Chemistry at the University of Arizona. In 2002 he became as assistant professor in the Department of Materials Science and Engineering at MIT (Cambridge, USA). There he became associate professor with tenure in 2009. In 2010, he moved as a full professor to EPFL where he holds the Constellium chair. Stellacci has published more than 130 papers and has more than 15 patent applications. He has won numerous awards, among the the Technology Review TR35 'top innovator under 35', the Popular Science Magazine 'Brilliant 10', and the EMRS EU40. He is a Fellow of the Royal Society of Chemistry, of the Global Young Academy, and of the European Academy of Sciences.



## Gert Storm

Full professor  
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Gert Storm, professor Targeted Drug Delivery at the Department of Pharmaceutics (80% employment), obtained his Ph.D. degree in 1987 at the Utrecht University. In 1988-1989, he was a visiting scientist at Liposome Technology Inc. in Menlo Park, USA, and visiting assistant professor at the School of Pharmacy, UCSF, San Francisco. In 1990-1991, he became senior research scientist at Pharma Bio-Research Consultancy B.V. in Zuidlaren, The Netherlands. During this period he contributed to the design, co-ordination and evaluation of clinical pharmacological studies. In September 1991 he took up his position at the Utrecht University. He is honorary professor (Biomacromolecular Drug Delivery) at the University of Copenhagen. From 2012 on, he is also professor (Targeted Therapeutics) at the MIRA institute of the University of Twente (20% employment). Furthermore, he keeps a position (seconded) at the University Medical Center Utrecht (UMCU) (Division Imaging).

## RESEARCH

The design, characterization and (pre)clinical testing of targeted nanomedicine formulations is the core activity. Additionally, the implementation of imaging-guided drug delivery protocols (using

e.g. SPECT, MRI, PET) is a major research objective. Over the last decade, clinical translation of academic results has become a vital element of Prof. Storm's ambition, as exemplified by the clinical development of liposomal corticosteroids (by the creation of the spin-off company Enceladus Pharmaceuticals BV in 2005), the clinical breast cancer study at the UMCU evaluating image-guided targeted doxorubicin delivery with hyperthermia (follow-up of the HIFU-CHEM project), his involvement in public-private partnership projects, i.e. EC (coordinator Meditrans (FP6), partnering in 4 FP7 projects and 2 Horizon2020 projects), CTMM (PI HIFU-CHEM), NanoNextNL (director Program Drug Delivery), TI-Pharma and AgentschapNL. Over the years, he also obtained significant funding for applied research from STW and several large industries (e.g. Astellas, Astra Zeneca, Novartis). He is on the Board of the CLINAM (European Foundation for Clinical Nanomedicine) organization in Basel, and the PRC (Phospholipid Research Center) in Heidelberg. From 2014 on, he is every year included in the lists of The World's Most Influential Scientific Minds of Thomson Reuters (Highly Cited Researchers) and Clarivate Analytics.



## Gleb B. Sukhorukov

Chair in Biopolymers and Bio-organic Interfaces  
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My research activities lie in Biomedical Science area covering disciplines of Biomaterials, Biophysics and Physical Chemistry. It comprises physics and (bio)-chemistry on submicron dimensions, design of multifunctional colloidal particles and capsules and nano-engineered biomaterials, elaboration of micron and submicron sized delivery systems with remote controlling and triggering properties including by light, magnetic field and ultrasound.

## HONOURS AND AWARDS

- Sofja Kovalevskaja Award of Alexander von Humboldt Foundation and German Ministry of Education and Research 2001-2005
- Oct'2011 Listed by Forbes in Top-10 world leading scientists of Russian origin

## ACADEMIC QUALIFICATIONS

1991 Master of Sciences Degree in Physics, Specialty Biophysics, Lomonosov Moscow State University, Moscow Russia  
1994 PhD degree at Division of Biophysics, Departments of Physics, Lomonosov Moscow State University. Ph.D. thesis "Formation and investigation of multilayer films containing nucleic acids"

More than 50 invited talks including ACS Meetings (2001, 2005, 2003, 2008, 2010, 2013, 2016), Controlled Release Society (2009), Biomaterials Congress (2012, 2016) and other international conferences. Publication in Scientific journals: More than 300 publications, overall citations >23 000, H-index 83.

## SELECTED RECENT PUBLICATIONS:

- Voronin, DV, Sindeeva, OA, Kurochkin, MA, Mayorova, O, Fedosov, IV, Semyachkina-Glushkovskaya, OV, Gorin, DA, Tuchin, VV and Sukhorukov, GB (2017): In Vitro and in Vivo Visualization and Trapping of Fluorescent Magnetic Microcapsules in a Bloodstream. ACS Appl. Mater. Interfaces, 2017, 9 (8), pp 6885–6893
- Gai M, Frueh J, Tao T, Petrov AV, Petrov VV, Shesterikov EV, Tverdokhlebov SI and Sukhorukov GB. (2017): Polylactic acid nano- and microchamber arrays for encapsulation of small hydrophilic molecules featuring drug release via high intensity focused ultrasound. Nanoscale vol. 9, (21) 7063-7070.

- Gao, H., Sapelkin, AV, Titirici, MM, and Sukhorukov GB (2016): In Situ Synthesis of Fluorescent Carbon Dots/Polyelectrolyte Nanocomposite Microcapsules with Reduced Permeability and Ultrasound Sensitivity. *ACS Nano*, 2016, 10 (10), pp 9608–9615
- Luo, D., Gould, D.J., Sukhorukov, G.B. (2016): Local and Sustained Activity of Doxycycline Delivered with Layer-by-Layer Microcapsules. *Biomacromolecules* 17 (4), 1466–76
- Yi, Q, Sukhorukov, GB (2013): “Externally Triggered Dual Function of Complex Microcapsules”, *ACS Nano*. 7 (10), 8693–8705



## Hulda Swai Shaidi

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Extraordinary Professor; University of Pretoria

President; African Materials Research Society(AMRS)

Prof. Hulda Swai, a Nanotechnology Scientist, holds a PhD in Biomaterials, from Queen Mary’s College, University of London, UK where she also worked as a Researcher for 9 years. In May 2018, Prof. Swai was appointed the President of the African Materials Research Society (AMRS) and in 2013 she was appointed as an Extra ordinary Professor in University of Pretoria. In 2015, Prof. Swai joined NM-AIST and managed to acquire funds from the World Bank for establishment of a newly formed “African Centre for Research, agricultural Advancement, Teaching Excellence and Sustainability” (CREATES) in Food and Nutritional Security in Africa which was established at the NM-AIST through the World Bank’s African Centers of Excellence (ACE II) initiative.

The ACE II initiative aims at building training and research capacity in the region by training and raising a critical mass of specialized and skilled human capital that can use a multidisciplinary approach to ensure a sustainable environment, food and nutritional security. Prof. Swai is the CREATES program Director at NM-AIST. The program aims to strengthening content, delivery, outputs and outreach for the MSc and PhD programs in Life Sciences and other related programs, with particular focus on core thematic areas of Sustainable Agriculture, Biodiversity Conservation and Ecosystem Management; Food and Nutrition Sciences; and Health and Biomedical Sciences. These are also Departmental and CREATES pillars in the School of Life Science and Bioengineering (LiSBE) at NM-AIST. The CREATES program is geared to contribute to food and nutrition security in East Africa and Sub Saharan regions. This is a 5 program launched in the year 2016 and it is worth 6 million USD.

Prior to joining NM-AIST, Prof. Swai has worked as a Senior Principal Researcher at the Council for Scientific and Industrial Research (CSIR), in South Africa, where she led the Encapsulation and Delivery Research Group for 11 years. In this capacity, she proven her ability to independently formulate and execute research projects and implement scientific research programmes, both in public and private sectors.

Prof. Swai also instituted and headed the Department of Science and Technology-(DST)/CSIR Pan-African Centre of Excellence in Applied Nanomedicine Research and Training. In this capacity, she won a grant in Research and Training on infectious diseases of poverty worth R 60 million (about US \$ 7 million)



## Janos Szebeni

Janos Szebeni, M.D., Ph.D., D.Sc., Med. Habil., immunologist, director of the Nanomedicine Research and Education Center at Semmelweis University, Hungary. He is also founder and CEO of a contract research SME “SeroScience”, and full professor of (immune) biology at Miskolc University. He has held various guest professor

and scientific positions in Hungary and abroad, mostly in the USA where he lived for 22 years. His research on various themes in hematology, membrane biology and immunology resulted ≈150 publications including peer-reviewed papers, book chapters, patents, etc. (citations: ≈6000, H index: 39 i10 index: 73), and a book entitled “The Complement System: Novel Roles in Health and Disease” (Kluwer, 2004). Three fields stand out where he has been most active: artificial blood, liposomes and the complement system. His original works led to the “CARPA” concept, i.e., that complement activation underlies numerous drug-induced (pseudo)allergic (infusion) reactions.



## Yuki Takechi-Haraya

Yuki Takechi-Haraya is a research scientist in Division of Drugs at National Institute of Health Sciences in Japan since 2016. His current interest is in analytical sciences, especially on evaluation of nanomedicines. Prior to his current post, he was in Japan Agency Medical Research and Development from 2015 to 2016 as a post-doctoral fellow, and worked on a research project to establish an atomic force microscopy method for characterization of nanosized liposomes as drug carriers. During his career as an assistant professor at Himeji Dokkyo University from 2012 to 2014, he studied the mechanism for biological membrane penetration of arginine-rich peptides. Through this academic study, he received the Membrane Paper Award in 2016, and Young Scientist Award in 2017 from The Membrane Society of Japan. He holds a Ph.D. in pharmaceutical sciences from the University of Tokushima.

### RECENT PUBLICATIONS

- Takechi-Haraya, Y.; Goda, Y.; Sakai-Kato, K. Imaging and size measurement of nanoparticles in aqueous medium. *Anal. Bioanal. Chem.* 2018, 410, 1525–1531.
- Takechi-Haraya, Y.; Goda, Y.; Sakai-Kato, K. Control of liposomal penetration into three-dimensional multicellular tumor spheroids by modulating liposomal membrane rigidity. *Mol. Pharm.* 2017, 14, 2158–2165.
- Takechi-Haraya, Y.; Sakai-Kato, K.; Goda, Y. Membrane rigidity determined by atomic force microscopy is a parameter of the permeability of liposomal membranes to the hydrophilic compound calcein. *AAPS PharmSciTech* 2017, 18, 1887–1893.
- Takechi-Haraya, Y.; Sakai-Kato, K.; Abe, Y.; Kawanishi, T.; Okuda, H.; Goda, Y. Atomic force microscopic analysis of the effect of lipid composition on liposome membrane rigidity. *Langmuir* 2016, 32, 6074–6082.
- Takechi-Haraya, Y.; Sakai-Kato, K.; Abe, Y.; Kawanishi, T.; Okuda, H.; Goda, Y. Observation of liposomes of differing lipid composition in aqueous medium by means of atomic force microscopy. *Microscopy* 2016, 65, 383–389.



## Yeshayahu Talmon

Prof. Emeritus of Chemical Engineering

Prof. Yeshayahu ("Ishi") Talmon holds a B.Sc. (summa cum laude) and M.Sc. from the Department of Chemical Engineering, Technion-Israel Institute of Technology, Haifa, and a Ph.D. from the Department of Chemical Engineering and Materials Science, The University of Minnesota, Minneapolis. He has been on the faculty of the Technion Department of Chemical Engineering since 1979, as Professor since 1992. Ishi served as the Dean of the Department of Chemical Engineering (2000 to 2005), and as the Director of the Technion Russell Berry Nanotechnology Institute (RBNI) in the years 2010-12. He held the Wolfson Chair of Chemical Engineering from 2002 to 2015. Since October 2015 he is a very active Emeritus Professor. Ishi has supervised 59 graduate students and postdocs, and has published over 300 papers and book chapters, which have been cited over 20,000 times at the end of May 2018 (H=71).

Since 2013 Ishi is a member of the Israel national "Committee for Planning and Budgeting" ("VATAT") of higher education; Member of the Board, United States-Israel Educational Foundation, since 2013; Member of the Council and Member of the Academic Board, Israel Science Foundation; Member of the Board, The Technion Research and Development Foundation (TRDF) 2008-13, and Member of the Board, The Samuel Neaman Institute 2008-14. He is a past Chairman of the Israel Microscopy Society; past member of the board, Israel Institute of Chemical Engineers; past member of the board, FIRST (Bikura) program of the Israel Science Foundation.

Prof. Talmon received The Ernst Ruska Award of the German Society for Electron Microscopy (1993), and held The George T. Piercy Distinguished Visiting Professor Chair, Department of Chemical Engineering and Materials Science, University of Minnesota (1997 and 2005). He received the The Meitner-von Humboldt Research Award in 2003, The Henry Taub Prize for Excellence in Research in 2005. He was made Honorary Fellow of The Israel Institute of Chemical Engineers (2011), Honorary Fellow of the Israel Microscopy Society (2016), and Doctor of Science honoris causa of The University of Lund, Sweden (2015).

Prof. Talmon received The Ernst Ruska Award of the German Society for Electron Microscopy (1993), and held The George T. Piercy Distinguished Visiting Professor Chair, Department of Chemical Engineering and Materials Science, University of Minnesota (1997 and 2005). He received the The Meitner-von Humboldt Research Award in 2003, The Henry Taub Prize for Excellence in Research in 2005. He was made Honorary Fellow of The Israel Institute of Chemical Engineers (2011), Honorary Fellow of the Israel Microscopy Society (2016), and Doctor of Science honoris causa of The University of Lund, Sweden (2015).

### RECENT PUBLICATION

- Ram-On, M., Cohen, Y., and Talmon, Y., Effect of Polyelectrolyte Stiffness and Solution pH on the Nanostructure of Complexes Formed by Cationic Amphiphiles and Negatively Charged Polyelectrolytes, *J. Phys. Chem. B* 120, 5907–5915 (2016).
- Shaharabani, R., Ram-On, M., Avinery, R., Aharon, R., Arnon, R., Talmon, Y., and Beck, R., Structural Transition in Myelin Membrane as Initiator of Multiple Sclerosis, *J. Am. Chem. Soc.* 138, 12159–12165 (2016).
- Koifman, N., Biran, I., Aharon, A., Brenner, B., and Talmon, Y., A Direct-Imaging Cryo-EM Study of Shedding Extracellular Vesicles from Leukemic Monocytes, *J. Structural Biol.* 198, 177-185 (2017)
- Kleinerman, O., Adnan, M. Marincel, D.H., Ma, A.W.K. Bengio, A., Park, C., Chu, S.-H., Pasquali, M., and Talmon, Y., Characterization of Boron Nitride Nanotubes Dissolved in Superacid, *Langmuir* 33, 14340–14346 (2017).
- Aharon, A., Rebibo-Sabbah, A., Issman, L., Berkovich, H., Stern, H., Abboud, Y., Talmon, Y., and Brenner, B., Effects of Low- and High-Dose Chemotherapy on Tumorigenic, Thrombogenic and Angiogenic Properties of Extracellular Vesicles Derived from Breast Cancer Cell Lines, *Journal of Thrombosis and Haemostasis* 18, 480-489 (2018).



## James Taylor

CEO & Co-Founder. Precision Nano-Systems

Dr. Taylor is a co-founder of PNI and has lead PNI's technology and commercialization team since the technology's invention. Dr. Taylor has a B.A.Sc. in engineering physics from UBC and a Ph.D. in genetics

from the Institute for Systems Biology. James worked at the venture capital firm, Accelerator Corporation, and has nearly a decade of experience in microfluidics, nanotechnology and systems biology. Dr. Taylor is a member of the Board of Directors of Life Science British Columbia.

### RECENT PUBLICATION

- N. Belliveau, J. Huft, P. Lin, S. Chen, A. Leung, T. Leaver, A. Wild, J. Lee, R. J. Taylor, Y. Tam, C. Hansen and P. Cullis. Microfluidic Synthesis of Highly Potent Limit-size Lipid Nanoparticles for In Vivo Delivery of siRNA. *Molecular Therapy-Nucleic Acids*, 2012. 1, e37
- D. Falconnet, A. Niemistö, R. J. Taylor, M. Ricicova, T. Galitski, I. Shmulevich and C. L. Hansen. High-throughput tracking of single yeast cells in a microfluidic imaging matrix. *Lab Chip*, 2011. 11, 466-473
- D Falconnet, M Ricicova, A Quiring, A Niemistö, R.J. Taylor, T Galitski, I Shmulevich and CL Hansen. Microfluidic High-Throughput Imaging and Automated Lineage Tracking of Yeast. *Microscopy and Microanalysis*, 16 (Suppl. 2). 2010. 1044-1045
- R.J. Taylor, D. Falconnet, A. Niemisto, S. Ramsey, S. Prinz, I. Shmulevich, T. Galitski, and C. Hansen. Dynamic analysis of MAPK signaling using a high-throughput single-cell imaging platform. *PNAS*. 2009. 106:3758-3763
- R.J. Taylor, A.F. Siegel, and T. Galtiski. Network motif analysis of a multi-mode genetic network. *Genome Biology*. 2007. 8:R160



## Tambet Teesalu

Head of Laboratory fo Cancer Biology, University of Tartu Ülikool

Prof. Tambet Teesalu works on affinity targeting of tumors with homing peptides and peptidomimetic compounds. Since 2012, he heads the Laboratory of Cancer Biology at the University of Tartu. The laboratory

uses phage display screens to identify homing peptides that bind to specific targets in the tumor vasculature. Corresponding synthetic peptides are developed for targeting drugs, biologicals, and nanoparticles into tumors to increase their therapeutic index.

Dr. Teesalu received his PhD in 1999 for studies on the extracellular proteases in cell invasion and tissue remodeling (supervisors: Profs. Francesco Blasi and Antti Vaheri). For his postdoctoral training (2005-2011), Dr. Teesalu joined the laboratory of Prof. Erkki Ruoslahti at Sanford Burnham Prebys Medical Discovery Institute in the USA to work on development of tumor homing peptides and characterization of vascular ZIP codes in vascular trees of normal organs.

Awards and recognitions to Dr. Teesalu include S. Komen for Cure Career development award (2010), ERC starting grant (2010), Wellcome Trust senior international fellowship (2010), EMBO installation grant (2010), and Estonian National Prize in Medicine (2017). He holds a visiting professorship at the Center of Nanomedicine of University of California Santa Barbara (USA) and visiting associate professorship at Sanford Burnham Prebys Medical Discovery Institute, La Jolla (USA). On non-academic side, he has founded 3



biotech companies including DrugCendR inc. (La Jolla, USA) that develops tumor penetrating peptides for solid tumor targeting.

## RECENT PUBLICATIONS

- Precision Targeting of Tumor Macrophages with a CD206 Binding Peptide. Scodeller P, Simón-Gracia L, Kopanchuk S, Tobi A, Kilik K, Säälik P, Kurm K, Squadrito ML, Kotamraju VR, Rinken A, De Palma M, Ruoslahti E, Teesalu T. *Sci Rep.* 2017 Nov 7;7(1):14655.
- Ratiometric *in vivo* auditioning of targeted silver nanoparticles. Toome K, Willmore AA, Paiste P, Tobi A, Sugahara KN, Kirsimäe K, Ruoslahti E, Braun GB, Teesalu T. *Nanoscale.* 2017 Jul 20;9(28):10094-10100.
- Targeting of p32 in peritoneal carcinomatosis with intraperitoneal linTT1 peptide-guided pro-apoptotic nanoparticles. Hunt H, Simón-Gracia L, Tobi A, Kotamraju VR, Sharma S, Nigul M, Sugahara KN, Ruoslahti E, Teesalu T. *J Control Release.* 2017 Aug 28;260:142-153.
- iRGD peptide conjugation potentiates intraperitoneal tumor delivery of paclitaxel with polymersomes. Simón-Gracia L, Hunt H, Scodeller P, Gaitzsch J, Kotamraju VR, Sugahara KN, Tammik O, Ruoslahti E, Battaglia G, Teesalu T. *Biomaterials.* 2016 Oct;104:247-57. doi: 10.1016/j.biomaterials.2016.07.023.
- Etchable plasmonic nanoparticle probes to image and quantify cellular internalization. Braun GB, Friman T, Pang HB, Pallaoro A, Hurtado de Mendoza T, Willmore AM, Kotamraju VR, Mann AP, She ZG, Sugahara KN, Reich NO, Teesalu T, Ruoslahti E. *Nat Mater.* 2014 Sep;13(9):904-11.



## Donald A. Tomalia

CEO/Founder  
NanoSynthons LLC  
National Dendrimer & Nanotechnology Center  
1200 N. Fancher Avenue  
Mt. Pleasant, MI 48858 USA

Dr. Tomalia is the CEO/Founder of NanoSynthons LLC and the National Dendrimer & Nanotechnology Center, Distinguished Visiting Professor (Chemistry Department) Columbia University, NY; Adjunct Professor (Department of Chemistry) University of Pennsylvania, PA and Affiliate Professor (Department of Physics) Virginia Commonwealth University, VA. He received his B.A. in Chemistry from the University of Michigan and Ph.D. in Physical-Organic Chemistry from Michigan State University while working at The Dow Chemical Company. He has founded three dendrimer-based nanotechnology companies; namely, NanoSynthons LLC (2010-present), Dendritic Nanotechnologies, Inc. (2001) (acquired by Starpharma, Melbourne AU) and Dendritech, Inc. (1992) (acquired by Dow Chemical, Midland MI). Other positions currently held by Tomalia include: Advisory Board CLINAM, European Foundation for Clinical Nanomedicine; Faculty Member, Faculty 1000 Biology; Associate Editor, *Journal of Nanoparticle Research* (Nature/Springer); Editorial Advisory Board, *Nanomedicine* (Elsevier) and *Biomolecules* (MPDI).

Tomalia is the pioneering scientist/inventor credited with the discovery of living cationic polymerizations leading to poly(oxazolines) (Industrial Research-100 Awards in 1978 & 1986) and the first synthesis of dendrimers. His 1979 discovery of dendrimers (dendritic polymer architecture) led to a third R&D-100 Award in 1991 and the Leonardo da Vinci Award (Paris, France) in 1996. He received the International Award of The Society of Polymer Science Japan (SPSJ) (2003) which recognized his discovery of the fourth major macromolecular architectural class; namely, dendritic polymers. He was the invited "Linus Pauling Memorial Lecturer", Portland, OR (2010), recipient of the Wallace H. Carothers Award (American Chemical Society) (2012) and elected AAAS Fellow (American Association for the Advancement of Science) (2016).

Tomalia has been granted >135 U.S. patents, authored over 270 peer-reviewed publications with more than >42,467 citations and an h-index=92 (Google Scholar, 6-28-18). Over 170 papers are focused in the dendrimer/dendritic polymer field including two monographs entitled: *Dendrimers and Other Dendritic Polymers* (J. Wiley) co-edited with J.M.J. Fréchet (2001) (>1951 citations) and more recently *Dendrons, Dendrimers, Dendritic Polymers* (Cambridge University Press (2012)). His original dendrimer paper entitled: "A New Class of Polymers: Starburst Dendritic Macromolecules", *Polym. J.*, (1985), 17(1), 117 has received >3974 citations, whereas, his review article entitled: "Starburst Dendrimers: Molecular Level Control of Size, Shape, Surface Chemistry, Topology and Flexibility from Atoms to Macroscopic Matter," D.A. Tomalia, A.M. Naylor W.A. Goddard III, *Angew. Chem. Int. Ed. Engl.*, 29(2), 138 (1990) has > 3,718 citations. Tomalia was inducted into the Thomson Reuters Hall of Citation Laureates in Chemistry (2011) (i.e., top 40 most highly cited scientists in the field of chemistry).



## Katherine Tyner

Acting Associate Director of Science,  
Office of Pharmaceutical Quality, CDER/  
FDA

Dr. Katherine Tyner is the Associate Director of Science (acting) in the immediate office of the Office of Pharmaceutical Quality (OPQ), Center for Drug Evaluation and Research at the United States Food and Drug Administration (FDA). As Associate Director, Dr. Tyner leads the OPQ Science Staff in coordinating the intersection between science, review, and policy in OPQ as well as facilitating interactions between other CDER offices and FDA Centers. She received her PhD in Chemistry from Cornell University and joined the Food and Drug Administration in 2007 as a chemist specializing in nanotechnology. While at the FDA, Dr. Tyner has investigated the quality, safety, and efficacy of drug products containing nanomaterials, and she currently leads the CDER nanotechnology working group and is active in other CDER and FDA nanotechnology initiatives. Dr. Tyner is the author of multiple book chapters and journal articles concerning the appropriate characterization and biological impact of nanoparticle therapeutics.

## RECENT PUBLICATIONS

- Khan S....Tyner KM\*. Investigating the susceptibility of mice to a bacterial challenge after intravenous exposure to durable nanoparticles. *Nanomedicine* 12(17) 2017.
- Zou P, Tyner KM, Raw A, Lee SL. Physicochemical characterization of iron carbohydrate colloid drug products. *AAPS J.* 19(5) 2017.
- D'Mello S...Tyner KM\*. FDA/CDER analysis of drug products containing nanomaterials, *Nature Nanotechnology* 12(6) 523-529 (2017).
- Tyner KM... Cruz CN. How Has CDER Prepared for the Nano Revolution? A Review of Risk Assessment, Regulatory Research, and Guidance Activities. *AAPS J.* 19(4) 1071-1083 (2017).
- Kapoor M, Lee SL, Tyner KM\*. Regulatory considerations for liposomal drug product development from a chemistry, manufacturing, and control perspective. *AAPS J.* 19(3) 632-641 (2017).



## Steliyan Tinkov

Steliyan Tinkov has graduated pharmacy in Sofia and finished his PhD education in pharmaceutical technology at the LMU-Munich. Later on, Steliyan studied MBA at the WHU-Otto Beisheim in Dusseldorf. He also holds several renowned qualifications in operations excellence and project management. Steliyan has more than thirteen

years of experience with the development and manufacturing of nanomedicines, of which eight in the pharmaceutical industry at leading international companies. Currently, his professional experience evolved to the implementation of digital technologies in the development and manufacturing operations.



## Mehmet Hikmet Ucisik

Academia

Mehmet H. Ucisik studied chemical engineering at Boğaziçi University, Istanbul, Turkey. Following his MSc study in Biotechnology at TU Delft, the Netherlands, he received a doctoral degree based on thesis work in nanobiotechnology from the

University of Natural Resources and Life Sciences, Vienna, Austria. His main research interests include drug delivery systems and drug targeting. As a faculty member of Istanbul Medipol University, currently he pursues collaborative research on various projects on cancer, neurodegenerative and parasitic diseases with particular interest in leishmaniasis.

### RECENT PUBLICATIONS

- Ucisik, M. H., Küpcü, S.\*, Debreczeny, M., Schuster, B. and Sleytr, U. B.\* (2013) S layer Coated Emulsomes as Potential Nanocarriers. *Small*, 9: 2895-2904.
- Ucisik M.H.\*, Küpcü S., Schuster B., Sleytr U.B. (2013) Characterization of CurcuEmulsomes: Nanoformulation for Enhanced Solubility and Delivery of Curcumin, *Journal of Nanobiotechnology*, 11:37:1-13.
- Ucisik M.H.\*, Küpcü S., Breitwieser A., Gelbmann N., Schuster B., Sleytr U.B. (2015) S-layer Fusion Protein as a Tool functionalizing Emulsomes and CurcuEmulsomes for Antibody Binding and Targeting, *Colloids and Surfaces B: Biointerfaces*, 128, 132-139.
- Ucisik M.H.\*, Schuster B., Sleytr U.B. (2015) Emulsomes meet S-layer Proteins: An emerging Targeted Drug Delivery System, *Current Pharmaceutical Biotechnology*, 16:4, 392-405.
- Ruff L.E.\*, Uzri D., Fecteau J.-F., Ucisik M.H., Messmer B.T.\* (2016) DeNANO: a novel multivalent affinity reagent, in: "Rolling Circle Amplification (RCA): Toward New Clinical Diagnostics and Therapeutics, Edited by Dr. V. Demidov", Springer International Publishing, 2016. 161-168.



## Rudolf Urbanics

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Rudolf Urbanics, MD, PhD, Head of the *in vivo* laboratory of Nanomedicine Research and Education Center of Semmelweis University, Budapest, and SeroScience Ltd., an

immunotoxicity CRO, since 2008 in Budapest, Hungary. He obtained MD diploma and the PhD degree at Semmelweis Medi-

cal School, Budapest, Hungary. He had teaching and research activity at the parent university and held in between various research/collaboration positions at MaxPlanck Institute of Systemphysiology, Dortmund, Germany (prof D.W. Lübbers), at University of Pennsylvania, Philadelphia, USA, Cerebrovascular Research Center (head prof. M Reivich, Dr. J. H. Greenberg), at Pennsylvania Muscle Institute (prof. A.P. Somlyo), in the Knoll AG, Central Nervous System Research Department, Ludwigshafen, Germany, working in the field of CNS regulation of blood flow/metabolism, ischemic/hypoxic disorders, stroke and chronic neurodegenerative disease animal models.

R. Urbanics was invited lecturer of three SMI's conferences, London, delivering talks about ischemic-hypoxic as well as chronic neurodegenerative disease animal models.

He was the Deputy R&D Director and Head of CNS Pharmacology Department at Biorex R&D Co.(1997-2003), worked at IVAX/Drug Research Institute Budapest, as Scientific Adviser, Leading researcher in Safety and CNS Pharmacology and later in IVAX/Drug Research Institute, Subsidiary of TEVA as Head of In Vivo Pharmacology Group (2003-2008).

Currently, he is working with *in vivo* models of nano drug - nano carrier induced, complement activation related pseudoallergic reactions (CARPA), clarifying their immune-toxicological and safety hazards.



## Hans van der Voorn

Hans van der Voorn is the CEO of Izon Science Ltd, based in Christchurch, New Zealand. He originally trained as an engineer in New Zealand. Hans was one of the founders of Izon and became its fulltime CEO in 2007. He has been the inventor on several Izon patents and has a particular interest in developing high quality and reliable nano-

measurement and nano-separation capabilities for biomedical use. Izon's expertise is in Tunable Resistive Pulse Sensing (TRPS) and its applications to nanomedicine development and SEC column and TRPS for isolation and measurement in extracellular vesicle research and EV based therapeutics and clinical diagnostics development.



## Peter van Hoogevest

Head Scientific Department, Lipoid GmbH, Ludwigshafen am Rhein, Germany

Peter van Hoogevest, is a pharmacist by training (Utrecht University in The Netherlands), who got his PhD degree in biochemistry 1984 at the Utrecht University in The Netherlands. In 1994 he received the degree of Privat Dozent (adjunct professor) in pharmacy at the University of Basel, Switzerland.

His industrial career started at the Biovet Group of the Animal Health Division of Ciba-Geigy Ltd. (Basel) in 1984. Shortly thereafter he obtained a position at the Novel Dosage Form Department of Pharmaceutical Development of the Pharmaceuticals Division of Ciba-Geigy Ltd. After having several positions at this department at Ciba Ltd. and Novartis Ltd. he founded in 1998 together with colleagues of the Pharmaceutical Development Department and reputed industrial managers and scientists the company ADD Advanced Drug Delivery Technologies (Muttentz, CH) and became CEO of this company and was member of the Board of Directors. In 2000 he joined Phares Drug Delivery AG (Muttentz, CH), a company spe-

cialized in the delivery of poorly water soluble drug substances, as Managing Director and COO and member of the Board of Directors. Since 2012 he is Managing Director of the Phospholipid Research Center, Heidelberg and Head of the Scientific Department (including the Development Department) of Lipoid GmbH, Ludwigshafen am Rhein, Germany.

His drug delivery expertise especially in the (phospho)lipid research and development area is underscored by 60 scientific publications, including 8 book chapters, 33 symposium posters, co-promotion of 48 PhD Theses, 13 patents and 45 patent applications.

## RECENT PUBLICATIONS

- Gautschi, N., Van Hoogevest, P., Kuentz, M., Amorphous drug dispersions with mono- and diacyl lecithin: On molecular categorization of their feasibility and UV dissolution imaging. *Int. J. Pharm.* (2015), 491(1–2), 218–230.
- Fülöp, A., Sasmour, D.A., Erich, K., von Gerichten, J., van Hoogevest, P., Sandhoff, R., Hopf, C., Molecular imaging of brain localization of liposomes in mice using MALDI mass spectrometry. *Scientific reports*, (2016), 6, 33791.
- Gautschi, N., Van Hoogevest, P., Kuentz, M., Molecular insights into the formation of drug-monoacyl phosphatidylcholine solid dispersions for oral delivery. *Eur. J. Pharm. Sci.* (2017), 108, 93–100.
- Van Hoogevest, P., Review – An update on the use of oral phospholipid excipients *Eur J. Pharm. Sci.* (2017), 108, 1–12.s.
- Rahnfeld, L., Thamm, J., Steiniger, F., van Hoogevest, P., Luciani, P., Study on the in situ aggregation of liposomes with negatively charged phospholipids for use as injectable depot formulation, *Colloids and Surfaces B: Biointerfaces* (2018), Available online 16 February 2018; <https://doi.org/10.1016/j.colsurfb.2018.02.023>.

on the World Economic Forum Global Agenda Council in Nanotechnology (2014–2016), as well as for the Max-Planck Society, A\*STAR Singapore and the Wyss Institute, Boston.



## Yuri Volkov

Prof. Yuri Volkov received his MD from I.M. Sechenov Moscow State Medical University and subsequently a PhD in biomedical sciences at the Institute of Immunology, Moscow. He has been working at the Department of Clinical Medicine, Trinity College Dublin (TCD) since 1995 and he is currently Professor and Chair of Molecular and Translational Medicine at the School of Medicine, TCD and Principal Investigator at the Trinity Translational Medicine Institute.

His research interests include nanomedicine and biomedical applications of nanotechnologies, molecular mechanisms of immune system functioning in health and disease, cell adhesion and migration in inflammation and cancer, intracellular signalling and cytoskeletal dynamics, advanced cell and molecular imaging. Within the Advanced Materials for Bio-Engineering Research Centre (AMBER) at TCD his group is pursuing the applications of nanomaterials for advanced research and assessment of environmental, health and safety impact of emerging nanomaterials. Prof. Volkov has an extensive experience as Coordinator and Lead partner investigator on a number of large-scale international grants focused on the development of innovative clinical diagnostics and treatment approaches implementing nanotechnologies.



## Viola Vogel

Laboratory of Applied Mechanobiology, Institute of Translational Medicine, Department of Health Sciences and Technology, ETH Zurich, Switzerland

Professor Viola Vogel studied Physics and Biology in Frankfurt, at the Max-Planck Institute for Biophysical Chemistry in Göttingen and in Berkeley before starting her academic career in the Department of Bioengineering at the University of Washington (1991). In Seattle, she was the Founding Director of the Center for Nanotechnology at the University of Washington (1997–2003). She moved to Switzerland in 2004, where she initially joined the Department of Materials. She is now heading the Laboratory of Applied Mechanobiology in the Department of Health Sciences and Technology at the ETH Zurich, and is the Founding Director of the new ETH Institute of Translational Medicine (since 2017). She is the Chair of the Department of Health Sciences and Technology (D-HEST) since 2018.

Viola Vogel pioneered the rapidly growing field of mechanobiology and its medical applications as she discovered many structural mechanisms how mechanical forces can turn proteins into mechano-chemical switches. Such mechanisms are exploited by bacteria, as well as by mammalian cells and tissues to sense and respond to mechanical forces, and if abnormal, can cause various diseases. Her research was recognized by major awards, including the Otto-Hahn Medal of the Max-Planck Society 1988, the “First Award” from the NIH Institute of General Medicine (1993–98), the Julius Springer Prize 2006 for Applied Physics, the ERC Advanced Grant (2008–13), the International Solvay Chair in Chemistry Brussels 2012, and an Honorary Degree Doctor of Philosophy from Tampere University, Finland 2012. She also serves on various international advisory boards in the fields of nanotechnology and bioengineering, including on the White House panel that finalized the US National Nanotechnology Initiative under the Clinton administration (1999) and



## Natalia Vtyurina

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I have obtained my PhD degree in biophysics from Delft University of Technology, Bionanoscience Department in 2016. Prior to my PhD I was trained as a physicist and this helped me during my doctorate to develop in a short period of time number of biophysical assays for microscopy. I successfully applied these assays for studying dynamics of DNA-protein interactions at the single-molecule level. I believe that the results from my PhD project (published in PNAS) are an excellent example of how physics can be applied to study kinetic features of cellular processes in biology. This experience coupled with the skills I developed in microscopy enabled me to proceed further towards biomedical research in the field of nanomedicine: with the ultimate purpose of discovering how to maximize the delivery efficiency of nano-sized drug carriers, in April 2017 I started my postdoctoral project in the group of Dr. Anna Salvati at Groningen Research Institute of Pharmacy (GRIP).





## Andreas Wagner

Dr Andreas Wagner is currently the Head of Liposome Technology at Polymun Scientific GmbH in Klosterneuburg, Austria. He has significant expertise in development and optimization of liposomal drug products. Over the last 15 years, his group guided approx. 15 different liposomal drug products into clinical trials. He studied

Biotechnology in Vienna, Austria and earned his Master and Ph.D. degrees in the field of liposomology at the Institute of Applied Microbiology supervised by Prof. Hermann Katinger and Prof. Karola Vorauer-Uhl. Dr Andreas Wagner is listed as inventor on several patents, like the liposome technology and some product patents of liposomal formulations and he has published several peer reviewed articles dealing with liposomes, the technology, products thereof and their application in preclinical and clinical studies. Since 2001, he built up the liposome technology unit at Polymun Scientific GmbH.

Polymun Scientific is a private Austrian company, located in Klosterneuburg, offering contract development and manufacturing of biopharmaceuticals as well as development and production of liposomal formulations. Its patented liposome/LNP technology allows efficient manufacturing of constantly high quality in small and large scale. Over the last 10 years, Polymun has guided more than 15 liposomal formulations into clinical trials, amongst them DNA and different kinds of RNA formulations. Polymun is an FDA- and EMA-inspected manufacturer conducting several own R&D projects. For more information, please visit [www.polymun.com](http://www.polymun.com)



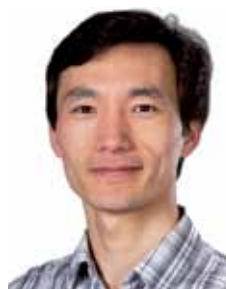
## Jing Wang

Ph.D. candidate

Jing Wang is a Ph.D. candidate at the University of Queensland. Her research interests include designing nanomaterials and developing nanotechnologies for disease diagnosis and monitoring. Her recent works mainly focus on using surface-enhanced Raman spectroscopy for liquid biopsy detection.

### RECENT PUBLICATION

- Simon Chang-Hao Tsao, ‡ Jing Wang, ‡ Yuling Wang, Andreas Behren, Jonathan Cebon, and Matt Trau. "Characterising the Phenotypic Evolution of Circulating Tumour Cells during Treatment." *Nature Communications*, DOI: 10.1038/s41467-018-03725-8.
- Jing Wang, Kevin M Koo, Eugene JH Wee, Yuling Wang, and Matt Trau. "A Nanoplasmonic Label-Free Surface-Enhanced Raman Scattering Strategy for Non-Invasive Cancer Genetic Subtyping in Patient Samples." *Nanoscale* 9, no. 10 (2017): 3496-503.
- Khondakar Kamil Reza, ‡ Jing Wang, ‡ Ramanathan Vaidyanathan, Shuvashis Dey, Yuling Wang, and Matt Trau. "Electrohydrodynamic-Induced Sers Immunoassay for Extensive Multiplexed Biomarker Sensing." *Small* 13, no. 9 (2017): 1602902.
- Junrong Li, ‡ Jing Wang, ‡ Yuling Wang, and Matt Trau. "Simple and Rapid Colorimetric Detection of Melanoma Circulating Tumor Cells Using Bifunctional Magnetic Nanoparticles." *Analyst* 142, no. 24 (2017): 4788-93.



## Jiong-Wei Wang

Principle Investigator

Dr Jiong-Wei Wang obtained his Ph.D. in Medicine from Leiden University Medical Centre, The Netherlands. He did his post-doctoral training in the Department of Cardiology, University Medical Centre Utrecht from 2012 to 2013 and in the Department

of Surgery at National University of Singapore from 2013 to 2016. Dr Wang is currently a faculty member in the Department of Surgery at Yong Loo Lin School of Medicine and Cardiovascular Research Institute at National University Heart Centre of Singapore. He also holds a joint appointment in the Department of Physiology at National University of Singapore. Dr Wang's research focuses on cardiac stress and remodeling, coagulation and inflammation in heart failure, and advanced drug delivery in cardiovascular disease. For his research, he has been awarded several times (e.g. Young Investigator Award from the International Society of Thrombosis and Haemostasis and the European Society of Cardiology).



## Julie Tzu-Wen Wang

Wang BSc, MSc, PhD

Senior Research and Teaching Fellow in Nanomedicine, School of Cancer and Pharmaceutical Sciences, King's College London, London SE1 9NH, UK  
E-mail: [tzu-wen.wang@kcl.ac.uk](mailto:tzu-wen.wang@kcl.ac.uk)

Dr Julie Wang obtained her BSc Degree in Biomedical Technology and MSc in Biomedical Engineering at the National Taiwan University in 2002 and 2004. She obtained her PhD in Photobiology in Cancer Therapeutics at the Department of Surgery and Interventional Science, University College London (Dr AJ MacRobert & Prof. SG Bown) in 2010. Her post-doctoral research moved to the field of nanomedicine after joining UCL School of Pharmacy in 2011 and then King's College London (KCL) (PI: Prof Al-Jamal) in 2012. She is currently a senior research and teaching fellow in Nanomedicine at the Institute of Pharmaceutical Science at KCL, and also a visiting scientist at the Centre for Molecular Oncology at Queen Mary University of London. Her research focus is on the pre-clinical translation of nanomedicines for image-guided delivery of drug/gene/radionuclide. She has utilised a range of nanoparticulate systems including dendrimers, polymeric nanoparticle, liposomes and carbon nanotubes (CNTs), mostly designed for cancer and brain delivery.

### RECENT PUBLICATION

- P M Costa, JT-W Wang, J-F Morfin, T Khanum, W To, JK Sosabowski, E Tóth, KT Al-Jamal. (2018) Functionalised carbon nanotubes enhance brain delivery of amyloid-targeting Pittsburgh Compound B (PiB)-derived ligands. *Nanotheranostics*. 2(2):168-183.
- NO Hodgins, JT-W Wang, KT Al-Jamal. (2017) Nano-technology based carriers for nitrogen-containing bisphosphonates delivery as sensitisers of  $\gamma\delta$  T cells for anticancer immunotherapy. *Advanced Drug Delivery Reviews*. 114:143-160
- HD Summers, P Rees, JT-W Wang, KT Al-Jamal (2017) Spatially-resolved profiling of carbon nanotube uptake across cell lines. *Nanoscale*. 9(20):6800-6807
- IFBM Suffian, JT-W Wang, NO Hodgins, R Klippstein, M Garcia-Maya, P Brown, Y Nishimura, H Heidari, S Bals, JK Sosabowski, C Ogino, A Kondo, KT Al-Jamal. (2016) Engineering hepatitis B Virus core particles for targeting HER2 receptors *in vitro* and *in vivo*. *Biomaterials*. 120:126-138
- HAFM Hassan, L Smyth, JT-W Wang, PM Costa, K Ratnasothy, SS

Diebold, G Lombardib, KT Al-Jamal. (2016) Dual stimulation of antigen presenting cells using carbon nanotube-based vaccine delivery system for cancer immunotherapy. *Biomaterials*. 104:310-322



## Frank F. Weichold

Dr. Weichold is the director for Critical Path and Regulatory Science Initiatives in the office of the Chief Scientist and the Office of the Commissioner for the US-Food and Drug Administration. The expertise he brings to the regulatory agency builds on his ability to advance, coordinate, and integrate scientific resources for FDA by addressing mission critical scientific regulatory challenges in a global environment. The FDA Centers of Excellence in Regulatory Science and Innovation (CERSI) network has been built under Dr. Weichold's leadership in collaboration with academic institutions to leverage scientific expertise, resources and capacity toward FDA's mission. He represents FDA at the Maryland Life Science Advisory Board and at the NIH National Center for Advancing Translational Sciences. He also chaired the FDA Senior Science Council and he was leading strategic partnership development and technology transfer. Health data liberation, value generation and knowledge management in the public health sector are the focus of his current work.

Dr. Weichold's experience includes execution of strategic and operational initiatives across the sciences' value chain. Dr. Weichold has led the development of international collaborations and public private partnerships for discovery and early medical product development, implemented global operating and development models, and executed large-scale business model transformations. He has accumulated more than a decade of industrial research and medical product development experience while leading teams in Clinical Pharmacology, DMPK, as a Director at MedImmune LLC, and AstraZeneca. Prior, he directed research and clinical development of vaccines at the Aeras Foundation (founded by The Bill and Melinda Gates Foundation).

As a tenured Professor in the University of Maryland system, he developed and managed independent research programs and trained graduate students. He also held faculty positions at the University of Maryland Biotechnology Institute to study signal transduction pathways that affect immune responses, as well as at the Humboldt University, Berlin (Germany) to teach and study microbial immune modulation. During the five years of postdoctoral education, Dr. Weichold worked at the National Institutes of Health in Bethesda, Maryland, first at the National Cancer Institute where he researched immune pathologies in HIV infection, then at the Hematology Branch of the National Heart Lung and Blood Institute where bone marrow pathologies, transplantation immunology and gene therapy were the focus of his clinical research studies. His medical practice and clinical experience includes Infectious Diseases and Immunology/Rheumatology.

As a tenured Professor in the University of Maryland system, he developed and managed independent research programs and trained graduate students. He also held faculty positions at the University of Maryland Biotechnology Institute to study signal transduction pathways that affect immune responses, as well as at the Humboldt University, Berlin (Germany) to teach and study microbial immune modulation. During the five years of postdoctoral education, Dr. Weichold worked at the National Institutes of Health in Bethesda, Maryland, first at the National Cancer Institute where he researched immune pathologies in HIV infection, then at the Hematology Branch of the National Heart Lung and Blood Institute where bone marrow pathologies, transplantation immunology and gene therapy were the focus of his clinical research studies. His medical practice and clinical experience includes Infectious Diseases and Immunology/Rheumatology.



## Klaus-Michael Weltring

I am a molecular biologist by training with a PhD and a Habilitation degree from the University of Münster. Since 2001 I am the managing director of bioanalytik-muenster responsible for the development of the Münster region into a leading nano-bioanalytic location at the European level. Between 2003 and 2008 I was the deputy-coordinator of the Nano2Life Network of Excellence and leader of the "ELSA" Board in this network. I co-managed the Nanomedicine

Round Table and the EuroNanoBio projects and participated in the NANOMED2020 project (FP7 CSA projects). Since 2009 I am a member of the Executive Board of the ETP Nanomedicine leading the ELSA Advisory Group of this platform. Since March 2015 I am the chair of the German platform NanoBioMedicine. At the local level I am the Chief Scientific Officer of the Nano-Bioanalytik-Zentrum Münster (NBZ) and manage the Nano-Characterization-Lab Muenster ([www.NCL-Muenster.de](http://www.NCL-Muenster.de)) interfacing 11 local companies which develop new and certified methods for characterization of Nanomaterials in consumer products and biological systems. Currently I am partner in the EU-projects NOBEL, REFINE and EU-NCL.



## Peter Wick

Head Particles-Biology Interactions

Peter Wick heads since 2014 the research laboratory for Particles-Biology Interactions at the Federal Laboratories on Materials Science and Technologies Empa in St. Gallen. He studied and received his PhD in cell- and molecular biology at the University in Fribourg (Switzerland). Thereafter (2002) he moved to Empa and began his research in nanobiomedical field among others. His laboratory enables particles-based solutions for diagnostics and therapeutics driven by clinical needs. His general research interest is to study the interactions of nanomaterials with human tissues including barrier tissue *in vitro* and *ex vivo* with the purpose to obtain detailed mechanistic understanding about their uptake, accumulation, transport and effect on different types of cells or entire tissue. He is author of over 140 publications, including around 100 peer-reviewed papers, in the field of nanosafety and nanomedicine.

He is a member of the advisory board of the Swiss Action Plan on Nanomaterials, Editorial Board Member of *Nanotoxicology*, associated editor of the newly launched *Journal NanoImpact* and since 2018 head of the national Contact Point *Nano.ch* for safe handling, regulation and transfer of engineered nanomaterials

### RECENT PUBLICATIONS

- Kucki M, Aengenheister L, Diener L Rippl AV, Vranic S, Newman L, Vazquez E, Kostarelos K, Wick P, Buerki-Thurnherr T (2018) Impact of graphene oxide on human placental trophoblast viability, functionality and barrier integrity (*2D Materials* 5(3):035014
- 91) Drasler B, Kucki M, Delhaes F, Bürki-Thurnherr T, Vanhecke D, Korejwo D, Petri-Fink Alke, Rothen-Rutishauser B, Wick P, (2018) Single exposure to aerosolized graphene oxide and graphene nanoplatelets did not initiate an acute biological response in a 3D human lung model (in press *Carbon*)
- 90) Aengenheister L, Kucki M, Keevend K, Muoth C, Schönenberger R, Diener L, Wick P, Bürki-Thurnherr T, (2018) An Advanced human *in vitro* co-culture model for translocation studies across the human placenta barrier *Scientific Reports* 8(1):5288
- Rösslein M, Liptrott N, Owen A, Boisseau P, Wick P, Hermann IK (2017) Holistic understanding of particle interactions with biological systems is imperative to rational particle design for biomedical applications *Nanotoxicol* 11:2:147-149
- Obarzanek-Fojt M, Curdy C, Loggia N, Di Lena F, Grieder K, Bitar M, Wick P (2016) Tracking immune-related cell responses to drug delivery microparticles in 3D dense collagen matrix, *Europ J Pharma Biopharma* 107:180-190



## Martin Wiemann

Chief Scientist, CEO of IBE R&D Institute for Lung Health gGmbH

Martin Wiemann (\*1960) studied zoology, botany and biochemistry at the Westfälische-Wilhelms-Universität, Münster, Germany, where he received his PhD in Zoology in 1990. During his postdoc period, from 1990-1993, he worked in the laboratories of Dr. Günter Ehret, Dept. of Comparative Neurobiology, University of Ulm, and in the Group of Dr. Floyd Bloom, Scripps Institute, La Jolla, California, before he became an assistant at the Institute of Experimental Epilepsy Research (Prof. Dr. Erwin-Josef Speckmann), Medical Faculty, Westfälische-Wilhelms-Universität, Münster. From 1995-2006 he worked as an Assistant Professor in the lab of Prof. Dr. Dieter Bingmann, Institute of Physiology, University of Duisburg-Essen, and qualified for a full professorship with a German Habilitation (in 2000) which was devoted to the neuronal control of breathing. Since 2007 he entered into the non-profit IBE R&D Institute of Lung Health gGmbH, and became its CEO and Chief Scientist in 2010. His current interest focusses on nanotoxicology, localization techniques for industrial and medical nanoparticles, and in developing appropriate *in vitro* models.

period, from 1990-1993, he worked in the laboratories of Dr. Günter Ehret, Dept. of Comparative Neurobiology, University of Ulm, and in the Group of Dr. Floyd Bloom, Scripps Institute, La Jolla, California, before he became an assistant at the Institute of Experimental Epilepsy Research (Prof. Dr. Erwin-Josef Speckmann), Medical Faculty, Westfälische-Wilhelms-Universität, Münster. From 1995-2006 he worked as an Assistant Professor in the lab of Prof. Dr. Dieter Bingmann, Institute of Physiology, University of Duisburg-Essen, and qualified for a full professorship with a German Habilitation (in 2000) which was devoted to the neuronal control of breathing. Since 2007 he entered into the non-profit IBE R&D Institute of Lung Health gGmbH, and became its CEO and Chief Scientist in 2010. His current interest focusses on nanotoxicology, localization techniques for industrial and medical nanoparticles, and in developing appropriate *in vitro* models.

### RECENT PUBLICATIONS

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## Christoph Wierling

Head of Bioinformatics and Modelling, Alacris Theranostics

Christoph Wierling studied biology at the University of Münster and holds a PhD in biochemistry obtained from the Free University Berlin. He was leading a research group for systems biology at the Max Planck Institute for Molecular Genetics, Berlin. Currently he is heading the bioinformatics and modelling unit at Alacris Theranostics, a Berlin-based company applying NGS and systems biology approaches for translational research and precision medicine. His research interests focus on modelling and simulation of biological systems and the development of systems biology software.

Max Planck Institute for Molecular Genetics, Berlin. Currently he is heading the bioinformatics and modelling unit at Alacris Theranostics, a Berlin-based company applying NGS and systems biology approaches for translational research and precision medicine. His research interests focus on modelling and simulation of biological systems and the development of systems biology software.

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**1979-1983:** Head of development and production, Bühlmann Laboratories AG, Switzerland

**1976-1978:** Research scientists, Institute of Biochemistry, University of Hamburg, Germany

**1973-1976:** Research scientists, Dep. of Internal Medicine, University of Zurich, Switzerland

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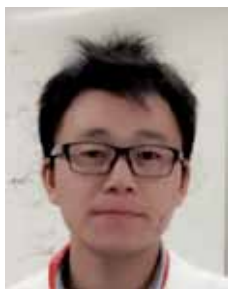


## Joy Wolfram

Assistant Professor, Mayo Clinic, Jacksonville, Florida (USA); Assistant Affiliate Member, Houston Methodist Research Institute, Houston, Texas (USA).

Dr. Joy Wolfram is an Assistant Professor of Medicine at Mayo Clinic in Florida, where she leads the Nanomedicine and Extracellular Vesicles Laboratory. She also holds affiliate faculty positions in the Department of Nanomedicine at the Houston Methodist Hospital in Texas, the Department of Biology at the University of North Florida in Florida, and the Wenzhou Institute of Biomaterials and Engineering at the Ningbo Institute of Industrial Technology in China. She received her bachelor's and master's degrees in biology from the University of Helsinki in Finland. In 2016, she completed her Ph.D. in nanoscience and technology at the University of Chinese Academy of Sciences in China. In the past five years, she has authored over 40 publications and received more than 25 scientific awards from seven different countries. She was included in the Amgen Scholars Ten to Watch List, which highlights the best and brightest up-and-comers in science and medicine across 42 countries. She has developed several nanoparticles for the treatment of various diseases, including cancer. Her goal is to bring new nanomedicines with increased therapeutic efficacy and safety to the clinic. Her mission is also to inspire and support underrepresented minorities in science. She is actively involved in community outreach and scientific education. For instance, she is the co-chair of the Physical Sciences-Oncology Network Education and Outreach Working Group of the National Cancer Institute in the United States.





## Lizhou Xu

Marie Curie Fellow

Lizhou Xu obtained his BS degree in Biopharmaceutical Engineering from Zhejiang University of Technology in 2011. He was then enrolled in a five-year PhD program in Biosystems Engineering at College of Biosystems Engineering and Food Science, Zhejiang University starting from 2011 to 2016 under the supervision of Prof. Yanbin Li. He was sponsored by the Chinese government as a joint-training PhD student in Biological Engineering at University of Arkansas (2013-2015). He joined the lab of Professor Khuloud Al-Jamal at the Institute of Pharmaceutical Science, King's College London in 2016 as a KC Wong Postdoctoral Fellowship from KC Wong Foundation. He is currently a Marie-Curie Fellow in the same institute. His current research interests include developing rapid detection methods for exosomes as biomarkers in health care using biosensing technology and exosome based drug delivery platform techniques for cancer therapies.

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witz Prize; Erice Peace Prize; Indian Prime-minister medal and the Nobel Prize for Chemistry



## Saeid Zanganeh

Senior Scientist at Sloan Kettering Institute for Cancer Research, New York (USA)

My research goals are directed toward developing clinical translational nanoscale technologies with particular emphasis on developing nanoscale biomaterials and immunoengineering systems for cancer immunotherapy, drug delivery and molecular/cellular imaging. My goal is to link the fields of nanotechnology, immunotherapy, cellular biology, and medical imaging towards more efficient and accurate diagnosis, personalized therapies and ultimately improving treatment outcomes and patient quality of life.

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## Ada Yonath

Ada Yonath focuses on genetic code translation by ribosomes, on antibiotics paralyzing this process, on antibiotic resistance, on designing novel antibiotics and on origin of life. She graduated from Hebrew University, earned her PhD from Weizmann Institute (WIS) and completed post-doctoral studies at CMU and MIT, USA. In

1971 she established the first biological-crystallography laboratory in Israel, which was the only lab of this kind in the country for almost a decade. Since then, she has been a faculty member and the Director of Kimmelman Center for Biomolecular Structures at WIS. In 1978 she spent a Sabbatical in the Chicago University, and during 1980-2004 she headed the Max-Planck-Research-Unit for Ribosome Structure in Hamburg in parallel to her WIS activities. Among others, she is a member of US-National-Academy-of-Sciences; Israel Academy of Sciences-and-Humanities; German Academy for Sciences (Leopoldina); European Molecular Biology Organization; Pontifical (Vatican) Academy of Sciences. She holds honorary doctorates from over 20 universities worldwide, in Israel, USA, Latin America, Europe and the Far East. Her awards include the Israel Prize; Linus Pauling Gold Medal; Albert Einstein World Award for Excellence; UNESCO-L'Oréal Award; Wolf Prize; Louisa Gross Hor-



## Rudolf Zentel

University Prof., Organic Chemistry, University Mainz, Germany

Prof. Rudolf Zentel studied chemistry at the Johannes Gutenberg-University Mainz (Germany) and received his PhD in 1983. During this time he got introduced to the concept of polymers for pharmaceutical applications in the lab of Prof. Helmut Ringsdorf. After a postdoctoral stay in Freiburg (Germany), and research stays at the "IBM Almaden Research Center" in San Jose (USA, 1989-1990) and in Düsseldorf (1990-1992) he got his first professorship in Mainz in 1992. Central topics of his research are self-organizing systems, materials for optoelectronics and - recently again - welldefined biocompatible polymers as nanocarriers for bioactive agents.

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## Daniel Zucker

Business Development Manager for Drug Delivery Systems, NOF EUROPE GmbH, Frankfurt, Germany.

Daniel Zucker has completed a Ph.D. at the age 26 in the field of drug delivery and biochemistry at the Hebrew University of Jerusalem in Israel. His thesis was about liposomes with chemotherapeutics and computational methods for remote loading of drugs into liposomes under the supervision of Prof. Yechezkel Barenholz. Next, he went for a postdoc at the Technical University of Denmark, at the Centre for Nanomedicine and Theranostics. He worked there on liposomes for cancer immunotherapy and vaccination against bacteria in the laboratory of Prof. Thomas Andresen. Afterwards, he worked as a senior scientist for RNA delivery at BioNTech RNA Pharmaceuticals GmbH in Germany. He developed there lipid and polymer formulations with antigen coding mRNA for cancer immunotherapy and vaccination against viruses. Currently, he is a business development manager for drug delivery systems at NOF Europe GmbH in Germany. NOF develops, manufactures and distributes innovative lipids, polymers, and surfactants for drug delivery. Daniel is a co-author of 7 peer-reviewed articles, 4 patent applications, and 1 book in the field of drug delivery. His publications were cited 379 times in Google Scholar.

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## Harald zur Hausen

Professor emeritus

Harald zur Hausen was born on March 11, 1936 in Gelsenkirchen-Buer, Germany. He studied Medicine at the Universities of Bonn, Hamburg and Düsseldorf and received his M.D. in 1960. After his internship he received the license to practice medicine and worked as a postdoc at the Institute of Microbiology in Düsseldorf, subsequently in the Virus Laboratories of the Children's Hospital in Philadelphia where he was later appointed as Assistant Professor. After a period of 3 years as a senior scientist at the Institute of Virology of the University of Würzburg, he was appointed in 1972 as Chairman and Professor of Virology at the University of Erlangen-Nürnberg. In 1977 he moved to a similar position to the University of Freiburg. From 1983 until 2003 he was appointed as Scientific Director of the Deutsches Krebsforschungszentrum (German Cancer Research Center) in Heidelberg. He retired from this position in 2003, and continues to work at the DKFZ until today.

He received a number of national and international awards, among them the Robert-Koch-Price, the Charles S. Mott Price of the General Motors Cancer Research Foundation, the Federation of the European Cancer Societies Clinical Research Award, the Paul-Ehrlich-Ludwig Darmstädter-Price, the Jung-Price, Hamburg, the Charles Rudolphe Brupbacher Price, Zürich, the Prince Mahidol Award, Bangkok, the Raymond Bourguine Award, Paris, the Coley-Award, New York, the Life Science Achievement Award of the American Association for Cancer Research, San Diego, and the Nobel-Prize for Medicine, 2008.

He received 36 Honorary Doctorates and 9 Honorary Professorships from international and national Universities. He is an elected member of various academies (LEOPOLDINA, Heidelberg Academy of Sciences, Polish Academy of Sciences, Venezuelan National Academy of Medicine, American Philosophical Society, Institute of Medicine of the National Academy of Sciences (USA), Foreign member of the US National Academy of Sciences and research organizations (EMBO, HUGO), National Academy of Sciences, USA, and became an Honorary Member of a number of biomedical scientific societies. A large number of Special Lectures and Visiting Professorships, Memberships in Editorial Boards and active involvements in the organization of international meetings complement his curriculum.

From 1989-1991 he was chairing the Association of National Research Centres, in Bonn, Germany. From 1993-1996 he was President of the Organization of European Cancer Institutes. From 2000-2009 zur Hausen was Editor-in-Chief of the International Journal of Cancer, and from 2006-2009 he was member of the Board of Directors of the International Union against Cancer (UICC). From 2003-2009 he was Vice-President of the German National Academy for Natural Sciences and Medicine LEOPOLDINA in Halle. From 2006 to 2015 he was a member of the National Science Transfer and Development Agency in Bangkok, Thailand

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CLINAM

European Foundation for Clinical Nanomedicine

11/2018

Basel, September 2–5

**ABSTRACTS  
SPEAKERS**



## SELECTIVE DRUG DELIVERY APPROACHES INTO LESIONED BRAIN THROUGH BLOOD BRAIN BARRIER DISRUPTION

ZAHRAA S. AL-AHMADY<sup>1</sup>, Dhifaf Jasim<sup>2</sup>, Sabahuddin Syed Ahmad<sup>2</sup>, Raymond Wong<sup>3</sup>, Michael Haley<sup>3</sup>, Graham Coutts<sup>3</sup>, Ingo Schiessl<sup>3</sup>, Stuart M. Allan<sup>3</sup> and Kostas Kostarelos<sup>2</sup>

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Stroke is a leading cause of death and disability worldwide, however, treatment options are extremely limited, and the development of new therapies continues to face repeated translational failures. Brain endothelial cells form paracellular and transcellular barriers to many blood-borne therapies. Although this barrier function is impaired in the event of a stroke, this impairment does not improve therapeutic delivery to the brain, therefore, the development of efficient delivery strategies is highly warranted. Here, we show, in experimental stroke model in mice, selective recruitment of clinically used nanoscale liposomes into the ischemic brain that correlates with the biphasic blood brain barrier (BBB) breakdown after stroke. We used a combination of both *in vivo* real-time imaging and histological analysis to study the contributions of transcellular and paracellular mechanisms to this process. Different time points of liposomal I.V administration into mice exposed to 20 minutes middle cerebral artery occlusion have been tested to cover the early (0.5h & 4h) and the delayed (24h & 48h) phases of BBB disruption. We found that selective liposomal brain accumulation coincides with biphasic enhancement in transcellular transport followed by the delayed impairment to paracellular barrier. Liposomal brain accumulation precedes neurological damage in the acute phase and maintains long-term co-localisation with the neurovascular unit which can have great potential for neuroprotection. Levels of liposomal uptake by glial cells are similarly selectively enhanced in the ischemic region late after experimental stroke which highlight their potential for blocking the inflammatory responses or shifting their polarisation towards brain repair.

These findings demonstrate the capability of liposomes to maximise selective translocation into the brain after stroke and identify for the first time two windows for therapeutic manipulation. This emphasizes the benefits of nanoscale drug delivery to achieve efficient tailoring of stroke treatment

## FORMULATION AND IN VITRO AND IN VIVO ANTI-MALARIAL EVALUATION OF ARTESUNATE-LOADED ETHOSOMES

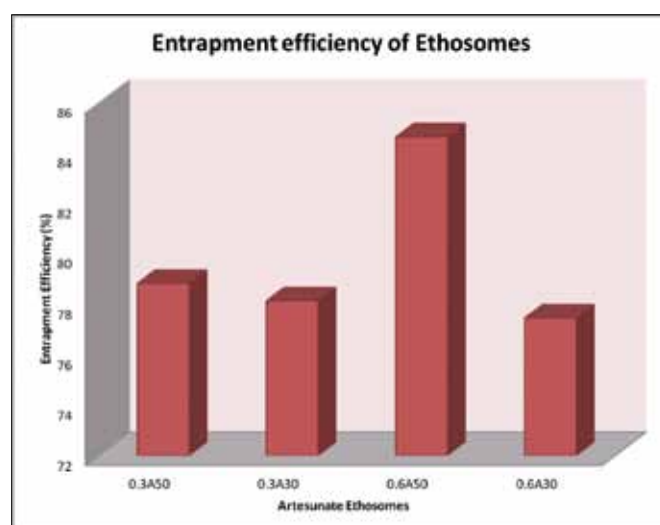
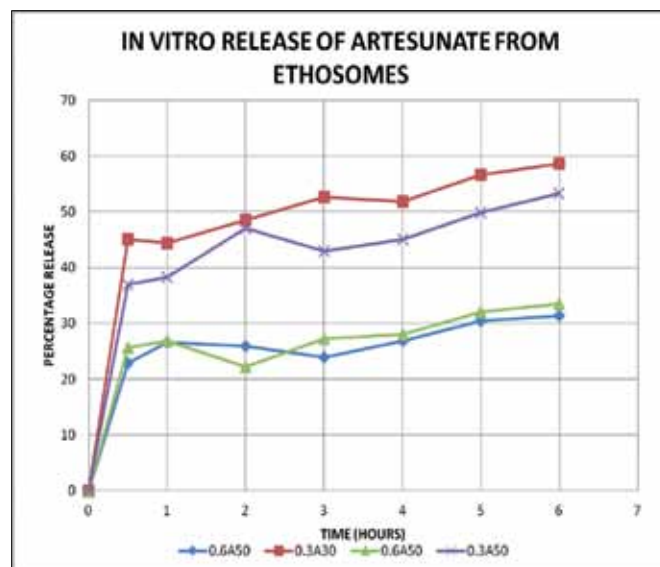
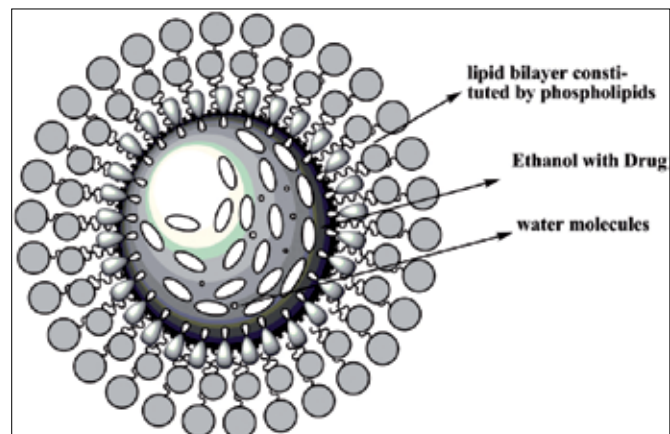
CHINAZOM PRECIOUS AGBO<sup>1</sup>, Offor E.N. <sup>1</sup>, Nwabueze H.U1, Attama A.A.<sup>1</sup>

<sup>1</sup> Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria

**Introduction:** Ethosomes are nano-sized drug delivery systems that can permeate biological barriers. Nasal delivery is becoming a promising technique considered as an alternative route to achieving non-invasive delivery of drugs directly and rapidly to the brain (by-passing the blood-brain-barrier) and the systemic circulation. Artesunate administered parenterally and orally is used for treating cerebral and server malaria and uncomplicated malaria respectively. Parenteral drug administration is difficult to achieve in some rural areas in Africa where availability to healthcare professionals

may be challenging. More so, the oral route may not be convenient for some groups of patients.

**Aim:** The aim of this research work was to formulate and investigate the intranasal delivery of artesunate-loaded ethosomes as an alternative, non-invasive route of administration for the treatment of cerebral, severe and uncomplicated malaria.



**Method:** Ethosomes containing artesunate were formulated using 30% and 50% ethanol, Phospholipon 90H<sup>®</sup> (2%W/V), Tween 80 (5.2%) and artesunate (2.4%). pH stability test, encapsulation efficiency, *in vitro* release and *in vivo* studies were carried out on all batches of ethosomes. The results obtained from *in vitro* release analysis were fitted into mathematical models. Commercial samples of these drugs were also investigated and comparisons were made by administering drugs through the intranasal and intramuscular routes.

**Results:** All batches of artesunate-loaded ethosomes showed high encapsulation efficiencies (77.46–84.63%). However, the pH stability study revealed reduction in pH after two months storage. In vitro release analysis of formulations showed sustained release of drugs with an initial burst release after 30 min. All batches of ethosomes followed zero order drug release. In vivo evaluation of ethosomes and commercial drug administered intramuscularly showed a significant decrease in parasitaemia ( $p < 0.05$ ). However, ethosomes and commercial formulations of artesunate could not reduce parasitaemia level when administered through the nasal route.

**Conclusion:** Artesunate-loaded ethosomes were successfully formulated and achieved sustained release of drug with significant clearance of parasitaemia when administered intramuscularly but not intranasally.

## PRIONS: DEADLY SELF-REPLICATING NANOMACHINES

**ADRIANO AGUZZI**, Institute of Neuropathology, University Hospital of Zürich

Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases of humans and many animal species caused by prions. The main constituent of prions is PrP<sup>Sc</sup>, an aggregated moiety of the host-derived membrane glycolipoprotein PrP<sup>C</sup>. Prions were found to encipher many phenotypic, genetically stable TSE variants. The latter is very surprising, since PrP<sup>C</sup> is encoded by the host genome and all prion strains share the same amino acid sequence. Here I will review what is known about the infectivity, the neurotoxicity, and the neuroinvasiveness of prions. Also, I will explain why I regard the prion strain question as a fascinating challenge – with implications that go well beyond prion science. Finally, I will report some recent results obtained in my laboratory, which is attempting to address the strain question and some other basic issues of prion biology with a “systems” approach that utilizes organic chemistry, photophysics, proteomics, and mouse transgenesis.

## MAGNETIC DRUG TARGETING: PRECLINICAL IN VIVO STUDIES, MATHEMATICAL MODELING, AND EXTRAPOLATION TO HUMANS

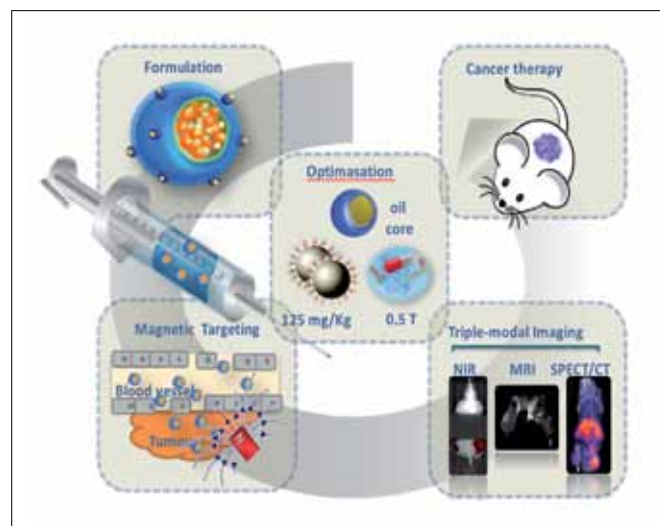
**KHULOUD T. AL-JAMAL**, School of Cancer and Pharmaceutical Sciences, Faculty of Life Science and Medicine, King’s College London, London SE1 9NH, UK, E-mail: khuloud.al-jamal@kcl.ac.uk

A sound theoretical rationale for the design of a magnetic nanocarrier capable of magnetic capture *in vivo* after intravenous administration could help elucidate the parameters necessary for *in vivo* magnetic tumor targeting. In this work, we utilized our long-circulating polymeric magnetic nanocarriers, encapsulating increasing amounts of superparamagnetic iron oxide nanoparticles (SPIONs) in a biocompatible oil carrier, to study the effects of SPION loading and of applied magnetic field strength on magnetic tumor targeting in CT26 tumor-bearing mice. Under controlled conditions, the *in vivo* magnetic targeting was quantified and found to be directly proportional to SPION loading and magnetic field strength. Highest SPION loading, however, resulted in a reduced blood circulation time and a plateauing of the magnetic targeting. Mathematical modeling was undertaken to compute the *in vivo* magnetic, viscoelastic, convective, and diffusive forces acting on the nanocapsules (NCs) in accordance with the Nacev–Shapiro construct, and this was then used to extrapolate to the expected behavior in humans. The model predicted that in the latter case, the NCs and magnetic forces applied here would have been sufficient to achieve successful targeting in humans. Lastly, an *in vivo* murine tumor growth de-

lay study was performed using docetaxel (DTX)-encapsulated NCs. Magnetic targeting was found to offer enhanced therapeutic efficacy and improve mice survival compared to passive targeting at drug doses of ca. 5–8 mg of DTX/kg. This is, to our knowledge, the first study that truly bridges the gap between preclinical experiments and clinical translation in the field of magnetic drug targeting.

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## PHOTOTHERMALLY ACTIVATED MAGNETOLIPOSOMES FOR CANCER THERAPY AND IMAGING

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## INTRODUCTION

Magnetic nanoparticles exhibit extraordinary properties, which make them excellent candidates for biomedical applications. Iron oxide nanoparticles have been used in magnetic resonance (MR) imaging, drug delivery, and cancer hyperthermia. In the present work, we report the engineering of novel temperature-sensitive magnetoliposomes for photothermally-induced drug release and MR imaging.

## METHODS

Magnetoliposomes were prepared using lipid film hydration and extrusion, and doxorubicin (DOX) was encapsulated in the liposome aqueous core using a remote loading method. The developed magnetoliposomes were characterized using dynamic light scattering (DLS), transmission electron microscopy (TEM), differential scanning calorimetry (DSC), inductively coupled plasma mass spec-

trometry (ICP-MS). DOX release was assessed in response to near-infrared radiation (NIR) laser. Magnetoliposomes were also studied to evaluate their capabilities as MR contrast agents.

## RESULTS

Structural characterization using TEM and DSC revealed that small (4.5nm) nitrodopamine palmitate (NDPM)-coated magnetic nanoparticles (NPs) were successfully incorporated into the bilayer of lysolipid-containing magnetoliposomes without compromising DOX loading. More interestingly, our data successfully showed that low-energy NIR laser induced hyperthermia at much lower magnetic NPs concentrations ( $\mu\text{g/ml}$ ), compared to magnetic hyperthermia ( $>10\text{mg/ml}$ ). In support of this, DOX release from our magnetoliposomes was triggered using low-energy NIR laser (800 nm), resulting in quick and efficient on-demand drug release ( $>90\%$  in 5 minutes). Furthermore, our lysolipid-containing magnetoliposomes revealed as a highly efficient T2 MRI contrast agent with 300-higher relaxivity ratio  $r_2/r_1$  compared to free hydrophilic iron oxide NPs.

Conclusion: We report the development of our novel magnetoliposomes that could be used in combination with NIR laser, offering efficient hyperthermia treatment, and on-demand drug release, besides being a highly sensitive T2 MRI imaging agent for image-guided drug delivery.

## ACKNOWLEDGMENTS

This work was supported by Prostate Cancer UK (Grant CDF-12-002), and the Engineering and Physical Sciences Research Council (EPSRC) (EP/M008657/1).

## UPSCALING OF IRON OXIDE NANOPARTICLES FOR BIOMEDICAL APPLICATIONS

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Figure 1: Facility of the Pharmacy Department, Erlangen University Hospital for the production of iron oxide nanoparticles in a scaled up manner under GMP conditions.

Nanoscience has now matured and has been transitioned from bench science to applied technology; superparamagnetic iron oxide nanoparticles (SPIONs) are widely used in various scientific fields, not only on commercial scale but their wide use in biomedical science are more significant. Due to their tremendous behavior and applications, much effort has been invested for more than a decade, but unfortunately, successful pharmaceutical developments are still rare, despite very promising results. The main stumbling blocks are insufficiently addressed toxicity and the lack of appropriate particle stability in biological media. Even if these factors are adequately addressed, another important challenge which hampers the way to the clinic is the need to upscale the synthesis process from the milliliter lab scale to the liter scale and by doing so to meet the requirements from the regulatory authorities. At

SEON we tackle exactly this issue and developed in the past a couple of iron oxide based formulations for drug targeting and contrast agents for MRI [1-3]. These systems are well-designed to conform to the physicochemical and toxicological requirements for their respective application. Due to a close collaboration with the Pharmacy Department of the Erlangen University Hospital, we are currently producing nanoparticles in the scaled up liter measure according to the highest possible international quality standard – the Good Manufacturing Practice (GMP) Guidelines. Based on the very promising preclinical results we are planning to bring our nanoparticle-based formulations to the clinic in the near future.

## ACKNOWLEDGEMENTS

This work was supported by the EU project Nanoathero (FP7-NMP-2012-LARGE-6-309820), by the Deutsche Forschungsgemeinschaft [German Research Foundation] (CI 162/2-1, CI 162/2-3, AL552/8-1), by the Bavarian State Ministry of the Environment and Consumer Protection and by the Manfred-Roth-Stiftung, Fürth, Germany.

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## EFFICACY AND SAFETY OF BOVINE LACTOFERRIN NANOCARRIERS FOR TREATMENT OF PLACENTAL MALARIA, ANEMIA, COMPLEMENT DYSREGULATION, PREECLAMPSIA IN MURINE MODEL

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**INTRODUCTION:** Lactoferrin is a milk protein known to have multiple therapeutic functions against diverse range of diseases. Bovine Lf (bLf) has been used as a dietary supplement and preclinical and subclinical studies have shown its role in maintaining intestinal microbial flora, iron metabolism in infants and pregnant women. However, its efficacy against congenital parasitic diseases are rarely studied.

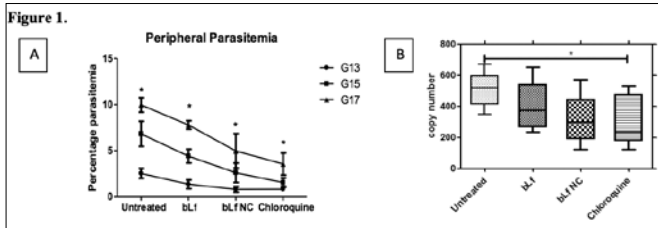
**AIM:** Present study was aimed to examine the efficacy of bLf nanocarriers against congenital model of murine malaria along with pregnancy induced complications.

**METHODS:** Pregnancy model of congenital malaria was developed using inbred Balb/c mice and Plasmodium berghei infection was given at gestation day 10 (G10) post pregnancy. bLf along with its nanoformulation (bL NC) was used at a concentration of 1.2% and approximately 3mg of bLf was given to each mice per day. Chloroquine was used at a concentration of 10mg/kg/day. Different treatments were started on day G11 and efficacy of various treatments were monitored in placenta by G19 using forced cesarean section. Molecular markers; parasite copy number, reactive oxygen species (ROS), nitric oxide (NO), C5a mediated placental lysis (CD88 ex-

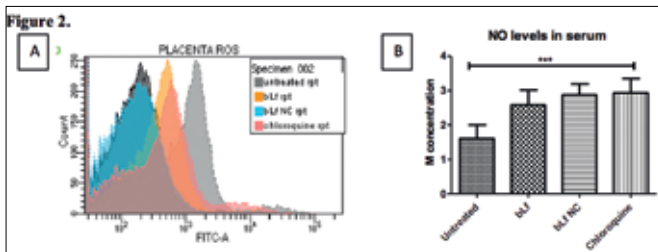


pression), preeclampsia related angiogenic factors; Angiotensin 1 & 2, vascular endothelial growth factor (VEGF), soluble fms like tyrosine kinase (sFlt1) and iron metabolism was studied. Vertical transmission of bLf NC to placenta was observed by immunohistochemistry (IHC).

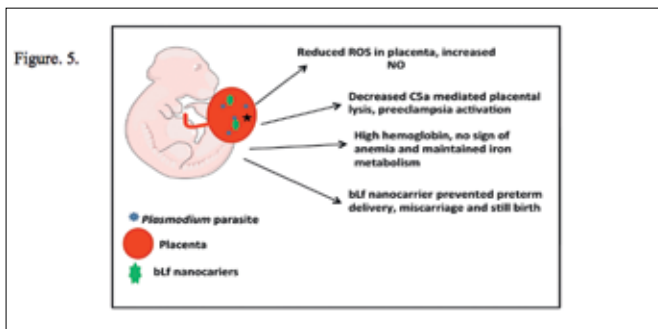
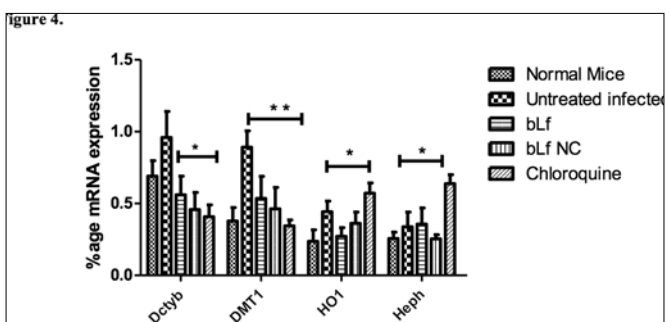
**RESULTS: Peripheral parasitemia and parasite copy Number:** Significant high parasite load was found in peripheral blood smear of untreated mice which was further confirmed by parasite copy number again observed from untreated group of mice followed by bLf, bLf NC and least number was found in chloroquine group. (Figure 1 A and B).



**Free radical ion production:** High ROS production was found in untreated group of mice showing placental damage along with low NO levels. Whereas, high NO levels were found in all treatment groups with low levels of ROS (Figure 2A & B).



**C5aR (CD88) expression:** Complement mediated placental damage was monitored by staining placental cells using CD88 PE labeled anti mouse antibody and expression was shown using histograms of mean fluorescence intensity (MFI). Highest MFI was found in untreated group representing the placental lysis compared to other treated groups (Figure 3A and B).



**Conclusion:** Present study has showed protective effects of bLf NC against congenital murine malaria model using *P. berghei*. bLf NC used in the study has shown protective efficacy as well as vertical transmission to the placenta with minimum toxicity to the developing fetus. As bLf is an iron binding protein, present study has demonstrated its

wide applications of reducing parasite burden, immunomodulation, iron metabolism, angiogenesis and controlling free radical ion damage to the placenta which can cause preterm delivery and abortion.

## NOVEL ORAL SELF-NANOEMULSIFYING DELIVERY SYSTEMS OF GENTAMICIN: NEW FRONTIER AGAINST CEREBROSPINAL MENINGITIS

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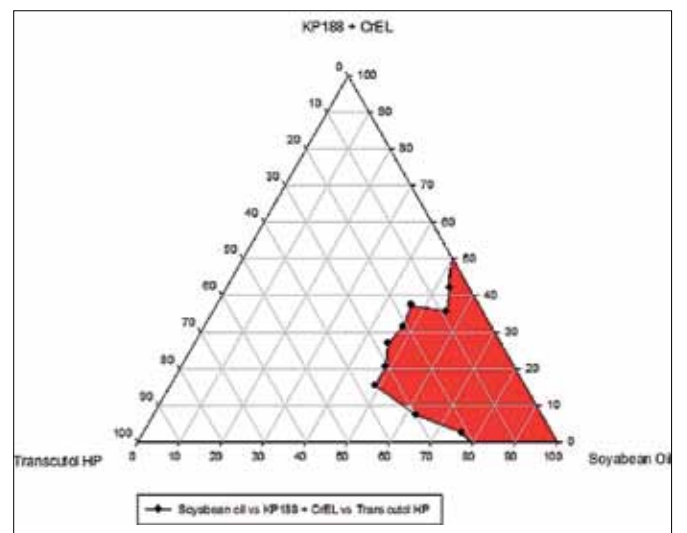
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The neuroprotective function of the blood brain barrier (BBB) is vital, but also represents an invincible obstacle for a multiplicity of therapeutically important drugs used in several central nervous system (CNS) diseases such as Alzheimer's disease, Parkinson's disease, cerebrospinal meningitis (CSM), brain tumors, etc [1,2]. Various CNS drugs on the market are administered in excessive doses to reach the brain in therapeutic concentrations with associated severe side effects in peripheral organs [3]. CSM remains a major therapeutic challenge with mortality between 12 and 75%. Aminoglycosides, e.g. gentamicin administered parenterally are often used to treat this disease, although these antibiotics penetrate the BBB poorly [4]. In adults, *S. pneumoniae* and *N. meningitidis* together cause 80 % of bacterial CSM cases [5]. Despite the availability of vaccines, CSM still kills a lot of people in developing countries. For instance, 489 people died in Nigeria with 4,637 suspected cases in 2017 across five states [6]. Informed by these facts, we developed self nano emulsifying formulations of gentamicin (GEN-SNEFs) capable of crossing the BBB to treat cases of bacterial CSM.

### EXPERIMENTAL

The critical pseudoternary phase diagram (Fig. 1) was developed using soybean oil (SBO), Transcutol<sup>®</sup> HP (THP) and Kolliphor<sup>®</sup> EL (CrEL)/Kolliphor<sup>®</sup> P188 (KP188) (1:1). Different batches of GEN-SNEFs were formulated (C1 – C9). The formulated GEN-SNEFs were characterized and further evaluated in vitro using strains of *Streptococcus pneumoniae*, and in vivo using Albino rats (Dose: 7 mg/kg).



In vivo studies were conducted on the optimized batches (C4 and C6) after obtaining permission from our Institution's Ethics Committee. For the determination of the concentration of gentamicin in CSF, the rats administered with the gentamicin or GEN-SNEFs (oral gavage) were anaesthetized in a chloroform chamber and a catheterized-needle used to extract the CSF carefully through the base of the brain at predetermined time intervals. The anti-pneumococcal activity of the extracts was determined by bioassay using Molten Columbia blood agar supplemented with 5 % defibrinated sheep blood.

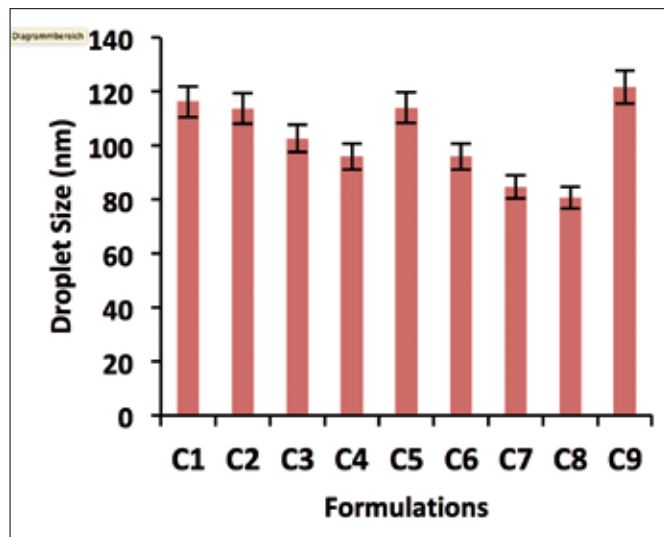


Fig. 2. Droplet sizes of the loaded (C1–C8) and unloaded (C9) SNEFs.

## RESULTS AND DISCUSSION

In vitro evaluation of GEN-SNEFs revealed nanometer-sized globules (80 – 210 nm (Fig. 2), polydispersity: 0.030 to 0.717, and zeta potential: -25.4 to -42.5 mV, after rapid (17 – 40 s) and high emulsification (efficiency ≈ 88 – 99%). These desirable properties were conferred on gentamicin by the unique choice and combination of the oil, surfactant and cosurfactants used in the formulation of GEN-SNEFs. Intact gentamicin structure was confirmed by Fourier transform infrared (FTIR) spectroscopy. In vitro antibacterial studies showed high susceptibility of *S. pneumoniae* to the GEN-SNEFs (IZD ≈ 20 – 35 mm) compared with the free drug solution in sterile water (IZD ≈ 13 – 18 mm) at equivalent concentrations.

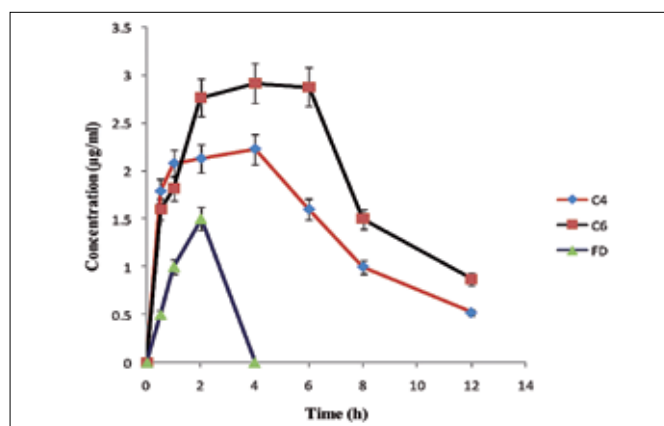


Fig. 3. Concentration of gentamicin in the serum after oral administration of GEN-SNEFs (C4 and C6). FD = Free drug.

In vivo study of GEN-SNEFs in albino rats indicated very high bioavailability in serum (AUC = 5 to 14 fold increase) compared with free drug solution in sterile water (Fig. 3). Cerebrospinal fluid (CSF) concentration of gentamicin achieved after oral administration was also very high compared with gentamicin solution in sterile water (Fig. 4). The increased serum and CSF bioavailability of gentamicin after oral administration of GEN-SNEFs was facilitated by formulation of gentamicin as SNEFs.

## CONCLUSIONS

The formulation technique employed yielded GEN-SNEFs with the desired targeted properties. This study has thus shown that it is possible to formulate oral nano delivery form of gentamicin giving hope for quicker intervention in acute bacterial meningitis.

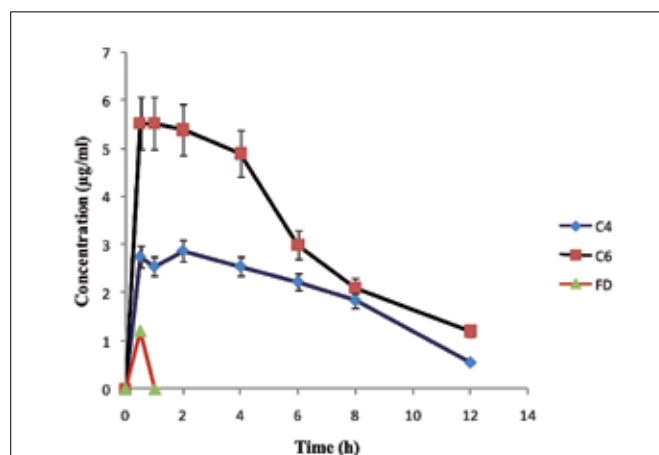


Fig. 4. Concentration of gentamicin in the CSF after oral administration of GEN-SNEFs (C4 and C6). FD = Free drug.

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## THE EUROPEAN NANOMEDICINE CHARACTERIZATION LAB INFRASTRUCTURE

SIMON BACONNIER

The use of nanotechnologies in healthcare promises to tackle major medical challenges. However, the manufacturing process of nanomedicines is potentially complex and inconsistencies must be carefully assessed before clinical applications can be considered. Furthermore, safety concerns related to the use of nanomaterials must be addressed as early as possible during product development.

The European Nanomedicine Characterization laboratory (EUNCL) will address these issues by providing the critical infrastructure and characterization services required to analyze physical and chemical attributes, *in vitro* biological properties, and *in vivo* characteristics of nanomedicines under development.

The EUNCL services will be accessible to all organizations developing candidate nanomedicines, whatever the development maturity. Product developers will benefit from a detailed and confidential characterization data set that supports their decision making for further product development

We will present you the access procedures, what should be a suc-

successful application, the lessons learned and the evolution to be implemented in the future Call campaigns.

### CHARACTERIZATION PLAN ACCESS PROCEDURES

The EUNCL characterization process will be conducted by the Core Expert Team (CET), a group of nanoparticles and nanomedicine experts within top-level European institutions using high-end methods. The Core Expert Team (CET) will be in charge of validating the adequacy of the sponsor proposal, material and development strategy and the EU-CNLC capacities and to define the most relevant characterization plan to be performed to provide the most reliable and relevant data-set for future development.

The access selection is organized in a two steps process: A first submission step based on a light proposal intended to give EUNCL external and internal reviewers an overview of the strategy, without requiring investigators to prepare costly, time-consuming proposals. This format will allow to briefly describe the background, the strategy or the concept of action of the innovative nanomedicine, a synthesis of the already accessible characterization data, a description of the innovation, the clinical impact and a scale-up compatibility description, *in vitro* and *in vivo* testing data.

In case of success at the first step the sponsor will be invited to submit a second step proposition. The level of details requested for the second step application is much higher and will be evaluated against more stringent criterion.

To fulfill its advisory role, the EUNCL will feedback to the sponsors successful or not. The objective is to motivate and support the sponsor to submit again in case of failure.



The selection process should not exceed 75 days after which the material transfer should start in accordance with the characterization plan and agreed sample typology and quantity.

### THE SUCCESSFUL APPLICATION PROFILE

There is no best sponsor profile, actually, any Med-NP provider, including Industry, academia or government agencies, can apply to the EUNCL services. But there are best applications.

Actually, some critical information should be detailed in the submission process among which the Med-NPs initial characteristics including inherent toxicity, or previous physico-chemical characterization data.

In addition, the Med-NP manufacturing environment should be fully mastered by the sponsor. Actually, two independent product batches will be requested. The detailed production process description and the related scaling up strategy should be described. Finally, the application should include a demonstrated efficacy in a biological system as well as the strategy to transfer the technology to the clinical environment and an anticipated impact on clinical application.

Globally, the maturity of the product as well as the production management and the anticipation of the translation to the patients will be accurately assessed.

### THE LESSONS LEARNED

After three TNA campaigns, EUNCL, the integrated nanomedicine characterization laboratory gathering 6 European institutions' expertise and capacities, has evaluated 24 application for compounds targeting different diseases.

As opposed to the NCI-NCL, our US cousin expert nanocharacterization infrastructure, EUNCL is distributed among Europe. This unique shared infrastructure offers a large variety of characterization techniques and equipment including novel and high-end technologies offering optimized correlation between *in vitro* data and *in vivo* NMPs behavior.

A key lesson learned, was to integrate the extensive diversity in the type of material to be analyzed. Actually, considering that the Med-NPS to be characterized can be organic, inorganic, metallic, drug associated or loaded, with therapeutic or diagnostic objectives, targeting specific target, charged or not, the EU-NCL partners will have to be able to adapt and optimize the characterization plan strategy and capacities.

A second lesson learned, is the compound and applicants maturity impact on the support EUNCL can provide. Actually, being a University spin-off or a big pharma does not require the same kind of support and involvement, however, the need for counseling is always high. EUNCL role has strongly evolve and its involvement early in the process of Med-NP is to be strongly considered.

Finally, one of the most important lessons learned from our close collaboration with the NCI-NCL is the strong need from sponsors for quality data produced through standardized methodologies in accordance with established standards and controls. The very stringent analysis framework is the only way to provide top quality data to the future providers of patients' innovative treatments and diagnostic tools.

### THE EVOLUTION TO BE IMPLEMENTED IN THE FUTURE CALL CAMPAIGNS.

Considering the strong awareness EUNCL is raising, the growing number of applications as well as the counseling role of this infrastructure, we will have to push forward the access strategy. From a starting community, EUNCL should evolve to an advanced community to ensure a better, a wider and stronger support to EU Med-NP developers.

## PRECISION NANOMEDICINE AND THE LANDSCAPE OF NANOMEDICINE PUBLICATIONS

LAJOS (LOU) BALOGH, PhD., Editor-in-Chief, Precision Nanomedicine (The official journal of CLINAM)  
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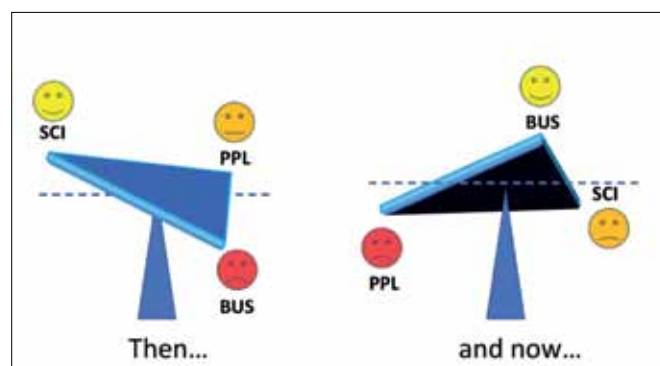


Figure 1: Balancing interests of Science (SCI), Business (BUS), and Scientists (PPL) – Then and now

Science publishing has many problems today most of them created by the imbalance between the interests of science, authors, and the publishing business.<sup>[1]</sup> Society needs reliable science now more than ever, but progress cannot exist without scientists and sharing reliable information. Science publishing can only move forward if the interests of all stakeholders are in balance. A publisher has to



run a sustainable business but needs the oversight of scientists to ensure that the primary interests of all parties are equally served. Scientists provide content, supply quality control, improve scientific merit, and as users they read, share, judge, and utilize content. This structure operates through board members, who guard the prestige and value of the journal. Science publications and in more general science communication is part of the global information ecosystem, which we will have to redesign in the 21st century.

There is a growing need for responsible publishing and sharing reliable results. This means publishing not only for the sake of having one more publication, but sharing information which is useful for peers, educates about research and development (R&D) knowledge, and converts information to knowledge translatable into practice. Forced to chase originality and novelty, many investigators now pursue only novel materials and complicated approaches to fulfill the criteria to make their manuscripts acceptable. [2] Originality and novelty are important, but without in-depth and reproducible studies and R&D knowledge, it is impossible to develop practical (nano)medicines for everyday use. While many societies hire for-profit publishers to run their media, academic-run publishing is on the rise, and we have also decided to create our own publishing company. In January 2018, and with the support of CLINAM. The speaker will describe the core values of this new endeavor and discuss them with the audience.

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## TARGETED NANO-THERAPEUTICS AIMING AT THE RESOLUTION OF LIVER DISEASES

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The treatment landscape is evolving rapidly for chronic liver diseases as significant improvements have been made towards therapies against chronic hepatitis B and hepatitis C infections. However, non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD), affecting the large proportion of population, is becoming the leading and growing cause of mortality worldwide. The prevalence of NAFLD and ALD will continue to grow progressively due to western life style, and increasing incidences of diabetes and obesity. The treatment strategies for liver diseases are currently focused on disease pathological pathways: hepatocytes damage, inflammation and fibrogenesis. Irrespective of disease etiology, hepatic stellate cells (HSCs) are recognized as the major cellular origin of fibrogenic myofibroblasts during chronic liver disease. Macrophages, found in close proximity of the activated HSCs have been shown to play a key role in fibrosis initiation and progression. Over past years, pro-inflammatory macrophages (Ly6Chigh or M1-like) have been shown to contribute significantly to the pathogenesis of liver diseases. Despite our increasing understanding of cellular and molecular mechanisms contributing to liver diseases, there are no effective and clinically approved therapies available. Therefore, there is an unmet need for the development of safe and effective therapies for the patients suffering from liver diseases mainly NAFLD and ALD.

## RESULTS AND DISCUSSION

Over past years, we have developed cell-specific targeted therapies for the treatment of liver diseases. These therapies are categorized as HSC-directed and macrophage-directed drug delivery strategies:

Figure 1: Receptors or cellular targets and different designed formulations for active targeting to the different cell types of liver (hepatic

stellate cells and macrophages). Nanoparticles or proteins are modified with specific surface ligands to be recognized by their receptors or cellular targets on a specific type of liver cells: hepatic stellate cells and macrophages.

Cell Type	Receptor or cellular target	Formulation
 Hepatic stellate cells	PDGFβ receptor	 pPB-HSA, pPB-PDG-IFN, pPB-HSA-IFN
	Relaxin receptor	 Iron-oxide NP, Relaxin
 Macrophages	Mincle (CLEC4E) receptor	 Liposomes
	FcGR1 (CD64) receptor	 Liposomes

HSC-specific targeted therapies (Figure 1): PDGFβR has been shown to be highly and selectively upregulated on activated HSCs during liver fibrosis. Therefore, using PDGFβR-binding peptides, we have delivered potent anti-fibrotic cytokine IFNγ and peptidomimetic IFNγ (IFNγ signaling peptide) for the treatment of fibrogenesis [1-4]. We have shown these delivered anti-fibrotic therapies significantly ameliorated liver fibrosis with minimal side effects [1-4]. We have recently developed relaxin-coated iron-oxide nanoparticles as therapeutic for liver diseases [5]. Relaxin is an anti-fibrotic hormone that binds to relaxin receptor which is highly expressed on activated HSCs and hence can be used for therapy and diagnosis. In advanced model of liver fibrosis, relaxin-coated iron-oxide nanoparticles attenuated liver fibrosis, angiogenesis and portal hypertension and showed increased contrast in MR-imaging [5].

Macrophage-specific targeted therapies (Figure 1): We and others have shown that Mincle and FcγR1 are receptors that are significantly upregulated in inflammatory macrophages [6-8]. We used these receptors as target receptors and designed targeted liposomes encapsulating anti-inflammatory steroid prednisolone for targeted inhibition of inflammatory macrophages. Targeted macrophages showed highly specific uptake in M1 macrophages and also showed potent anti-inflammatory effects in M1 macrophages *in vitro*. *In vivo* study using acute liver inflammation murine model, we demonstrated that strong inhibition in macrophage infiltration, activation and inflammation with targeted liposomes (with prednisolone) as compared to non-targeted liposomes (with prednisolone) and free prednisolone. Additionally, we have recognized activation of key signaling pathways in M1 macrophages i.e. SRC and SYK. These pathways when inhibited using selective small-molecule inhibitor, showed significant reduction in M1 activation markers. We thereafter investigated the potential of PLGA-mediated delivery of these potent inhibitors *in vitro* and *in vivo* in MCD-murine model of NAFLD.

## CONCLUSION

In conclusion, there is an absolute need for the development of effective and safe therapies for the treatment of rapidly progressing liver diseases. The novel targeted therapies are currently evolving with promising outcome, these therapies should be explored further for their prospective use in liver diseases.

## ACKNOWLEDGEMENT

This work is supported by VENI innovation grant 916.151.94 from Netherlands Organization for Health Research and Development ZonMw, NWO.

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## **BUPIGELTM: A FORMULATION BASED ON TWO-STAGE LIPOSOMES-IN-HYDROGEL SLOW-CONTROLLED RELEASE LOCAL ANESTHETIC WITH A PROLONGED DURATION OF ANALGESIA: BASIC, ANIMAL, AND HUMAN STUDIES**

**ECHEZKEL (CHEZY) BARENHOLZ# AND RIVKA COHEN**

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There is a compelling need for an ultra-long acting local anesthetic to treat local or post-operative moderate-to-severe pain. Standard local anesthetics have a short (1 to 3 h) duration of analgesia, and their potential toxicity limits their administered dose and treatment frequency. Currently moderate-to severe pain is currently treated with systemic opioids which produce many untoward effects including respiratory depression, ileus, urinary retention, pruritus, nausea, vomiting and potential misuse, abuse and addiction issues. Thus, long acting local anesthetics have the potential to substitute for or spare opioid use and would fill a large unmet need in pain care.

Bupigel<sup>TM</sup> is an ultra-long acting local anesthetic composed of a hydrogel that entraps large amount of Bupisomes. These are multivesicular vesicles (LMVV) containing a high entrapped aqueous volume and are remotely loaded with a large amount of bupivacaine (an amphipathic weak base) via a trans-membrane ammonium gradient. The use of hydrogel ensures longer retention of the Bupisomes at the site of administration. Two Phase I human studies of Bupisomes in healthy volunteers administered subcutaneously demonstrated large prolongation of local analgesia, and a prolonged pharmacokinetic release-profile exhibiting low plasma levels (consistent with slow release of bupivacaine from the LMVV depot), and excellent safety. Subcutaneous administration of Bupigel in a pig wound-healing model demonstrated analgesic duration of greater than 96 hours with low- but almost unchanged plasma level for longer than the final 72 hours measurement point thus supporting the slow and controlled bupivacaine release. Maximum tolerated dose (MTD) in rats was >100mg/kg, which was more than 5 times better than the MTD of free Bupivacaine. In Beagle dogs MTD was > 60mg/kg. Electrocardiogram and troponin/CPK results demonstrated lack of cardiotoxicity. Bupigel also lack irreversible neuro-toxicity determined in a sciatic-posterior tibial nerve conductance test in which local administration of free bupivacaine and lidocaine exhibited neuro-toxicity. Over all our data should sup-

port the claim that single administration of Bupigel<sup>TM</sup> will eliminate/strongly reduce moderate-to-severe pain for at least 72 hours in humans without major side effects. Hebrew University licensed this formulation to Lipocure LTD ([www.lipocurerx.com](http://www.lipocurerx.com)) who has selected Virpax Pharmaceuticals Inc. to help moving forward with clinical development for US and worldwide use.

\*The authors acknowledge with pleasure: (i). Yaelle Bavli for performing the sciatic-posterior tibial nerve conductance tests, and Hiba Kanaan for her technical help, both from the Barenholz lab; also,(ii). Lipocure Ltd team for partial scale up, QC and help in coordination of the animal studies

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## **EDUCATIONAL ECOSYSTEMS IN RAPIDLY CHANGING ENVIRONMENTS**

**JACK BAROKAS**

The concept of 'ecosystem' originates from biology, and usually refers to a complex system involving different species that work together to provide the conditions necessary for their survival. In recent years, this concept has acquired a more general meaning, and has expanded to include human actors or organizations, including educational institutions that collaborate as a system of interconnecting and interacting parts. In today's constantly changing world, educational institutions can survive and thrive only if they respond rapidly enough to adapt to new demands. However, successful adaption is an extremely complex process: Each component in the ecosystem must accurately assess its own unique situation to ensure optimal mobilization of its limited resources in order to meet new challenges and changing conditions.

The NanoEL ERASMUS+ project and Horizon 2020 Up2University (UP2U) project are two examples of EU- funded projects in which industry, universities and high schools formed an educational ecosystem to help stakeholders respond to the challenges of change. The NanoEL project focused on Nano-Microelectronics, which Tzanova, Stankovski and Schintke (2013) [2] have referred to as the one of the most rapidly developing areas of the science, in which learning materials should be innovated every year. In order to adequately equip their students for their future roles in the Nano industry, university lecturers must be able to respond in real time and constantly adapt their courses to keep pace with the new knowledge fields, technological and entrepreneurial skills the industry requires. Clearly, the Nano industry also depends on highly skilled graduates to continue the path-breaking advances that until now have characterized this industry. Consequently, these mutual dependencies and shared interests between industry and academia formed ideal conditions for interrelationships that resulted

in mutual benefits for all. The second use case, Up2U, aims at collaboration between high schools, universities as well as small and medium-sized enterprises from the hi-tech sector in order to bridge the gaps between high school and university. Although Up2U is still a work-in-progress, the first year of this project saw the completion of four gap analysis surveys that targeted school principals, high school teachers, school IT personnel and university lecturers in an attempt to identify how best to bridge the high school – university gap.

## POLYPEPT(O)IDES: FROM NOVEL MATERIALS TO SYSTEMS FOR DRUG AND NUCLEIC ACID DELIVERY

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The enormous potential of polymeric nanomedicines arises from the possibility to combine desirable material properties with compartmentalized functionalities in one distinct nanoparticle.<sup>[1]</sup> The price to pay for this multi-functionality is the intrinsic complexity of these systems. This synthetic complexity, however, is a major drawback for clinical translation and can prevent even the most effective nanomedicines from becoming drugs, since it complicates scale up and can affect reproducibility of material properties, being key requirements for transfer.<sup>[2]</sup> Consequently, a major task for polymer chemists is the development of synthetic pathways to biomaterials and functional systems, which intrinsically reduces chemical complexity, while preserving the desired multi-functionality.

With respect to these needs, we have developed reactive polypept(o)ides (polypeptoid-block-polypeptide copolymers),<sup>[3]</sup> which can be synthesized with precise control over molecular weight, low polymer dispersity, high end group integrity, and a variety of available reactive groups.<sup>[4,5]</sup> Furthermore, this class of materials is completely based on endogenous amino acids and combines the “stealth”-like properties of polysarcosine with the multi-functionality of polypeptides.

These polymers enable the straightforward synthesis of core cross-linked micelles (PeptoMicelles) and nanohydrogels (PeptoNanoGels),<sup>[6]</sup> bottlebrush polymers (PeptoBrushes)<sup>[7]</sup> and star-like polymers (PeptoStars),<sup>[8]</sup> with control over morphology and core as well as corona functionality, permitting us to systematically investigate the influence of particle properties on biological responses and ultimately to tailor particle properties to medical needs in diagnosis and therapy. An overview on preclinical research on polypept(o)ide-based nanoparticles in immune and chemo therapy of cancer,<sup>[9,10]</sup> inflammatory liver diseases,<sup>[11]</sup> gene therapy,<sup>[12,13]</sup> and on *in vivo* click chemistry for pre-targeting in relevant preclinical models will be presented.

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## INNOVATOR'S PERSPECTIVE ON UNDERSTANDING REGULATORY REQUIREMENTS FOR NANOMEDICAL PRODUCTS PRIOR TO INNOVATOR-REGULATOR INTERACTIONS

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Advances in engineering polymer chains at the molecular level have pushed the development of synthetic strategies for the controlled compaction of single polymer coils into unimolecular soft nano-objects, named single-chain polymer nanoparticles (SCPNS)<sup>[1]</sup>. SCPNs based on natural polysaccharides like Dextran, which mimic proteins folding mechanism, have shown promising results in different fields including nanomedicine<sup>[2]</sup>.

Our group has developed an environmentally-friendly strategy to easily obtain water-dispersible Dextran-based SCPNs (DXT-SCPNS) of around 20 nm (Figure 1)<sup>[3]</sup>.

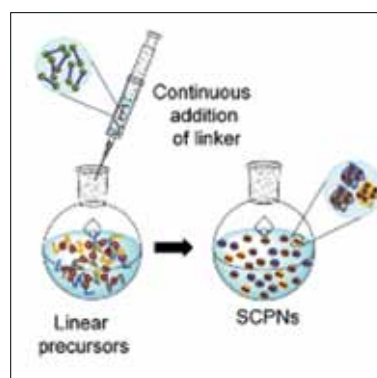


Figure 1. Schematic representation of the production of DXT-SCPNS.

*In vivo* distribution studies for DXT-SCPNS functionalized with contrast agent by single-photon emission computed tomography (SPECT) after aerosol administration to healthy rats showed very promising results to the use of SCPNs for nanomedicine applications (Figure 2).

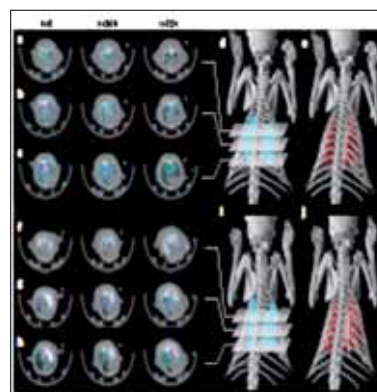


Figure 2. SPECT-CT images after administration of [67Ga]Gallium citrate and 67Ga-labelled SCPNs

These results confirmed the high potential of innovation for the SCPN technology, but clinical validation is required and the production of innovative nanopharmaceuticals

under Good Manufacturing Practices-GMP to enter clinical trials remains a challenge in most of the cases. In that sense, CIDETEC is establishing a flexible and adaptable pilot plant operating under GMP for the production of small batches of polymer-based nanopharmaceuticals in the framework of the Programme for Research and Innovation Horizon 2020 as NanoPilot project (Figure 3). In this context, our group worked from the very beginning of this development taking into account GMP guidelines, and the experience and knowledge gained during NanoPilot project implementation, in order to accelerate the translation into the clinic of the SCPN technology.

Thus, for future translation of the SCPNs technology to patients, special attention has been focused on characterization, as it re-



mains a big challenge for nanosystems, and in parallel the identification of the critical process parameters (CPPs) that can influence the physical-chemical characteristics and/or Critical Quality Attributes (CQAs) of our final product, i.e. a development based on Quality-by-Design (QbD) approach.

Figure 3. Focus of NanoPilot project in the product development of new nanopharmaceuticals.



As first action to achieve GMP compliance, using a pharma grade Dextran as starting polymer was identified as critical issue. Actually, changing the grade of the starting material (narrower molecular weight distribution) appeared to affect all the characterization previously set up in R&D, demonstrating the relative difficulty to transfer technology from laboratory to the requirement for GMP production (Figure 4). In that sense, reliable nanoparticle characterization techniques, such as Taylor dispersion analysis (diffusion coefficient, ViscosizerTD) and SEC-AF4 techniques in addition to DLS, TEM and GPC-SEC have been especially scrutinized to establish quality control and identify Critical process parameter during nanoparticles synthesis.

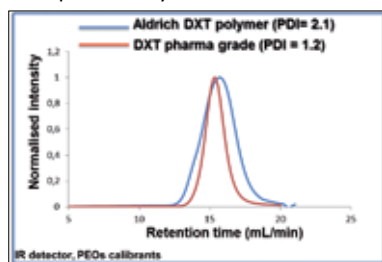


Figure 4. Molecular weight distribution of R&D grade dextran (blue, Aldrich) and pharma grade dextran (red) studied by GPC.

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## COMBINED THERAPEUTIC MEDICAL DEVICE AND STEM CELLS FOR REGENERATIVE NANOMEDICINE

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In our group we explore a new generation of smart living implants combining not only active therapeutics but also stem cells, as a novel strategy to regenerate stabilized cartilage and avoid prosthesis, by achieving regeneration of its subchondral bone foundation, requirement which is failing today in the clinic.

In our group, a unique nanotechnology strategy is used to entrap, protect, and stabilize therapeutic agents into polymer coatings: nanoreservoirs, covering nanofibres of implantable nanofibrous membranes for bone and cartilage regeneration. Upon contact with cells, therapeutic agents become available through enzymatic degradation of the nanoreservoirs. As cells grow, divide, and infiltrate deeper into the porous membrane, they trigger slow and progressive release of therapeutic agents that, in turn, stimulate further cell proliferation. The nanoreservoirs technology enables to reduce the quantities of required therapeutic agent (compared to soaked membranes for instance) thereby reducing costs.

### CLINICAL TRIAL: PHASE 1, (FR, UK, SP, SW) WILL BE SUBMITTED

Feasibility and safety assessment of a therapeutic implant based on an active polymeric wound dressing and autologous mesenchymal stem cells derived from bone marrow for the treatment of femoral cartilage isolated lesions

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## BE OF LIPOSOMAL PARENTERALS: SUGGESTIONS OF THE GLOBAL BIOEQUIVALENCE HARMONIZATION INITIATIVE CONFERENCE 2018

HENNING BLUME, SocraTec C&S, Frankfurt/Germany

The “Global Bioequivalence Harmonization Initiative” was started in 2010 by EUFEPS (European Federation for Pharmaceutical Sciences). Three main conferences have been organized so far with significant contribution by the European Authorities as well as US-

FDA and other Agencies around the world. BE of liposomal parenterals was one of the main topics of the third conference organized in May 2018 in Amsterdam/The Netherlands.

Two aspects have intensively been discussed based on experimental data presented during the conference, (1) the question of the most appropriate analyte – the active ingredient encapsulated in liposomes, the “free” compound or the total drug – to be measured and (2) the problem of different doses administered – due to patient’s advice in the labelling – to be considered for intraindividual comparison.

This discussion is an excellent example of establishing regulatory requirements based on scientific evidence and the essential contribution of pharmaceutical scientists from academia and industry to science-driven regulations. Suggestions derived from these contributions will be summarized and presented for further debate.

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## EUNCL: LESSONS LEARNED IN CHARACTERISATION OF NANOMEDICINES

**PATRICK BOISSEAU**, Coordinator, European Nanomedicine Characterisation Laboratory CEATech

In order to support the 500+ European SMEs and the 1500+ academic labs in nanomedicine in the development of their innovative nanoformulations, Europe initiated a unique infrastructure powered by an unprecedented international partnership to moving efficiently new nanoformulations from labs into products validated in clinics are established.

EUNCL, the European Nanomedicine Characterisation Laboratory, aims at fostering innovation in nanomedicine by providing access to state of the art full characterisation of nanomaterials intended for medical applications, developed by public labs, spin –offs and innovative SMEs. The EUNCL also serves as a European knowledge base for researchers and industry ensuring that European knowledge is documented in Europe for the benefit of the European economy, healthcare systems and patients.

EUNCL provides a trans-disciplinary comprehensive testing infrastructure covering a comprehensive set of preclinical characterisation assays (physical, chemical, in-vitro and in-vivo biological testing) allowing researchers to fully comprehend the biodistribution, metabolism, pharmacokinetics, safety profiles and immunological effects of their medical nanoparticles. But EUNCL is also fostering the use and deployment of standard operating procedures (SOPs), benchmark materials, and quality management for the preclinical characterisation of medical nanoparticles (nanoparticles used for medical applications) to promote inter-sectorial and inter-disciplinary communication among key drivers of innovation, especially between developers and regulatory agencies.

To fulfill its mission EU-NCL aims to achieve 4 major objectives:

- **Objective 1:** To qualify a comprehensive portfolio of medical nanoparticles preclinical characterisation assays (more than 40 assays) within an efficient collaborative environment over the first year of EUNCL.
- **Objective 2:** To provide preclinical characterisation of medical nanoparticles to researchers, developers and inventors from academia and industry developing Med-NPs by opening transnational access (TNA).
- **Objective 3:** To constantly refine and upgrade the assay portfolio and processes of EU-NCL.

As nanomedicine is a fast evolving field of research, it is a key objective for EUNCL to constantly refine and adapt its assay portfolio and processes in order maintain the provision of state-of-the-art TNA to the scientific community. Therefore, we will progressively

implement additional assays to increase our characterisation capacity, for instance in terms of medical application or route of administration.

4. **Objective 4:** To disseminate the EU-NCL findings to the nanomedicine stakeholders in order to strengthen the innovation potential in that field.

The emphasis of EUNCL is to serve as a nexus for trans-disciplinary research, development and clinical applications of nanotechnology. Therefore, lessons-learned, best practices, knowledge, tools and methods will be made available to the scientific community such as academic researchers, industry, regulatory bodies, metrology institutes and others. However, care will be taken to ensure that proprietary information and materials disclosed to the EU-NCL by the TNA users are protected.

EU-NCL provides a comprehensive set of characterisation tests (physical, chemical, *in vitro* and *in vivo* biological properties) allowing researchers and SMEs to better understand or predict the clinical *in vivo* effects of their medical nanomaterials. On top of that, a full characterisation is required by regulation agencies before approval of any tests on human beings. The knowledge base being developed by EUNCL helps the European Medicines Agency (EMA) or other relevant agencies (e.g. notified body) to adapt the current regulation and approval process to Nanomedicine products.

The EU-NCL also has a strategic and political role in helping newcomers, like spin-offs or SMEs, in getting an easy access to nano-characterisation and further to prepare their submission for product approval.

EUNCL is supported by 8 partners, connecting their 7 analytical platforms over Europe and USA. EUNCL is in operation since May 2015. Its Transnational Access is open to all developers of nanomedicines from August 2016.

More info at : [www.euncl.eu](http://www.euncl.eu)

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## ENGINEERING NANOPARTICLES FOR A CONTROLLED REDUCTION OF INTRATUMORAL cAMP LEVEL

**TOBIAS BOPP**<sup>1,2,3</sup>

- 1 Institute for Immunology, University Medical Center Mainz, Germany,
- 2 Research Center for Immunotherapy (FZI), University Medical Center Mainz, Germany
- 3 German Cancer Consortium (DKTK)

The vertebrate immune system has evolved to a specialized network of different cell types protecting the organism against foreign invading pathogens while at the same time ensuring tolerance to self-tissues. In addition, the immune system is believed to keep self-tissues under surveillance with the aim to identify and eliminate malignant cells. Yet, tumors can develop in the presence of a functioning immune system, demonstrating the imminent medical need to generate novel immunotherapies for the treatment of cancer. A main obstacle for such an endeavor is the fact that tumor cells have developed sophisticated immune evasion strategies leading to inefficient anti-tumor immune responses. Here, we have identified cyclic AMP as a major driver of tumor immune evasion in melanoma. Due to its central role in a great variety of different physiological processes systematical modulation of cyclic AMP not only considerably improves anti-tumor immune responses but inevitably evokes severe side effects. Therefore, sophisticated therapeutic strategies that ensure targeted drug delivery to tumor invading immune cells are required to maintain self-tissue tolerance while at the same time strengthening anti-tumor immune responses. In this presentation I will highlight the molecular mechanisms underlying cAMP-driven tumor immune evasion and how these mechanisms can be targeted by nanoparticle-based drug delivery to enforce efficient anti-tumor immunity.

## EVALUATION OF NANOMEDICINE: PHYSICAL-CHEMICAL INVESTIGATION OF IRINOTECAN BASED LIPOSOMES

SVEN EVEN BORGOS<sup>2</sup>, Fanny Caputo<sup>1</sup>, Neill Liptrott<sup>3</sup> and Adriele Prina-Mello<sup>4,5</sup> on behalf of the EUNCL consortium

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EU-NCL consortium has developed a full set of standard operating procedures (SOPs) for the characterization of Med-NPs, including the characterization of their physico-chemical attributes, *in vitro* and *in vivo* safety. This section would like to offer an in-depth discussion on the pre-clinical characterisation of Med-NPs, focusing on the lessons learned from the characterization of a liposomal formulation of irinotecan.

### PHYSICAL CHARACTERISATION (F CAPUTO):

Particle size, particle size distribution (PSD) and particle concentration are key factors for the manufacturing quality, as well as the efficacy and safety of Med-NPs. The most widespread tool for the determination of PSD, batch mode dynamic light scattering (batch-DLS), completely fails in characterising complex systems such as multimodal populations or not spherical particles. Moreover, it is not able to resolve mixtures of pristine particles and small aggregates (such as dimers and multimers), or mixtures of Med-NPs and plasma proteins. In the EU-NCL consortium the analysis of PSD and morphology of complex systems is performed by using a combination of orthogonal techniques, including Asymmetrical Field Flow Fractionation (AF4) combined with online light scattering (AF4-DLS-MALS), nanoparticle tracking analyzer (NTA), analytical ultracentrifugation (AUC) and electron microscopy (EM). A typical examples of a liposome formulations of irinotecan will be presented. Batch mode DLS showed one population with a polydispersity index (Pdl) < 0.2, which is usually obtained for well monodisperse samples. Analyzing samples with AF4-MALS-DLS and NTA indicated the presence of particles of different sizes and shapes, also confirmed by direct imaging of the sample using cryo-TEM (spherical liposomes vs coffee beans like particles). The simple batch mode DLS analysis provided somewhat misleading results, while only by the combination of high resolution orthogonal techniques, we were able to reveal the real nature of the samples.

### CHEMICAL CHARACTERISATION (SE BORGOS):

Chemical composition, e.g. the concentration of API and excipients, is critical for any medicinal product. Med-NPs, however, come with some particular challenges. The API can exist both in an encapsulated and free form, and only the latter exhibits biological activity. Both the total API loading, the amount of free API and the release rate of the encapsulated fraction into different liquid media then becomes interesting attributes.

For the liposomal irinotecan formulation studied, an additional complexity is introduced by the fact that irinotecan is a prodrug, which is decarboxylated into the compound SN-38 with a much stronger (> 1000x) antineoplastic activity. SN-38 is further deactivated and prepared for excretion by glucuronidation. We verified the lipid composition and API loading in the liposomal formulation, and showed that metabolic conversion and effective API exposure in *in vitro* cytotoxicity assays was formulation-dependent. Furthermore, release of irinotecan into plasma was investigated. The ana-

lytical methods were further extended to investigate biodistribution and pharmacokinetics *in vivo* in rats (see the complementing EUNCL contribution).

## 34. EU-NCL EUROPEAN NANOMEDICINE CHARACTERIZATION LABORATORY – LESSONS LEARNED

### The Regulators' Needs; Towards Standardization DR. SUSANNE BREMER-HOFFMANN

A detailed understanding on regulatory information requirements and accepted test methods for the physical, chemical and biological characterisation of nanomedicines is essential to fully exploit the potential of nanotechnology in health applications. Appropriate documentary standards and reference materials are crucial building blocks in this process as they will reduce the uncertainty for product developer and support the mutual acceptance of data in various markets. However, robust datasets allowing firm conclusions on regulatory demands are not yet available due to a lack of regulatory experience with such innovative products. In order to resolve this catch-22 situation, an iterative process monitoring continuously the scientific evidence and promoting intensive knowledge exchange between all involved stakeholders is required. In order to review the current situation of existing standards, we have brought together recommendations proposed by regulatory scientists<sup>1</sup>, in reflection papers published by the European Medicines Agency (EMA)<sup>2</sup>, or which have been identified in surveys organised by the EU-NCL with the regulatory community<sup>3</sup>. These information requirements have been mapped against existing standards. In our analysis, we observed that only a very limited number of standards were designed for nanomedicines and other existing standards have not been systematically assessed for their suitability to evaluate nanomedicines. We could demonstrate that there is a need to evaluate existing standards as they might have some shortcomings when it comes to the testing of nanomedicines<sup>4</sup>. For other necessary endpoints such as drug release/loading and the interaction of nanomedicines with the immune system no standards are available so far. However, the emerging nanomedicine sector could benefit from cross-sector collaboration and review the suitability of standards that have been developed for nanomaterials used for other industrial applications. Only a concerted action of all parties can lead to a smooth translation of nanomedicines to clinical applications and to the market which is in particular important since nanotechnology based drug delivery systems are a key element for the development and implementation of personalised medicine. *Nanomedicine* 13 (4), pp 539-554

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## DISSEMINATION FOR LAY PUBLIC IN NANO-MEDICINE: A GAME FOR NANOMEDICINE IN ATHEROSCLEROSIS

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Clinam brings together scientists and clinicians applying nanotechnology to clinical medicine. Its underlying assumption is that nanomedicine will help treat sick or injured people that we struggle to treat at present, or can forewarn us of a medical condition in time to intervene, maybe even prevent it. We assume that most people would agree that it's a good thing, and will welcome the drugs, clinical procedures, devices and tests that nanomedicine is enabling. But acceptance may not happen automatically. The 'nano' word is still unfamiliar to many lay people: isn't it something to do with fancy high tech materials, and strange particles? That sounds OK for paints, solar panels and tennis rackets, but it could be a bit scary if it comes close to my body. As nanomedicine becomes more established in clinical use, it is important that we build bridges to the general public and patients.

In the recently completed EC FP7 NanoAthero project, to demonstrate the clinical feasibility of nanosystems for imaging and treating atherosclerotic plaques, we sought to address this is to engage with people interactively in the form of a Democs\* card game. The Democs concept is a group activity for 6-8 people on a new technology, using cards from which people learn, discuss together and come to their own views. It has been widely used for over 15 years, on different subjects as diverse as climate change and tuberculosis. We created a game to explore nanomedicine in general and its application to atherosclerosis. In this talk we will present the concept, some of the first results from playing the game, and explore its potential for wider use.

The value of Democs is that it is a grassroots method, without needing experts or any prior technical knowledge - the cards are the 'expert'. It can be played by groups of people anywhere, something to do with your friends one evening. It uses three sets of cards: case studies and factual cards to introduce the scientific topic in ways understandable to lay people, and then 'issue cards' to present ethical and social implications for discussion. The group context is of great value in helping people to learn together, benefit from each other's viewpoints, and to seek to come to both individual and collective understandings. At the end, participants are invited to give written statements and opinions, and these qualitative outputs can then be analysed.

\* Democs (also known as Decide) was invented in 2001 by Perry Walker, then of the New Economics Foundation. The NanoAthero game is available via [www.nanoathero.eu](http://www.nanoathero.eu) and [www.edinethics.co.uk/democs/nanoathero](http://www.edinethics.co.uk/democs/nanoathero)

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## THE FIRST EXTRACELLULAR VESICLE-MADE SUPPORTED LIPID BILAYER BIOSENSOR

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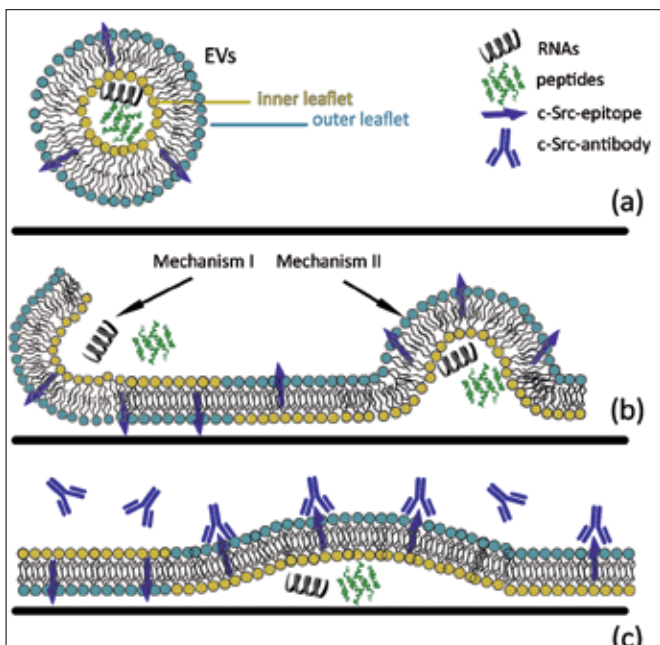
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Extracellular vesicles (EVs) are nanoparticles delimited by a cell-derived lipid bilayer, which encloses, transports, and protects bioactive cargo. EVs include microvesicles (or ectosomes), which are 100-1000 nm in size and bud from the plasma membrane, and exosomes that are of endosomal origin with a size ranging from 30 to 150 nm<sup>[1]</sup>. EVs are produced to carry cell-derived compositional signatures (e.g. proteins and nucleic acids) that mediate communication between cells, supporting both physiological and pathological processes<sup>[2, 3, 4]</sup>. Pathophysiological roles for extracellular vesicles are highly recognized in diseases such as cancer, infections, and neurodegenerative disorders. For instance, it has been shown that the levels of circulating EVs increase more than twofold in patients with multiple myeloma<sup>[5]</sup>. Furthermore, it has been demonstrated that pancreatic ductal adenocarcinoma-derived exosomes induce liver pre-metastatic niche formation in mice and consequently increase the metastatic burden. The importance of EV-mediated signaling in cancer progression makes these biological nanoparticles a novel class of therapeutic targets. Consequently, the development of therapeutic interventions to reduce the load of circulating EVs or block crucial components of EVs is underway. Therefore, various possibilities can be envisioned for inhibiting EV-mediated crosstalk, although they are still largely theoretical<sup>[6]</sup>. However, it is important to emphasize that interfering with EV biogenesis could result in undesirable off-target effects as EVs are important for the regulation of normal biological processes. Increasing knowledge of EV biology (release, targeting abilities, uptake processes etc.) will help address these issues and should also elucidate novel therapeutic strategies for inhibiting EV-mediated intercellular communication in cancer<sup>[7, 8]</sup>. Therefore, there is a growing need for high throughput analytical drug discovery techniques incorporating EV membranes. Moreover, the majority of drugs applied in clinical practice or under development target membrane markers on cells, making the analysis of molecular membrane interactions a key part of the drug discovery process<sup>[9, 10]</sup>. To develop a personalized and EV-centered approach to drug discovery, we have designed the first EV supported lipid bilayer (SLB) biosensor, where EV properties are projected onto a 2D platform. Our EV-SLB has structural membrane characteristics that are linked to the function of the originating cells. We have conducted a proof of concept study based on membrane formation kinetics, structure, and properties of the SLB obtained from TRAMP-C2 murine prostate cancer cell-derived EVs.

In particular, we investigated if the 2D topology of the EV-SLB retains the properties of the native 3D EV membrane. For instance, we show that the proto-oncogene tyrosine-protein kinase Src (c-Src) is easily detectable on our membrane biosensor. This protein is involved in approximately 50% of colon, liver, lung, breast, and pancreas cancers, and plays a key role in signaling, endocytosis and exocytosis processes<sup>[11]</sup>. Specifically, c-Src has been found to be highly enriched in exosomes produced by prostate cancer cells, where it is associated with the outer leaflet of the EV membrane<sup>[12]</sup>. The presence, recognition capability, and distribution pattern of c-Src indicated that the biosensor displays regions that preserve the native orientation and characteristics of EVs. We propose a "parachute-type" mechanism for membrane formation, where the inner leaflet of the EV becomes the lower leaflet of the bilayer, as shown in Figure 1.

*Figure 1. Formation and structure of a supported lipid bilayer (SLB) from extracellular vesicles (EVs). (a) Schematic representation of an EV derived from TRAMP prostate cancer cells. (b) EV fusion and SLB completion occurs through two distinct mechanisms. Mechanism I results in the exposure of the inner leaflet to the aqueous solution and concomitant release of the biomolecular content of EVs. Mechanism II results in the exposure of the outer leaflet to the aqueous solution in parachute mode. (c) On the final EV-SLB, tyrosine-protein kinase Src (c-Src) antibody recognition is possible.*



This study demonstrates that we can produce SLBs on a solid surface that mimic key features of prostate cancer cell-derived EVs. This biogenic surface system represents the first example of 2D EV engineering<sup>[13]</sup>.

From a translational perspective, EV-SLBs can provide streamlined fabrication of surfaces that reproduce the structure and function of biological membranes, which traditionally poses fundamental and technical challenges. This platform can contribute to a breakthrough in surface biotechnology through the development of simpler, more specific, and personalized biosensors. Although further investigation into membrane mobility and functionality is desirable to better understand the potential of EV-SLB, we believe that this study provides crucial information on EV-surface interactions and fosters the study of inaccessible EV properties.

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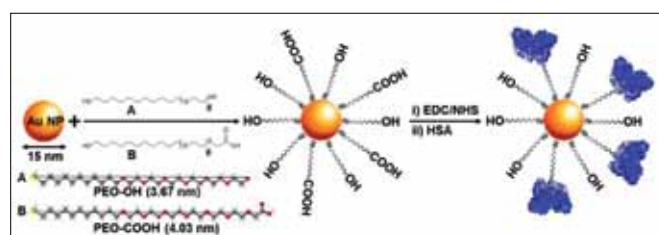
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## RATIONAL DESIGN AND IN-DEPTH CHARACTERIZATION OF TARGETED, MULTI-FUNCTIONAL GOLD NANOPARTICLES

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Multi-functionalized nanoparticles are of great interest in biotechnology and biomedicine, especially for diagnostic and therapeutic purposes. However, at the moment the characterization of complex, multi-functional nanoparticles is still challenging and this hampers the development of advanced nanomaterials for biological applications. We have designed a model system consisting of gold nanoparticles functionalized with two differentially-terminated poly(ethylene oxide) ligands, providing both “stealth” properties and ligand-binding capabilities to the nanoparticles. We use a combination of techniques (SDS-PAGE, Centrifugal Liquid Sedimentation, Dynamic Light Scattering, Flow Field Flow Fractionation, Transmission Electron Microscopy, and Circular Dichroism) to:

- Monitor and quantify the ratios of ligand molecules per nanoparticle;
- Determine the effect of coating density on non-specific protein adsorption;
- Assess the number and structure of the covalently-bound proteins.

This presentation will provide a suite of analytical methods for the systematic characterization on functionalization of AuNPs.

This suite of analytical methods is easily applicable to different classes of nanodrug delivery systems and could serve as an excellent reference for all researchers who work in the field.

I Ojea-Jiménez et al., Rational design of multi-functional gold nanoparticles with controlled biomolecule adsorption: a multi-method approach for in-depth characterization. *Nanoscale*. In press.

## GOLD NANOPARTICLE-BASED ARTIFICIAL ANTIBODY CREATED BY CONFORMATIONAL ENGINEERING

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**Keywords:** Artificial Antibody; Conformational Engineering; EGFR; Goldbody; gold nanoparticle.

Proteins are the most miraculous nano-machines in nature. To mimic protein-like specific interactions and functions is a long-pursuing goal for nanotechnology. The key challenge is to precisely organize non-functional surface groups on nanoparticles (NPs) into specific 3D conformations to function in a concerted and orchestrated manner.

Interestingly, the function of a protein might be reproduced by grafting its key residues onto another heterologous protein skeleton that is suitable to anchor them in the right conformation. Inspired by this fact, we hypothesized that nanoparticles (NPs) could serve as such skeletons, and the key residues of a protein could be installed conformation-correctly onto NPs to reconstruct the function of the original protein [1]. After almost 10 years of efforts, we have finally developed a “conformational engineering” method to graft the complementary-determining regions (CDR) of natural antibodies onto NPs and reconstruct their “active” conformation to create gold NP (AuNP)-based artificial antibody, denoted as Goldbody. Upon anchoring the both terminals of the free CDR peptide, which has no defined conformation and function alone, on AuNPs, we managed to reconstruct the original “active” conformation of the CDR peptide in the natural antibody by tuning the span between the two terminals. As a result, the original specificity of the natural antibody has been successfully reconstructed on the AuNPs. Two Goldbodies have been created by this “conformational engineering” method to specifically bind with hen egg white lysozyme (HEWL) and epidermal growth factor receptor (EGFR, a common cancer target) (see Fig. 1 for the binding model), respectively, with affinities several orders of magnitude stronger than the natural antibodies [2]. Goldbodies have several advantages as compared to the natural antibodies, including stronger affinity, much better stability and no humanization problem, and thus are very promising for biomedical applications.

Our work demonstrates that it is possible to create protein-like functions on NPs in a protein-like way, i.e. organizing multiple surface groups into the correct conformation to work in a concerted and orchestrated manner. Given the apparent merits of Goldbodies, we anticipate that a category of Goldbodies could be created to target various antigens, thus could be used as good substitutions for natural antibodies for various applications.

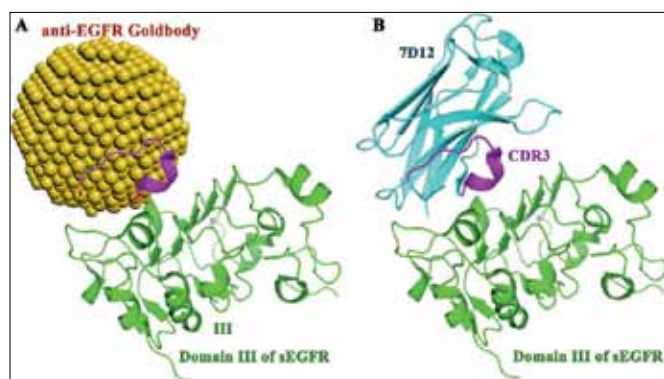


Fig. 1. A) Model of the anti-EGFR Goldbody (with the grafted peptide loop shown in magenta) in complex with the domain III of sEGFR (green). B) Structure of antibody 7D12 (cyan, with the CDR3 shown in magenta) in complex with the domain III of sEGFR (green) (PDB code: 4KRL).

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## EVALUATION OF NANOMEDICINE: PHYSICAL-CHEMICAL INVESTIGATION OF IRINOTECAN BASED LIPOSOMES

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EU-NCL consortium has developed a full set of standard operating procedures (SOPs) for the characterization of Med-NPs, including the characterization of their physico-chemical attributes, *in vitro* and *in vivo* safety. This section would like to offer an in-depth discussion on the pre-clinical characterisation of Med-NPs, focusing on the lessons learned from the characterization of a liposomal formulation of irinotecan.

### PHYSICAL CHARACTERISATION (F CAPUTO):

Particle size, particle size distribution (PSD) and particle concentration are key factors for the manufacturing quality, as well as the efficacy and safety of Med-NPs. The most widespread tool for the determination of PSD, batch mode dynamic light scattering (batch-DLS), completely fails in characterising complex systems such as multimodal populations or not spherical particles. Moreover, it is not able to resolve mixtures of pristine particles and small aggregates (such as dimers and multimers), or mixtures of Med-NPs and plasma proteins. In the EU-NCL consortium the analysis of PSD and morphology of complex systems is performed by using a combination of orthogonal techniques, including Asymmetrical Field Flow Fractionation (AF4) combined with online light scattering (AF4-DLS-MALS), nanoparticle tracking analyzer (NTA), analytical ultracentrifugation (AUC) and electron microscopy (EM).

A typical examples of a liposome formulations of irinotecan will be presented. Batch mode DLS showed one population with a polydispersity index (Pdl) < 0.2, which is usually obtained for well monodisperse samples. Analyzing samples with AF4-MALS-DLS and NTA indicated the presence of particles of different sizes and shapes, also confirmed by direct imaging of the sample using cryo-TEM (spherical liposomes vs coffee beans like particles). The simple batch mode DLS analysis provided somewhat misleading results, while only by the combination of high resolution orthogonal techniques, we were able to reveal the real nature of the samples.

### CHEMICAL CHARACTERISATION (SE BORGOS):

Chemical composition, e.g. the concentration of API and excipients, is critical for any medicinal product. Med-NPs, however, come with some particular challenges. The API can exist both in an encapsulated and free form, and only the latter exhibits biological activity. Both the total API loading, the amount of free API and the release rate of the encapsulated fraction into different liquid media then becomes interesting attributes.

For the liposomal irinotecan formulation studied, an additional complexity is introduced by the fact that irinotecan is a prodrug, which is decarboxylated into the compound SN-38 with a much stronger (> 1000x) antineoplastic activity. SN-38 is further deactivated and prepared for excretion by glucuronidation. We verified the lipid composition and API loading in the liposomal formulation, and showed that metabolic conversion and effective API exposure in *in vitro* cytotoxicity assays was formulation-dependent. Further-



more, release of irinotecan into plasma was investigated. The analytical methods were further extended to investigate biodistribution and pharmacokinetics *in vivo* in rats (see the complementing EUNCL contribution).

## CHALLENGES AND OPPORTUNITIES OF NANOMEDICINE APPROACHES TO INDUCE IMMUNE TOLERANCE IN TYPE 1 DIABETES

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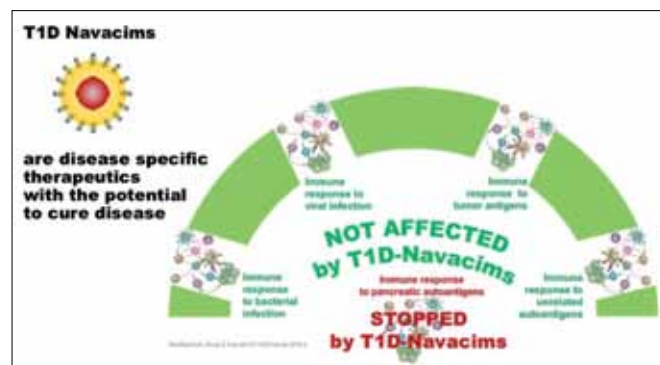
Banting and colleagues pioneered insulin therapy in 1922 and provided the first effective treatment to control type 1 diabetes (T1D). Nearly 100 years later, we do not have alternatives to insulin, which although enables control of glucose homeostasis does not impact the underlying pathological process.

T1D, like other autoimmune diseases, develops when immune tolerance breaks down. Immune tolerance is an active process of specific unresponsiveness to self-antigens, which is different from non-specific immune suppression and immune deficiency. Immune tolerance is initiated in the thymus by the process of clonal selection, which eliminates all T cells displaying strong reactivity for self-antigens and only exports those T cells reacting poorly to self-antigens to the periphery. In individuals with a genetic predisposition to T1D, the threshold of activation of these thymic escapees in response to pancreatic beta cell autoantigens is reduced. Diminished stringency for T cell activation facilitates the expansion and differentiation of effector T cells that attack pancreatic-tissue and promote life-long autoimmunity. The autoimmune process of T1D could remain unrecognized for years. However, the progression into full-bloom disease is reliably predicted by the presence of autoantibodies to pancreatic antigens. Once overt T1D is diagnosed, the only currently available therapeutic solution is insulin replacement. Unfortunately, despite all advances in formulation and dosology, exogenous insulin therapy cannot control glucose homeostasis to the degree achieved with endogenous insulin secretion. As a result, patients are at risk of developing a whole host of serious complications, including blindness, kidney failure, stroke, myocardial infarction, which result on a significant lower life expectancy.

The immunological approaches aiming to ameliorate T1D can be grouped into three major categories: i.) immune intervention with broadly acting agents, which are not specific to the disease and often increase the risk of infections or malignancies; ii.) transplantation of ex-vivo differentiated beta cells, which need sophisticated encapsulation devices to shield them from the ongoing immune attack; iii.) restoration of immune tolerance, which offer the most promising choice to control, and perhaps even cure, the disease. In recent years, many academic and biotech groups have proposed clinical interventions to induce immune tolerance. Among those, engineering the delivery of autoantigen peptides in a tolerogenic form, with the help of cells, biologics and particularly nanoparticles, seem to be a superior choice. We will review the different approaches reported in the literature, with particular emphasis on Navacims: peptide-major histocompatibility complex- (pMHC)-based nanomedicines (Clemente-Casares et al., 2016).

Navacims prevents disease in pre-diabetic non-obese diabetic (NOD) mice and reverses disease in diabetic NOD mice, without compromising systemic immunity. Human-specific Navacims expand human regulatory T cells in immune deficient mice reconstituted with peripheral blood mononuclear cells from T1D patients. Navacims represent a very attractive therapeutic option for T1D patients with recent disease onset. Since the disease is strongly

linked to a few specific MHC haplotypes, it is envisioned that in the future this approach could also be applied prophylactically to patients at risk. Finally, studies from the Network for Pancreatic Organ Donors with Diabetes (nPOD) suggest that pancreata of T1D patients with long-standing disease retain regenerative capacity and thus, we could speculate that beta cell function in these patients could recover and sustain significant insulin production once all the inflammatory autoimmune stress is released, which will extend the benefit of Navacims also to this patient's population. In summary, nanomedicine therapies, capable of promoting immune tolerance to pancreatic antigens, offer the potential to ameliorate T1D in already diseased patients and prevent its outbreak in predisposed individuals, which in the long term could result in a complete eradication of T1D.



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## CLARITHROMYCIN-PLGA NANOCAPSULES: A PROMISING STRATEGY TO TARGET INTRACELLULAR STAPHYLOCOCCUS AUREUS AND MYCOBACTERIUM ABSCESSUS INFECTIONS

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**Background:** Nanoparticle drug delivery systems are promising for targeting antibiotics directly to infected tissues. Of note, severe chronic pulmonary infections usually are a mark in diseases like cystic fibrosis (CF), which is characterized by polymicrobial infections that frequently led to chronic inflammation and decline of lung functions. Besides biofilm formation, some CF pathogens may also become intracellular, like *Staphylococcus aureus* and *Mycobacterium abscessus*. Due to the extensive use of antibiotics and their limited intracellular bioavailability, those bacteria have developed resistance. Clarithromycin (CLARI), a hydrophobic macrolide, is an antibiotic recommended in respiratory diseases, not only due to its immunomodulatory effect but also due to a broad antimicrobial activity against respiratory pathogens. However, CLARI limited aqueous solubility and low oral bioavailability lead to several side effects and pathogen resistance. Therefore, our hypothesis is to overcome these problems by designing nanocarriers for improving the intracellular delivery of anti-infective agents like CLARI. **Aim:** to encapsulated clarithromycin in chitosan-coated poly(Lactic-co-Glycolic Acid) nanocapsules (NC-Clari), suitable for aerosol delivery by nebulization of an aqueous dispersion, to target *S. aureus* and *M. abscessus* inside macrophages. **Methods:** PLGA-NCs with non-ionic

## SUGAR BASED SURFACTANTS FOR BIOTHERAPEUTICS PERMEABILITY-ENHANCEMENT

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Surface-active agents characterized by a carbohydrate polar head group, linked to alkyl or acyl chains, are classified as non-ionic sugar-based surfactants and they are considered promising candidates for a wide range of applications due to their attractive physicochemical properties<sup>[1]</sup>.

As regards the pharmaceutical perspective, this class of amphiphilic compounds demonstrated the capacity to modify bioavailability of drugs in different dosage forms by influencing dissolution and absorption of payload.

They can in fact represent a suitable alternative to the commonly employed non-ionic surfactants (e.g. polysorbates). For instance, alkyl glycosides have been proposed as replacers of polysorbates in biologics commercial formulations, because of their ability not to induce progressive protein degradation or increased immunogenicity, during manufacturing or storage time prior to administration<sup>[2]</sup>.

Moreover, such molecules could be used to formulate nanotechnology-based drug delivery systems with the potential to reach a specific target by interact with sugar receptor overexpressed in some pathogenic cells.

In our group we recently explored this class of surfactants as epithelial permeation enhancers for biotherapeutics<sup>[3]</sup>. We chemically or enzymatically synthesized a library of sugar-based surfactants, which after chemico-physical characterizations have been applied to formulate innovative drug delivery systems.

By varying the polar head and the hydrophobic tail, surfactants with different physicochemical characteristics can be easily prepared. While many research papers have focused on sucrose derivatives, relatively few studies have been carried out on lactose-based surfactants.

Thermal, surface, and aggregation properties of the synthesized molecules were studied in detail. Later we performed on them biological in vitro tests to determine their safety profile versus various cell lines and successively tested them as excipient able to allow the overcoming of biological barriers (Trans Epithelial Electrical Resistance and permeability assays), particularly for therapeutics macromolecules. Ex-vivo experiments (Ussing chambers) have been also conducted before to test them in vivo.

Interestingly sugar-based surfactant displayed a satisfactory anti-microbial activity over a range of Gram-positive and Gram-negative bacteria.

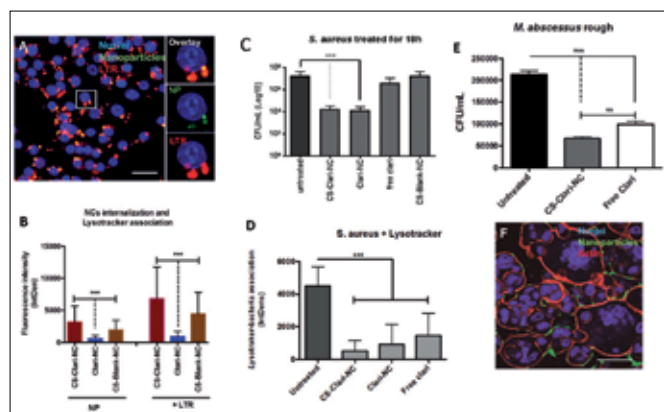
Overall, the obtained results can be considered promising for a further development in drug delivery systems as alternative biodegradable excipients for pharmaceutical applications.

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(CLARI-NC) and cationic surface (CS-CLARI-NC) were prepared using the interfacial deposition of the preformed polymer technique<sup>[1]</sup> and loaded with the antibiotic clarithromycin (CLARI). The drug permeability and effects on epithelial barrier function were assessed after aerosol deposition on air-liquid cultured Calu-3 monolayers. Anti-bacterial efficacy was assessed in infected RAW macrophages. **Results:** The particle size of both nanocarriers was in the order of 100 nm with low polydispersity indexes (~0.15). Low pH (4.2) and positive zeta potential (+16.5 mV) of CS-CLARI-NC confirmed the cationic surface owing to the presence of chitosan around the nanocapsules, while non-ionic NCs showed a negative zeta potential (-28.2 mV). For both formulations, clarithromycin loading rate was around 23 mg/g and the drug released approximately 65% after 24h (data not shown). Nanocapsules were efficiently phagocytosed in RAW macrophages, in which chitosan coated nanocarriers showed the highest internalization and nanocarriers were found in lysosomes, after 18 hours (Figure 1A, B). NCs internalization did not show any toxic effect neither on macrophages nor in lung epithelial cells (data not shown). Compared to the same dose of the free clarithromycin, nanoencapsulation reduced the *S. aureus* survival in murine RAW macrophages by about 1000 X (3 logs) (Figure 1C). While untreated *S. aureus* was located in acidic compartments, the treatment with Clari-loaded NCs abrogated such acidification (Figure 1D), thus disrupting bacteria survival and the regular cycle of infected macrophage-lysis. Cs-Clari-NCs were also effective against intracellular *M. abscessus* (Figure 1E). In comparison to free Clari, NC-Clari increased drug permeability in Calu-3 monolayers, without affecting cell viability and trans-epithelial resistance (data not shown). Contrasting with the high uptake in RAW macrophages, NC-Clari were less uptaken and were preferentially bounded to the plasma membrane (Figure 1F).

Figure 1. A) Representative confocal pictures showing lysosome (red), nanoparticle (green) and channels overlay. N= cell nucleus. Bar 10µm. B) Intracellular NCs quantification in RAW cells measured by green fluorescence per cell and quantification of lysotracker red association with NCs. C) Survival of intracellular *S. aureus* measured by CFU at 18h hours after treatment. D) Quantification of lysotracker red association with intracellular *S. aureus* in untreated cultures and treated with NCs or free drug. E) Survival of intracellular *M. abscessus* measured by CFU at 18h hours after treatment. F) Fluorescent confocal microscopy of Calu-3 cells showing nanocapsules association with Calu-3 cells. Bar: 20 µm. \*\*\*  $p < 0.0001$ , 1-way ANOVA with Bonferroni's post-hoc test.



**Conclusion:** These data reveal that such nanotechnology-based strategy may improve the intracellular delivery of antibiotics in cystic fibrosis lung infection. The anti-microbial activity of CLARI-PLGA NCs against intracellular *S. aureus* and *M. abscessus* might add value to the treatment of polymicrobial infections.

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## ONGOING PHASE 2 TRIAL OF LEAD CANDIDATE, SEL-212, IN DEVELOPMENT FOR CHRONIC SEVERE GOUT

**DR. WERNER CAUTREELS**, CEO, Selecta Biosciences, Watertown/Boston, MA (USA)

**Background:** Pegylated uricases are promising therapies for the treatment of severe chronic gout, but are limited by their immunogenicity. We have previously shown that synthetic vaccine particles encapsulating rapamycin (SVP-Rapamycin) co-administered with pegsiticase prevented the formation of anti-drug antibodies (ADAs) in a dose-dependent manner. A Phase 1 study of SEL-212, a novel combination product consisting of pegsiticase, a pegylated uricase, and SVP-Rapamycin, demonstrated sustained control of serum uric acid (sUA) levels for at least 30 days after a single dose. Here we report data from an ongoing Phase 2 dose ranging trial on the safety, tolerability, and effects on sUA, ADAs, and gout flares of repeated monthly doses of SEL-212 in symptomatic gout patients treated with different doses of SVP-Rapamycin in combination pegsiticase.

**Methods:** Patients with symptomatic gout ( $\geq 1$  tophus, gout flare within 6 months, and/or gouty arthropathy) and elevated sUA (sUA  $\geq 6$ mg/dL) were enrolled in different SEL-212 treatment cohorts. Patients received three monthly doses of SEL-212 (pegsiticase combined with SVP-Rapamycin) followed by two monthly doses of pegsiticase alone. Safety, tolerability, sUA, and ADAs were monitored, and clinical data were collected.

**Results:** As of 1 June 2018, about 150 patients had been dosed in the Phase 2 dose ranging study. Approximately 80% of evaluable patients in the clinically active dose range receiving maintained sUA levels substantially below 6 mg/dl at week 12 after three monthly doses of SEL-212. The sustained reduction of sUA correlated with low or no ADAs. SEL-212 was generally well tolerated and associated with a low rate of gout flare rates. Only 27% of all current patients in the SEL-212 Phase 2 trial, experienced gout flares after the first month of treatment with continued reduction of gout flare rates over months two to five. This low rate of gout flares appears to be in contrast with higher incidence of gout flares reported in clinical trials involving other urate lowering therapies. SEL-212 has been generally well tolerated at clinically active dose levels and infusion reactions observed with repeat dosing were reduced with increasing doses of SVP-Rapamycin.

**Conclusions:** SEL-212 has been well tolerated, mitigated immunogenicity in a dose-dependent manner, and enabled sustained control of sUA levels in patients with symptomatic gout and hyperuricemia.

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## UPDATES ON THE DEVELOPMENT OF SELF-ASSEMBLING ANTIBODIES

**MARK CHIU**

Therapeutic Biologics designed to bind to multiple targets drastically alter the landscape of drug development by achieving previously unattainable levels of affinity and specificity. Antibodies that bind to respective antigens individually are being used to create novel bispecific antibodies that manipulate complex biological systems to deliver novel activities that are found only when binding

to two or more targets simultaneously. We utilize the controlled Fab arm exchange technology to generate scalable amounts of bispecific antibodies. A brief review of the self-assembly mechanism will be described and is being used to foster better process control. In this work, a combination of Förster resonance energy transfer (FRET), non-reducing SDS-PAGE, and strategic mutation of the Ab hinge region was employed to identify and characterize the individual steps of cFAE. Fluorescence correlation spectroscopy (FCS) was used to determine the affinity of parental (homodimer) and bispecific (heterodimer) interactions within the CH3 domain, further clarifying the thermodynamic basis for bsAb formation. The result is a clear sequence of events with rate constants that vary with experimental conditions, where dissociation of the K409R parental Ab into half-Ab controls the rate of the reaction. This technology is amenable to being able to engineer properties such as solubility, pharmacokinetics, and Fc effector function that can be critical to the clinical efficacy of a protein therapeutic molecule. This talk will also highlight a recent example of how novel architecture of bispecific biologics can be used to design highly potent molecules with sometimes unexpected activities.

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## PHYSICOCHEMICAL CHARACTERIZATION OF NANOPARTICLES INTENDED FOR THERAPEUTICS

**JEFFREY D. CLOGSTON**

Principal Scientist, Section Head, Physicochemical Characterization Nanotechnology Characterization Laboratory, Cancer Research Technology Program, Leidos Biomedical Research, Inc., Frederick National Lab, Frederick, MD 21702 USA

The National Cancer Institute's (NCI) Nanotechnology Characterization Laboratory (NCL) conducts preclinical characterization including physicochemical (analytical), *in vitro*, and *in vivo* of nanoparticles intended as cancer therapeutics and diagnostics. This presentation will highlight the methodology and analytical techniques related to the sizing of nanoparticles from the testing of more than 375 nanotechnology-based candidate cancer treatments and diagnostics. Characterization techniques such as dynamic and static light scattering, transmission electron microscopy, laser diffraction, and resistive pulse sensing will be featured. As particle size is a critical quality attribute of a nanomedicine drug product, the current gaps and needs in particle sizing and techniques will be discussed along with the current state of sizing standards and references.

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## NANOMEDICINES FOR THE TREATMENT OF SEVERE DISEASES

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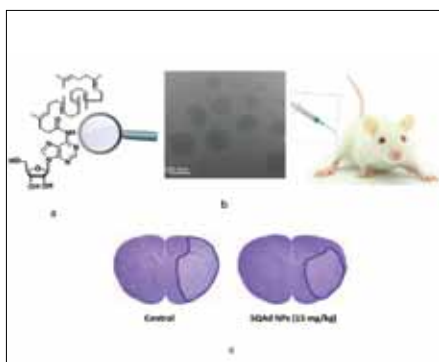
Even if new molecules are discovered to treat severe diseases like cancers, the clinical use and efficacy of conventional chemotherapeutics is hampered by the following limitations: (i) drug resistance at the tissue level due to physiological barriers (non-cellular based mechanisms), (ii) drug resistance at the cellular level (cellular mechanisms), and (iii) non-specific distribution, biotransformation and rapid clearance of the drugs in the body. It is therefore of importance to develop nanodevices able to overcome these limitations. This will be illustrated by various nanomedicine platforms developed in the laboratory:

- The design of biodegradable doxorubicin-loaded polyalkylcyanoacrylate nanoparticles for the treatment of the multidrug re-



sistant hepatocarcinoma (a nanomedicine with phase III clinical trials ended)<sup>[1]</sup>.

- The construction of nanoparticles made of metal oxide frameworks (NanoMOFs)<sup>[2,10]</sup>, a highly hyperporous material obtained by the complexation of iron oxide clusters with diacids. The nanopores of this material may be designed according to the molecular dimension of the drug molecule to be encapsulated.
- The “squalenoylation”<sup>[3,4]</sup>, a technology that takes advantage of the squalene’s dynamically folded molecular conformation, to link this natural and biocompatible lipid to anticancer drug molecules<sup>[5]</sup> to achieve the spontaneous formation of nanoassemblies (100–300 nm) in water, without the aid of surfactants. Surprisingly, these squalene-based nanoparticles are using the circulating LDL as “indirect” carriers for targeting cancer cells with high expression of LDL receptors<sup>[6]</sup>. The application of the “squalenoylation” concept for the treatment of brain ischemia and spinal cord injury will be discussed too (Figure 1). The possibility to use other terpenes (natural or synthetic) than squalene to design nanoparticles for the treatment of cancer will be discussed, too<sup>[9]</sup>.



*Figure 1: Adenosine-Squalene bioconjugate (a) spontaneously self-assembles in water as nanoparticles (SQAd NPs) of ca. 100 nm (b). When injected into mice subject to brain ischemia, nanoparticles induce reduction of ischemic zone (c)*

The design of “multidrug” nanoparticles combining in the same nanodevice chemotherapy and imaging (i.e., “nanotheranostics”) or various drugs with complementary biological targets will be also discussed<sup>[7]</sup>. Finally, it will be shown that the construction of nanodevices sensitive to endogenous (i.e. pH, ionic strength, enzymes etc.) or exogenous (i.e., magnetic or electric field, light, ultrasounds etc.) stimuli may allow the spatio-temporal controlled delivery of drugs and overcome resistance to current treatments<sup>[8]</sup>.

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## ENGINEERING THE IMMUNE SYSTEM WITH SYNTHETIC BIOMATERIALS

BRUNO DE GEEST

The field of immuno-engineering has vastly expanded the last decade and offers new avenues for the treatment of infectious diseases and cancer. My research group works at the interface between life sciences and materials chemistry with a special interest in polymer chemistry, nanotechnology and immunology. In my talk, I will give an overview of our recent findings in how we can engineer the immune system using synthetic nanomaterials to fight cancer. In

this regard, we are developing biomaterials strategies that allow for activating adaptive in innate anti-cancer immune responses.

In the context of adaptive immune engineering we are developing biomaterials-strategies for efficient delivery of molecular adjuvants and tumour associated antigens to antigen presenting cells in lymphatic tissue. These strategies comprise the synthesis of particulate and amphiphilic polymers linked with small molecule TLR agonists.

In the context of innate immune engineering we are developing biomaterials-strategies for decorating the cancer cell surface with motifs that trigger innate immune mechanisms that lead to cancer cell eradication. These strategies comprise polymers composed of a cancer cell surface anchoring ligand and multiple copies of ligand that binds to endogenous antibodies present in human serum.

## INTRODUCTION

JON DE VLIAGER

Coordinator NBCD Working Group hosted at Lygature, the Netherlands, [www.lygature.org/nbcd](http://www.lygature.org/nbcd), [jon.devlieger@lygature.org](mailto:jon.devlieger@lygature.org)

The rise of bio- and nanotechnology has resulted in rapid development of complex drug products and their copy versions. One of the key questions for these products is how to grant market authorization, since their complexity provides challenges for current regulatory pathways.

It is difficult and sometimes impossible to fully characterize complex drugs, and minute variations in the manufacturing process can substantially change the composition of final products. Biologics exemplify one category of complex drugs. Another category is non-biological complex drugs (NBCDs). This class of products includes – but is not limited to – nanomedicines, such as liposomes and iron-carbohydrate complexes.

For biologics and their biosimilars, clear legislation and global guidance policies are in place to aid the development of high-quality products. For NBCDs, on the other hand, no specific legislation was written, and guidance documents for NBCDs and their copies differ both between products and across the globe. This complicates requests for approval and may result in different decisions taken for the same NBCD product.

One of the key questions remains how to assess equivalence or similarity of these complex products. Essential for regulatory guidance alignment, is the definition and understanding of the critical quality attributes (CQAs) – those product characteristics that essentially ensure similar product efficacy and safety in humans.

In the introductory lecture, I will lay out the complex drug landscape, reflect upon outstanding challenges and discuss recent steps that have been taken by different stakeholders to provide science based frameworks for the evaluation of NBCDs and their similars

## MODULATING CELL SEQUESTRATION, BLOOD LONGEVITY AND BIODISTRIBUTION BY TUNING NANOCONSTRUCT RIGIDITY

PAOLO DECUZZI, Laboratory of Nanotechnology for Precision Medicine; Italian Institute of Technology – Genova  
<https://www.iit.it/research/lines/nanotechnology-for-precision-medicine>

Multifunctional nanoconstructs are particle-based nano-scale systems designed for the ‘smart’ delivery of therapeutic and imaging agents. The Laboratory of Nanotechnology for Precision Medicine

at IIT-GE synthesizes polymeric nanoconstructs with different sizes, ranging from a few tens of nanometers to a few microns; shapes, including spherical, cubical and discoidal; surface properties, with positive, negative, neutral coatings; and mechanical stiffness, varying from that of cells to rigid, inorganic materials, such as iron oxide. These are the 4S parameters – size, shape, surface, stiffness – which can be precisely tuned in the synthesis process enabling disease- and patient-specific designs of multifunctional nanoconstructs. In this lecture, the role of manipulating these 4S parameters over different temporal and length scales will be elucidated in the context of improving particle interaction with professional phagocytic cells, blood longevity and tissue specific accumulation.

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## ENGINEERING THE IMMUNE SYSTEM WITH SYNTHETIC BIOMATERIALS

HELEEN DEWITTE

The field of immuno-engineering has vastly expanded the last decade and offers new avenues for the treatment of infectious diseases and cancer. My research group works at the interface between life sciences and materials chemistry with a special interest in polymer chemistry, nanotechnology and immunology. In my talk, I will give an overview of our recent findings in how we can engineer the immune system using synthetic nanomaterials to fight cancer. In this regard, we are developing biomaterials strategies that allow for activating adaptive in innate anti-cancer immune responses. In the context of adaptive immune engineering we are developing biomaterials-strategies for efficient delivery of molecular adjuvants and tumour associated antigens to antigen presenting cells in lymphatic tissue. These strategies comprise the synthesis of particulate and amphiphilic polymers linked with small molecule TLR agonists. In the context of innate immune engineering we are developing biomaterials-strategies for decorating the cancer cell surface with motifs that trigger innate immune mechanisms that lead to cancer cell eradication. These strategies comprise polymers composed of a cancer cell surface anchoring ligand and multiple copies of ligand that binds to endogenous antibodies present in human serum.

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## COMPLEMENT ACTIVATION RELATED PSEUDOALLERGY (CARPA) AS A POSSIBLE REACTION BEHIND HYPERSENSITIVITY SIGNS DURING HEMODIALYSIS

LÁSZLÓ DÉZSI<sup>1,3,4</sup>, Pethő Ákos<sup>2</sup>, Fülöp Tamás<sup>1,4</sup>, Mészáros Tamás<sup>1,4</sup>, László Rosivall<sup>3</sup> and János Szabó<sup>1,2,3</sup>

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### INTRODUCTION

Patients suffering from chronic kidney insufficiency undergo repeated hemodialysis (HD) treatment. During HD acute life threatening hypersensitivity (anaphylactic) reactions with unknown origin may occur<sup>(1)</sup>. Based on the clinical background of this phenomenon a pre-clinical animal model may be established. A frequent manifestation of the anaphylactic reactions is known as the IgG mediated, complement activation related pseudoallergy (CARPA). This may occur in humans, as well as in animals following iv. administration of various nanomedicines. In recent years a high sensitivity pig model was developed and successfully applied by our research group<sup>(2)</sup>. In our present study, with the combination of the above two methods (HD and pig CARPA), a novel model has been developed to study hypersensitivity signs during HD.

### MATERIALS AND METHODS

Pigs: Domestic male Yorkshire pigs (20-25 kg) were sedated with Calypsol/Xilazine (10 and 2 mg/kg respectively) and anesthetized by isoflurane (2-3% in O<sub>2</sub>). Animals were breathing spontaneously. PAP was measured using a Swan-Ganz catheter introduced into the pulmonary artery via the right external jugular vein, while systemic arterial pressure (SAP) and heart rate (HR) were measured in the femoral artery. The left femoral vein was cannulated for blood sampling. Test agents were injected in bolus (< 10 sec) via the left external jugular vein (n=3/group). Hemodynamic changes were continuously monitored using an AD Instruments (ADI) PowerLab System. Mean PAP, SAP and HR data were evaluated by the ADI LabChart software.

Experimental protocol. HD was performed according to human protocol using a 4008 S (Fresenius) dialysis machine. The equipment was attached to the anaesthetized pig via the femoral vein using a double lumen catheter. The effects of a 60 min HD treatment and the subsequent reinfusion were studied. At the end of the experiment zymosan was utilized for direct complement (C) activation. Blood sampling: Blood samples of 2 ml, each were collected from the pigs before (time 0), and at pre-determined time points (15-30-45-60 min) during HD, as well as at 5 and 10 min during blood reinfusion. Samples were collected into K2-EDTA Blood Tubes, of which samples for TXB2 analysis were containing Indomethacin. At the same time points another samples were collected for C analysis. Aliquots of 100 µl blood were drawn into tubes with K2-EDTA for haematological analysis. Blood was centrifuged at 1500 rpm for 10 min at 4°C, and plasma was stored at -80°C until analysis.

Thromboxane B2 levels: Plasma TXB2 (the stable metabolite of plasma TXA2) levels were measured with an ELISA kit (Cayman Chemicals).

### RESULTS AND DISCUSSION

Cardiopulmonary effects of HD showed somewhat variable, but insignificant changes in PAP, SAP or HR during the hemodialysis process itself. On the other hand, during reinfusion of the extracorporeal blood a 30 to 50 % increase in PAP was unequivocally found in all experiments. At the end of each experiment the i.v. injection of 0.1 mg/kg zymosan induced strong CARPA reactions. TXB2 data solely follow this pattern, namely small to medium rises during reinfusion and large elevations upon zymosan administration were observed. Data from serum complement reflect C activation during the procedure, however individual differences largely influence these results.

In the present study combining the method of hemodialysis with our well established pig CARPA model a new experimental method has been established. This method is applied to model hypersensitivity reactions during human HD treatments. Our data partly confirm previous suspects, that the mechanism of the observed anaphylactic phenomena might be the CARPA reaction. However, these changes were observed not during HD, but rather upon reinfusion. This raises the possibility that these changes are mainly attributable not to the dialyzer membrane material properties themselves, but rather to noxious effects (e.g. surface contact with plastics, formation of free radicals etc.) of the extracorporeal circulation. The clarification of the exact mechanism of these changes requires further investigations.

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## CRITICAL ISSUES IN DEVELOPMENT OF GUIDELINE FOR NANOSAFETY REGULATION: THE ROLE OF ALTERNATIVE TEST STRATEGY

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Nanotechnology is an enabling technology with a great potential for incremental innovation in the field of medicine with development of new product and processes with high economic and social impact. However, we lack a clear understanding about how the nanomaterial reacts with the living system with different kind of applications ranging for biosensors, imaging and therapy. There are still difference of opinion about the method of assessment of the relevant “end point” of toxicity of a particular nanosystem. Issues become more complicated when complex multifunctional nanoparticles are used both for imaging/diagnosis and therapy (theragnosis). Among the several application of nanotechnology in medicine the nanodrug delivery system has generated maximum interest. Engineered multifunctional nanoparticles as drug delivery system may have complex composition with metal and organic compound with surface functionalization. The safety assessment of these multifunctional system may not be complete with conventional toxicity studies.

In a long term in-vivo biokinetics and toxicity study of gold nanoparticle (GNP) we demonstrated redistribution and translocation of GNP among blood and different tissue compartments at different time points following single intravenous injection in mice<sup>1</sup>. Reticulo-endothelial system (RES) macrophage uptake may give rise to high sequestration of drug loaded NP in organs like liver, spleen which can act as reservoirs of drug. In several polymer based drug delivery systems we observed after intravenous injection first drug level peak in serum followed by fall due to RES sequestration. We noted rise in blood level of drug after few hours which was likely due to release of drug from RES system. This pattern is significantly different that the Pk of the free drug injected intravenously. The kinetics of redistribution of the drug among blood and tissue compartment depends on degradation kinetics of the NP which in turn is related to its composition. For nondegradable NP like GNP the situation may be more complex. The Superparamagnetic iron-oxide nanoparticles (SPION), which is regarded as safe can cause oxidative stress to cells if accumulated in high concentration<sup>2</sup>.

The conventional Pk studies as performed for free drug may not be comparable with the nanodrug delivery system specially with slowly degrading or nondegradable systems. Physiologically based pharmacokinetics (PBPK) modeling may be an alternative method of Pk studies for nanoformulation. However there are several barriers in developing this system which need further exploration. The orally non-absorbable drugs can be administered orally with nano-drug delivery systems. However the Pk/Pd of the drug will be very different with nano-intervention which can alter toxicokinetics and safety profile of the drug with altered tissue sequestration. Similarly the lymphatic targeting of the drug through oral or parenteral route also alter the conventional Pk/Pd pattern. The potential advantages of nano system has been demonstrated in large number of well documented publications. The regulatory research should complement and help to devise new methods of assessment of short and long term safety of these nanoformulations. There is a tremendous challenge in integrating in-vitro, ex-vivo and in-vivo data for assessment of toxicity/safety profile.

The situation may be much more complex to assess immunological safety of the formulation. The standard immune-safety studies currently recommended for formulation needs to be supplemented with additional tests depending upon composition, properties of the nanocarrier, route of administration, sequestration pattern and kinetics of degradation. Different test approach may be needed for administration through respiratory route compared with oral or systemic route. Effect of nanomaterial on components of innate and adaptive immunity should be explored according to the risk

perception. The degradation product may have potential for hapten like activity or abnormal activation of complement system.

There is a need for development of new test strategy based on science based exploration with a regulatory consequence. The greater challenge is to develop multidisciplinary collaboration integrating nanotechnology with pharmacology, patho-physiology, cell and molecular biology for devising the guideline for nano-safety regulation.

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## IMMUNE CHECKPOINT BLOKERS (ICB) DRIVE CURRENT REVOLUTION IN IMMUNOTHERAPY FROM MELANOMA ACROSS MULTIPLE TUMOR TYPES

ALEXANDER M.M. EGGERMONT MD, PHD, Gustave Roussy Cancer Campus Grand and Univeristy Paris-Saclay, France

- **ICB History:** Melanoma has been the most important cancer to drive immunotherapy development in the field of solid tumors. Where immune stimulating approaches with cytokines in the 1980's and 1990's lead to approvals of interferon-alpha and interleukin-2, the clinical impact was rather small. Since 2010 immunotherapy has been revolutionized by the concept of breaking tolerance with ICs. It represents a major paradigm shift that marks the beginning of a new era. The impact of the first ICs, i.e. anti-CTLA-4 (Cytotoxic T Lymphocyte Antigen-4) and anti-PD1 / anti-PDL1 (Programmed death-1 receptor and its ligand PD-L1) is unprecedented.
- **Advanced Disease:** In only 5 years advanced melanoma has been transformed from an incurable disease into a curable disease in over 50% of metastatic patients. We are only at the beginning of discovering its transversal impact throughout oncology. For the treatment of advanced disease approvals were obtained for the immune checkpoint inhibitors ipilimumab (2011), nivolumab (2014), pembrolizumab (2014) and the combination ipilimumab + nivolumab (2015).
- **Adjuvant Therapy:** Ipilimumab is the first checkpoint inhibitor that has also been approved as adjuvant therapy for high risk stage III melanoma (2015). In 2017 spectacular results were reported on nivolumab and the combination of dabrafenib plus trametinib, and in 2018 similarly on the adjuvant use of pembrolizumab. All these adjuvant therapies are expected to be FDA and EMA approved in 2018.
- **Combinations:** Further developments in the field of melanoma are focused on combination therapies between various immunotherapeutic agents such as vaccines and antibodies, and combination therapies between immunotherapeutic agents with chemotherapeutic or targeted agents, or even radiation therapy.
- **Transversal Development:** Thanks to in particular anti-PD1/anti-PDL1- based immunotherapies and the activity of the combination of anti-PD1/anti-CTLA4 immunotherapy is now developed in a transversal manner across multiple tumor types (a.o lung, head&neck, oesophageal and gastric, liver, MSI-colorectal, MSI-any tumor type, renal, bladder, and Merkel cell cancers and Hodgkin-lymphoma with unprecedented success.
- **Toxicities:** Success however does come at a price, both in terms of side-effects, in particular immune-related adverse events (irAEs), as well in terms of financial toxicity. irAEs come in many forms. With ipilimumab at 10mg/Kg the most frequent are Gastro-Intes-



tinal (diarrhea, colitis, perforations); Hepatic (grade 3-4 hepatitis in 16%); Endocrinopathies (hypophysitis in up to 16 %, grade 3-4 in up to 5%) and hyper/hypothyroiditis; Dermatitis; Arthralgia; Rare but important are neurodegenerative irAEs, Guillain-Barré syndrome, pneumonitis, and myocarditis. Anti-PD1/PDL1 agents are much less toxic in general. The combination of anti-CTLA-4 and anti-PD1/L1 are associated with increased irAEs. Algorithms have been established including administration of high dose corticosteroids and anti-TNF agents. All treatment regimens with ICBs have reported drug-related fatalities range.

- **Smart Combos:** Anti-PD1/PDL1 has become the central drug in all further development strategies to be combined with other ICBs, agonists, cytokines, vaccines, targeted agents, chemotherapeutics and radiotherapy.

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## THE PERSONALIZED SWISS SEPSIS STUDY

ADRIAN EGLI

Sepsis is associated with high morbidity and mortality. Early diagnosis and treatment can significantly influence the heterogeneous outcome. Within this collaborative network of all Swiss University Hospitals, Universities and ETH Zürich, we will generate a highly interoperable research network on novel digital and molecular – omics based biomarkers with the final goal to recognize a bacterial sepsis earlier and to predict its course more precisely than currently possible for an individual patient.

Bacterial infection progressing to sepsis is associated with high morbidity, mortality, reduction of quality of life in survivors and health care costs. The course and outcome of sepsis is highly heterogeneous and depends on the causative pathogen and varies from patient to patient. The individual outcome is significantly influenced by various complex host- and pathogen-related factors.

Increasing rates of multi-drug resistant bacteria further complicate the diagnostic process and clinical management and may lead to treatment failure. Therefore, patients with sepsis would greatly benefit from personalized diagnostic assessment and treatment strategies evaluating and integrating the host and the pathogen.

The PSSS Driver project aims to build an interoperable infrastructure among the intensive care units of the Swiss university hospitals and several research groups, to gather complex information on the host and pathogen during the entire course of a sepsis. The integration of continuous monitoring data from intensive care units will result in digital biomarkers. Combined with the molecular data from bacterial pathogens (metagenomics and whole genome sequencing) and from the host (metabolomics, immunophenotyping, genotyping), new avenues for sepsis research will emerge. These very comprehensive and complex data will be combined via the SPHN data hubs to enable multi-dimensional analyses through machine learning. The goal is to recognize a bacterial sepsis earlier and to predict its course more precisely than currently possible for an individual patient.

The heterogeneous course of sepsis and associated fatal outcome may greatly benefit from a personalized approach in the diagnostics and treatment. Due to low sensitivity and specificity of current biomarkers, sepsis is recognized relatively late, which leads to a reduced efficacy of antibiotic treatment and high mortality. Therefore, novel digital, molecular and hybrid biomarker will help to (i) recognize sepsis at a much earlier state and (ii) predict the likelihood of mortality. Critical for exploring and validating novel types of biomarkers will be a sufficiently large study cohorts including multiple centers. The proposal offers the opportunity to (i) form a collaborative network with well-established research groups in the field and (ii) reach sufficient patient numbers within a few years, and (iii) to statistically identify biomarkers that hold the potential

to drastically improve the early diagnosis of sepsis and prediction of mortality due to sepsis. The analysis of these complex multidimensional datasets requires specific expertise in bioinformatics, statistics and machine learning - the PHRT will also help to improve the overall data quality and integrate complex -omics data back into routine clinical workflows. To conclude, the Personalized Swiss Sepsis Study will put Switzerland at the forefront of personalized diagnostic and treatment research on sepsis in the world.

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## DOXORUBICIN ELUTING INTRA-ARTERIAL THERAPY FOR PEDIATRIC EXTRA-ABDOMINAL DESMOID FIBROMATOSSES – A PROMISING APPROACH FOR A PERPLEXING DISEASE

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**Purpose:** Desmoid fibromatoses (DF) are rare and clinically enigmatic soft tissue tumors with high propensity for local invasion but no metastatic potential. Surgery remains the cornerstone of primary treatment but postoperative recurrence is common and tends to behave more aggressively than the primary tumor. Systemic options are widely varied and overall show marginal benefit, with the exception of doxorubicin. Doxorubicin has proven clinical benefit but its use is limited by dose-dependent toxicities. To maximize the local efficacy of doxorubicin while minimizing systemic toxicity we developed a protocol for super-selective intra-arterial doxorubicin delivery via drug eluting beads (DEB-DOX).

**Materials and Methods:** Here we present the results of selective DEB-DOX in four children with recurrent or refractory DF using cumulative bead-loaded doses of 27, 45 and 133 mg/m<sup>2</sup>. We assessed efficacy by tumor response on MRI and by symptomatic improvement.

**Results:** Tumor volumes were reduced by 54% - 97% within three to twelve months. Treatment was accompanied by reduction or complete elimination of MRI T2 signal in all DFs. Symptomatic relief was accomplished within three months for all patients. A single patient experienced transient lower extremity paresthesia (CTCAE Grade I) which resolved within two months. No other adverse effects were encountered over a post treatment follow up of 6-32 months.

**Conclusion:** Trans-arterial doxorubicin-eluting bead embolization should be considered for patients with DF amenable to endovascular treatment. Further study is needed to validate these first promising results.

## THE BEHAVIOR OF NANOPARTICLES IN COMPLEX ENVIRONMENTS

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Nanoparticles possess unique properties which can be used for designing specific functionality for biomedical applications, however, several crucial challenges remain to be addressed for nanomedicines to make clinically important progress. To exert its mode of action, the nanoparticles / nanocarriers need to reach the organ, tissue or cell of interest. Independent of the routes by which nanoparticles are applied, e.g. inhalation, injection, ingestion and application to the skin, the particles inevitably encounter a complex physiological fluid populated with a wide range of biomolecules, e.g. proteins, vitamins, lipids and salts/ions<sup>[1]</sup>. Upon contact with physiological fluids the formation of a surface-bound protein layer, particle dissolution or aggregation might occur, which are expected to have a crucial impact on cellular interaction<sup>[2]</sup>. Once inside the cells the particles initially are localized in early and eventually late endosomes, which then fuse with lysosomes, complex digestive organelles that have a low pH (~4.5) and a salt rich environment filled with hydrolytic enzymes<sup>[3]</sup>. Such an acidic and enzyme rich environment also might strongly impact the stability and aggregation behavior of nanoparticles and needs to be studied by a combination of different techniques to gain some indications as to their potential biological impact, as well as how to design nanoparticles for a specific cell response to take advantage of nanotechnology applications in biotechnology and in medicine.

Characterization of nanoparticles in complex environments has therefore become of paramount importance. In this presentation, we will give an overview about the diversity of physiological fluids as well as present an inventory of the most relevant experimental techniques used to characterize nanoparticles.

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## DEVELOPMENT OF BIOHYBRID MULTISTAGE NANOVACCINES FOR MELANOMA

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The treatment of melanoma has been revolutionized by the discovery of immune check-point inhibitors, antibodies that act at the level of the immunological synapsis or at the tumor level.<sup>[1]</sup> Active immunotherapy involves the reactivation of the patient's immune system against the tumor.<sup>[2]</sup> Vaccines against tumor antigens are regarded as a mean to prime the immune system against the tumor cells.<sup>[3]</sup> Some of the challenges associated with the efficacy of the cancer vaccine are related to the poor adjuvancy of the antigenic peptides.<sup>[4]</sup> Thereby, newer adjuvants, in the form of particulate (micro or nano) systems, have been investigated.<sup>[5]</sup>

In this context, thermally oxidized porous silicon (TOPSi) nanoparticles (NPs) induce the activation of antigen presenting cells, with a potential function as adjuvants.<sup>[6]</sup> In order to increase the efficacy of the adjuvant particles, we employed glass capillary microfluidics to encapsulate TOPSi NPs in a polymeric layer composed of acetylated dextran (AcDEX). AcDEX is a biocompatible polymer derived from the modification of dextran, with innate immunostimulative properties. TOPSi@AcDEX particles were able to induce the maturation of antigen presenting cells, as signified by the increased expression of co-stimulatory signals (CD80 and CD86; Figure 1) and by the secretion of pro-inflammatory cytokines (interferon gamma).<sup>[7]</sup> We added the antigenic component to the system by coating, through a process of membrane extrusion, the NPs with cellular membranes derived from cancer cells of interest, thereby formulating biohybrid nanovaccines.<sup>[7, 8]</sup>

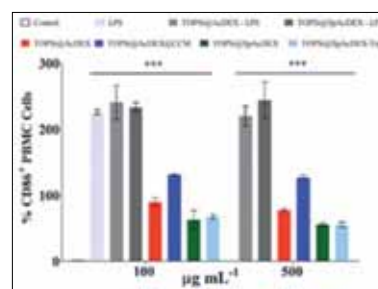
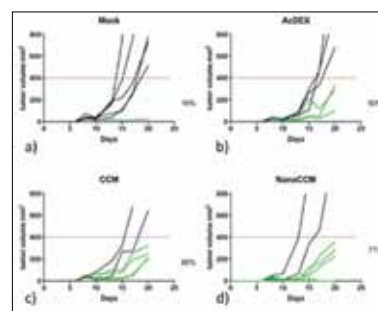


Figure 1. Percentage of CD86+ peripheral blood monocytes after incubation with nanosystems at two different concentrations (100 and 500 µg/mL). The results are presented as mean±s.d. (n=3) and have been analyzed with one-way ANOVA,

followed by Bonferroni post-test. The level of significance was set at \*\*\*p<0.001.

We then evaluated the efficacy of the particles *in vivo*, in an immunologically relevant murine model (B16.OVA). As shown in Figure 2, the administration of the complete vaccine (NanoCCM) enables a higher control of the growth of established tumors, when compared to the single components (adjuvant nanoparticles or cancer cell membranes).

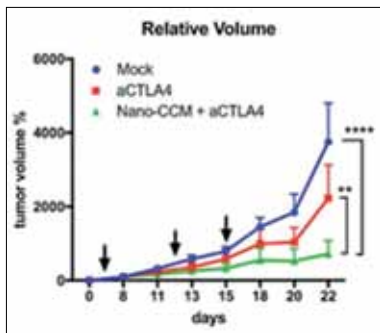
Figure 2. Tumor growth single curves after the administration of a) mock, b) adjuvant component, particles only, c) antigenic component only, cell membranes, and d) the final vaccine formulation, subcutaneously.



Furthermore, a synergistic effect between the nanovaccines and checkpoint inhibitors (anti CTLA-4) was also established (Figure 3), with promising results for a future translation of the biohybrid nanovaccines to the clinic, in a personalized medicine optic.

Figure 3. Tumor growth curves after treatment with mock, checkpoint inhibitor (aCTLA4) or combination therapy (checkpoint inhibitor and nanovaccines). The results are presented as mean±s.d. (n=6) and were analyzed with two-way ANOVA with Tukey's multiple comparison correction.

The levels of significance were set at  $**p < 0.01$  and  $****p < 0.0001$ .



In conclusion, we developed an innovative bio-hybrid nanovaccine and investigated its efficacy both *in vitro* and *in vivo*. The vaccine controls tumor growth when used in monotherapy and improves the efficacy of the standard of care (treatment with immune checkpoint inhibitor).

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## PROGRAMMING SUPRAMOLECULAR MATERIALS FOR REGENERATIVE MEDICINE

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A major challenge of regenerative medicine is to recapitulate the dynamics of the native extracellular matrix in which signals can be displayed dynamically and reversibly, positioned with nanoscale precision, and combined synergistically to control cell function. Most efforts in this area have effectively addressed only one of these three key phenomena, and focused mainly on static cell adhesion or irreversible switching of bioactivity. I will describe a molecular system that can be programmed to control the dynamics, spatial positioning, and combinatorial synergies of signals in extracellular matrices. In this approach, a biological signal is immobilized to a surface through DNA tethers. By engineering a series of tethers responsive to different stimuli, we show that cells adhered and spread on the surface reversibly. The DNA was also used as a molecular ruler to control the distance-dependent synergy between two adhesion peptides. Finally, orthogonal DNA tethers enabled the regulation of two different bioactive signals, with the ability to independently up- or downregulate each over time.

Another unique feature of the native extracellular space is its hierarchical architectures. Creating soft materials with the tunable hierarchical structures observed in nature using supramolecular self-assembly remains an enormous challenge. Synthetic hierarchical systems have been reported, but strategies to reversibly modulate their structure and function are largely unknown. I will describe our recent discovery of such a system based on peptide-DNA supramo-

lecular fibers that can organize reversibly into hydrogel networks containing bundles of twisted fibers. Molecular dynamic simulations explain that weak inter- and intra-fiber interactions promote hierarchical assemblies by enabling large scale redistribution of monomers. By exploring the desired energy scales of the cohesive interactions, we were able to derive design rules to encode similar dynamic hierarchical behavior in other supramolecular materials. Lastly, I will show how we utilized these reconfigurable materials to induce reversible changes in the phenotypes of neuronal cells.

## EXOSOME-HYDROGELS AS MACHINERY FOR ANTI-INFLAMMATORY THERAPEUTICS' SYNTHESIS

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Extracellular vesicles (EVs) are natural lipid-based membranous particles produced by almost any cell<sup>[1]</sup>. They may transfer nucleic acid and protein-cargoes selectively between cells locally and at distance, which has created excitement in the drug delivery field<sup>[2]</sup>. Although initial clinical trials are ongoing, the use of EVs for therapeutic applications may be limited due to potential "dilution effects" upon systemic administration or undesired off-target activity which may affect their ability to reach their target tissues. To tap their full therapeutic potential in a localised manner, we created a biomedical hydrogel containing EVs designed to achieve local delivery of therapeutics<sup>[3]</sup>. Due to the challenge of incorporating EVs into hydrogels without compromising their biological constitution, such approach had not been developed yet.

## RESULTS AND DISCUSSION

EVs from human mesenchymal stem cells (MSC) were loaded with a glucuronidase enzyme using our established saponin-assisted technique<sup>[4]</sup>, and compared to liposomes (egg phosphocholine/cholesterol mol-60/40%). EVs and liposomes were incorporated into polyvinyl alcohol (PVA) hydrogel (12 wt%) and crosslinked with PEG. Incorporation of glucuronidase-loaded EVs or liposomes into biocompatible PVA hydrogels did not impact on hydrogel biomechanical properties and preserved the enzyme's stability compared to free enzyme during two weeks of incubation at 37°C, as assessed using glucuronide-fluorescein.

To visualise EVs within hydrogels, uranyl acetate-labelled vesicles were imaged in carefully dehydrated gels by density-dependent colour scanning electron microscopy (DDC-SEM)<sup>[5]</sup>. Taking advantage of density-sensitive backscattered imaging we spatially localised EVs within 3D-hydrogels. When incubating hydrogels with curcumin-glucuronide prodrug and murine RAW264.7 macrophages, all gels showed significant anti-inflammatory activities, as assessed by cell viability measurements. Recycling of gels after 7 d showed that this effect was lost for gels containing free enzyme but not when encapsulated into vesicles, indicating that both natural and synthetic vesicles may protect enzyme activity in the hydrogels during enhanced periods. Interestingly, without addition of curcumin-prodrug only gels containing EVs showed an inherent anti-inflammatory effect, as detected by TNF-alpha gene expression analysis in bone marrow-derived primary macrophages. Our results indicate that hydrogels with MSC EVs are anti-inflammatory per se, which has not been reported to date.

## CONCLUSION

We have presented the first example of incorporation of EVs into a biomedical hydrogel and applied DDC-SEM, a powerful technique to resolve hydrogel morphology and spatial EV distribution. Furthermore, we revealed the inherent anti-inflammatory activity of EV-hydrogels in various cell models, creating the important basis towards their therapeutic application.



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## LIPID-NANOPARTICLE FORMULATIONS FOR MRNA DELIVERY: A FOCUS ON CELLULAR UPTAKE AND TRAFFICKING MECHANISMS

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RNA-based drugs have enormous potential to transform modern medicine and to provide new opportunities to treat and cure a wide variety of previously undruggable human diseases, but their poor cellular delivery (bioavailability) is impeding progress. We have studied the cellular uptake, intracellular trafficking and cytosolic release of an eGFP-encoding Cy5-labelled mRNA loaded into lipid-based nanoparticles (LNPs) of different sizes (diameter) as well as surface lipid composition. Our aim is to obtain fundamental insights into the molecular and biological factors that determine delivery efficacy. Our approach relies on applying robust analytical methodology, such as flow cytometry and live cell confocal imaging to obtain quantitative data on LNP uptake, subcellular localisation and cytosolic release leading to eGFP production. We have also performed cytotoxicity screening in human hepatic cell lines to evaluate LNP biosafety. By overexpressing fluorescent protein tagged trafficking markers mRFP-Rab5, DsRed-Rab11, mRFP-Rab7, and Lamp1-RFP, we have been able to track the intracellular location of the Cy5-mRNA and its release into the cytoplasm overtime following LNPs exposure. This work is conducted within the Swedish Industrial Research Centre FoRmulaEx which has the long-term mission to generate the fundamental knowledge required for the design of safe and efficient drug vehicle formulations for the next generation of functional nucleotide based drug modalities.

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## REDEFINING CANCER BY INTEGRATING THE IMMUNE SYSTEM: TRANSFERRING CUTTING EDGE MEDICINE TO THE PATIENTS

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To date the anatomic extent of tumor (TNM-classification) has been by far the most important factors to predict the prognosis of cancer patients. However, this classification provides limited prognostic information and does not predict response to therapy. We redefined cancer by integrating the immune system, to transfer cutting edge medicine to the patients. We showed that tumors from human colorectal cancer with a high-density of infiltrating memory

and effector-memory T-cells (Tem) are less likely to disseminate to lymphovascular and perineural structures and to regional lymph-nodes. We demonstrated the critical tumor-microenvironment parameters determining the dissemination to distant metastasis. We showed that the combination of immune parameters associating the nature, the density, the functional immune orientation and the location of immune cells within the tumor was essential to accurately define the impact of the local host-immune reaction on patients prognosis. We defined these parameters as the “immune contexture”. We characterized the immune landscape within human tumors, and showed the importance of adaptive immune cells including, cytotoxic T-cells, Th1-cells, B-cells and T-follicular-helper (Tfh) cells. We described the immunopenotype and antigenome associated with immune escape mechanisms and demonstrated mechanisms associated with pre-existing and proliferating intratumoral T-cells.

Based on the immune contexture, a standardized, simple and powerful digital-pathology-based immune stratification-system, termed “Immunoscore”, was delineated having a prognostic power superior to that of the currently used cancer staging-system. Tumor invasion parameters were statistically dependent on the host-immune reaction. A worldwide consortium is validating the prognostic value of Immunoscore, using a standardized-assay. Recent data are supporting the significant role of Immunoscore within lung, liver, and brain metastases. Thus, tumor progression, invasion and recurrence are dependent on pre-existing immunity and on Immunoscore.

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## RISK MANAGEMENT FOR NANOMEDICAL PRODUCTS IN RELATION TO SAFE INNOVATION AND EMERGING REGULATORY REQUIREMENTS

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The safe innovation approach combines the concepts of safe-by-design (responsibility of innovators) and regulatory preparedness (responsibility of regulators), emphasising the need for interactions between innovators and regulators, preferably already in early stages. This could be in more general terms during conferences such as CLINAM, but also more specifically in a one-on-one interaction on their own product. More or less formal interactions could both be useful. Both innovators and regulators will benefit from this. Several mechanisms for such interactions already exist or are under development.

In general, regulatory requirements as well as regulatory/scientific guidance on nanotechnologies and nanomaterials applied in medicinal products as well as medical devices are emerging. A new regulatory framework for medical devices was recently published in Europe. The new regulation contains several provisions for nanomaterials, including a definition, specific attention for safety of nanomaterials and classification rules leading to different routes for conformity assessment. For the implementation of these provisions, more guidance is needed. Work is currently ongoing in European Commission Working Group for New & Emerging Technologies to develop guidance. Also in standard development organisations like ISO, CEN and ASTM are working on standards that can help implementation of emerging regulatory requirements for nanomedicinal products and medical devices. Furthermore, work done in European projects such as REFINE (Regulatory Science Framework for Nano(bio)material-based Medicinal Products and Medical Devices) will provide important contributions for this purpose.

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## AN IMPROVED AND INTEGRATED STRATEGY FOR NON-CLINICAL EVALUATION OF POTENTIAL IMMUNOTOXICITY OF NANOMEDICINAL PRODUCTS

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Various nanomedicinal products (NMPs) have been reported to induce adverse immunotoxic effects. This may be related to their tendency to accumulate in cells of the immune system. Therefore, immunotoxicity should be one of the major endpoints in the safety evaluation of NMPs before their market authorization.

Non-clinical regulatory immunotoxicity testing of non-biological medicinal products, including NMPs, is generally performed by following the guideline S8 “Immunotoxicity Studies for Human Pharmaceuticals” of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). Over the past years, several reflection papers (RP) for specific NMPs and for surface coatings of NMPs were developed, with recommendations for immunotoxicity testing included in only one of them.

Previously, we identified a number of gaps between the immunotoxicity effects reported for NMPs in the literature and the immunotoxicity endpoints included in the tests recommended by ICH-S8. Examples of such gaps are Complement Activation Related Pseudo Allergy, Hypersensitivity and Immunosuppression. In addition, ICH S8 does not provide any nanospecific testing considerations, which is important given their tendency to interfere with many commonly used toxicity assays. The goal of the current study was therefore to recommend a predictive battery of tests for the missing endpoints, taking into account known pitfalls related to the testing of NMPs. Here, we propose a strategy (summarized in Figure 1), consisting of a number of *in vitro*, *in vivo* and ex vivo tests, which were evaluated for their nano compatibility using a variety of NMPs (liposomes, iron oxides and dendrimers). The strategy starts with information on the physicochemical and pharmacokinetic properties to identify the NMP and to define its intended use. Subsequently, the route of exposure will determine the endpoints to be addressed, which will start with an *in vitro* assay cascade. This forms the basis for a weight of evidence review, which will be used to shape the design of further *in vivo* investigations. The final outcome of the immunotoxicity assessment can be included in the overall risk assessment of the NMP and/or give alerts for relevant endpoints to address during clinical investigation.

## REDEFINING THERAPEUTIC AGENTS BIOAVAILABILITY VIA TRANSIENT LIVER SATURATION BY NANOPARTICLES

MATTHIEU GERMAIN

**Introduction:** The benefit of a nanomedicine is due to its bioavailability, its intrinsic efficacy balanced with its toxicity profile. The nanomedicine should exhibit sufficient blood bioavailability for efficient accumulation at the target site. So far, a large part of the administered dose remains useless due to the high rate of clearance by the mononuclear phagocytic system (mainly by Kupffer cells). Enhanced bioavailability can be achieved by modifying physico-chemical properties of the nanomedicine but such modifications affect also its efficacy and toxicity profiles. Ultimately, nanomedicines design results usually in a compromise between bioavailability, efficacy or toxicity. Here we propose a new approach to change the way nanomedicines are biodistributed, by priming the body to receive the treatment. This approach relies on the sequential administration of a nanoprimer before the nanomedicine. The nanoprimer is a nanoparticle designed to transiently occupy the main pathway responsible for the limited bioavailability of the nanomedicine. As such, the nanoprimer allows to redefine the bioavailability of the nanomedicine. **Methods:** For proof of concept implementation, we designed a liposomal nanoprimer with specific physico-chemical properties and that was also prepared with a fluorescent labelling. Its biodistribution was assessed after intravenous (IV) administration in mice using *in vivo* imaging system (IVIS). Toxicity evaluation was performed by body weight and clinical signs monitoring over one week after 3 IV injections of nanoprimer spaced of 24h in mice. Evaluation of the impact of nanoprimer on different nanomedicines bioavailability was performed *in vivo* by tracking fluorescent nanomedicines IV injected 10min after nanoprimer. Tumor growth delay experiment was performed by comparison between IV administration of nanoprimer 10min before irinotecan loaded liposomes and irinotecan loaded liposomes alone, in HT29 xenografted mice (colorectal adenocarcinoma) once the tumor reached 150mm<sup>3</sup>. Treatment was repeated one week later. **Results:** The liposomal nanoprimer presents a preferential hepatic accumulation. No signs of systemic or hepatic toxicity were observed with maximized dose of this nanoprimer. Bioavailability studies showed a transient nanomedicine blood bioavailability increase correlated with a lower hepatic accumulation when combined to nanoprimer compared with nanomedicine alone. Finally, efficacy study showed that nanoprimer markedly enhanced anti-tumor efficacy of irinotecan loaded liposomes in the HT-29 model when compared to the nanomedicine alone.

Body priming may benefit to a wide variety of existing products modulating their bioavailability and may open perspectives to design new nanomedicines by decreasing the notion of compromise between bioavailability, efficacy and toxicity.

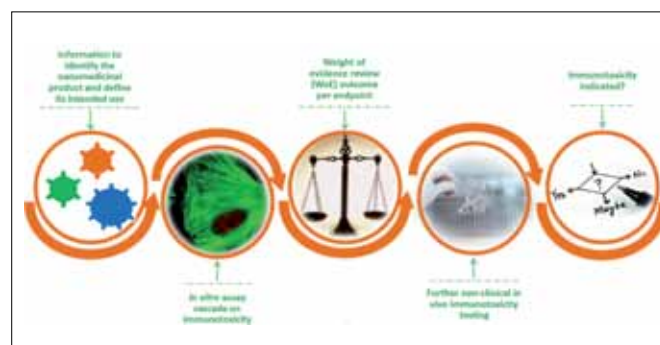


Figure 1: An integrated strategy recommended for assessing the risk of potential immunotoxicity after administration of nanomedicinal products.

## TOWARDS PREDICTION OF IN VIVO BEHAVIOR OF NANOPARTICLES: NEW METHODS FOR EVALUATION AND SCREENING OF NANOPARTICLES FOR THERAPEUTIC APPLICATIONS

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Proteins and other biomolecules in human biological fluids interact with the surface of nanoparticles. These interactions generate a coating (corona) around the nanoparticles which is the nanoparticles' interface in the human body.

In addition to traditional nanoparticle characterization techniques, a more biologically like investigation of the biomolecular corona is desirable. The long-established physico-chemical characterization of nanoparticles considers the interaction without the influence of the corona and hence, new methods are needed to describe and/or predict nanoparticle-protein, nanoparticle-protein-protein, and nanoparticle-cell interactions. Here we characterize the corona-coated nanoparticles' interactions with different biomolecules and assess orientation and functionality of specific key molecular features. The obtained information is a valuable tool to optimize nanoparticles for therapeutic applications and to improve the predictability of *in vivo* performance of the nanoparticles.

More specifically, we present a method for the rapid screening of exposed protein recognition motifs on the surface of nanoparticles exploiting quartz crystal microbalance (QCM). We quantify accessible functional epitopes of transferrin-coated nanoparticles and correlate them to differences in nanoparticle size and functionalization. The target recognition occurs label free in flow, thereby pushing our investigation into a more *in vivo* like scenario. Our method is applicable to a wide array of nanoparticles. Moreover, the possibility to perform the analysis in situ (including highly complex dispersions and relevant biological milieu) represents an important step for the acquisition of molecular detailed information in realistic biological scenarios. The developed characterization assays enable to determine the specific orientation of the proteins and therefore will predict the functionality and availability of specific epitopes of interest.

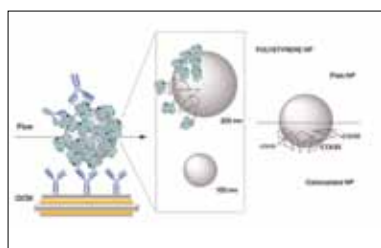
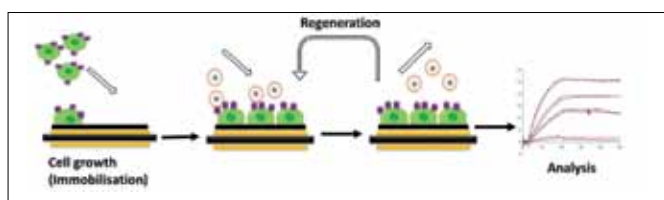


Figure 1. Schematic illustration of the experimental set-up for the bio-recognition assay and of the nanoparticles employed (two different sizes and two different surface chemistries for the same type of nanomaterial have been used).

The platform also allows studies of the effect of surface modifications of NPs on the interactions with cells grown on QCM sensor surfaces. The results highlight the influence of corona formation on interaction properties of NPs with cells as revealed by kinetic rate constant profiling and demonstrate the potential of the platform to predict NP - cell interactions within a biological system.

Figure 2. Schematic illustration of the experimental set-up for the cell-based assay



## DEVELOPING CELL-TARGETED, BRAIN-PENETRATING POLYMERIC NANO-MICELLES TO TREAT NEURODEGENERATIVE DISEASES

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Due to the aging world population, age-related neurodegenerative diseases such as Alzheimer's disease (AD) will become a huge medical and economic burden on society if they are not properly addressed. Due to the poor brain penetration of therapeutic drugs, the number of current available therapeutic candidates for neurodegenerative disorders is inadequately low. Poor drug brain penetration is mainly due to the presence of the blood-brain barrier (BBB), a highly impermeable endothelial cell layer lining the brain microvessels which prevents unspecific entry of molecules larger than 500 daltons. Hence, developing nano-vehicles capable of efficiently crossing the BBB and target individual brain cells will allow for delivery of therapies to specific cell populations in sufficient levels to have success in the clinic. Our research group has recently developed glucose-conjugated polymeric micellar nanoparticles (gPMN) (fig. 1a) capable of efficient and specific BBB transport and brain penetration by targeting the glucose transporter (Glut)-1 protein<sup>1</sup> (fig. 1b). In the present work, we have advanced this achievement by targeting the gPMN to individual brain cell types through functionalization with antibody fragments capable of recognizing cell-specific membrane markers.

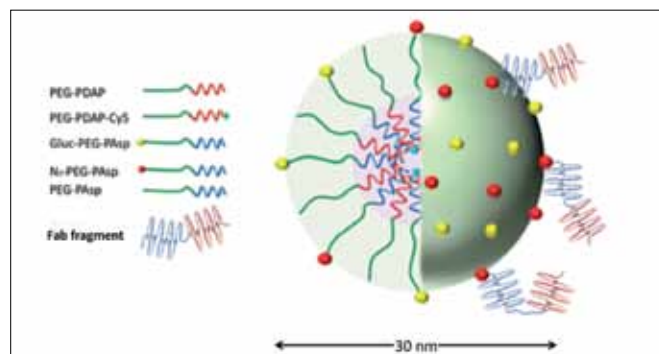


Fig 1a. Composition of glucose- and azide- displaying polymeric micellar nanoparticles functionalized with Fab fragment antibodies

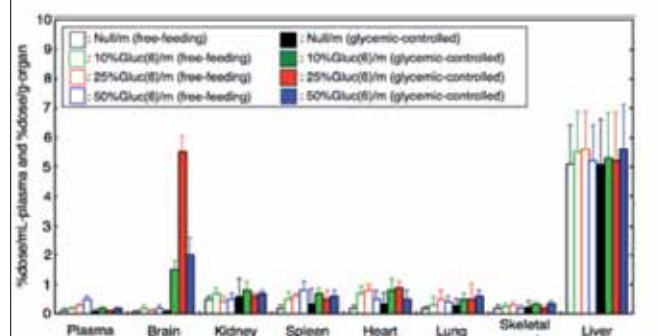
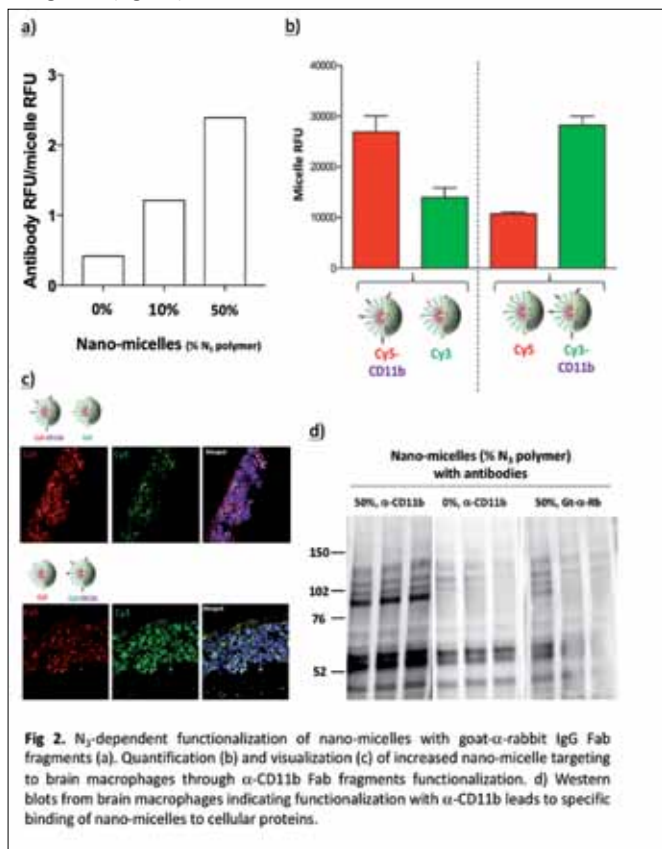


Fig 1b. Brain penetration of glucose-displaying polymeric micellar nanoparticles specifically under glyceamic control conditions

In order to conjugate antigen binding (Fab)-fragments onto the gPMN, we firstly modified the polymer composition of the nano-micelles to include an azide (N3)-capped polymer capable of binding to DBCO-conjugated proteins through copper-free Click chemistry. To this end, Fab-fragments generated from full antibodies were DTT-reduced and reacted with maleimide-DBCO to generate DBCO-Fab proteins. Employing a model antibody (Goat  $\alpha$ -Rabbit-Alexa 488), we have successfully shown conjugation of DBCO-Fab



proteins onto N3-displaying gPMN, with the number of conjugated proteins correlating to the percentage of N3 polymers composing the gPMN (fig. 2a).



Because of the crucial role inflammation plays in driving the progressive neuronal loss seen in AD<sup>2,3</sup>, we were interested in targeting the gPMN to the resident macrophages of the brain, the microglia. To this end, we created Fab-fragments from antibodies recognizing the microglia marker protein CD11b. Similarly to the model antibodies,  $\alpha$ -CD11b Fab fragments associated with the gPMN in an N3-polymer-dependent manner, indicating tunable protein loading onto the nano-micelles. To examine whether the  $\alpha$ -CD11b-functionalization successfully localized nano-micelles to microglia cells, two combinations of micelles labelled with either Cy5 or Cy3 fluorophores and alternating  $\alpha$ -CD11b functionalization were prepared and incubated with microglia cells *in vitro* (BV2 cell line). Fluorescence quantification indicated microglia displayed higher fluorescence corresponding to the  $\alpha$ -CD11b functionalized micelle in each combination (fig. 2b). This was corroborated by confocal microscopy visualization of cell-bound nano-micelles (fig. 2c). In order to examine whether the microglia-localization of  $\alpha$ -CD11b nano-micelles was specific for CD11b protein and not due to unspecific interaction with the cell membrane due to antibody functionalization, Western blots from microglia cell lysates were incubated with nano-micelles and their localization visualized through Cy5 fluorescence detection. A strong band around 100 kDa was detected in blots incubated N3-micelles functionalized with  $\alpha$ -CD11b. Importantly, this band was absent in blots incubated with non-N3-micelles ( $\alpha$ -CD11b) or with N3-micelles functionalized with a control antibody, indicating the increased micelle binding was due to specific  $\alpha$ -CD11b interaction with microglia proteins.

In conclusion, the present work lays the foundations to generate brain-penetrating nano-micelles capable of targeting individual cell types to increase the spatial specificity of drug delivery. Targeting cell populations would have clear benefits to treat neurodegenerative diseases by minimizing cellular side-effects and decreasing the required drug dose to achieve impactful clinical outcomes.

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## TARGETING OF ANTIGEN PRESENTING CELLS FOR TUMOR IMMUNOTHERAPY

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The induction of potent, T cell mediated tumor immunity requires presentation of tumor antigens to T cells by antigen-presenting cells (APC). The most efficient APC are dendritic cells (DC), which effectively engage naive T cells and are able to present antigen via MHC class II to CD4+ T cells and cross-present antigen via MHC class I to CD8+ T cells, resulting either in potent immunity or in specific immune tolerance, depending on their subtype and functional state. Thus, nanoparticle (NP)-based vaccination approaches for induction of potent T cell responses have mostly focussed on codelivery of antigen and adjuvant to DC. There are various ways to target DC by NP, among these are strategies to engage surface molecules on DC by their natural or synthetic ligands, by monoclonal antibodies or antibody fragments, or by synthetic molecules such as aptamers, cystine-knot miniproteins, DARPin and others. In addition, DC can be targeted functionally by transmitting plasmids that express genes under the control of DC-specific promoter constructs. Many of these approaches have proven effective in inducing antigen-specific T cell responses, and some of these approaches are in clinical trials at present.

In contrast to DC, specific targeting of B cells which generate antigen-specific antibodies has rarely been addressed. Here we show that iron oxide nanoparticles coated with dextran (DEX-NP) after i.v. injection predominantly targeted B cells in spleen. B cell targeting was observed *in vitro* after preincubation of DEX-NP with native mouse serum. The resulting protein corona of DEX-NP contained key components of the lectin pathway of complement activation and complement C3 at high level. Preincubation of DEX-NP with serum from C3-/- mice and from MBL-/- mice which are unable to mount lectin-dependent complement activation yielded no B cell targeting. Antibodies specific for the B cell complement receptors CR-1/2 blocked binding of C3-opsonized DEX-NP *in vitro* and *in vivo*. To exploit inherent B cell targeting of DEX-NP for vaccination, the model antigen ovalbumin (OVA) and an immunostimulatory CpG-rich oligonucleotide (ODN) were conjugated. Derived multifunctionalized DEX-NP activated B cells *in vivo*, and induced much higher levels of OVA-specific IgG2a antibodies than obtained after immunization with soluble OVA plus CpG-ODN. Vaccination of C3-/- and MBL-/- mice, and blockade of CR-1/2 in wild type mice yielded strongly diminished antibody production. Our study shows that lectin surfaces of NP may initiate lectin-dependent complement activation which results in specific B cell targeting. This phenomenon must be considered in the design of NP surfaces, and may be exploited to induce profound humoral immune responses.

## LINKS BETWEEN BACTERIAL COMPOSITION AND BREAST CANCER TISSUE

STEPHEN GROBMYER

Breast cancer (BC) remains the most common cancer among women globally and the number of BC cases are projected to increase significantly over the next 15 years. Current clinical efforts at BC risk reduction are focused on surgical prophylaxis, hormonal modulation and life style modification. These strategies have had only a minor impact on reduction of BC incidence. Over 50% of women who develop BC have no known risk factors, and most of the known risk factors for BC development are only weakly associated with sporadic BC risk. The lack of identification of risk factors for BC development and metastasis has severely hampered efforts to prevent and treat the disease. Therefore, identification of new factors associated with BC risk, progression and treatment resistance, such

as the microbiome, represents an unique opportunity for designing new preventive and therapeutic strategies and reducing suffering associated with BC. Environmental effects are known to have a major role in cancer development and progression for cancers including cervical cancer, gastric cancer, and lung cancer.

Microbiomics is the emerging field utilizing massively parallel sequencing focused on characterization of microbes that colonize human tissue broadly including bacteria, non-human Eukaryotes, and viruses. With bacteria in the human body outnumbering human cells 10:1, it is rapidly becoming clear that the microbiome represents the undiscovered frontier in the understanding of human biology. There is a growing understanding of and interest in the relationship between these microbial communities, inflammation, and human cancer. "Dysbiosis" refers to microbial imbalance within a specific milieu. Dysbiosis has been associated with colorectal disease progression and resistance to chemotherapy in pancreatic cancer. Our group has recently completed a study breast cancer patients and non-cancer patients having breast surgery using 16s rRNA gene sequencing <sup>(1)</sup>. Metagenomic analysis of breast tissue from cancer patients and non-cancer patients was performed to test whether overall bacterial taxa composition was different between cancer and non-cancer tissue, we used principal coordinates analysis of weighted Unifrac distances. We found that the patient samples clustered distinctly from the extraction and environmental controls ( $p = 0.004$ ). Among patient samples, cancer and non-cancer samples clustered differently ( $p = 0.03$ ), and among cancer patient tumor samples, samples clustered by molecular subtype (ER+ vs. ER-) and (HER-2+ vs. HER-2-).

These findings are supported by several other studies which have suggested presence of bacteria within human breast cancer. Taken together, these findings suggest the potential role for specific interventions aimed at intra-tumoral bacteria to prevent or treat human breast cancer.

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## ADVANCED CHARACTERIZATION FOR DEFINING QUALITY ATTRIBUTES OF RNA NANOPARTICLES

**HEINRICH HAAS**, Vice President Drug Delivery, BioNTech RNA Pharmaceuticals, GmbH, Mainz (D)

RNA delivery vehicles for organ-selective targeting can be engineered by appropriate assembly routes and vehicle compositions. For pharmaceutical development of clinically applicable products, criteria to define quality and specifications must be defined. In addition to biological evaluation, physicochemical characterization can provide valuable data for defining critical product parameters and help to justify specifications for quality controls such as in release testing. Here we present results from advanced characterization of RNA lipoplex products, which are assembled from RNA and liposomes in different ratios. Small angle X-ray scattering (SAXS) measurements, in combination with binding studies and assays to derive information on aspects of colloidal organization were applied. This enabled to obtain insight into ranges were certain structural characteristics were correlated with biological activity and targeting selectivity. Such information can be helpful for defining and justifying quality criteria for pharmaceutical products.

## INTRAVENOUSLY INJECTABLE MRNA-LIPOPLEX NANOPARTICLES FOR TUMOR IMMUNOTHERAPY: CLINICAL UPDATE FROM A FIST-IN-HUMAN PHASE I/II TRIAL

**HEINRICH HAAS**, Vice President Drug Delivery, BioNTech RNA Pharmaceuticals, GmbH, Mainz (D)

Intravenously injectable mRNA-lipoplex nanoparticles for tumor immunotherapy: clinical update from a fist-in-human phase I/II trial

Approaches for therapeutic vaccination against cancer using tumor antigen-encoding RNAs are gaining increasing interest, and several products have successfully being translated up to the level of clinical trials. BioNTech has developed a technology platform of intravenously injectable mRNA nanomedicines, which was engineered to enable selective delivery of the cargo of antigen-encoding RNA to APCs in lymphoid compartments. The RNA-lipoplex products, denoted RNA(LIP) account for high biopharmaceutical availability as well as efficient and organ-selective expression of the RNA.

A first-in-human phase I/II dose escalation Lipo-MERIT trial was initiated (NCT02410733) to assess the safety, tolerability, and biological efficacy of RNA(LIP) for cancer immunotherapy. Here an update on the preliminary data obtained from this ongoing trial is given. The results confirm the safety and tolerability of the RNA(LIP). Patients experience mostly only mild to moderate adverse drug reactions typically associated with immune activation. Immunological assessment so far reveals a high rate of vaccine-induced immunity and point towards de novo induction of antigen-specific immune responses as well as potent expansion of pre-existing immunity upon the multiple applications of the Lipo-MERIT vaccine. Following these encouraging results, several further studies for cancer vaccination based on this universally applicable technology platform have been initiated or initiation is underway.

## LIPOSOME PLATFORM ADDRESSING UNMET MEDICAL NEEDS IN ONCOLOGY AND NEUROLOGY

**DR. STEFAN HALBHERR**, Manager Research and Development, InnoMedica

InnoMedica has established an economically efficient method for GMP compliant industry-scale manufacturing of ultra-small liposomes as low as 30nm in diameter. This small size along with other physico-chemical features of the nanoparticles give rise to previously unseen pharmaceutical effects and favorably affect drug bio-distributions. In essence, InnoMedica is exploiting the liposome-derived biodistributions to address a broad spectrum of unmet medical need, predominantly in oncology and neurology, but also including bacterial diseases. All the pipeline projects are summarized in the infographic below (Fig.1).

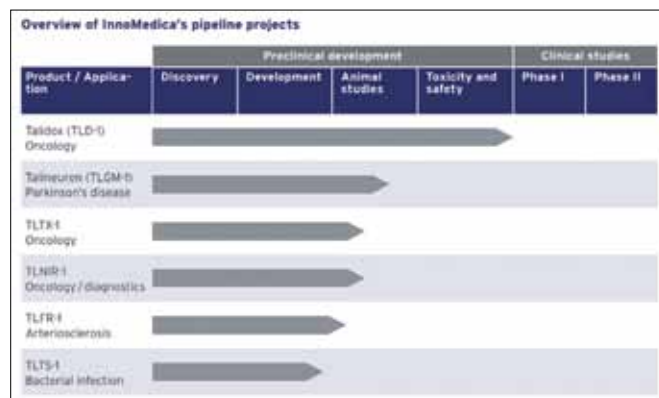


Fig.1: Pipeline projects of InnoMedica (May 2018). Talidox is a liposomal formulation of doxorubicin. Talineuren is a liposomal

formulation of GM1 ganglioside. TLTX-1 is a liposomal formulation of docetaxel. TLNIR-1 is a liposomal formulation carrying near-infrared dye. TLFR-1 is a liposomal formulation of forskolin and rolipram. TLTS-1 is a liposomal formulation carrying both glycans and phospholipids native to the human body.

Talidox (Targeted Liposomal Doxorubicin) is InnoMedica's most advanced liposome product whose application for phase 1 clinical trial has been submitted to SwissMedic. Talidox aims at improving drug performance relative to competitors by maximizing antineoplastic activity while reducing side-effects such as the hand-foot-syndrome (Caelyx/Doxil) and cardio-toxicity (free drug doxorubicin). Talidox holds the potential to become the new best-in-class anthracycline.

Further pipeline projects in Oncology include a liposomal formulation of docetaxel (TLTX-1), another widely used cytostatic drug as well as a diagnostic liposome containing a near-infrared fluorescent dye (TLNIR-1) for specific labelling of tumor tissue in order to assist fluorescence-guided surgery.

Talineuren (Targeted Liposomal Neuroregeneration) delivers neuroprotective GM1 ganglioside to the brain after oral intake thereby preserving dopamine levels in the striatum in a Parkinson mouse model of disease. Due to the broad neuroprotective effects of GM1, Talineuren could also be effective in treating other diseases of the central nervous system. Talineuren does not directly address any known specific cellular targets such as alpha-synuclein plaques in Parkinson's disease, or amyloid-beta plaques in Alzheimer's disease, but supports neuronal function and survival. Talineuren could become a valuable compound to address many age-related diseases of the CNS.

InnoMedica's TLTS-1 liposomes have proven useful in neutralizing different bacterial toxins. As the world is inevitably moving into the post-antibiotic era, these liposomes could be used to treat bacterial infections resisting conventional antibiotics. The mechanism of action of these liposomes is of a completely different nature than bactericidal drugs, as these liposomes are essentially non-bactericidal. Instead, the liposomes mimic native cell surfaces, thereby effectively sequestering bacterial toxins. No evolutionary pressure is applied, no resistance arises, and the indispensable symbiotic microbiome is unaffected.

The continuous development of a proprietary technology platform allows InnoMedica to specifically target a broad range of organs and tissues enabling advantageous treatments in the field of oncology and neurology by increasing efficacy and reducing side-effects as well as administered dosages. Moreover due to the experience of InnoMedica additional indication areas such as bacteriology are opening their doors.

## SPECIFIC AND QUANTITATIVE ANALYSIS OF NANOPARTICLES, LIPOSOMES, MICROVESICLES, EXTRACELLULAR VESICLES AND VIRUSES WITH ZETAVIEW TWIN NTA

CLEMENS HELMBRECHT

Nanoparticle Tracking Analysis (NTA) of bionanoparticles, such as liposomes, virus, microvesicles and extracellular vesicles, is an efficient technique for quantification of size and total concentration. Traditionally, NTA instruments were equipped with only one laser for analysis of nanoparticle in scatter and fluorescence mode. The fluorescence detection option F-NTA allows phenotyping on single particle level of typical samples such as liposomes, microvesicles, extracellular vesicles and viruses. However, shifting between excitation wavelength was a tedious task including manual steps and realignment.

We present an NTA instrument equipped with two laser sources. For the first time, it is possible to perform double estimation of biomarkers (e.g. tetraspanins CD63/CD81) on the same sample. The alignment free switching between laser sources allows the detection of membrane dyes or fluorescently labeled antibodies within minutes. Quantification of absolute vesicle and biomarker concentrations were performed on selected samples.

## NANOGELS AS ANTITUMOR VACCINES

WIM E. HENNINK, D. Li, C. F. van Nostrum, and T. Vermonden  
Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, 3584ED, the Netherlands.

An attractive approach to induce a strong immune response against a certain antigen is its targeting to dendritic cells (DCs). Targeting can be established by loading the antigen in a nano-sized carrier and keeping the antigen encapsulated or associated with the particles until they are internalized by DCs<sup>[1, 2]</sup>. We designed reduction-sensitive cationic dextran nanogels in which an antigen (ovalbumin, OVA) is reversibly immobilized to the hydrogel network via disulfide bonds. These bonds are stable in the extracellular environment but are cleaved in the cytosol of DCs due to the presence of glutathione resulting in triggered release of the loaded antigen after internalization of the nanogels by DCs. These nanogel OVA conjugates showed intracellular release of the antigen when the nanogels were internalized by DCs and boost the MHC class I antigen presentation<sup>[3]</sup>. The prophylactic and therapeutic vaccination potential of conjugated OVA nanogels was studied by analyzing their efficiency to induce anti-tumor immune responses.

To investigate the antitumor efficacy of the OVA nanogel conjugates, we tested their efficacy in both a prophylactic and a therapeutic tumor model using B16OVA melanoma (expressing OVA antigen). C57BL/6 mice (10 animals per group) received various formulations subcutaneously (s.c.) before (prophylactic vaccination) or after (therapeutic vaccination) challenge with s.c. administered tumor cells. Mice immunized with OVA nanogel conjugates effectively provoked an antigen specific T cell immune response as well as showed a strong increase of antigen specific antibody production. In the prophylactic model, 90% of the mice vaccinated with OVA nanogel conjugates+ poly I:C did not develop a tumor in 55 days (Figure 1). In the therapeutic model, 40% mice eliminated their tumor cells which is remarkable compared to other groups (Figure 1). In summary, the nanogels described in this study are promising formulations to induce antigen specific immunity against cancer.

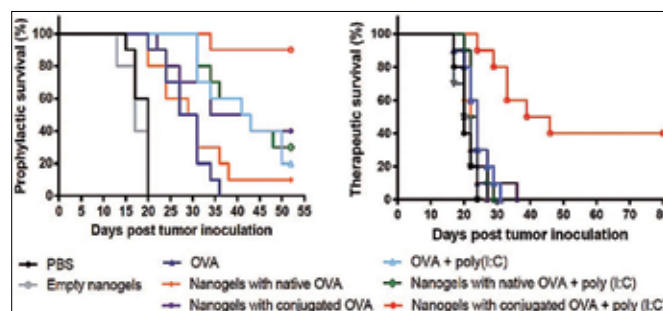


Figure 1. Survival of mice after prophylactic and therapeutic immunization<sup>[4]</sup>.

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## ACTIVATION OF INNATE IMMUNITY: TOLL-LIKE RECEPTORS AND OTHER RECEPTORS OF THE INNATE IMMUNE SYSTEM

JULES HOFFMANN

Insects are a formidable zoological group, representing an estimated 80 % of extant species and putting one third of humanity at risk of severe morbidity or deaths through their vector capabilities for various types of microbes. For a long time, insects have been known to be themselves particularly resistant to infection. We and others have undertaken an in-depth analysis of the mechanism underlying this resistance, and essential results will be given in the presentation. Unexpectedly, it appeared that stringent similarities exist between *Drosophila* antimicrobial defenses and innate immune reactions in mammals – extending to such hallmark molecules as the Toll/ Toll-like receptors, the roles of which in immune defenses were first uncovered in *Drosophila*. We can now trace back the essential characteristics of innate immunity to the appearance of the first multicellular organisms, probably a billion of years ago. Adaptive immune defenses however are absent from invertebrates (95 % of species to-day) and are believed to have appeared in cartilaginous fishes some 450 millions years ago.

Of major importance in the field of biomedical sciences are the recent demonstrations that innate immunity to a large extent activates and orients the adaptive immune response. Furthermore, it has become apparent during the last decade that several other families of innate immune receptors in addition to the Toll-like receptors sense microbial infections, and that these various receptors can also be activated by some non microbial ligands (even endogenous molecules) which often signal stress or danger to the hosts. The presentation will attempt to integrate these various concepts which have to a large extent changed our views on immune immunological defense in animals in recent years.

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## COMPUTATIONAL ONCOLOGY & MOLECULAR MODELING – THE DEVELOPMENT OF NOVEL STRATEGIES

KRISZTIAN HOMICKO

Oncology is being revolutionized by technological breakthroughs that allow deep molecular interrogation of tumor tissue, bringing unprecedented opportunities to understand oncogenesis but also the innate and adaptive tumor resistance mechanisms. Within personalized medicine, oncology is in a privileged situation to move forward quickly as some of the molecular alterations are directly actionable with specific treatment strategies. Multi-dimensional data is collected both in clinical application as well as by research. Also, the evaluation of new multidimensional molecular data requires the capture of high-quality clinical data. An additional, novel layer of this data is coming from molecular modeling of mutant proteins and potential inhibitors.

Together, these rich datasets require novel methods of integration and interrogation. In my presentation, I will review the complex-

ity of the currently available, as well as future data and the novel strategies that are implemented and developed to understand the wealth of information and to provide interpretable outputs.

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## TRANSFORMING THE FUTURE OF CANCER: MANUFACTURING OF CAR-T CELL THERAPIES

ALEXANDER HUBER

Reprogramming T-cells for adoptive cell therapies are now everywhere in the news. Such chimeric antigen receptor T cells (CAR-T cells) are used in a “living” treatment with very special needs in a highly personalized manner. Presently several types of therapies are advancing or have already received regulatory approval. One of these therapies is Kymriah, manufactured by Novartis at several locations worldwide. In this therapy, patients’ T-cells are equipped with a chimeric antigen receptor that recognizes CD19. Thus, Kymriah reprogrammed T-cells recognize CD19+ B-cell malignancies and they can be used to treat cancer indications such as acute lymphoblastic leukemia and non-Hodgkin’s lymphoma (NHL).

CAR-T cell therapies like Kymriah need a new business model since one batch produced is only for one patient. A decentralized production is required with significant investments in facilities that are able to deal with cell processing in large scale under GMP. Overall, a novel supply chain needs to be established to supply worldwide demand with the same raw materials and consumables. Many materials and the whole manufacturing process needed to be adapted from an academic setting into a fully approved GMP process that is able to supply the global demand for the indications mentioned. In this presentation, challenges for manufacturing and supply for large-scale manufacturing of CAR-T cells will be explained using Kymriah as an example case.

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## PERSONALIZATION BY PRECISE KNOWLEDGE AND PRECISE THERAPIES, ENABLED BY NANOMEDICINE

PATRICK HUNZIKER

Oncology is being revolutionized by technological breakthroughs that allow deep molecular interrogation of tumor tissue, bringing unprecedented opportunities to understand oncogenesis but also the innate and adaptive tumor resistance mechanisms. Within personalized medicine, oncology is in a privileged situation to move forward quickly as some of the molecular alterations are directly actionable with specific treatment strategies. Multi-dimensional data is collected both in clinical application as well as by research. Also, the evaluation of new multidimensional molecular data requires the capture of high-quality clinical data. An additional, novel layer of this data is coming from molecular modeling of mutant proteins and potential inhibitors.

Together, these rich datasets require novel methods of integration and interrogation. In my presentation, I will review the complexity of the currently available, as well as future data and the novel strategies that are implemented and developed to understand the wealth of information and to provide interpretable outputs.

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## THE VARIOUS FACES OF PRECISION MEDICINE

PATRICK HUNZIKER

The various faces of precision medicine- Hunziker Precision medicine is a simple term for a challenging task. While analyzing the

„-omics“ of an individual is becoming increasingly feasible and payable, interpreting its relevance for a given patient at a given time having a given disease and facing several treatment options is much less clear. This talk will explore the landscape of topics relevant to individualize diagnosis and therapy, including aspects from the macro down to the nano scale of a human being. This approach will require the most recent technologies available, including nano-medical analyses and therapies, targeted therapies, artificial intelligence exploiting supercomputer capabilities, predictive models in terms of physiology, pharmacology, and health economy. As animal models and large randomized studies often lack the capability of predicting outcome in specific individuals, new ways of studying therapies and new regulatory paths are increasingly needed.

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## ATHEROSCLEROSIS ERADICATION

PATRICK HUNZIKER

This talk gives an update 2018 to the desirable perspective of eradicating

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## ENGINEERING ENHANCED ADOPTIVE T CELL THERAPIES

DARRELL J. IRVINE, MIT, Koch Institute for Integrative Cancer Research; Ragon Institute of MGH, MIT, and Harvard; Howard Hughes Medical Institute

Adoptive cell therapy (ACT) using autologous T cells genetically modified with chimeric antigen receptors or synthetic TCRs has shown significant promise in early clinical trials, but strategies to enhance ACT T-cell functionality *in vivo* are needed. To this end, we developed a strategy combining nanomedicine with ACT, based on the chemical conjugation of drug-loaded nanoparticles (NPs) as synthetic “backpacks” to the surfaces of live lymphocytes for ACT. To maximize the drug cargo associated with each cell, nanoparticles were formed by reversibly crosslinking protein drugs into nanogels comprised >90 wt% of cytokine drug cargos of interest. We further interfaced drug release from these particles with the biology of the T-cells by designing reduction-sensitive crosslinks, that allow drug to be selectively released as T-cells are triggered through the T cell receptor and upregulate cell surface redox activity. ACT T-cells backpacked with these cytokine nanogels are capable of massive *in vivo* expansion and robust anti-tumor responses, while avoiding side effects commonly observed with systemically-administered immunomodulatory drugs. In a second approach, we have developed a synthetic vaccine strategy to boost CAR T cells *in vivo*, allowing small numbers of ACT cells to be expanded in the native microenvironment of lymph nodes for enhanced functionality and persistence.

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## NANOCARRIER DELIVERY OF STING AGONISTS FOR TUMOR IMMUNOTHERAPY

DARRELL J. IRVINE, MIT, Koch Institute for Integrative Cancer Research; Ragon Institute of MGH, MIT, and Harvard; Howard Hughes Medical Institute

Ligands for the intracellular Stimulator of Interferon Genes (STING) receptor are potent inducers of anti-tumor immunity, via immunostimulatory effects on the tumor stroma, intratumoral immune cells, and cytotoxic effects directly to tumor cells. Local administration of cyclic dinucleotide (CDNs) to syngeneic tumor models of a variety of cancers in mice leads to rapid vascular disruption and immune cell activation. As small, hydrophilic drugs, naked CDNs

are rapidly cleared from the tumor site, leading to the undesirable circumstances of decreased intratumoral potency and the potential for harmful, nonspecific systemic inflammation. We designed self-assembled lipid nanodiscs (LNDs) that reversibly complex CDNs in order to modulate their biodistribution and pharmacokinetics. Lipids bearing peptide nucleic acid (PNAs) headgroups were synthesized, which noncovalently associate with CDNs via base stacking and hydrogen bonding. By complexing the CDNs with lipid nanodiscs including these PNA-functionalized amphiphiles, we are able to increase retention at the tumor site and promote delivery to the proximal lymph node following systemic or intratumoral injection. Through *in vitro* studies, we have demonstrated that when loaded onto LNDs, the CDNs remain active and are able to stimulate STING signaling in a mouse macrophage reporter cell line. In a murine model of adenocarcinoma, we found that intratumoral administration of CDN-loaded lipid nanodiscs increases the antitumor activity of STING agonists over injection of the uncomplexed CDN, especially in combination with the approved checkpoint inhibitor anti-PD1. The potentiated STING signaling induced by CDN delivery via LND carriers results from increased nanoparticle uptake by tumor-infiltrating leukocytes and prolonged drug residence within both stromal and tumor cells. Our studies suggest that exploiting noncovalent interactions of CDNs with synthetic nucleic acid receptors can be used to enhance their functional delivery *in vivo*.

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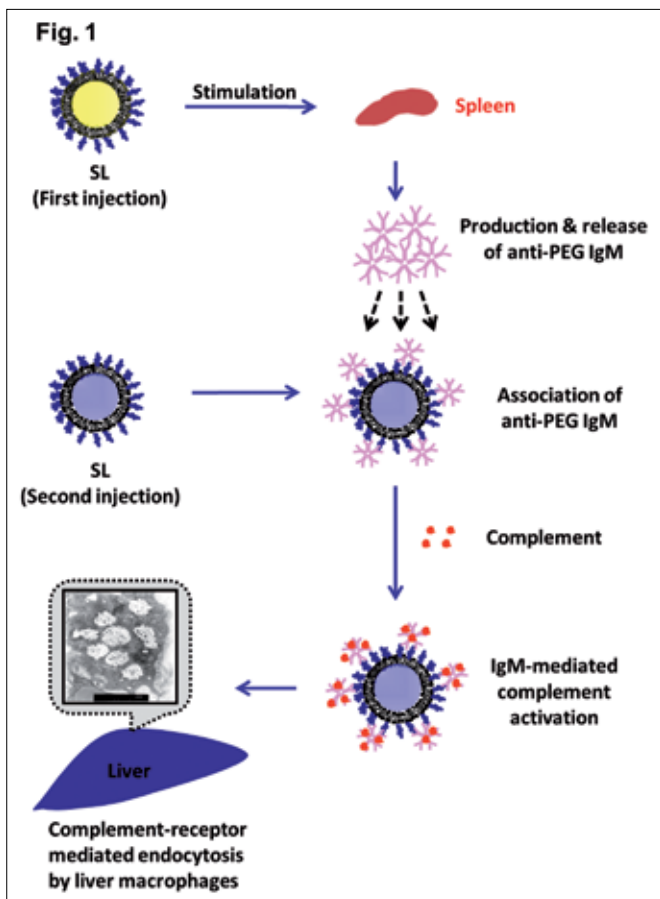
## ANTI-POLYETHYLENE-GLYCOL ANTIBODY RESPONSE TO PEGYLATED NANOPARTICLES

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Polyethylene glycol (PEG) is considered as non-toxic and non-immunogenic material, and surface modification with it can improve the immunogenicity and pharmacokinetics of nanocarriers. However, we have reported that PEGylated liposome (SL) loses their long circulating properties when they are administered twice in same animal with certain interval (accelerated blood clearance (ABC) phenomenon)<sup>[1]</sup>. We elucidated that anti-PEG IgM, secreted in response to the first dose of SL, is responsible for the rapid clearance of the second dose via initiation of complement activation (Fig. 1). We further elucidated that such anti-PEG IgM production is caused in nude mice (no T-cells), while it was not caused in SCID mice (no B and T cells) and splenectomized mice (no spleen)<sup>[2]</sup>. These suggest that spleen B cells produce the anti-PEG IgM in a T-cell independent manner. It appears that SL activates the immunity in spleen as T-cell independent antigens do.

In addition, we have reported that PEG-OVA elicits an anti-PEG IgM response, while it did not elicit antibody against OVA<sup>[3]</sup>. The mice pretreated with PEG-OVA showed rapid clearance of test PEG-OVA from blood circulation. Interestingly, the anti-PEG IgM induced by SL did not affect the blood concentration of subsequent dose of PEG-OVA. These suggest that anti-PEG IgM also plays an important role in the accelerated blood clearance of PEG-conjugated proteins, although it seems the presence of anti-PEG IgM in blood circulation does not necessarily affect circulating property of entire PEGylated materials. We very recently observed similar phenomenon upon NEULASTA- pegfilgrastim (PEG-G-CSF) in animal experiment.

Of interest, an emerging body of evidence is emphasizing the existence of naturally occurring anti-PEG antibodies in normal individuals who have never received PEGylated therapeutics. Any PEGylated formulations might display unexpected pharmacokinetic behavior under such condition, resulting in less therapeutic efficacy or even cause undesirable side-effects. Therefore, a deep understanding of the prevalence and clinical implications of anti-PEG immunity is a prerequisite for the continual clinical application of PEGylated therapeutics.



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## ACKNOWLEDGEMENT

This work was partially supported by the Japan Society for the Promotion of Science, a Grant-in-Aid for for challenging Exploratory Research (16K15108), a Grant-in-Aid for Scientific Research (B) (15H04639), the Takeda Science Foundation, the Uehara Memorial Foundation, the Takahashi Industrial and Economic Research Foundation

## MONITORING OF PROTEIN AGGREGATION IN INJECTABLE VACCINES SYRINGES: A NEW DIRECT IN SITU & CONTACT-LESS TECHNIQUE

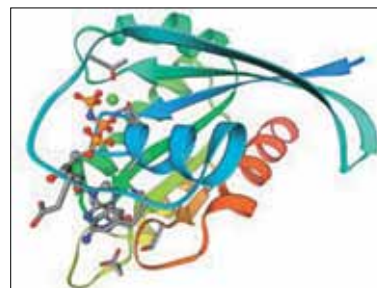
DAVID JACOB<sup>1</sup>, B. Pedrono<sup>1</sup>, S. Boj<sup>1</sup>

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**Key Words:** Protein aggregation, Biopharmaceutical API, Injectable, nano-particle sizer, In situ, contactless measurements, optical fiber remote probe, Dynamic Light Scattering (DLS).

## INTRODUCTION

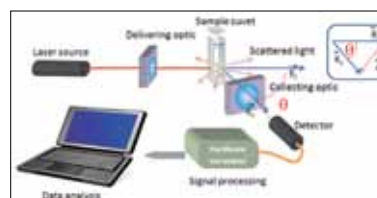
Aggregation of proteins and active principle ingredients (API) in injectable biopharmaceutical products remains a major concern impacting the stability and usability of a product. Indeed, Protein aggregation can occur during all stages of the lifetime of a protein therapeutic, including expression, refolding, purification, sterilization, shipping, storage, and delivery processes<sup>[1, 2]</sup>. The mechanism



of protein aggregation is still not well understood; but it is known that certain manufacturing stages like formulation composition, presence of microbial or vial contaminants during cell culture, and storage influence the risk of chemical degradation, which increases the risk of

physical degradation and the formation of aggregates. In particular, it has been shown that the storage container environment plays a role like in prefilled syringes for example where leaking silicone oil from the rubber stopper, traces of tungsten oxides and glass delamination can induce aggregates. In a context of more and more stringent international health regulations about the control of biopharmaceutical products, the in-situ monitoring of the denaturation and degradation process of therapeutic proteins during production and storage can be a key competitive advantage for manufacturers and researchers.

## CURRENT MEASUREMENT TECHNIQUES AND LIMITATIONS



Many different analytical techniques are used today to study protein aggregates in the one to few hundreds  $\mu\text{m}$  range like light obscuration (LO), dynamic imaging particle analysis (DIPA) techniques,

micro-flow imaging (MFI), and Coulter counter (CC). For early stage detection and quantification of submicron aggregates, other techniques such as multi-angle static light scattering (MALS) coupled to separative techniques like size-exclusion chromatography (SEC), analytical ultracentrifugation (AUC) and asymmetrical-flow field-flow fractionation (AF4) are routinely used<sup>[3]</sup>. Alternatively, batch Dynamic Light Scattering (DLS) is another useful optical technique to characterize aggregates when the dilution or shear encountered in SEC or FFF causes the aggregates to disassociate or when aggregation is to be measured under many conditions and/or temperatures. All these recognized techniques are quite complementary and efficient in their range of use but they require some specific preparation, conveying and handling process of the sample before or during measurement which can modify the sample aggregation state. That's why measuring directly into the storage medium like hermetically sealed vial or syringe without any need of sample manipulation and handling would be preferred in many cases. But none of the previously mentioned techniques are fitted to make measurements directly in injectable. A change of paradigm is then needed: if you cannot bring your sample to the measurement, you have to bring the measurement to your sample!

## IN SITU CONTACTLESS DLS MEASUREMENT CONCEPT

Dynamic Light Scattering (DLS), a mature and very powerful indirect optical technique is one of the prevalent method of choice in colloidal sciences and proteins characterization studies; Its principle (see schematic on right) is based on the measurement of the time fluctuation of a coherent laser light scattered at a given angle by particles undergoing Brownian motion in a liquid,<sup>[4]</sup>. The auto-correlation of the measured light fluctuation allows to calculate the self-diffusion coefficient  $D$  of the particles which is related (see eq. 1) to the size of the particle  $\varnothing H$  (hydrodynamic diameter) by the Stokes Einstein equation:

$$\varnothing H = \frac{KT}{3\pi\eta D} \text{ (eq. 1) ,}$$

Where  $T$  is the temperature of the samples (in Kelvin),  $K$  is the Boltzman constant, and  $\eta$  is the viscosity of the liquid phase. Standard DLS allows accurate particle size measurements from one nanometer up to a few microns in a minute. Though different mea





urement configurations are available on commercial DLS systems today, they all require the user to batch the sample with a pipette or an automatic pump and to place/in-

ject the sample into the instrument prior to the measurement, potentially impacting the sample protein aggregation state. In order to circumvent this important limitation, a new measurement system based on real time in-situ contactless remote DLS measurements has been developed by replacing the standard cuvette configuration by an Optical Fiber Remote Probe (OFRP). The OFRP, an optimized and highly robust optomechanical assemblies is designed to make direct and contact less measurements without any need for sample batching process. Connected to an Optical Unit by mean of a special optical fiber umbilical, the OFRP injects a laser beam into the sample and collect the light scattered by the sample in the backward direction at an angle 170°(see picture on left ) A highly sensitive single photon Avalanche Photodiode Detector (APD) connected to a dedicated fast acquisition electronic board monitors in real time the scattered light intensity fluctuations which are converted into time-resolved particle size kinetic analysis with powerful mathematical algorithms.

### MEASUREMENT IN VACCINES SYRINGES



In order to demonstrate the capabilities of this new measurement system to achieve contactless in-situ measurement into syringe, we have made series of particle size measurement on a commercial injectable flow vaccine (see fig 1- left

). This vaccine is a complex medium made of a mixture of many different ingredients : desactivated and fragmented Flew Virus from three different stem cells, several excipients like ppi water, Potassium chloride, Sodium chloride, buffered saline solution, and traces of chicken eggs proteins (ovalbumin), Formaldehyde, Neomycin, Octoxinol 9, etc. For the purpose of the measurement the vaccine syringe was removed from its plastic blister (see figure 1) and placed in a dedicated mount at a distance of 6 cm in front of the prob (see fig 1-insert right) ;

Figure 1: The commercial vaccine syringe out of its blister (left); Measurement setup principe (right) with the remote head mounted on a dedicated translatable stage and placed in front of a syringe holder designed for the purpose of the experiment (see insert)

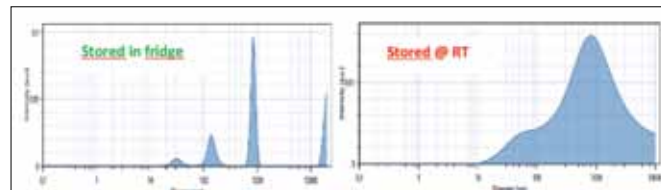


For comparison purpose and to evidence possible vaccin aging effects, we have stored one vaccin in a fridge at 7°C while another vaccin was stored at room temperature for 8 months. The two vaccins were then measured in the same conditions the same day. The particle size distribution results are presented below:

1 For verification purpose, some tests have also been made with-

out removing the plastic blister giving very consistent results with the measurements outside the blister.

Figure 2: Particle size distribution measurement results (X axis: size in nm; Y axis Amplitude in arbitrary unit) of a vaccine stored in a fridge (top) and of a vaccine stored at room temperature for 8 months (bottom)



For the vaccine stored in a fridge (fig 2 -top) one can notice the relative complexity of the sample particle size distribution exhibiting three distinct populations ranging from 30 nm up to 800 nm. These peaks correspond to virus fragments and proteins. One can also notice the presence of few aggregates beyond 10 µm. By comparison (fig 2- bottom), the vaccine stored at room temperature clearly shows some noticeable changes in the particle size distribution which now appears like a broad continuum from 10 nm to 10 µm and beyond. This variation would certainly deserve further investigation to understand the reason for this change. But, to the best of our knowledge these preliminary results are the first demonstration of an in situ DLS measurement into injectable syringe.

### CONCLUSION

We have presented the first practical demonstration of a contactless in situ particle size measurement of a commercial injectable vaccine directly into a syringe. By eliminating sample batching steps, the new DLS set up based on an innovative optical fiber remote head is opening up new fields of application to particle size measurement systems, in particular for the in-situ monitoring of protein aggregation in biopharmaceutical injectable products. This new setup could also be used to monitor in real-time nano-particle synthesis Kinetic in various type of reactor configuration (double jacket glass reactor, high pressure & high temperature Super Critical CO2 autoclaves, microwave reactors, micro fluidic chips, etc.) or for instrumental coupling [5].

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## FOLLOW-ON NBCDS AT HEALTH CANADA: CURRENT PERSPECTIVES AND FUTURE CONSIDERATIONS

MICHAEL JOHNSTON

This presentation outlines Health Canada's current approach to regulating nanomedicines and the agencies approach for the assessment of follow on/generic versions of currently marketed products. A summary of Health Canada's internal working groups and international regulatory efforts will be presented as well.

## HEALTH CANADA'S APPROACH TO NANOTECHNOLOGY AND NANOMEDICINE

MICHAEL JOHNSTON

This presentation provides an outline for Health Canada's approach for the regulation of nanomedicines. An overview of the structure and responsibilities of the department will be provided as well as the departments national and international cooperative efforts regarding nanotechnology/nanomedicines.

## BIODEGRADABLE SILICON NANOPARTICLE FOR TARGETED TREATMENT OF BACTERIAL INFECTION

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Bacterial resistance to antibiotics is a growing problem and has made it necessary to resort to antibiotics that have considerable toxicities. We reasoned that targeted delivery of an antibiotic to the site of infection would result in increased antibacterial activity, and the lower dosing made possible by the targeting would reduce side effects.

To test this concept, we set out to explore peptide-based targeting on infections. We have used phage display on *Staphylococcus aureus* bacteria and *in vivo* screening in mice with *S. aureus*-caused lung infections to identify peptides that would recognize *S. aureus*-infected tissue. A cyclic 9-amino acid peptide (sequence: CARG-GLKSC), named CARG, identified from the screening bound to *S. aureus* bacteria *in vitro* and selectively accumulated *in vivo* in *S. aureus*-infected lungs and skin in mice.<sup>1</sup> The specificity of the tissue recognition was evidenced by lack of accumulation in non-infected and in *Pseudomonas*-infected tissues, and lack of binding to *Pseudomonas* bacteria *in vitro*. A *Pseudomonas*-binding peptide had the opposite specificity.

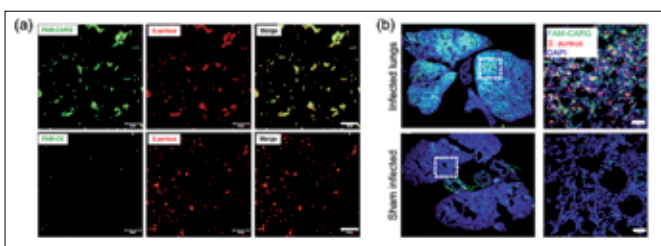


Figure 1. (a) CARG peptide shows selective binding to cultured *S. aureus* *in vitro* and homes to infected lungs *in vivo*.

To investigate translational potential of the targeted therapeutics, we then examined CARG-mediated delivery of nanoparticles *in vivo*. Since porous silicon nanoparticles (PSiNP) have attracted great attention as a biocompatible drug delivery platform utilizing large surface area and high pore volume to carry therapeutic payloads,<sup>2</sup> we use PSiNP conjugated with the targeting peptide to deliver the therapeutic nanoparticles to the infected site with high specificity, resulting in the extent of the drug payloads to be released at the targeted tissue. Vancomycin, a glycopeptide antibiotic used clinically to treat infections involving *S. aureus*, was incorporated into the nanoparticles via self-sealing chemical process derived by calcium silicate formation on the silicon surface as

reported recently.<sup>3</sup> Substantial quantity of vancomycin was loaded (> 12wt%) in the porous silicon matrix, along with intrinsic antibacterial activity retained as-is. The drug payload is gradually released from the nanoparticles over 20 hr period. The PSiNP were further modified with the CARG peptide, which guides systemic delivery of the therapeutic nanoparticles.

Accumulation of the CARG-nanoparticles was in excellent agreement with the localization of the free CARG peptide, suggesting targeted PSiNP also mimic the homing ability of the free peptide. Time-gated luminescence imaging of silicon nanoparticles clearly visualizes long-lived photoluminescence signal, thus allows quantification of nanoparticles in each tissue. The CARG-nanoparticles accumulated in the infected lung at markedly higher levels than the control nanoparticles. In addition, intravenously injected nanocarriers coupled with CARG peptide specifically recognize *S. aureus*-caused infection and spread into the extravascular site of the infected lung lesion, rather than uninfected lung. Fluorescence microscopy on the lung tissue section exhibited widely spread nanoparticles over the whole infected tissue, whereas lung from mice treated with control nanoparticles shows significantly lower intensities. It has been known that nanoparticles usually have potential to be accumulated at the site of infection due to localized increment in vascular permeability mediated by both cellular inflammatory response and direct activation of the kinin-kallikrein system bacterial proteases. Nevertheless, it is clear that the control nanoparticles have only limited ability to exploit the potential targeting to the infected tissue.

The CARG peptide enhanced the accumulation of intravenously injected nanoparticles loaded with vancomycin in *S. aureus*-infected lung tissue. These targeted nanoparticles more effectively suppressed *Staphylococcal* infections than untargeted vancomycin nanoparticles and free vancomycin. Peptides selected for bacterial binding *in vivo* may be valuable tools in combatting difficult-to-treat infections. In our infection model of mice, intratracheally introduced *S. aureus* causes serious pneumonia, resulting in ~60% mortality rate between 24 - 48 hr post-infection, and only 15 % survival by day 6. Treatment of infected mice with intravenous injections of the CARG-targeted vancomycin-PSiNPs one day post-infection resulted in 100% recovery and long-term survival, whereas only 30% survival was seen when free vancomycin was administered (Fig. 2). Complete survival required a ~10-fold higher dose of free vancomycin than was delivered in the nanoparticles. The targeted PSiNP formulation significantly improved the therapeutic efficacy of vancomycin, and tissue histology supported the conclusion that the lung infection had completely resolved, with no infectious foci or toxic damage observed in any of the major organs.

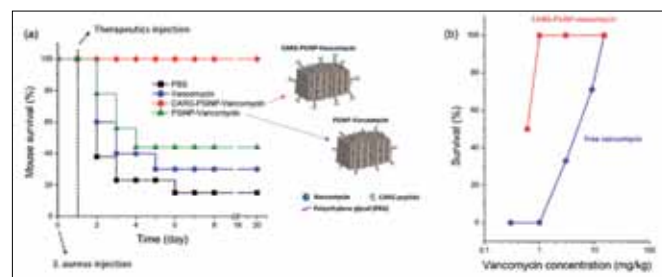


Figure 2. Targeted, infection-homing nanoparticles enhance antibiotic therapy.

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# BIOCOMPATIBLE, AMINOCAPROIC ACID STABILIZED CERIA NANOPARTICLES REDUCE BRAIN INJURY AFTER SUBARACHNOID HEMORRHAGE BIOCOMPATIBLE, AMINOCAPROIC ACID STABILIZED CERIA NANOPARTICLES REDUCE BRAIN INJURY AFTER SUBARACHNOID HEMORRHAGE

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Subarachnoid hemorrhage (SAH) is the most devastating type of stroke, largely caused by rupture of cerebral aneurysm. About 10% of patients die before hospital arrival, and about 57% of the patients expire within 6 months from onset. In the early phase of SAH, abundant amount of free heme derived from extravasated blood in the subarachnoid space is a major source of massive free radical toxicity, leading to global brain injury. Despite this disastrous cascade, few treatment modalities are currently available, except for simply repairing ruptured aneurysm.

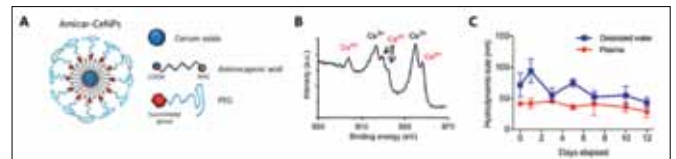
Ceria nanoparticles (CeNPs) has both Ce<sup>3+</sup> and Ce<sup>4+</sup> oxidation states on their surfaces, and redox cycling between the two states enables self-regenerative and multi-functional reactive oxygen species (ROS) scavenging activities. With their high surface-to-volume ratio, CeNPs have been highlighted as therapeutic agents for various diseases. However, toxicity still has been remained an issue for CeNPs to become biocompatible. Herein, we synthesized CeNPs in aqueous phase, not in organic solvent, and used aminocaproic acid as a stabilizer which is a FDA-approved drug per se. Then, we coated CeNPs with polyethylene glycol (PEG) to increase biocompatibility. We investigated whether our custom-made aminocaproic acid-coated CeNPs (Amicar-CeNPs) improve mortality and neurological outcomes in the SAH animal model.

We synthesized uniform water-dispersed Amicar-CeNPs from short sol-gel reaction of cerium (III) ions in aqueous phase with aminocaproic acid and PEGylated it (Fig 1A). Transmission electron microscopy (TEM) observation showed 3-nm discrete nanoparticles and lattice planes. In X-ray photoelectron spectroscopy (XPS), calculated Ce<sup>3+</sup>-to-Ce<sup>4+</sup> ratio based on the identified XPS peaks was 57% to 43% (Fig 1B). Amicar-CeNPs showed high colloidal stability with hydrodynamic diameter of 38.5 ± 3.1 nm in plasma for over 10 days (Fig 1C).

Amicar-CeNPs showed macrophage uptake in dose- and time-dependent manner *in vitro*. In a massive ROS generating condition induced by hemin, an oxidized form of the heme, Amicar-CeNPs reduced intracellular ROS in dichlorodihydrofluorescein diacetate (DCF-DA) assay. Moreover, in lactate dehydrogenase (LDH) assay, which detects LDH release from plasma membrane rupture and subsequent cell death, Amicar-CeNPs reduced LDH release. DNA fragmentation determined by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) positive nuclei were also re-

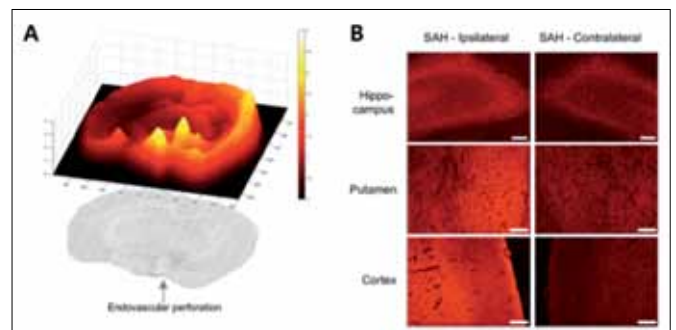
duced with Amicar-CeNPs treatment.

Fig 1. A. Schematic representation of Amicar-CeNPs. B. X-ray photoelectron spectroscopy analysis. C. Dynamic light scattering analysis.



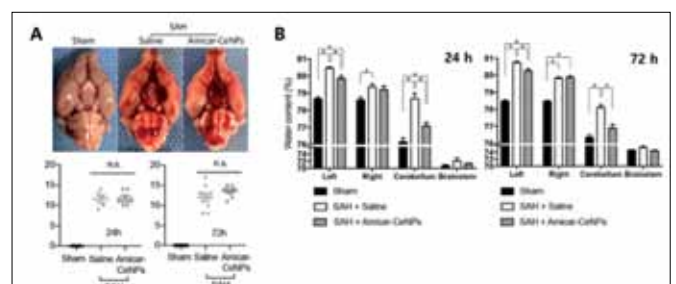
We induced SAH by endovascular perforation of the middle cerebral arteries of rats. Rhodamine B isothiocyanate (RITC)-conjugated Amicar-CeNPs (RITC-Amicar-CeNPs) were intravenously injected 1 hour after SAH, and brain was harvested after 72 hours from SAH. RITC-Amicar-CeNPs were more distributed in ipsilateral hemisphere than in contralateral hemisphere, in gray matter than in white matter, and in cortex than in deep structure (Fig 2A, 2B). More specifically, RITC-Amicar-CeNPs reached the cytoplasm of the neuronal cells and microglia/macrophages.

Fig 2. Distribution of Amicar-CeNPs in the brain after subarachnoid hemorrhage. A. 3-dimensional kernel density plot of RITC-conjugated Amicar-CeNPs in the brain at 72 h after SAH. B. Regional distribution of RITC-Amicar-CeNPs in hippocampal, putaminal, and cortical area of SAH. scale bar, 200 μm.



To investigate neuroprotective effects of Amicar-CeNPs, randomized, investigator-blinded animal trial was conducted. Single dose of Amicar-CeNPs (0.5 mg/kg) or saline control was intravenously injected 1 hour after SAH. SAH severity grades were comparable between two groups at both 24 hours and 72 hours, suggesting SAH models were well established (Fig 3A). Amicar-CeNPs significantly reduced neuronal cell death at the basal cortex at 72 hours after SAH (246.9 ± 14.1 per mm<sup>2</sup> vs. 2.2 ± 1.3 per mm<sup>2</sup>, P < 0.001). The number of CD68-positive macrophages were also decreased in the Amicar-CeNPs treated group (368.7 ± 30.1 per mm<sup>2</sup> vs. 144.3 ± 70.7 per mm<sup>2</sup>, P = 0.008), suggesting anti-inflammatory effect of Amicar-CeNPs. Brain water content, which reflects cytotoxic and vasogenic edema after SAH, also decreased significantly after treatment with Amicar-CeNPs in the ipsilateral hemisphere and cerebellum at 24 and 72 h (Fig 3B).

Fig 3. A. Comparison of the amount of SAH between sham, saline-treated and Amicar-CeNPs treated groups. B. Effect of Amicar-CeNPs treatment on brain edema measured at 24h and 72h after SAH. values, mean ± s.e.m. \* P < 0.05.

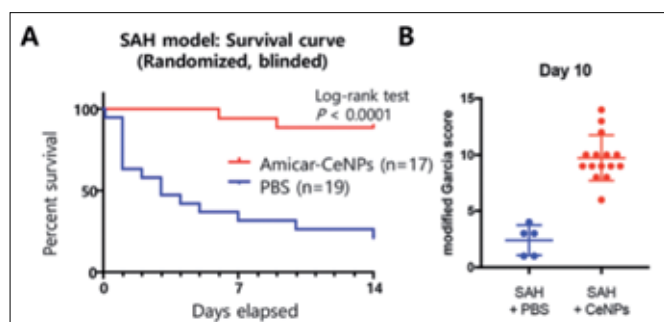


Finally, mortality rate and neurological score, the hard end-points of this study, were investigated. Mortality rate for 14 days was sig-



nificantly improved with Amicar-CeNPs treatment (Fig 4A, 78.9% vs. 11.8%,  $P < 0.001$ ). Modified Garcia score, neurological score of the rats, were much better in the Amicar-CeNPs group than in the control group (Fig 4B, median 3 vs. 9,  $P < 0.01$ ).

Fig 4. A. Survival curve of the rats with SAH for 14 days after the Amicar-CeNPs or saline treatment. B. Neurologic assessment of the survivors at day 10 after SAH induction.



In conclusion, Amicar-CeNPs, totally synthesized in aqueous phase, reduced mortality and improved neurological outcomes in the SAH model via potent anti-oxidative and subsequent anti-inflammatory effects. Given the lack of treatment modalities for SAH, this study suggests the potential of Amicar-CeNPs as a new therapeutic agent for patients with SAH.

## MULTIMODAL IMAGING FOR EVALUATING THE BEHAVIOR OF NANOMATERIALS IN VIVO

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Nanoparticles can be used as *in vivo* imaging agents or a drug delivery system. Some of them are now approved for commercial use. The nanoparticles introduced into the body should be eliminated from the body, either by degradation or by excretion. While many inorganic nano-carriers are very stable and difficult to metabolize, many of organic nanoparticles are biodegradable. Radiolabeling of biomolecules enables tracing these molecules *in vivo*. Biodistribution and autoradiography studies validate tissue distribution of the nanoparticles in animals. Nuclear medicine imaging such as single photon emission computed tomography (SPECT) or positron emission tomography (PET) allows non-invasive longitudinal monitoring of the *in vivo* pharmacokinetics and tissue distribution of the nanoparticles even in human subjects. On the other hands, *ex vivo* tissue imaging of fluorescently labeled nanoparticles reveals their patterns of microscopic distribution. *In vivo* tracking of nano-carriers using radionuclide imaging techniques enables a theranostic approach as well, not just being a drug carrier. The combination of diagnostic and therapeutic capabilities in a single drug delivery system can be used for precision personalized therapies.

## HOW HAS IT EVER COME TO THIS? A FRESH LOOK AT HEALTHCARE

PETER KAPITEIN

Inspire2Live is convinced that we can get cancer under control by the cooperation of patients, researchers and clinicians and with the combination of emotions and arguments. Emotions because it are we patients that are dying and need the better treatments. Arguments because we can only cooperate when we patient advocates know everything about the way the researchers and clinicians work and are an equal partner in this fight. If about us, not without us.

We know how to do the things that will benefit the patient, we want to do these things and we can do it. So what is preventing us from doing it? Yes there are barriers like data, law & regulation, money, privacy and of course the publication culture that determines the reward structure; publish or perish. But there is more.

The way the Medical Industrial Complex works has great similarities with the Financial and Military industrial complexes. The way the academic world works together with government and industry does not necessarily benefit the patient and her loved ones as does it not in the Military and Financial Industrial Complex where the citizen and owner of saving accounts also see that their interest is not always served in a proper way.

I stopped believing that there is bad intention in all this. There is not. It is my conviction that in the past decennia we've lost the possibility of having good discussions about good and bad. We do not challenge each other any more. When we have good discussions then the outcome determines the rules and regulations that we want to follow. It should be the Morality that defines the Law, but it is now the Law that defines Morality. The cause of this is the stopping of thinking: 'The sad truth about evil is that it is been done by people who never make their minds up about good and bad' (Hannah Arendt). This is why a doctor is honest and thinks she's doing the right thing when she tells a dying patient that she can't give this drug because she does not know what the long term effects of this drug are. The patient would have been very happy with long term effects after 5 years ...

Now, we can bring back the right order of Morality and Law by 4 rules:

- Put different people in a room.
- Go for the root cause.
- Scale up fast.
- Be independent.

By doing this in the cooperation between patients, researchers and clinicians and use the emotions and arguments to define the Why, What and How, we are able to get cancer under control and inspire people to lead Happy and Healthy lives in Harmony with cancer.

And the beauty is that it works for the other complexes as well!

## SELF-ASSEMBLED SUPRAMOLECULAR POLYMERIC NANOSYSTEMS FOR SMART DIAGNOSIS AND TARGETED THERAPY OF INTRACTABLE DISEASES

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Nanotechnology-based medicine (Nanomedicine) has received progressive interest for the treatment of intractable diseases, such as cancer, as well as for the non-invasive diagnosis through various imaging modalities. Engineered polymeric nanosystems with smart functions play a key role in nanomedicine as drug carriers, gene vectors, and imaging probes. This presentation focuses present status and future trends of supramolecular nanosystems self-assembled from designed block copolymers for therapy and non-invasive diagnosis of intractable diseases.

Nanosystems with 10 to 100 nm in size can be prepared by programmed self-assembly of block copolymers in aqueous entity. Most typical example is polymeric micelle (PM) with distinctive core-shell architecture. Compared with conventional formulations, such as liposomes, PMs have several advantages, including controlled drug release, tissue penetrating ability, and reduced toxic-

ity<sup>1,2</sup>. Furthermore, smart functionalities, such as pH- and/or redox potential responding properties, can be integrated into the PM structure<sup>3</sup>. These smart PMs loaded with various chemotherapy reagents were evidenced to have a significant utility in the treatment of intractable and metastatic cancers, including pancreatic cancer<sup>4</sup>, glioblastoma<sup>5</sup>, and tumors harboring recalcitrant cancer stem cells (CSCs)<sup>6</sup>. Eventually, five different formulations of the PMs developed in our group have already been in clinical trials world-wide, including Japan, Asia, USA and European countries<sup>7</sup>.

Versatility in drug incorporation is another relevant feature of PMs for drug delivery. Nucleic acid-based medicine can be assembled into PM through the electrostatic interaction with oppositely-charged polycationic block copolymers<sup>8</sup>. In this way, siRNA- or antisense oligo (ASO)-loaded PMs were prepared, and their utility in molecular therapy of cancer has been revealed<sup>9,10</sup>. Recently, PM-based imaging reagents were developed, opening new avenue for the novel type of theranostic nanomedicines<sup>11</sup>.

PM-based nanosystems hold promise for the treatment of intractable diseases other than cancer. Very recently, we developed PMs decorated with glucose to crossing blood-brain barrier by recognizing glucose-transporter overexpressing on brain endothelial cells, indicating a novel route to deliver versatile drugs into brain for the treatment of neurodegenerative diseases, including Alzheimer's disease<sup>12</sup>.

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## PRECISION MEDICINE IN THE TREATMENT OF BREAST CANCER OF RELATING TREATMENTS TO MUTATION

DANIELA KATZ

Precision medicine is a term in Oncology designated to reflect the precise nature of current personalized diagnosis and therapy. Breast cancer is one of the first malignancies for which targeted therapies have been used successfully, with endocrine therapy and anti ERBB2-targeted agents being the cornerstone of this approach. Recent advances in molecular technology lead to the discovery of novel driver pathways and oncogenic somatic aberrations in breast cancer. Oncogenic aberrations such as ERBB2, PIK3CA and AKT1 mutations or FGFR1 amplifications have been the target of novel therapies in metastatic breast cancer. Enriching clinical trials with patients carrying these biomarkers has improved patient outcome in early clinical trials. The main hurdle of this targeted approach is the rarity of breast cancer patients carrying these highly selected aberrations. This presentation, will demonstrate that testing patients for somatic molecular aberrations along with informed realistic expectations from these tests, may assist patients with recruitment to clinical trials designed to adapt the new paradigm of precision medicine, moving away from lumping heterogeneous patient populations in one size fits all clinical trials.

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## THE FUTURE OF CLINICAL CANCER DIAGNOSIS AND ITS ROLE IN PRECISION THERAPY

FABIAN KIESSLING

Despite intense research in the last decade, only a few new diagnostic probes and hardly any nanoparticulate diagnostics have been approved for clinical cancer diagnosis. Major reasons are big uncertainties about their performance in the daily practice, true clinical need and thus, market potential. It can be assumed that many current diagnostic shortcomings can be addressed substantially by advanced feature extraction from conventional imaging data (radiomics) as well as by omics analysis from blood, urine and histopathology. In addition, automation in image analysis and data management will render diagnostic processes more reliable as well as more time and cost efficient. However, these competing and complementary tools and technologies have not yet been evaluated in a systematic manner and new translational infrastructures are required disrupting the borders between classical diagnostic disciplines. In this context, systematic and comprehensive approaches critically considering all diagnostic data for complementarity and competitiveness will enable us important steps in precision medicine and elucidate the profound needs in research and diagnostic drug development.

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## THE ROLE OF PET AND RADIONUCLIDE THERAPY IN CANCER IMMUNOTHERAPY

ANDREAS KJAER, Department of Clinical Physiology, Nuclear Medicine & PET, Rigshospitalet, University of Copenhagen, Denmark (DK)

Cancer immunotherapy has been described as a revolution in cancer treatment. However, despite impressive results in selected cancer types the response rates even in the most promising applications is only around 20%. Furthermore, in many cancer forms immunotherapy is not applied as tumors are low immunogenic ("cold") and therefore therapies as check-point inhibitors are not effective. Accordingly, there is room for improvement in the use of immunotherapies.

To broaden the applicability of immunotherapy, nuclear medicine may offer support in two areas. First, the possibility to non-invasively monitor the immune response is of importance. This includes imaging of CD4+ and CD8+ T cells. Furthermore, the imaging of targets for immune checkpoint inhibitors, e.g. PD-L1 and PD-1 may predict whether the therapies will be successful. Secondly, the application of radiation therapy is known to induced an immune response in cancer types that are low immunogenic probably making them sensitive to subsequent immune check-point inhibitors. Whereas, external radiation therapy may be used for this application, in disseminated disease radionuclide therapy may be more effective as the whole tumor burden is activated.

The presentation will discuss the above-mentioned concepts and how they may become game-changing in the future practice of cancer immunotherapy.

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## FLUORESCENT POLYMERIC NANOPARTICLES FOR ULTRASENSITIVE DETECTION OF NUCLEIC ACID CANCER MARKERS

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Detection of nucleic acids, especially messenger RNA (mRNA) and micro-RNA (miRNA), in biological fluids, cell and tissues is a rapidly expanding field important for diagnostics of diseases, such as cancer, viral infections, etc. Due to ultra-low concentration of RNA in biological samples its detection requires molecular multiplication techniques, such as PCR. However, PCR is a multi-step process that requires complex mixture of expensive reagents, sophisticated equipment and well-trained staff. Our goal is to develop alternative signal amplification strategy to achieve simple one-step ultrasensitive detection of RNA cancer markers. To this end, we developed ultrabright dye-loaded polymeric nanoparticles,<sup>1,2</sup> which feature controlled small size and exceptional brightness because they encapsulate >1000 dyes per particle. We found that these nanoparticles operate as light-harvesting nanoantenna capable to amplify emission of single dye molecules >1000 fold, which enables for the first time detection of single molecules in sunlight excitation conditions.<sup>2</sup> To convert these nanoantennas into RNA probes, we covalently modified our polymer nanoparticles with nucleic acids. The obtained nanoprobe enable detection of nucleic acids (encoding cancer biomarker survivin) in solution and on surfaces at <5 pM concentration, which is >1000-fold lower than the detection limit of conventional fluorescence probes for nucleic acids. Currently, their capacity to detect survivin and other cancer markers in biological samples is being evaluated. The developed nanoprobe constitute a new powerful tool for rapid and low-cost detection of biomarkers, which can accelerate the development of assays for early diagnosis of diseases.

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## MECHANO-RESPONSIVE NANO-MEDICINES FOR TARGETED DRUG DELIVERY

### NETANEL KORIN

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In the cardiovascular system, hemodynamics, and transport phenomena are key factors in the development of efficient targeted nano-medicine. Additionally, cardiovascular disease sites are characterized by a locally abnormal mechanical environment. Leveraging such an abnormality, we developed a drug delivery approach that utilizes the high shear stress in regions of artery stenosis as a means to selectively deliver target drugs to these disease sites. In order to rationally design such carriers, we use *in silico/in vitro* microfluidic models which emulate vascular stenosis conditions and allow us to study the behavior of mechano-responsive nano-medicines under relevant dynamic conditions. Using such models we study design parameters as well as drug targeting capabilities of targeted nano-medicines. So far, we have utilized this approach to efficiently deliver a thrombolytic drug to dissolve clots at sites of stenosis. Altogether, our work illustrates biomechanics can be used to design and develop novel cardiovascular therapeutic strategies.

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## SARAH NANOTECHNOLOGY: A NOVEL APPROACH FOR METASTATIC CANCER TREATMENT

SARAH KRAUS<sup>1</sup>, Rephael Hof<sup>1</sup>, Raz Khandadash<sup>1</sup>, Ektarina Sigalov<sup>1</sup>, Pazit Rukenstein<sup>1</sup>, Adam Antebi<sup>1</sup>, Lihi Levy<sup>1</sup>, Rana Kassem<sup>1</sup>, Moshe Eltanani<sup>1</sup>, Shifra Hoch<sup>1</sup>, Gil Reuven<sup>2,3</sup>, Abraham Nyska, and Ofer Shalev<sup>1</sup>

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<sup>2</sup> Almog Diagnostic Ltd., Shoham, Israel;

<sup>3</sup> Bar Ilan University, Givat Shmuel, Israel

## INTRODUCTION:

The Sarah Nanotechnology, developed by NewPhase Ltd., Israel, is a medical device that comprises Sarah Nanoparticles (SaNPs) and a radiofrequency (RF) machine. Sarah Nanotechnology is aimed primarily for the treatment of small cell lung cancer (stage IV), while being studied in parallel for potential treatment applications in other cancer types. The 4T1 triple negative mammary carcinoma is a transplantable tumor cell line that can be grown *in vivo* as a primary tumor in BALB/c mice. A major advantage of the 4T1 tumor is that 4T1 spontaneously metastasizes in a pattern that is analogous to human mammary cancer. When injected intravenously (i.v.), 4T1 cells are capable of metastasis to several organs affected in breast cancer and form metastases in the lungs. SaNPs consist of a phase change material iron oxide nanoparticle (PCM NP) core surrounded by an encapsulating polymer crosslinker. The SaNPs are administered i.v. to the patient and localize on cancer cells. Following delivery and attachment of the SaNPs to malignant cells, the patient undergoes partial body RF non-ionizing irradiation (300 kHz) with the system's RF machine. The SaNPs convert the applied RF electromagnetic field (EMF) to thermal energy whereas the PCM NP absorbs energy without heating above the melting enthalpy point, controls, and stabilizes the temperature of the SaNP to 500C thereby, causing the attached malignant cells hyperthermic cell death in the primary tumor and tumor metastases. The main innovation of the SaNP is in the inner ability to control its temperature. The PCM core of the SaNP allows temperature control without inducing thermal ablation. The Sarah Nanotechnology offers two major advantages:

1. Systemic treatment - The SaNPs are administered i.v. and localize on cancer cells through the use of passive targeting based on the Enhanced Permeability and Retention (EPR) effect that enables the preferential retention of SaNPs in the tumor.
2. Total body irradiation - Because of the SaNP's unique properties, any tumor larger than 20 microns will be sensitive to the treatment, including vascular tumors. At this tumor size, the EPR effect is expected to be significant.

For its therapeutic effect, the SaNP needs to accumulate in the tumor. However, the accumulation of SaNP in healthy organs is an important risk consideration and therefore, potential toxic effects and the biodistribution of the SaNPs were assessed in mice in the following experiments. In addition, the efficacy of treatment as well as the effect of Sarah Nanotechnology on the survival of mice bearing 4T1 metastatic tumors were demonstrated.

## AIM:

Safety, biodistribution, efficacy, and survival studies were performed in order to evaluate the effects of SaNPs in both healthy and 4T1 mCherry breast cancer bearing BALB/c mice.

## METHODS:

All protocols were reviewed and approved by an Institutional Animal Care and Use Committee (IACUC) and followed officially approved procedures for the care and use of laboratory animals.

### 1. Safety study

The potential toxic effects of SaNPs were assessed following an i.v. bolus injection to BALB/c healthy mice. Mice (n=60) were subjected to observation and terminated at 3 different timepoints, 3, 14, and 30-days after treatment. Measurements post-sacrifice included blood analyses (hematology, chemistry), necropsy, gross pathol-



ogy, and histopathology of vital organs conducted by a Board-certified study Pathologist.

## 2. Short distribution study

The distribution of SaNPs in vital organs, blood, and in tumor tissue over time, was assessed at 3 different timepoints (2, 4, and 8 hours) following a single i.v. injection of SaNPs, in the murine 4T1 mCherry breast cancer metastatic model in BALB/c female mice (n=12). The iron oxide content in the tissues and blood was determined by SQUID (Superconducting Quantum Interference Device) analysis that measures electromagnetic properties and can therefore detect the presence of the magnetic iron oxide nanoparticles of the SaNP in organic samples.

## 3. Long distribution study

The long distribution and clearance of SaNP in vital organs and blood was evaluated over time. Healthy BALB/c female and male mice (n=30) were treated with Sarah Nanotechnology (SaNP injection, followed by 30 min. EMF application at 8 hours post injection). The animals were sacrificed at 14, 30 and 60/90 days post-treatment and the samples were analyzed by SQUID.

## 4. Efficacy study

The efficacy of Sarah Nanotechnology was evaluated following 3 treatment cycles, in the murine 4T1 mCherry breast cancer metastatic model in BALB/c female mice (n=10). The treatment cycles (SaNP injection, followed by 30 min. EMF application at 8 hours post injection) were applied within 2-days intervals between each cycle. The primary endpoint of the study was the number of metastases in the lungs. At the end of the study, the mice were sacrificed and the lungs were subjected to macro analysis by visual count of the metastases, fluorescence imaging of the lungs using the Cri Maestro™ multispectral imaging system, in order to determine the fluorescence intensity of the lung metastases, and histopathology analysis.

## 5. Survival study

In this study, the survival of BALB/c mice (n=10) bearing mCherry breast cancer metastatic tumors was assessed. The mice were treated with 5 cycles of Sarah Nanotechnology (SaNP injection, followed by 30 min. EMF application at 8 hours post injection) and followed-up until the last mouse died. The primary endpoint of the study was the survival of the mice; treated vs. untreated control.

## RESULTS:

The safety study indicated that no mortality occurred in none of the animals throughout the 3, 14, and 30-days observation periods. No abnormal clinical signs in response to treatment were observed throughout the study in any of the animals and they exhibited normal body weight gain. The histopathology evaluation did not find any treatment related changes in the organs of the mice, except of sporadic cases of pigment laden macrophages seen in the liver and lungs of treated animals, at all timepoints. As these changes were of minimal degree and were thought to reflect the accumulation of the pigment itself in these organs, were not associated with necrosis, significant inflammation, or any other pathological finding/s, these changes were not considered as adverse.

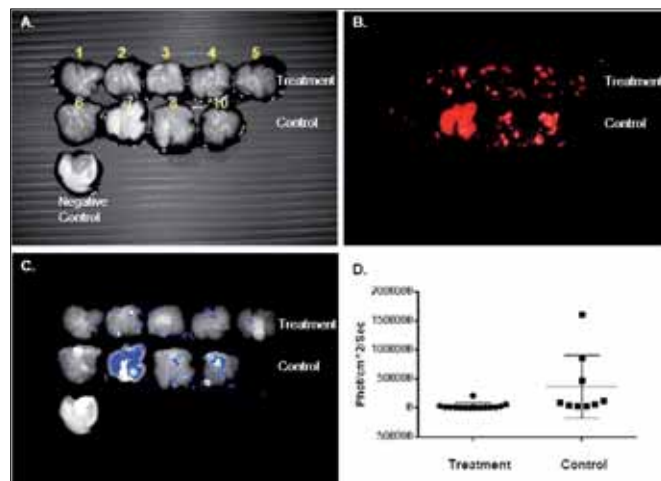
The results of the short distribution study showed that the amount of SaNP that reaches the lungs and lung metastases was the highest at 8 hours post injection. Based on this study, the time of EMF application post SaNP injection was set to 8 hours.

The long distribution study is still in progress. However, the same experiment with similar designs demonstrated 20-30% residual SaNP in the liver at 30-days post-treatment and therefore we expect to see similar results with the current design. At 60/90 days post-treatment most of the SaNPs are cleared from the body.

The main findings of the efficacy study demonstrated a 49.6% reduction in the number of lung metastases, based on the macro visual count; 22.5±10.7 metastases in the control group vs. 13.6±4.6

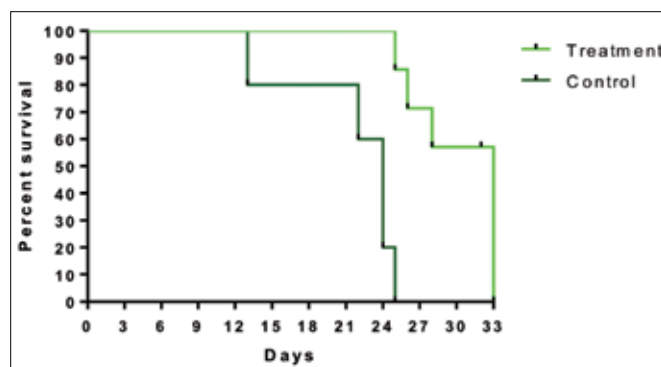
in the treatment group. Histopathology analysis demonstrated that there was a 70% reduction in the relative size of metastases in the lung sections of the treated compared to the control mice; 3.3795±1.219 mm<sup>2</sup> in the control vs. 1.0116±0.602 mm<sup>2</sup> in the treatment group. The fluorescence imaging analysis (Fig.1) showed a 91.3% reduction in the fluorescence intensity of metastases in the lungs thus, further supporting a reduction in the size of metastases due to treatment (i.e. efficacy).

Figure 1: Maestro fluorescent ex-vivo imaging of lungs Lungs (A); mCherry fluorescent imaging (red spots) (B); Heat map of viable metastases (blue spots); Quantitation of fluorescence intensity (D).



The results of the survival study showed that the number of mice surviving at the end of the experiment was significantly greater by more than 40% in the treated compared to the control mice group. Results are shown in Fig.2

Figure 2: Sarah Nanotechnology treatment in metastatic breast cancer significantly prolongs survival Kaplan-Meier analysis demonstrates a significant improvement in survival after treatment compared to the control group (p-value <0.005\*\*\*).



## CONCLUSIONS:

1. Systemic administration of SaNP followed by EMF application to mice, that were terminated at 3 different timepoints after treatment (3, 14, and 30 days), was not associated with any evident adverse reactions.
2. Based on the short distribution study, the optimal time for EMF application after i.v. injection of SaNP was 8 hours.
3. The percentage of residual SaNP at 30-days post treatment is expected to be ~20-30%.
4. The results of the efficacy study, following 3 treatment cycles of Sarah Nanotechnology treatment, showed that the primary endpoint of the study was reached as a significant reduction in the number of metastases was demonstrated as well as a significant reduction in their size.
5. The survival of treatment mice was significantly improved compared to untreated control mice, following 5 treatment cycles of Sarah Nanotechnology treatment.

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## CHEMOTHERAPEUTIC DRUG SELECTIVE NANOPARTICLES AND THEIR APPLICATION IN A POINT-OF-CARE THERAPEUTIC DRUG MONITORING DEVICE

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The decision-making in chemotherapy nowadays depends on standard methods that are liquid chromatography followed by mass spectroscopy (LC-MS/MS)<sup>1</sup> or capillary chromatography; both are labour- and cost-intensive and can be performed only in dedicated hospitals and laboratories. This leads to a minimal therapeutic drug monitoring in patients and hence that 30-60% of drugs are administered without clinical benefits.

We developed approaches for point-of-care devices for quantification of chemotherapeutic drugs in small body fluid samples and in clinically relevant concentrations. Some based on the combination of highly selective nanoparticles extracting the drug from plasma samples and liquid crystal phase separation incorporated in a microfluidic lab-on-a-chip device (optofluidics based)<sup>2</sup>. Others based on highly sensitive fluorescence read-out of differences in emission of parental drug and its metabolite<sup>3</sup>. These techniques will allow the real-time drug monitoring while the patient undergoes treatment. The proposed therapeutic drug monitoring should reduce adverse effects by too high drug dose, detect under-treatment as the administered drug dose does not reach the minimum effective concentration the therapeutic outcome and reduced health care costs.

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## HIGH-THROUGHPUT SCREENING OF NEW LIPID MARKER FOR CORONARY ARTERY DISEASE

**REIJO LAAKSONEN**

Patients with established coronary heart disease (CHD) have an increased risk for CHD events. However, currently used risk calculators rely heavily on age and cholesterol measurements and do not necessarily identify patients at risk early enough for successful preventive measures. Thus, novel analytical methods have used to develop biomarkers for more precise identification of high risk patients.

Recent developments in analytical mass spectrometry have enabled development of high throughput methods for quantitative

analyses including complex bioactive sphingo- and phospholipids. Thus, detailed lipidomic screenings have been performed in a number of cardiovascular patient populations in order to identify new biomarkers.

Studies have revealed the presence of hundreds of different lipid species in circulation. Thus far distinct ceramides and phospholipids have proven to have predictive value over and above currently used lipid biomarkers in predicting CHD outcome risks. Importantly, distinct ceramide ratio appears to be a sensitive marker of incident type 2 diabetes and these novel lipid markers seem to work independent of traditional risk markers and even in low risk cohorts. Published data shows that analysis of lipid metabolites offers an opportunity to increase our knowledge of the biological processes related to development and progression of atherosclerotic coronary disease. The current lipidomic findings are modernizing the lipid based risk stratification as particularly the ceramide lipids and their distinct molecular ratios are superior over currently used lipid markers in identification of CHD patients at high risk for future cardiovascular events.

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## PROTEIN NANOCAPSULES FOR TARGETING AND CONTROLLED RELEASE OF DRUGS

**KATHARINA LANDFESTER**, Max-Planck-Institute for Polymer Research, Mainz, Germany

Our vision is to construct multipotent drug-loaded nanocapsules of high homogeneity in size and surface functionality, which find their target cells in the desired organs and release the drug in a controlled manner in the cytoplasm of these cells. For the delivery of bioactive compounds to a specific cell, it is not only vital to improve the stability of the therapeutic agent during passage through the blood stream, but also to extend the circulation time in the body. The encapsulation of sensitive drugs into nanocarriers retaining their bioactivity and achieving selective release is a challenging topic in current drug delivery design. Established protocols rely on metal-catalyzed or unspecific reactions to build the (mostly synthetic) vehicles which may inhibit the drug's function.

For the formation of nanocapsules different click reactions as biorthogonal reactions of miniemulsion droplets have been successfully proved to be highly suitable for the encapsulation of functional drug molecules like siRNA. Catalyst-free reactions were designed to take place at the interface of aqueous nanodroplets in miniemulsion to produce core-shell protein-based nanocapsules with more than 90% encapsulation efficiency. Click reactions allow also a specific functionalization of the protein nanocarriers for further efficient targeting of cells. Additionally, many interactions to biological matter have to be considered and tuned: the interaction with blood components (proteins etc.) has to be controlled to limit aggregation processes. Furthermore, the interaction to cell membranes and uptake in blood cells like macrophages has to be minimized. Only then the drug can reach the target cells. And then the specific interaction to target cells have to be tuned. Together with the thorough colloidal analysis of the nanocapsules, their stability in human blood plasma and the detailed protein corona composition, the results underline the high potential of such naturally derived drug delivery vehicles.

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## TWO JOURNEYS: FROM BASIC SCIENCE EXOSOME RESEARCH TO LISTING ON STOCK EXCHANGE. "WHY DO THIS"? "WHAT DO WE WANT IN LIFE"?

CHUEN NENG LEE

For our scientific research efforts to reach and benefit our patients, it is imperative to produce and distribute real world products. The processes required to move from laboratory to the market is daunting, requiring time, efforts, persistence, money, support, system and good teams.

Two efforts (Mesenchymal Stem Cell derived Exosomes and Umbilical cord lining stem cell derivatives) supported by the Surgery group in National University of Singapore illustrate issues encountered by the teams:

- Science, publications, presentations. Directions of research work
- Research Funding management and options
- Patent processes, strategies, cost
- Animal trials, clinical trials
- Exploring with regulatory authorities on issues related to "first in kind" therapeutics
- Gathering of key opinion leaders to formulate guidelines
- Formation of companies, recruitment of executives, investments, strategies minimising dilutions of the founders, directions of the companies, "low lying fruits" for revenue (Liposomes)
- Utilising resources within the country's support systems to reduce financial burden
- Processes for listing on stock exchanges, working with bankers and lawyers

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## RADIONANOMEDICINE: CERIA-BASED ONE

DONG SOO LEE

Reactive oxygen species (ROS) contribute to a wide range of pathologies and ceria (cerium oxide) nanoparticles yield free-radical scavenging activity. Auto-catalytic nature of ceria particles act as enzymes and maintain antioxidant effects for a sustained period. Ceria was also applied to neurodegenerative disease models.

In 6-OHDA mouse model of Parkinson's disease, encapsulated ceria nanoparticles using 'click chemistry', we control surface feature of ceria nanoparticle and try to enhance the targeting ability and in-vivo stability. On PET/CT, biodistribution of the encapsulated ceria nanoparticle are examined. Using the time activity curve of ceria nanoparticles, pharmacokinetic is understood to enable us understand safety issues to critical organs. Whether the labeled radio-nuclides are in situ during excretion with or without detachment from the core particles are investigated. Amount of accumulated nanoparticles in the organs such as liver is to be calculated and might be the basis of further in vitro verification of possible effect or toxicity of ceria nanoparticles. The benefits of biorthogonal click method to maintain ceria anti-ROS effect or preventing detachment is also examined..

Despite the conflicting report of beneficial and toxic effect of ceria nanoparticles, click-reaction based encapsulation methods, the therapeutic or non-toxic beneficial effect of ceria nanoparticles are going to be elucidated. This will fasten the translation of ceria into clinical theranostics sooner.

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## VIRTUAL SELF AS THE BASIS OF TRULY PERSONALISED THERAPY AND PREVENTION

HANS LEHRACH, Max Planck Institute for Molecular Genetics Dahlem Center for Genome Research and Medical Systems Biology Alacris Theranostics GmbH

Every patient is different. In particular, every tumor is different. Even subgroups of tumor cells can react differently to specific therapies, due to the heterogeneity of many tumors. Drug therapies therefore typically only help a fraction of patients; many patients do not respond, with some suffering sometimes severe side effects of ineffective treatments.

The ability to identify effects and possible side effects of different drugs on individual patients will, in our view, require highly detailed molecular, clinical, imaging and sensor analyses ideally covering a wide range of different techniques of every individual patient and his/her individual disease; data that is integral to generating individualized computer models, which can then be used to test the effects of drugs (or other therapies) on the individual. In oncology, for example, we minimally need data on low coverage genome, deep exome and transcriptome of the tumor, low coverage genome and deep exome of the patient, but many types of additional information could help a lot (e.g. epigenome, proteome, metabolome, spatially resolved transcriptome of the tumor, deep immune status of the patient, sequences and methylation status of free DNA etc.).

This will, on one hand, provide a basis for a truly personalized selection of therapies optimal for the individual patient, first in cancer patients, but increasingly also in other areas of medicine and prevention. It will, however, also open the way to an increasing virtualization of the drug development process, by e.g. virtual clinical trials of drug candidates carried out throughout the development process.

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## NANO-STRUCTURED AND NANO-SIZED SHAPE-MEMORY POLYMERS FOR BIOMEDICAL APPLICATIONS

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Polydepsipeptides, alternating copolymers of an  $\alpha$ -amino acid and a  $\alpha$ -hydroxy acid, are an interesting group of degradable polymers. Pure oligodepsipeptides have been selected as a hydrophobic block in segmented polymers in order to achieve strong physical interactions stabilizing nanoparticles during their formation. These degradable block copolymers have shown great potential to combine transfection capability with low toxicity of the transfection agent<sup>[1]</sup>. Depsipeptide containing block copolymer can be obtained by ring-opening or polyaddition of suitable oligomeric precursors. The incorporation of a polydepsipeptide segments in multiblock copolymers enables the combination of the advantageous degradation behavior of the depsipeptide segment with the shape-memory capability of multiblock copolymers<sup>[2]</sup>.

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# TARGETED LIPOSOMES FOR PRETERM LABOR MANAGEMENT: DEVELOPMENT, OPTIMIZATION AND SCALE UP TOWARDS CLINICAL TRANSLATION

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Preterm labor poses the highest risk in neonatal morbidity and mortality. Premature birth, defined as birth prior to 37 weeks of complete gestation, accounts for 5 million preterm infants and causes about 1 million neonatal deaths worldwide annually. Additionally, premature babies are at increased risk for both acute and chronic health problems, and developmental deficiencies. Current treatments for preterm labors include short-term administration of tocolytic agents, which can reduce uterine contraction and prolong pregnancy. Unfortunately these compounds can cross the placenta, causing side effects to the fetus.

In particular, indomethacin, a tocolytic agent most frequently used in the US from the non-steroidal anti-inflammatory drugs family which acts by reducing prostaglandin production in the maternal uterus, can cross the placenta freely and has been commonly associated with fetal side effects, including antenatal closure of the ductus arteriosus, oligohydramnios, necrotizing enterocolitis, and intraventricular hemorrhage in human and animal models.

Nanomedicine in the obstetric field is still in its infancy, although potentially can significantly enhance the safety during the pregnancy. In this study, we aim to increase the tocolytics concentration in their site of action, the maternal uterus, while limiting the transplacental passage and thus, reducing toxicity to the fetus. For this purpose, we have developed and tested liposomes directed to pregnant uterus. The liposomes termed LIPINDORA (LIPosomes loaded with INDomethacin and conjugated with Oxytocin Receptor Antagonist-ORA, Fig 1), were designed and evaluated in-vitro, ex-vivo and in-vivo.

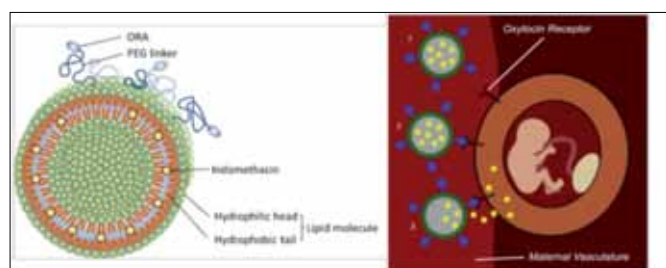


Figure 1. Illustration of LIPINDORA and its mechanism to specifically target uterus and reduce accumulation in the fetus.

LIPINDORA was shown to be able to double the drug concentration in the uterus, reduce in the drug levels detected in the fetus, while maintaining indomethacin's tocolytic efficacy in the pregnant mice model. The mechanism involve limiting prostaglandin level in the uterus, which further inhibit uterine contraction, ultimately prolonging pregnancy by 31% and reducing the rate of preterm birth by 15% when compared with free indomethacin.

Based on these promising and aiming to translate this technology to the clinic in the near future, we are currently planning large animal studies (in GLP and non-GLP setting), while working on the scaling-up and establishing the GMP manufacturing processes. Scaling up and stability of nanoformulations during the long-term storage are considered two of the main challenges in the clinical translation. LIPINDORA were previously produced by thin layer evaporation technique followed by hydration to allow for spontaneous formation of liposome vesicles. Although simple, the thin layer vaporation

technique followed by hydration and extrusion is time consuming, suffer from batch to batch variations and are not easily being scaled up. Thus, we conducted a scale up study with the microfluidics instrument NanoAssemblr™ (Precision NanoSystems, Inc), which has been tested and used in various studies to improve reproducibility and scalability of liposome production, in accordance with the GMP practice. The microfluidic system allows for the translation of the same parameters from the small batch to be applied in the larger batches during the scale up process. We have tested various solvent flow rates, lipid concentrations, as well as various aqueous phase components to be utilized for LIPINDORA production. The optimal flow rate for the synthesis of these liposomes to be 4mL/min and ratio of 1:1 (organic phase:aqueous phase). Concentration of phospholipids between 4 to 20 mg/mL did not affect the average diameter. The final size and polydispersity index of the LIPINDORA produced conventionally (diameter: 105.7nm, PDI: 0.027) were slightly smaller than NanoAssemblr-generated liposome with the previously described parameters (diameter: 122.4nm, PDI: 0.148) (Fig 2). Zeta potential of liposomes were  $-18.1 \pm 15.7$  mV for liposome generated with conventional method and  $-22.8 \pm 8.68$  mV with NanoAssemblr.

To enable a long-term storage of the system, it cannot be kept in the liquid environment and should be lyophilized. To optimize lyophilization process various cryoprotectants were tested. Sugar cryoprotectants such as trehalose, sucrose, mannitol, sorbitol, glucose, lactose, and dextrose were added in varying concentration of 1 to 10% and the optimal conditions were selected.

These promising approach opens new horizons for use of nanomedicine in the field of obstetrics that could greatly impact preterm birth, which currently has no successful treatments.

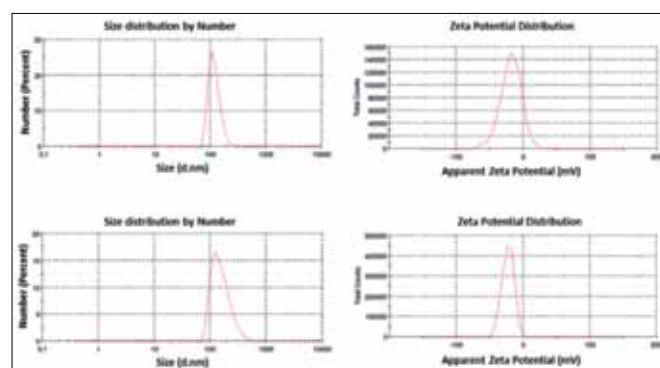


Figure 2. Size distribution and zeta potential of liposomes produced with conventional method (thin layer evaporation and hydration method)- upper panels, and comparison with liposome produced with NanoAssemblr™ – bottom panel.

## NANOMEDICINE FOR IMAGING AND TREATMENT OF ATHEROTHROMBOSIS – THE EU-FUNDED PROJECT “NANOATHERO”

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Atherothrombotic diseases remain the main cause of morbidity and mortality. There is a need for new approaches for early diagnosis and improved therapies. This is the focus of NanoAthero, an European large scale project to demonstrate that nanotechnologies can be developed and clinically proven by Phase-I Clinical Trials to be effective in tackling cardiovascular diseases (Chauvierre & Letourneur, 2015; Zheng & Stroes, 2016). NanoAthero combines in-depth knowledge of nano-carrier bioengineering and production with state-of-the-art expertise in imaging and treatment of

cardiovascular patients providing a full framework of 16 partners within one collaborative European consortium (16 partners from 10 countries - <http://www.nanoathero.eu/>). NanoAthero project integrates several key elements: GMP production, the initiation of clinical investigations in patients, including the preparation of dossiers on regulatory issues, nanotoxicology, risk and ethical assessments, and the evaluation of the performance of optimized diagnostic and therapeutic compounds. In NanoAthero, several systems (Matuszak et al., 2016) were studied and evaluated *in vitro* and *in vivo* (Suzuki et al., 2015; van der Valk FM et al., 2016). Using GMP liposomal nanoparticle (Lobatto et al., 2015), the clinical studies of nanosystem in atherosclerosis were already performed (van der Valk FM et al., 2015). Another Phase I clinical trial started in June in Amsterdam for the molecular imaging of thrombus with fucoidan.

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## NOVEL LAYERED DOUBLE HYDROXIDE NANOPLATFORM AS ULTRASENSITIVE T1-MRI CONTRAST AGENT AND THERANOSTIC AGENT FOR MICE TUMOR DIAGNOSIS AND COMPLETE TREATMENT

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Recently, Mn(II)-containing nanomaterials<sup>1</sup> have been explored widely as attractive alternatives to Gd(III)-based T1-weighted magnetic resonance imaging (MRI) contrast agents (CAs)<sup>2</sup> for cancer diagnosis. However, to our best knowledge, no Mn-based MRI CAs have been reported to sensitively respond to the very weakly acidic environment (pH 6.5~7.0, i.e. pH range in actual tumor microenvironment) with satisfactory imaging performance. This talk will present our recent work on devising a novel Mn-based layered double hydroxide (Mn-LDH) with outstanding T1-MRI performance ultrasensitive to the very weak acidity (pH 6.5~7.0), and show clear MR imaging of tumor tissues in mice for at least two days (Figure 1). Thus, this novel Mn-LDH nanoparticle with ultrasensitive pH response, high relaxivity, and prolonged imaging time, has high potential for cancer CAs. This research has been published in *Adv. Mater.* (2017, 29, 1700373). Based on this work, a further MnAl-LDH dual-functional platform has been constructed successfully as well for MRI and siRNA delivery, which has been reported and highly recommended by Chemistry Views and Dual-Function Theranostic Nanoparticles as a hot paper in *Chem. Eur. J.* (2017, 23, 14299).

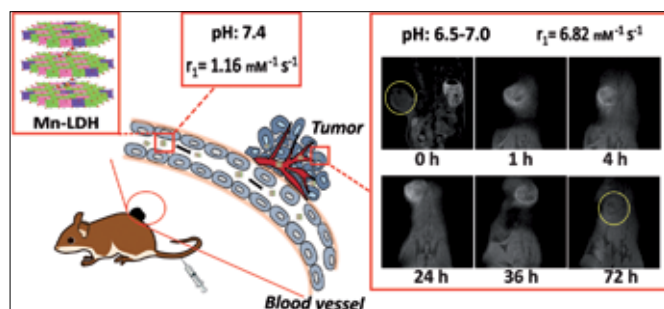
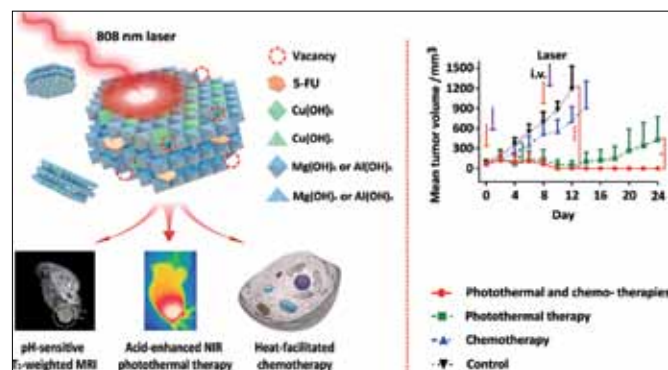


Figure 1. Schematic illustration of structure related multifunctional properties of Mn-LDH nanoparticles.

Furthermore, the development of biomaterials has accelerated a shift from isolated tumor diagnosis or therapy towards non-inva-

sive imaging-guided combinational therapy.<sup>3</sup> In our second exploration, novel defect-rich multifunctional Cu-LDH nanopatform was devised delicately to perform on-target tumor theranostics via excellent T1 weighted MR imaging-guided synergistic photothermal/chemo-therapy with minimal impacts on normal tissues (Figure 2). As characterized with extended X-ray absorption fine structure and X-ray photoelectron spectroscopy, smaller Cu-LDH nanoparticles possess a considerable amount of defects around Cu cations, an advantageous microstructure that enables a high photothermal conversion of 808 nm NIR laser (53.1%). The exposure of Cu-OH octahedra on the LDH surface makes the photothermal conversion significantly acid-enhanced (53.1% at pH 7.0 vs. 81.9% at pH 5.0). This Cu peculiar microstructure also makes T1-MRI very pH-sensitive, a desirable guide for subsequent tumor photothermal therapy. Combined photothermal therapy and chemotherapy lead to complete elimination of tumor tissues *in vivo* with a low agent injection dose. Therefore, this novel defect-rich Cu-LDH nanopatform is one of promising tumor-specific nanotheranostic agents for non-invasive imaging-guided combinational therapy. This research is under revision for *Biomaterials* with very positive comments from three reviewers.

Figure 2. Schematic representation of theranostics of defect 5-FU/Cu-LDH nanoparticles.



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## NOVEL THERMOSENSITIVE LIPOSOMES FOR NEOADJUVANT TREATMENT OF HIGH-RISK SOFT TISSUE SARCOMA; FROM COMPARATIVE ONCOLOGY TRIALS TOWARDS CLINICAL APPLICATION

LARS LINDNER

Neoadjuvant anthracycline and ifosfamide based chemotherapy in patients with high-risk STS has shown to improve overall survival. However, response to doxorubicin (DOX) and ifosfamide combination chemotherapy is limited to less than 30% for most STS subtypes. One important shortcoming of standard drug application is the limited drug penetration. This can be overcome with intravascular release of DOX from thermosensitive liposomes (TSL) based on a novel phospholipid excipient showing an improved pharmacokinetic profile and heat-mediated drug delivery to tumors.

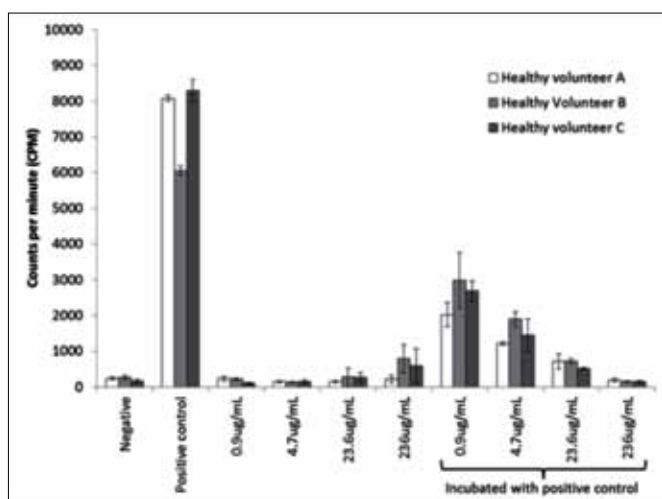
## EVALUATION OF NANOMEDICINES: IN VITRO AND IN VITRO INVESTIGATION OF AN IRINOTECAN LOADED LIPOSOMAL FORMULATION

NEILL LIPTROTT<sup>1</sup>, Anna Bogdanska<sup>2</sup>, Oliviero Gobbo<sup>2</sup>, Adriele Prina-Mello<sup>2,3</sup>, Fanny Caputo<sup>4</sup>, Astrid Hyldbakk<sup>5</sup>, Sven Even Borgos<sup>5</sup> on behalf of the EUNCL consortium

- 1 Advanced Pharmacological Strategies Group, Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, The University of Liverpool, Liverpool, UK;
- 2 Laboratory for Biological Characterisation of Advanced Materials (LBCAM), Department of Clinical Medicine, Trinity Translational Medicine Institute (TTMI), School of Medicine, Trinity College Dublin, Dublin 8, Ireland;
- 3 AMBER Centre and CRANN Institute, Trinity College Dublin, Dublin 2, Ireland;
- 4 CEA, LETI, Minatec Campus, F-38054 Grenoble, France;
- 5 Department of Biotechnology and Nanomedicine, SINTEF Industry, Trondheim, Norway

Liposomes are the most common form of drug delivery nanosystem and have been reported to improve the pharmacokinetic properties of their cargo [1]. In the current study, we have investigated a liposomal formulation of irinotecan. Following a robust physico-chemical characterisation carried out under the EUNCL project, we performed an *in vitro* immunotoxicological assessment of common acute toxicities prior to examining the pharmacokinetics, bio-distribution and side effects of this formulation in comparison to irinotecan (CPT-11) *in vivo*. The assays used to assess *ex vivo* biocompatibility were selected from the EUNCL suite of assays which have previously been shown to have good correlation with *in vivo*, human, endpoints [2]. No impact on plasma coagulation, complement activation or haemolysis was observed following treatment with the current liposomal formulation. Leukocyte proliferation in response to mitogens was significantly lower when co-incubated with liposomal materials, possibly as a result of cytotoxicity attributed to the loaded irinotecan (figure 1). The highest concentration of liposomes tested showed lower concentrations of IFN $\gamma$  suggesting a particular toxicity to IFN $\gamma$  producing cells, possibly neutrophils. Liposomal formulations have previously been shown to induce neutropaenia.

Figure 1. Leukocyte proliferation in response to treatment with liposomal formulated irinotecan. PBMC were incubated with various concentrations of nanomaterials for 48 hours prior to assessment of proliferation by 3H-thymidine incorporation. Positive control consisted of PHA-M (10 $\mu$ g/mL). PBMC were also coincubated with PHA-M and nanomaterials in order to assess possible suppression of mitogen activation. Data presented as mean  $\pm$  standard deviation of 4 technical replicates per volunteer.



Since Irinotecan is known to cause chemotherapy-associated ste-

atohepatitis, drug induced tissue toxicity was investigated, up to 48h post injection, in three main organs of interest which were processed for histopathological examination. We employed techniques that were previously used in similar studies [3], and also introduced different approaches to gain insights into the effects of irinotecan and its liposomal formulation *in vivo*. Lipid type bodies were observed in the liver hepatocytes. This was confirmed by mass spectrometry analysis carried out in tissue and plasma. To the best of our knowledge, this study is first in its kind since it looks at those parameters after a single i.v. dose.

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## IN VIVO MULTIPLEX MOLECULAR IMAGING OF VASCULAR INFLAMMATION USING SURFACE-ENHANCED RAMAN SPECTROSCOPY

PASQUALE MAFFIA<sup>1,2,3</sup>

- 1 Centre for Immunobiology, Institute of Infection, Immunity and Inflammation, University of Glasgow, United Kingdom;
- 2 Institute of Cardiovascular and Medical Sciences, University of Glasgow, United Kingdom;
- 3 Department of Pharmacy, University of Naples Federico II, Italy.

My talk will focus on the development a surface-enhanced Raman scattering (SERS) platform for multiplex molecular imaging of vascular inflammation. Vascular immune-inflammatory responses play a crucial role in atherosclerosis and the ability to detect multiple vascular inflammatory biomarkers would significantly improve cardiovascular risk assessment and management; however, no multi parameter molecular imaging technologies have been established to date.

A series of antibody functionalized gold nanoprobcs (BFNP) were designed containing unique Raman signals in order to detect intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and P-selectin using SERS. We were able to utilize SERS and BFNP to concomitantly detect ICAM-1, VCAM-1 and P-selectin *in vitro* on human endothelial cells and *ex vivo* in human coronary arteries. Finally, we were able to image adhesion molecules *in vivo*, in a non-invasive multiplexing manner, in a humanized mouse model following intravenous injection of the nanoprobcs. This study demonstrates the feasibility of using SERS for *in vivo* multiplex molecular imaging of vascular inflammation.

## FUNDING

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## EMPLOYING THE PROTEIN CORONA TO ADDRESS DENDRITIC CELLS

VOLKER MAILÄNDER, Center for Translational Nanomedicine, University Medicine of the Johannes Gutenberg University Mainz, Mainz (D)



To promote drug delivery to exact sites and cell types, the surface of nanocarriers are functionalized with targeting antibodies or ligands, typically coupled by covalent chemistry. Once the nanocarrier is exposed to biological fluid like plasma however, its surface is inevitably covered with various biomolecules forming the protein corona, which masks the targeting ability of the nanoparticle. We show that we can use a pre-adsorption process to intentionally convey targeting antibodies to the surface of the nanocarrier. Pre-adsorbed antibodies or also ligands like interleukin-2 (IL-2) remain functional and are not completely exchanged or covered up by the biomolecular corona, whereas coupled antibodies are more affected by this shielding. We conclude that pre-adsorption is potentially a versatile, efficient and rapid method of attaching targeting moieties to the surface of nanocarriers. We demonstrate that the pre-adsorption of antibodies is an easier, more flexible and highly effective process in targeting nanocarriers towards the cell compared to the widely performed process of chemical coupling.

But also the other proteins which are adsorbed to nanocarriers will remodel the targeting and the fate of the nanocarriers in the organism. For this we demonstrate how the biomolecular corona can contribute to a mistargeting and how we can one day may be able to predict the effect of the mistargeting in the end.

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## NEW FACETS OF CARDIOVASCULAR DISEASE IN PERSONS LACKING CONVENTIONAL RISK FACTORS NEW FACETS OF CARDIOVASCULAR DISEASE IN PERSONS LACKING CONVENTIONAL RISK FACTORS

**HARALD MANGGE**, Dr. Oksana Thelivets\*

Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz

\*Institute for Molecular Biosciences, Karl-Franzens University of Graz

Myocardial infarction and stroke are caused by unstable, vulnerable atherosclerotic (AS) plaque lesions of the vascular wall. An early diagnosis and therapeutic stabilization of these lesions may effectively prevent fatal clinical endpoints. As the scenario of vulnerable AS-plaques encompasses interlocked pathological conditions, the individual clinical outcome differs significantly. An improved recognition of the individual risk will give a better basis for a personalized medicine of atherosclerosis.

Vulnerable AS plaques are frequently non-stenotic, they remain undetectable by conventional imaging modalities. Blood lipids, C-reactive protein, and interleukin-6 may be increased, but are insufficient for a personalized diagnostic assessment. Some biomarkers indicate acute coronary syndrome or cardiac insufficiency, but not the preclinical phase of a critical destabilization of AS lesions. Thus, valuable time that could be used to treat the patient is wasted.

Immune-inflammatory activation including macrophage and T-cell polarization is critically involved in the process of destabilization. Biomarkers of interest comprise Pentraxin 3, Trimethylamine N-oxide (TMAO) and microbiome interactions, and homocysteine (Hcy), an "old" biomarker with new topicality. Homocysteine is a sulfur-containing, non-proteinogenic amino acid. Hyperhomocysteinemia

(HHcy) is a risk factor for atherosclerosis and correlates with cardiovascular mortality. In a pilot joint project between the University of Graz and the Medical University of Graz we found out that HHcy induced by dietary intervention also in the absence of Hypercholesterolemia (HCL) resulted in the formation of atherosclerotic plaques. This HHcy induced type of atherosclerotic vessel disease had an alteration of collagen organization and longitudinal stiffening of the aorta. Combination of HHcy- and HCL-inducing diets triggered even faster and more severe plaque development than HCL alone. Notably, these plaques were much softer, indicating their more vulnerable nature that can lead to plaque rupture. Hence, Hcy gains new interest as biomarker for plaque vulnerability.

Nevertheless, the main challenge of a feasible personalized management remains: which person should be screened? At which time? Furthermore, it is essential to act therapeutically specific, effective, without side effects because the "patient" feels healthy at the time of prediagnosis.

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## RELIABLE, REPLICABLE KNOWLEDGE & DATA ANALYSIS TOWARDS PRECISE "DISEASE SIGNATURES"

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The promise of personalized and precision medicine is facing nowadays many barriers.

Among these is the huge criticism regarding the reliability, replicability and reproducibility of published outcomes; through ensuring the privacy and security of medical knowledge & data; and the need to define a comprehensive precise "Disease Signature". The term "Disease Signature" is widely used these days, but a systematic literature review reveals no proper definition and no clear concept for this term. Many articles define disease signature as a single (often-genetic) biomarker, mainly in neurology and oncology.

One of the main targets of the Medical Informatics sub-project of the European Human Brain Flagship project is a comprehensive definition of "Brain Disease Signature" that relates to all relevant micro and macro environmental features of the human body functioning in its surroundings. It might include features such as age, gender, clinical tests, biological markers, medical history, genetics, imaging, lifestyle, as well as physical, sociological and cultural environment, etc.

New advanced technologies have profoundly enhanced our insights on the human body functioning, enabling the capturing, adjusting and analysis of big data while encountering many barriers. For example, the integration of knowledge and data from different domains originating in different sources and the bridging of conceptual and technical barriers such as missing values, and the lack of consistency in the final diagnosis.

Furthermore, privacy and security of all data and knowledge has to be ensured through all procedures while tackling data base leakage (Eloyan et al (2012), Brown et al (2012) - combining Big Data related to small samples, replicability (Baggerly & Coombes-2011, Rosenblatt, Vink & YB-2013), reproducibility, etc. For example, hospital databases suffer from leakage and replicability barriers, constructed for different purposes with limited availability of healthy records and different diagnostic tests that the healthy and sick people underwent.

Additional challenges are the growing sensitivity and specificity needs in representing the medical data variance and its relevance and contribution to personalized and precise medicine.

A 3C- Categorization, Classification & Clustering- strategy, developed in our lab, aims to provide a comprehensive insight through a stepwise process, incorporating medical expert knowledge, in a structured way, into the analysis process of the disease manifestations and potential biomarkers - towards reliable precise medicine. The 3C strategy applied to the Alzheimer's disease Neuroimaging Initiative (ADNI) cohort went beyond the discovery of association to new sub-type identification.

These subtypes were later verified with hospital clinical data collected from 14 various Medical centers. Another case study application is the Post Traumatic Stress Disorder (PTSD) carried out at the Tel Aviv Sorasky Medical Center.

## NEW DRUGS FOR NEGLECTED TROPICAL DISEASES: NEEDS, GAPS, AND THE POTENTIAL OF NANOMEDICINES TO OVERCOME THEM

PASCAL MÄSER, Swiss Tropical and Public Health Institute, Basel

New drugs for neglected tropical diseases and for malaria will be required to attain the United Nations' Sustainable Development Goals. The shortcomings of the current drugs are manifold, including poor oral bioavailability, unfavorable pharmacokinetics, high cost of goods, and the looming threat of drug resistance. Nanoformulations may help to overcome some of these issues. A further problem in the control of parasitic infections are dormant stages such as the hypnozoites of *Plasmodium vivax* or quiescent forms of *Trypanosoma cruzi*. These are less responsive to chemotherapy, and they are of epidemiological and pathological importance. Here, too, nanomedicines may support novel developments. I shall try to point out needs and gaps in drug R&D in the form of target-product-profiles, and the potential of nanotechnology to meet these profiles with new approaches in parasite chemotherapy.

## CHARACTERIZATION CHALLENGES FOR NON-BIOLOGICAL COMPLEX DRUGS AND THEIR FOLLOW-ON VERSIONS

SCOTT E. MCNEIL

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Non-biological complex drugs (NBCD) are intricate formulations that frequently constitute a range of physical and chemical properties, making characterization of these formulations challenging. Establishing chemical and/or therapeutic equivalence of follow-on versions of these complex formulations is often even more demanding, as identifying the critical quality attributes (CQA), and developing appropriate assays to evaluate the CQA, is frequently a major hurdle. As an increasing number of follow-on NBCD formulations are being developed, there is an urgent need for establishing robust techniques for their translational development. Having evaluated more than 400 different nanoformulations, including comparison of follow-on NBCD to the innovator products, the Nanotechnology Characterization Laboratory (NCL) has insight into the nuances required in detailed physical, chemical, and biological characterization. This talk will highlight some of the unique characterization challenges for NBCD, and offer analytical and bioanalytical techniques that may prove helpful in establishing similarity of follow-on NBCD.

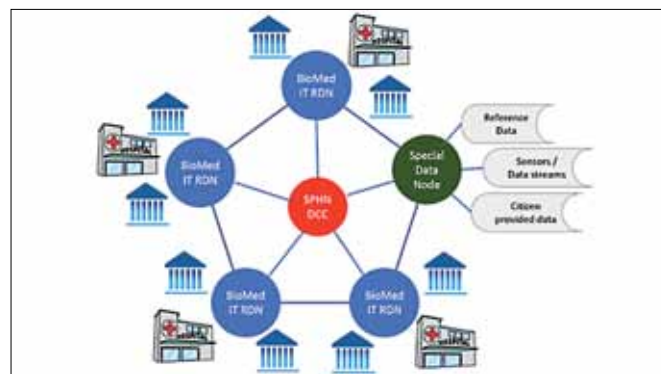
Funded by NCI Contract No. HHSN261200800001

## THE SWISS PERSONALIZED HEALTH NETWORK (SPHN)

PETER J MEIER-ABT

Swiss Academy of Medical Sciences (SAMS)

Personalized medicine/health aims to prevent, diagnose and treat diseases by taking into account individual variability of genes, environment and lifestyle for each person. In order to tap the full potential of the increasing amount of molecular, biological and clinical health data, new infrastructures are required that permit collection, storage and analysis of high quality data and adequately link biobank samples to clinical data. Many countries have started to build interconnected "precision/personalised medicine ecosystems", but the federalistic and heterogeneous healthcare system has so far prevented nationwide coordinated activities in Switzerland. Therefore, the Swiss Personalized Health Network (SPHN) initiative has been launched. Its mission is to lay the foundations for personalised health on a national level by the development of a nationally coordinated interoperable data infrastructure to enable nationwide accessibility and exchange of health-related data. We present the goals of SPHN, its current procedures and funding regulations, its organisation, its ethical and legal framework for responsible data processing, its data management infrastructure and information security and its relevant partnerships with other organisations and institutions. Although the SPHN initiative starts with the institutions of higher education such as university hospitals, universities and the ETH domain, other hospitals, public health institutions and medical practitioners, as well as private institutions such as industry and health insurances, will be included into the initiative at a later time point. Ultimately, the SPHN initiative shall lead to the development of an effective Swiss personalised health ecosystem, which is required to advance effective individual prevention, diagnosis and treatment of disease states and to push Switzerland to the international forefront of personalised health-related research and health care.



## PRECLINICAL AND CLINICAL DEVELOPMENT UPDATE OF INTRAVENOUS PEG-LIPOSOMAL CORTICOSTEROIDS

JOSBERT M METSELAAR

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Glucocorticoids (GC) are potent anti-inflammatory and immunomodulatory drugs but their systemic (parenteral/oral) use in diseases such as rheumatoid arthritis and inflammatory bowel disease is limited by poor target localization and toxic effects in healthy organs. Targeted delivery of GCs to the pathologic lesions with long-circulating pegylated liposomes can improve the therapeutic index. This approach has proven successful in many rodent models of inflammatory disease but also in some preclinical cancer stud-

ies. Furthermore, over the past few years we conducted trials with pegylated liposomal GC formulations in patients with a variety of diseases of inflammatory origin and with cancer. Doses of 37.5 up to 300 mg prednisolone in long-circulating pegylated liposomes have been studied in patients with rheumatoid arthritis (RA), multiple sclerosis (MS), colitis ulcerosa (UC), and patients with inflamed and instable atherosclerotic plaques, while pegylated liposomal dexamethasone was studied in patients with multiple myeloma and prostate cancer. Besides obtaining first hints of therapeutic efficacy and targeting, these studies allowed us to carefully assess the safety profile and study the pharmacokinetics of liposomal GC in humans and to compare these results with previously obtained animal data. Interesting observations include (lipid) dose dependent PK, the effect of particle size, but also differences among animal species and gender. A discussion will be initiated about what product properties and PK parameters are important for efficacy and safety assessment, and what consequences this may have for drug product quality control. So far we can conclude that liposomal GC targeting is a safe and efficacious novel treatment strategy for several diseases with an immune component.

**Keywords:** liposomes, drug targeting, glucocorticoids, clinical trials  
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## IMAGING BIOMARKERS TO PREDICT THE EFFICACY OF CANCER IMMUNOTHERAPY

MATTHIAS MIEDERER

To image the morphology and biology of organs and tumors is a key method for clinical management and development of therapy in oncology. Due to the well-established ability of non-invasively measuring biomarkers by imaging of radiolabeled compounds with Positron Emission Tomography (PET) and Single Photon Emission Tomography (SPECT) these methods have great potential to complement further preclinical and clinical development of new therapeutic approaches.

For sensitive localization of tumor manifestations and their metabolic response to therapy PET imaging with [18F]-2-fluorodeoxyglucose (FDG) is a fundamentally important imaging method in oncology. With the expanding implementation of immunotherapies, tools to monitor the immune system by imaging are increasingly requested for example to depict mechanisms of action or to personalize therapies to the individual patient. Since both malignant tissue and activated immune cells share the metabolic principle of shift from mitochondrial oxidative phosphorylation to glycolytic metabolism FDG-PET imaging can also be used to monitor the status of the immune system. Here, close metabolic similarities between different types of immune cells are evident and preclinical findings can be translated to clinical situations by imaging the glucose metabolism of the spleen.

Additionally, PET imaging is increasingly capable to obtain imaging information by means of radiolabeled monoclonal antibodies. For example with Zirconium-89 labeled PD-L binding antibodies PD-L1 expression can be non-invasively imaged to visualize immune checkpoint pathways. The highly promising properties of cellular immune therapy are the potential of T-cells to home to target tissue and then proliferating at target sites. Thus, imaging of T-cell migration and proliferation can be achieved by PET-imaging with Zirconium-89 labeled anti CD3, CD4 and CD8 antibodies. Imaging of the pan T-cell marker CD3 enables for example non-invasive visualization of homing of T cells to tumor sites or depicting the cellular basis of development of different stages of graft versus host disease in mouse models.

Thus, PET imaging is the imaging modality with greatest potential to translate imaging research along with new therapeutic approaches to clinical trials.

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## NOVEL NEURO-NANOPHARMACEUTICALS FOR ALZHEIMER'S DISEASE

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The Delphi study (Lancet 2005; 336:2112–2117) estimated that there were 24.3 million people with dementia in the world in 2001, with majority suffering from Alzheimer's disease (AD) and predicted a rise to 42.3 million cases in 2020 and 81.1 million by 2040. The Alzheimer's Association (United States) in 2010 estimated that one in eight people aged 65 and older have AD and every 70 seconds, someone in America develops Alzheimer's and by mid-century, someone will develop the disease every 33 seconds. Currently, AD is the forth-leading cause of death in adults after heart disease, cancer, and stroke. Realistically, therapeutic options are limited and to date none have provided a cure. AD is characterized by a progressive loss of cognitive function and with two established pathophysiological hallmarks. These include extracellular accumulation of pathologic amyloid-beta (A $\beta$ ), also referred to as senile plaques, and intracellular neurofibrillary tangles of hyperphosphorylated tau protein. It has been argued that A $\beta$  in the brain is in equilibrium to that in the peripheral blood. This led to hypothesis that shifting this equilibrium towards the blood by enhancing peripheral clearance might reduce A $\beta$  levels in the brain. This is known as the 'sink effect'. We are addressing AD improvement through two approaches. The first approach is through development of functional immunoliposomes with high binding avidity for circulating A $\beta$ , where A $\beta$ -bound immunoliposomes are cleared rapidly by hepato-splenic macrophages. Indeed, this treatment has dramatically improved AD condition in APP/PS1 transgenic mice on repeated intraperitoneal injection compared with free monoclonal antibody treatment. The second approach is based on a patented peptidic-based supramolecular assembly platform technology, derived through "simple-by-design" and "safe-by-design" concepts, capable of rapidly and efficiently targeting cerebral endothelial cells *in vivo* and delivering reporter and modulating agents (including small-molecules, peptides and nucleic acid medicines) to these cells as well as crossing the blood-brain-barrier (BBB). Here, our approach is not only based on crossing the BBB, but also on genetic manipulation of barrier molecules involved in escalation of AD condition. The further development of these technologies can overcome current limitations in brain drug delivery and may improve many neurological conditions and disorders such as Parkinson's disease, Huntington's disease, multiple sclerosis and glioblastoma. Moghimi, SM and Howard, KA (2018) Mol Ther 26:933–934; Ordonez-Gutierrez, L et al. (2017) Biomaterials 112:141–152.

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## MONITORING AND MODULATING INNATE IMMUNITY USING NANOMATERIALS

WILLEM MULDER

Immunotherapy is revolutionizing the sophistication with which cancer is treated and the success with which it is cured. However, the numerous immunotherapies that are currently being developed typically engage the adaptive immune system. In recent years, emerging evidence has shown that the innate immune system displays long-term changes in its functional program, through epigenetic programming of primarily macrophages and monocytes. This innate immune memory has been termed 'trained immunity'. In this lecture, an innovative treatment paradigm in which innate immune cells are specifically targeted – and 'trained' – by drug-



loaded nanobiologics. A trained immunity-engaging nanobiologic library, containing high density lipoprotein derived nanomaterials with different immunomodulatory payloads, will be discussed. The application of nanobiologics that promote organ acceptance will be this presentation's main focus.

## SHEDDING LIGHTS ON CANCER CELLS AND THEIR MICROENVIRONMENT: A 3D MODEL TO MIMIC PANCREATIC TUMOR BARRIERS

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Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease which represents the fourth leading cause of cancer-related death in Europe and North America. The median survival is less than 6 months and the maximum is 5 years for 6% of patients only. This poor prognosis is mainly due to the absence of specific risk factors hindering an effective prevention and a late diagnosis that reveals an unresectable or metastatic cancer in nearly 90% of patients for which only chemotherapy treatments are available. Clearly, pancreatic cancer represents an unsolved medical need that urgently requires new therapeutic strategies. Application of nanotechnology to medicine (i.e., nanomedicine), holds the promise to overcome the limits associated to conventional chemotherapy.<sup>[1,2]</sup> In the past years a plethora of various engineered nanomedicines has been designed driven by the idea to improve the therapeutic efficacy of anticancer drugs. However, despite the wide enthusiasm and the promises associated with the presentation of each new nanomedicine, no significant progress in pancreatic cancer therapy has been achieved.

Mainly, the failure of even highly promising systems is related to the unique physio-pathological complexity of the pancreatic tumor and its microenvironment.<sup>[3-5]</sup> The latter consists of different cell types (e.g., fibroblasts, endothelial cells, macrophages, immune cells) embedded in a dense stroma (extracellular matrix, ECM), which can account for as much as 90% of the tumor tissue. This stiff extracellular matrix acts as a tortuous barrier, which sequesters nanomedicines blocking their diffusion, dramatically hampering the drug bioavailability and therefore limiting the effectiveness of the treatment. Accordingly, the overcoming of the stromal barrier is required to achieve better exposure of the tumor to the therapeutics agents and thus obtain clinical benefits.

However, a crucial issue is that the tumor transport barriers are generally not taken into account during *in vitro* preclinical evaluation of nanomedicines, which is still routinely carried out mainly on two dimensional (2D) monocultures of isolated cancer cells. Despite their relative ease of handling, these cultures do not show any structural architecture and lack the complex physiology and the microenvironment of the real tumor tissues. As consequence, results obtained *in vitro* on 2D cell culture models often do not translate to similar results *in vivo*. In this context, a great emphasis

has been directed in recent years toward three-dimensional (3D) culture methodologies, proposed as capable to better simulate the tumor phenotype and therefore suitable to overcome the aforementioned limitations.<sup>[6-8]</sup>

In this context, we have developed an organotypic tumor model of PDAC capable to reproduce the heterogeneity of the tumor tissue (i.e., the presence of multiple cell types), the complex cancer cells/stroma interactions and to mimic the drug permeability in fibrotic tissues.<sup>[9]</sup>

A 3D tissue composed of pancreatic cancer cells (MiaPaCa-2) and fibroblasts (NHDFs) has been constructed by cell surface coating with nanometer sized ECM films of fibronectin-gelatin via a layer-by-layer (LbL) assembly process.<sup>[10]</sup> These films provide a cell-adhesive surface similar to naturally ECM.<sup>[11,12]</sup> The constructed 3D-tissues displayed permeability to dextran 200 kDa, used as a model drug, inversely proportional to the content of cancer cells. This result well correlated to the higher amount of collagen I and other proteins of the extracellular matrix detected in the 3D tissue containing 90% of cancer cells compared to the one containing 10% only of cancer cells. To be noted that in the former, up to 9 times higher secretion of ECM protein from a single fibroblast was measured, thus revealing the strong influence of cancer cells on the ECM secretion. (Figure 1)

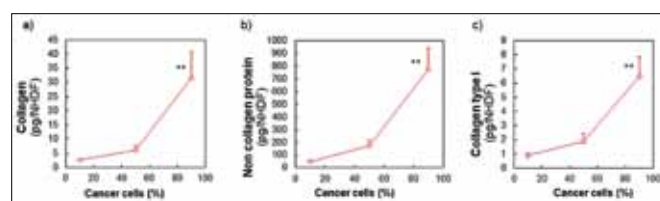


Figure 1. Quantification of the secretion of (a) collagen; (b) non-collagen proteins and (c) collagen type I by each NHDF fibroblast in an 8 layers cancer cells: fibroblasts tissue. Values represent mean  $\pm$  SD. \*\*  $p < 0.05$ .

Thanks to the capacity to reproduce the crosstalk between cancer cells and fibroblasts, this model might represent a relevant tool to understand more detail the molecular cross-talk between cancer and stromal cells and to provide knowledge on the penetration capacity of drug delivery systems.

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## SCHISTOSOMIASIS: NEW DATA UNCOVERING A MAJOR HEALTH PROBLEM IN EASTERN KONGO

**MAURICE MUTRO NIGO**, PhD Student, Bunia, COD, CLINAM Lab and University of Basel, Basel (CH)

Nano technologies should not only improve healthcare in the developed world, but should also contribute in a major way to healthcare in developing countries, thereby serving the poorest.

To achieve these goals, understanding a poverty disease's biology, epidemiology as well as the socioeconomic background and the working modes of local health care is of key importance.

With the long-term goal of contributing nanomedicine-based and computational modeling tools for diagnosis and therapy, we therefore performed two large field trials in a large area of one of the poorest regions of the world, eastern Democratic Republic Congo, focusing on the prevalence and predictors of the invasive worm disease, Schistosomiasis. This region has not seen large-scale epidemiologic analyses since colonial times and after two decades of war still presents major security risks for healthcare workers. Approximately 3000 individuals were tested. First results of the survey will be presented and a requirements for development of new diagnostic and therapeutic tools will be discussed.

## USE OF NANOCARRIERS FOR CHEMOTHERAPY DELIVERY AND IMMUNOTHERAPY IN PANCREATIC CANCER

**ANDRÉ NEL<sup>\*</sup>**, MD/PhD; Jianqin Lu, PhD; Xiangsheng Liu, PhD; Huan Meng, PhD.

<sup>\*</sup>Distinguished Prof. of Medicine, University of California, Los Angeles, California

Pancreatic ductal adenocarcinoma (PDAC) is essentially a death sentence and is currently treated by two major drug regimens, namely gemcitabine, and a 4-drug combination known as FOLFIRINOX (oxaliplatin, 5-fluorouracil, Irinotecan, and leucovorin). In addition to the contributions of late diagnosis and early metastatic spread to mortality, a major treatment obstacle is the abundant dysplastic stroma, which provides a barrier to vascular access of chemotherapeutic agents at the tumor site as well as participating in drug resistance. While FOLFIRINOX leads to better survival outcome, the high toxicity levels of irinotecan and oxaliplatin often prevent its use as a first-line treatment regimen. We have recently developed a mesoporous silica nanoparticle (MSNP) carrier, coated by a lipid bilayer, which can effectively deliver (i) a synergistic combination of paclitaxel and gemcitabine, or (ii) irinotecan as a single drug. These "silicasome" carriers outperform commercial nanocarriers (Abraxane and Onivyde) in a rigorous orthotopic PDAC model from an efficacy as well as a survival perspective, including the ability to suppress metastases. Moreover, the irinotecan carrier could also significantly reduce bone marrow and gastrointestinal toxicity compared to a liposomal equivalent. The efficacy of the irinotecan-silicasome is enhanced by involving a novel transcytosis pathway that can be triggered by iRGD co-administration. We have obtained ultrastructural demonstration of this transport pathway, which complements the role of the EPR effect. The transcytosis pathway is active in human PDAC cancer xenografts, commensurate with the level of NRP-1 expression (which acts as the receptor that interacts with iRGD). In addition to improving survival through chemotherapy delivery, we have also developed a series of nanocarriers for immunotherapy of PDAC, with highly successful outcome through induction of immunogenic cell death, coupled with interference in the immunomodulatory tumor microenvironment. This has allowed the development of local administration, systemic administration and vaccination platforms for pancreas cancer immunotherapy.

## TUMOR MICROENVIRONMENT TARGETING NANOROBOTS: A PROMISE FOR A CURE OF CANCER

**GUANGJUN NIE**, Ph.D, Professor, National Center for Nanoscience and Technology, China, niegj@nanoctr.cn

It was witnessed the rapid development on precision design and fabrication of intelligent next generation nanomedicine. It is clear that tumor microenvironment plays critical roles on promotion of primary tumor rapid growth and metastasis. Combination of the two key items, precision nanomedicine for regulation of tumor microenvironment, offers a great promise of a feasible and fruitful strategy to improve the therapeutic outcomes for cancer treatment. In this talk, I will report our recent development on using peptide, protein and DNA based nanorobots or nanomachines as intelligent nanomedicines to regulate tumor microenvironment and to block tumor microvessels or re-store the homeostasis of tumor stroma. Given the robust functionality, exceptional designability, potent antitumor activity and minimal *in vivo* adversity, the nanorobots represent the next generation of nanomedicine and a promising strategy for precise drug design for cancer therapeutics.

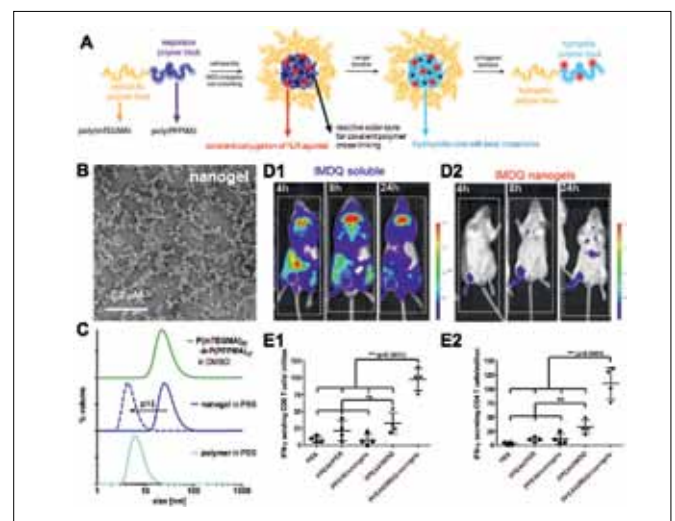
## PH-DEGRADABLE IMMUNE MODULATING NANOGELS FOR CANCER IMMUNOTHERAPY

**LUTZ NUHN<sup>1</sup>**, Bruno G. De Geest<sup>2</sup>, Tanja Weil<sup>1</sup>

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<sup>2</sup> Department of Pharmaceutics, Ghent University, Ghent (Belgium)

Engineering stimuli-responsive nanomaterials can provide novel opportunities in medicine to address unmet medical needs, e.g. also in (cancer) immunotherapy.<sup>1</sup> However, facile and straightforward access to multifunctional as well as biodegradable carriers is required. Self-assembly of amphiphilic block copolymers is often utilized to obtain micellar nanoparticles readily, yet with limited degree of functionalization, drug load or stability under physiological conditions. To that respect, we utilize reactive precursor block copolymers that can be functionalized and stabilized during their self-assembly process, e.g. by covalent incorporation of hydrophilic cross-links, fluorescent dyes or drugs into the micelle core affording functional pH-degradable nanogels (Fig1A-C).<sup>2</sup>



**Figure 1:** TLR7/8-agonist covalently loaded nanogels for lymph node focused immune activation. **A:** Schematic overview of the synthesis of IMDQ loaded, pH-degradable nanogels. **B:** TEM image of the nanogels. **C:** DLS studies of self-assembled precursor polymers and the resulting cross-linked nanogels before and after degradation upon endosomal acidification affording water-soluble single polymer chains. **D:** Monitoring INF- $\beta$  based immune stimulation upon footpad injection of soluble TLR7/8 agonist IMDQ

(D1) and nanogel-bound IMDQ (D2). E: Vaccine adjuvant properties of IMDQ-nanogels triggering superior CD8 (E1) and CD4 (E2) T-cell responses.

## RESULTS AND DISCUSSION

Agonists of Toll-like receptors (TLRs) are potent activators of the innate immune system and hold promise as vaccine adjuvant and for anticancer immunotherapy. Unfortunately, in soluble form they readily enter systemic circulation and cause systemic inflammatory toxicity (Fig 1D1). By covalent ligation to 50-nm-sized degradable polymeric nanogels a lymph node restricted immune activation can be observed while fully abrogating systemic inflammation (Fig 1D2).<sup>3</sup> Immunization studies in mice showed that relative to soluble TLR7/8 agonist only imidazoquinoline-ligated nanogels induce superior Th1-mediated immune responses (Fig 1E) and trigger protection against infections like e.g. the hard-to-vaccinate respiratory syncytial virus (RSV).<sup>4</sup> After peritumoural injection, imidazoquinoline-ligated nanoparticles are also capable to activate dendritic cells in the tumour-draining lymph nodes and induce tumour-specific CD8+ T-cells that inhibit tumour growth, especially in combination with anti-PD-L1 checkpoint inhibitors.<sup>5</sup> Therefore, next generation of nanogels will target the immunosuppressive milieu of the tumour microenvironment, for instance, by modification of dangling polymeric chain ends on the nanogel surfaces with single chain antibodies (nanobodies) addressing regulatory immune cell subpopulations (e.g. tumour-associated macrophages TAM).<sup>6</sup> In summary, our approach opens avenues to enhance the therapeutic benefit of small molecule TLR agonists for a variety of applications in cancer immunotherapy.

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## LONG-ACTING INJECTABLE NANOMEDICINES FOR ADDRESSING ADHERENCE ISSUES IN INFECTIOUS DISEASES

**ANDREW OWEN**, PhD, FRBS, FBPhS, Professor of Pharmacology, Molecular and Clinical Pharmacology University of Liverpool (UK)

The reasons for non-adherence to infectious disease treatments are complex involving numerous patient- and drug-related factors. In chronic infectious diseases such as HIV, these issues are further exacerbated by the need for a life-time commitment to therapy and “pill fatigue” which is experienced by many patients. When patients do not take their medications regularly and on time, plasma drug concentrations fall below necessary levels for controlling replication of the pathogen, and this can create a sub-therapeutic environment that favours the emergence of drug resistant strains. The overwhelming majority of current therapies for infectious diseases require strict adherence to oral medicines, sometimes drug combinations, and sometimes requiring more than once-daily dosing. Importantly, the overwhelming burden of diseases such as malaria,

HIV and tuberculosis reside in low- and middle-income countries, and so advanced therapeutic strategies need to be cost effective in these contexts. Recently, two long-acting injectable (LAI) nanomedicines have progressed through Phase II trials for treatment or prevention of HIV. These LAI nanomedicines provide therapeutic plasma exposure for a period of weeks or months from a single intramuscular administration, with the ability to effectively overcome issues with adherence to daily oral medicines. Moreover, the infectious diseases scientific community are expanding investigation of this novel therapeutic approach to other infectious diseases. This presentation will address the mechanistic basis for how drug release is modified through creation of a nanoparticle depot within the muscle, recent advances in the development of such formulations across disease such as malaria and tuberculosis, and the challenges in further implementation of this new paradigm for infectious disease control.

## DEGRADATION OF PARTICLES EFFECTS THEIR COLLOIDAL PROPERTIES AND THUS INTERACTION WITH CELLS

**WOLFGANG PARAK**, Fachbereich Physik und Chemie, CHyN, Universität Hamburg, Germany; CIC Biomagune, San Sebastian, Spain

Composite nanoparticles comprising an inorganic core and an organic shell, which provides water-solubility can be enzymatically degraded. This is relevant for particles that are endocytosed by cells. As enzymes can be specifically present in certain intracellular compartments degradation can depend on the intracellular location of particles. Degradation can be tuned by different surface chemistries, to make particles labile in one environment or stable in other environments.

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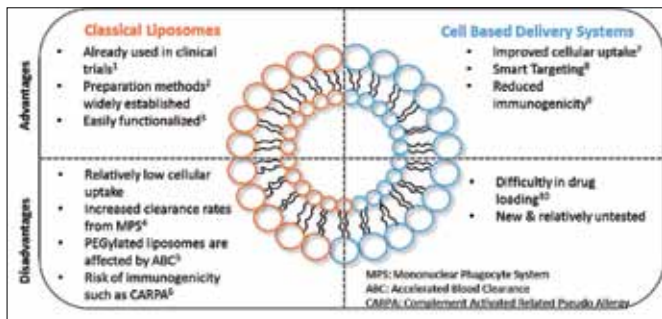
## NANO CELL VESICLE TECHNOLOGIES (NCVTS): USING BIOMIMICRY TO DESIGN NOVEL DRUG DELIVERY SYSTEMS

**GIORGIA PASTORIN**

Efficient methods of drug delivery are of paramount importance in the successful treatment of medical conditions. Problems associated with drug’s limited solubility, tissues damage etc., can be overcome and/or ameliorated by the use of a drug delivery system (DDS). Current DDS mainly include synthetic nanoparticles like liposomes, dendrimers, polymeric & metal nanoparticles. Several efforts on these materials have resulted in approximately 250 nanomedicines currently approved or in various stages of (pre)clinical evaluation.



However, major drawbacks of these nanoparticles include weak specific tissue targeting properties and non-optimal local drug delivery, limiting the therapeutic outcomes. Hence, the current project attempts to use biomimicry-based DDS, ranging from exosome mimetics to nano Cell Vesicle Technologies (nCVTs): this generation of DDS is designed to exploit our body's own cells to generate novel DDS that provide the benefits of intrinsic targeting towards cancer cells (due to the preservation of surface cues on nCVTs from their original parent cells) and nano-size dimensions (which enable the accumulation at the diseased area), thus paving the way towards new advances in the field of personalized nanomedicine.



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## GLOBAL COLLABORATIONS IN REGULATORY SCIENCE AND STANDARDS DEVELOPMENT

**ANIL K. PATRI**, Chair, Nanotechnology Task Force, Director, Nanocore, National Center for Toxicological Research (NCTR), U.S. Food and Drug Administration (FDA), USA.

Regulatory Science<sup>1</sup> is the science of developing new tools, standards and approaches to assess the safety, efficacy, quality and performance of regulated products. FDA conducts regulatory research to support its mission to protect and promote public health by advancing much needed novel medical products for human use while making sure they are safe and effective. The complexity involved in developing these novel medical products, including those that contain nanomaterial, require evaluation through standardized methods and approaches to assure reproducibility. This presentation will highlight regulatory science research at FDA and provide an update on global collaborations for standards development through stakeholder involvement.

Disclaimer: The views expressed in this presentation do not necessarily represent those of the U.S. Food and Drug Administration

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<https://www.fda.gov/downloads/ScienceResearch/SpecialTopics/RegulatoryScience/UCM268225.pdf>

## STATE OF THE ART REPORT ON ELECTRONIC INFORMED CONSENT (ECONSENT)

**CHRISTIANE PAULI-MAGNUS**

According to the Swiss Law on Research in Humans, the reuse of routinely collected genetic and non-genetic data and samples from patients for research purposes requires the consent of patients. The paper-based consent processes so far established at all five

Swiss University Hospitals are intrinsically linked to the hospital admission process. The resulting percentage of in- and outpatients asked for consent ranges from less than 10% up to 20%. If not addressed, general consent will therefore likely develop into a major obstacle for personalized health research in Switzerland and jeopardize the success of the whole initiative

## GOALS

The above-mentioned issues could be resolved by complementing the current general consent process by a more flexible and admission-independent electronic way to contact patients and collect general consent information. Results from a pilot project with electronic consent presentation suggest that this approach is well accepted by patients. Encouraged by this positive outcome, the aim of this project is to develop an interoperable professional grade electronic general consent solution

## SIGNIFICANCE

This project builds on the possibilities arising from digitalization to propose new technical solutions to present general consent information in an understandable way and to collect general consent independent from hospital admission. It takes into consideration the Swiss legal requirements, specific needs of the involved University Hospitals as well as values and preferences of affected patients.

By addressing a central bottleneck of the SPHN initiative, the project has the potential to significantly advance the development of a functioning Swiss Personalized Health Network and to tailor one of the central prerequisites allowing data exchange and interoperability for research purposes

## PRECISION THERAPY BASED ON A MOLECULAR PLATFORM FOR RNA THERAPEUTICS

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Accumulating work points out relevant genes and signaling pathways hampered in human disorders as potential candidates for therapeutics. Developing nucleic acid-based tools to manipulate gene expression, such as siRNAs, mRNA and genome editing strategies, open up opportunities for personalized medicine. Yet, although major progress was achieved in developing RNA targeted delivery carriers, mainly by utilizing monoclonal antibodies (mAbs) for targeting, their clinical translation has not occurred. In part because of massive development and production requirements and high batch-to-batch variability of current technologies, which relies on chemical conjugation. Here we present a self-assembled modular platform that enables to construct theoretically unlimited repertoire of RNA targeted carriers. The platform self-assembly is based on a membrane-anchored lipoprotein, incorporated into RNA-loaded lipid nanoparticles that interact with the antibody Fc domain. We show that a simple switch of 8 different mAbs, redirects specific uptake of siRNAs by diverse leukocyte subsets *in vivo*. The platform therapeutic potential is demonstrated in an inflammatory bowel disease model, by targeting colon macrophages to reduce inflammatory symptoms, and in Mantle Cell Lymphoma xenograft model, by targeting cancer cells to induce cell death and improve survival. This modular delivery platform can serve as a milestone in turning precision medicine feasible.

## CELLULARIZED SCAFFOLDS FOR THE TREATMENT OF MYOCARDIAL INFARCTION: FROM BENCH TO BEDSIDE

BEATRIZ PELACHO

In recent years, regenerative cell therapy has been widely explored for the treatment of myocardial infarction (MI). Transplantation of the stem cell population ADSC (adipose-derived stem cells) in chronic MI has been associated with functional improvement, however, its therapeutic value has been limited by the poor cell engraftment and survival. The first aim of our study was to examine whether transplantation of ADSC seeded on collagen scaffolds (CS) could enhance their engraftment and improve cardiac function. Chronically infarcted Sprague-Dawley rats were transplanted with the CS seeded with rat ADSC (CS-ADSC). Animals implanted with unseeded CS or injected media or rat ADSC where also included as control groups. Cell engraftment, histological changes and cardiac function were assessed 4 months after transplantation. Transplantation of CS-ADSC was associated with increased cell engraftment, significant improvement in cardiac function, myocardial remodeling, and revascularization.

The ADSC-patch therapeutic potential was next tested in a preclinical model of CMI. Göttingen minipigs were subjected to MI and then transplanted 2 months later with CS or CS seeded with autologous pig ADSC. Functional and histological assessments were performed 3 months post-transplantation demonstrating an improved cardiac function associated with decreased fibrosis and increased vasculogenesis. Hence, transplantation of autologous CS-ADSC resulted in enhanced cell engraftment that was associated with a therapeutic benefit. In order to get into the clinical scenario, CS were seeded with allogeneic ADSC and manufactured under GMP standards. Their safety was confirmed under GLP conditions in different rodent models of toxicity, tumorigenicity and biodistribution and their putative immunogenic action analyzed in a pig chronic MI model. Toxicity studies showed no adverse effects 2, 10, 30 and 90 days post-implantation in infarcted and healthy Nude rats. Clinical biochemistry, hematological and coagulation blood parameters together with urine analysis did not show significant changes. Necropsy and anatomo-pathological analysis did not reveal significant alterations due to the patch, neither. Importantly also, tumorigenicity was evaluated in Rag2<sup>-/-</sup>gc<sup>-/-</sup> immunodeficient mice subcutaneously implanted with the cellularized patch. No tumoral or differentiated cells were detected after 3 and 8 months of implantation. Also, ADSC distribution was shown to be confined to the cardiac tissue when implanted in the infarcted rat hearts. Cells were no detected in reproductive organs or brain (among other organs) 7 or 30 days post-implant.

Finally, the inflammatory and immune response towards the allogeneic patch was analyzed in chronically infarcted pigs implanted with 50 million ADSC injected or implanted in combination with the collagen scaffold. Untreated infarcted animals were included as control group. The immune response was analyzed before implantation and 15, 30 and 90 days post-implant, determining the evolution of lymphocyte populations, monocytes, granulocytes and leukocytes in blood as well as levels of immunoglobulins and serum proteins. No significant changes were globally detected in any of the parameters analyzed. Moreover, blood biochemical parameters indicated a well preserved hepatic and renal function in all groups thorough the study.

In conclusion, treatment of the heart with an allogeneic Cg-ADSC patch is a safe treatment and do not induce an adverse inflammatory reaction, which could be a promising therapy for the treatment of ischemic patients.

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## ENHANCED EFFICACY OF STIMULI-RESPONSIVE LIPOSOMES OF A DOXORUBICIN-PSA CLEAVABLE PRODRUG (L-377202) FOR ADVANCED PROSTATE CANCER

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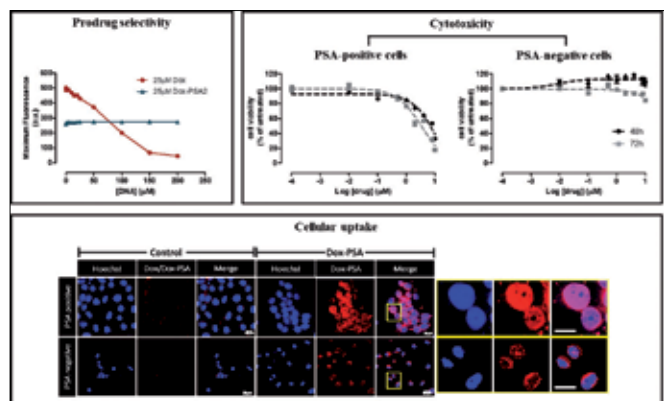
**Background:** Prostate cancer (PC) is the most common cancer in men. Approximately 44,000 men are diagnosed with prostate cancer each year in the UK alone. The serine protease, prostate specific antigen (PSA) is clinically the best serological marker for PC, as PSA levels are elevated in PC. Moreover, PSA has been exploited as a therapeutic target in advanced PC patients. L-377202 prodrug, developed by Merck, was in phase II Clinical trial for patients with metastatic hormone-independent PC. It consists of doxorubicin (Dox) conjugated to a PSA-cleavable peptide that can be selectively cleaved and activated by enzymatically active PSA at the tumour site. It is mainly used to reduce the systemic toxicity of Dox, specifically cardiotoxicity. However, it suffers from low water solubility and unfavourable pharmacokinetics, which results in a limited tumour accumulation in metastatic PC lesions. Therefore, in this work we aim to encapsulate Dox-PSA prodrug into stimuli-responsive nanomedicines, to alter its pharmacokinetics, while maintaining its biological activity. pH- and temperature-sensitive liposomes will be used for the controlled drug release in response to the acidic endosomal system and to temperatures around 41-43°C.

**Methods:** A series of androgen-dependent and independent PC cell lines, which represent different stages of PC, were screened for their PSA expression levels by Western blot and RT-qPCR. Free PSA levels in LNCaP and C4-2B cell lysates and supernatants were quantified by ELISA. Dox-PSA prodrug was synthesized and selectivity was assessed by drug-DNA binding assays. The prodrug was loaded into pH- and temperature-sensitive liposomes. An array of liposomal loading methods including passive and remote loading were investigated to select the formulation that rendered the highest encapsulation efficiency. Cytotoxicity of free and liposomal Dox-PSA prodrug was determined in PSA positive and negative 2D and 3D PC models. Cellular uptake was evaluated by flow cytometry and confocal laser scanning microscopy (CLSM).

**Results:** Prodrug selectivity was supported by drug-DNA binding assays which revealed that as opposed to Dox, Dox-PSA did not interact with DNA. Additionally, Dox-PSA prodrug demonstrated dose- and time-dependent toxicities in PSA-expressing PC cells, whereas in PSA-negative cells toxicity was minimal. On the contrary, free Dox exhibited dose-dependent toxicities in both PSA-positive and negative cells, suggesting that Dox-PSA prodrug was effectively being activated by PSA present in these cells. We have shown that the encapsulation of Dox-PSA into liposomes did not compromise its cytotoxic activity. Dose- and time-dependent toxicities were obtained with the nanoformulation in 2D PSA-secreting cells. Strikingly, in 3D PC spheroids, Dox-PSA-loaded liposomes showed improved cell kill compared to the free prodrug. Flow cytometry studies showed a time-dependent uptake of the encapsulated prodrug, which was further confirmed by CLSM. The latter revealed a time dependent accumulation of the encapsulated drug in the cell nuclei of PSA-secreting cells, supporting the notion that Dox-PSA was efficiently released from the liposomes, hydrolysed by PSA and entering the cell nuclei as free Dox. Importantly, PSA-negative cells did not show prodrug nuclear uptake, once again indicating good selectivity of the prodrug, which relies on the enzymatically active PSA in PSA-secreting cells to be converted into Dox.

**Conclusions:** This is the first study to investigate the encapsulation of Dox-PSA prodrug in a delivery system. We have shown that the prodrug was selective towards PSA-secreting cells and that Dox-PSA was able to be cleaved by PSA and exert its cytotoxic activity. This work further demonstrates that the encapsulated prodrug was capable of being released from the environmental-responsive

nanocarriers and exert its cytotoxic activity both in 2D and 3D PC models. Prodrug-loaded liposomes displayed a superior toxicity compared to the free prodrug in 3D PC spheroids, likely due to an enhanced permeability and penetration. Uptake studies revealed a time-dependence uptake of the prodrug, which is localized in the cytoplasm at earlier time points and, upon PSA hydrolysis, gradually accumulates in the cell nuclei. Further studies are warranted to assess the *in vivo* therapeutic advantage of Dox-PSA liposomes.



## CHARACTERISATION OF PARTICLES IN SOLUTION: A PERSPECTIVE ON CURRENT TECHNOLOGIES.

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In my talk I will provide a perspective detailing the current state-of-the-art technologies for the characterization of nanoparticles in liquid. From assess the current technologies and their applications in the determination of nanoparticle size and concentration; to the identification of the parameters that can influence the results.

The objective of this talk is to provide to and assist manufacturers of nanomaterials in complying with regulatory agencies' demands for accurate and reliable nanoparticle size and concentration data. Outcome of this talk could represent a possible perspective document of the overall Characterisation workshop of the 2018 CLINAM summit.

## PROTEIN-SIZED AND ULTRABRIGHT DYE-LOADED POLYMER NANOPARTICLES FOR INTRACELLULAR IMAGING

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Is there an ideal size for nanoparticles for intracellular applications? Despite its importance for such diverse applications as intracellular delivery or single molecule imaging of biomolecules inside living cells, answering this question remains a challenge. The major reason for this is that the majority of non-deformable nanoparticles are relatively large and their size cannot be finely tuned to match that of proteins.

Figure 1. Example of polymer, dye, and counterion used to assemble dye-loaded nanoparticles

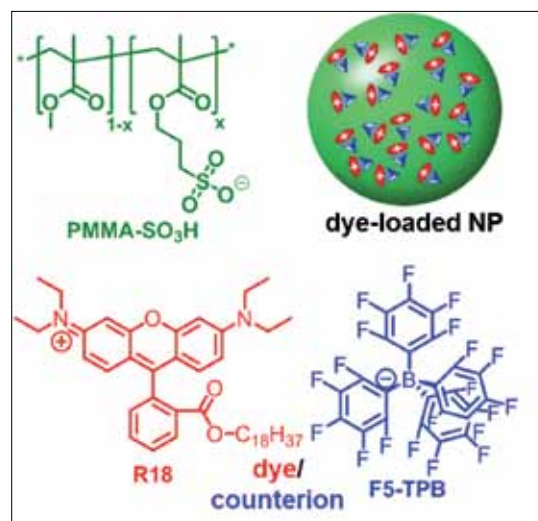
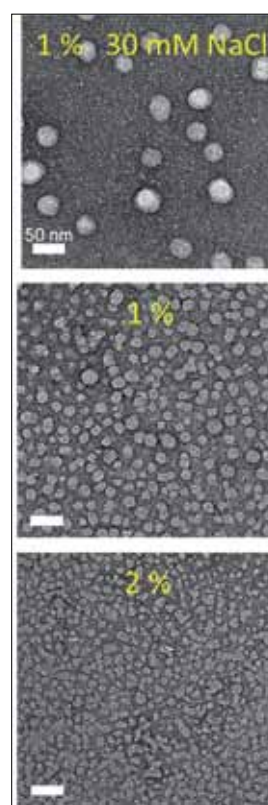


Figure 2. TEM images of NPs made from PMMA-SO<sub>3</sub>H 1 or 2% in mQ and NaCl solution.



In this work, we develop poly(methyl methacrylate) copolymers with varied fraction and type of charged groups (carboxylate, sulfonate and trimethylammonium), which enable preparation of polymer nanoparticles with controlled sizes from 50 nm down to 7 nm through nanoprecipitation. These particles can be made brightly fluorescent through encapsulation of large amounts of fluorescent dyes. Indeed, dye-loaded polymer nanoparticles appeared recently as systems with exceptional brightness.<sup>[1]</sup> In particular, we used cationic dyes together with bulky hydrophobic counterions to achieve efficient fluorescence with various emission colors.<sup>[2-5]</sup> The obtained particles were up to tenfold brighter than quantum dots, which enabled their tracking at the single-particle level in the cytosol of living cells. In contrast to particles  $\geq 32$ -nm, smaller particles of  $\leq 17$  nm diameter show faster diffusion and

much larger spreading throughout the whole living cell upon microinjection. These results show distinct particle sieving in the cytosol for non-deformable particles of the same nature in the size range from  $<10$  to 50 nm, and suggest a critical particle size of  $\leq 17$  nm for free diffusion and spreading in the cytosol. The proposed concept of polymer design opens the route to organic nanoparticles of ultrasmall sizes and high loading with cargos, which should enable applications ranging from high-speed tracking of single biomolecules with high localization precision to site-directed intracellular drug delivery.

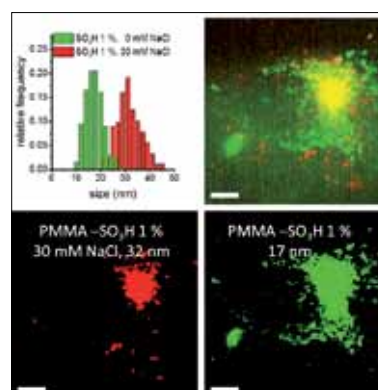


Figure 3. Two-color imaging of PMMA-SO<sub>3</sub>H NPs 32 nm (red, Cy5/F12-TPB) and 17 nm (green, R18/F5-TPB) co-microinjected into the cytosol. Top left: size distributions from TEM; top right: superposition of maximum projections; middle: threshold images obtained from the projections. Scale bars, 10  $\mu$ m.



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## CROSSING THE VALLEY OF DEATH: MOVING MEDICAL PRODUCTS TOWARD COMMERCIALIZATION AND CLINICAL PRACTICE

FRANCES RICHMOND

The “Valley of Death” is a term often used to describe the challenges faced by researchers as they translate new pharmaceuticals and medical devices through a highly regulated environment to become commercial products. Because the path is long and costly, medical products typically are developed by established companies with access to the resources needed to cross the Valley of Death. For those companies, the decision to acquire a novel technology or to form a joint venture with an academic or small start-up can be fraught with risk. Companies attempt to control those risks by assessing not only the potential of the product itself but also the capabilities of the research entity with whom they must form a relationship.

We identify two gaps that contribute to the attrition that often occurs during the translation of medical products. One is a resources gap, because meeting regulatory and quality requirements involves expensive clinical trials and controlled manufacturing. The second is a competency gap. Academic scientists often do not understand what needs to be done to develop a commercial product or to work with a company in a regulated industry. This competency gap diminishes the potential of a novel product to progress from the bench to the bedside. Appropriate educational programs can improve competency. However, academic scientists often do not recognize their own needs and few programs provide the particular skillsets to navigate this difficult stage of product development. Most academic researchers have never worked in a regulated research environment and have a fairly steep learning curve if they are to be successful in the development of these types of products. By misunderstanding the development, regulatory and commercialization processes, academic champions may get in the way of success and risk the loss of promising therapies for patients. This problem is particularly acute for highly innovative fields such as nanotechnology and stem cells where scientists, clinicians and regulators must often work together to forge new methodologies for manufacturing and clinical trials. In this lecture, I will discuss strategies to enrich the training and skillsets of those responsible for the development of innovative medical products in the academic environment and thus to foster the translation of these promising medical technologies.

### PANEL: THE GREAT DIVIDE: DEVELOPING REGULATORY COMPETENCE IN ACADEMIA VERSUS INDUSTRY

Clinical scientists, academic product developers, regulators and medical-product companies belong to an integrated ecosystem bound by a common system of regulatory requirements and constraints. However, the perception of what is and is not important from a regulatory perspective varies significantly from one group of individuals to another and from product to product. Clinicians and

academic researchers often consider it enough to understand the narrow set of regulatory elements related to clinical trials as box-checking activities divorced from the mainstream considerations of science and business. For successful regulatory teams in industry, however, the regulatory strategy is much broader in scope and must take into account the entire lifecycle of the product. Regulatory personnel in industry must anticipate the demanding pre-clinical and clinical activities and submissions that dominate market registration, reimbursement and manufacturing compliance. The type of training appropriate for each group therefore differs. Discovery scientists and clinicians do need education about the narrower requirements for preclinical and clinical development, but they must also have an awareness of all aspects of new product development that can contribute to the success or failure of a new medical product. This understanding of the entire product-development pathway will become an important tool as they begin to interface with industry. At least a modicum of knowledge about the challenges faced during later stages of development, reimbursement and marketing would improve these interactions. Drug and device development is too expensive and competitive for academic researchers to “go it alone” without the experience and resources that a company can provide. At the same time, companies rely increasingly on the innovative research undertaken in academic institutions to help provide new technologies for their product pipelines. Our goal should be to prepare each side to understand the needs of the other so that we do not waste time and opportunities to translate discovery science into clinical practice.

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## CRIPeC® NANOMEDICINES: THE TRANSLATIONAL PATH FROM PRECLINICAL TO CLINICAL STUDIES

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Cristal Therapeutics is a clinical stage pharmaceutical company developing the next generation of nanomedicines based on its proprietary CriPec® platform to treat various diseases, including cancer. CriPec® is perfectly suited for the rational design of (targeted) nanomedicines with superior efficacy and safety profiles<sup>[1-4]</sup>. The most advanced product in development is CriPec® docetaxel for the treatment of solid tumours, while other CriPec® products are in (late-stage) preclinical development. CriPec® products are fully customisable and biocompatible, with a robust manufacturability at clinical scale.

## RESULTS AND DISCUSSION

### CriPec® docetaxel

Significantly enhanced anti-tumour efficacy of 65 nm sized CriPec® docetaxel has been demonstrated in preclinical breast, prostate and gastric cancer mice xenograft models. The superior efficacy is attributed to an improved pharmacokinetic profile, confirmed higher tumour uptake and improved tolerability of CriPec® docetaxel as compared to Taxotere®.

Upon clinical evaluation, CriPec® docetaxel demonstrates a half-life of approximately 33 hours with encouraging signs of target response in patients with advanced solid tumours. In addition, the significant reduction in certain dose limiting toxicities provide clinical evidence for altered tissue disposition of CriPec® docetaxel. In addition to the ongoing phase 1b study to determine the recommended phase 2 dosing, the biodistribution and the extent of clinical tumour uptake versus Taxotere are being determined both invasively as well as non-invasively.

### CriPec® oligonucleotides

The potential of therapeutic compounds such as peptides and oli-

gonucleotides is still hindered by effective tissue and cellular targeting. CriPec<sup>®</sup> has successfully been combined with a variety of small molecules, peptides and oligonucleotides. The surface can be modified with targeting ligands, and *in vitro* evaluation has demonstrated increased cellular uptake.

CriPec<sup>®</sup> ds-siRNA are ~ 55 nm nanoparticles with a half-life of ~ 14 hours and an enormous increase of tumour uptake in PC3 tumour-bearing mice compared to naked ds-siRNA.

### Conclusion

CriPec<sup>®</sup> allows for the rational design of nanomedicines for a superior therapeutic performance in several indications. Results of the clinical phase I evaluations of CriPec<sup>®</sup> docetaxel are promising. Moreover, additional CriPec<sup>®</sup>-based products carrying different types of payloads are being developed, both internally and in co-development with external parties.

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## TECHNOLOGY PLATFORM FOR THE PRODUCTION OF LIPID-BASED NANOVESICLES AS NEW NANOMEDICINES

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Molecular self-assembly has enabled the fabrication of biologically inspired, advanced nanosystems as lipid-based nanovesicles (L-NVs) [1]. The oldest type of L-NVs, liposomes, have been widely proposed as potential candidates for drug delivery, diagnostic and/or theranostic applications and some liposome-based drug products have already stepped from the lab-bench to the market. This success is attributed to their ability to encapsulate both hydrophobic and/or hydrophilic molecules, efficiently carry and protect them within the body and finally deliver them at the target site. These positive features are also coupled with high biocompatibility. However, liposomes still present some unsolved drawbacks, such as poor colloidal stability, short shelf-life, restricted and expensive conditions of preparation because of the inherent nature of their fundamental constituents (phospholipids). The new tools available in the controlled self-assembly of molecules have significantly advanced in the field of L-NV design and synthesis, and new kind of nanovesicles are appearing once other surface-active ingredients are considered [2]. This new generation of nanovesicles can represent a paradigm shift in nanomedicine: they may complement liposomes, showing their advantages and overcoming most of their

drawbacks. Clearly, being still young, their rocky way to the clinic first and then to the market has just started and it is still long, but they have all the potentialities to reach their objective target.

The arrival of a nanomaterial to the market is strongly dependent on the availability of technological-scale preparation methods. This is especially relevant in the Pharmaceutical sector, where scaling up of nanopharmaceuticals under cGMP conditions is challenging. Green technologies show the potential to represent a game-change in the production of L-NVs, favouring the step of L-NVs from the bench to the clinical use. The DELOS-susp platform is an eco-efficient compressed fluid-based technology that allows the reproducible preparation of colloidal systems such as L-NVs with remarkable physico-chemical characteristics, in terms of homogeneity and particle size, and a high versatility to integrate different active compounds. These features make DELOS-susp a very suitable production process for cGMP scale up, as is being demonstrated with several production capacity increases without affecting product quality. This platform allows the formulation of innovative nanoconjugates for therapeutic and diagnostic applications, [3], and in the present work some nanomedicines under development by using DELOS-susp platform will be presented.

Smart-4-Fabry is the acronym of a H2020 funded Project that stands for "Smart functional GLA-nanoformulation for Fabry disease". Fabry disease is a rare inherited disease caused by the loss of function of the  $\alpha$ -Galactosidase A (GLA) enzyme. One of the commercially available treatments is based on the intravenous administration of GLA. This enzyme replacement therapy (ERT) demonstrated positive short-term effect reducing the progression of the disease and improving the quality of life in patients. However, ERT exhibits drawbacks such as the degradation of the exogenously administered enzyme, its limited efficacy in patients with an advanced stage of the disease and the extremely high cost of the treatment. Smart-4-Fabry is a collaborative project which has been conceived and created to obtain a new nanoformulation of GLA, that will improve the efficacy and toleration compared to the actual treatment with non-formulated GLA. The final benefit will be seen as considerable reduction on the Fabry disease treatment cost and a substantial improvement in the life-quality of Fabry disease patients. The nanotechnological approach followed in Smart4Fabry project is to increase the plasma half-life and improve the delivery efficacy of the current treatment, and is based on the production of nanovesicles L-NVs containing GLA as novel drug delivery systems (DDS) [4]. The incorporation of GLA in nanovesicles was obtained following the DELOS-SUSP methodology. Moreover, a c(RGDfK) peptide ligand is incorporated in the membrane bilayer of the vesicles to enhance the targeting and the uptake efficiency of the GLA-loaded conjugates to the affected cells. The latter constitutes a major challenge in the treatment of lysosomal storage disorders. Other objectives to be fulfilled by the realization of this project are to achieve strong quality control over the self-assembly of nano-GLA molecular components and its physicochemical parameters, to develop this new GLA nanoformulation following the requirements of the pharmaceutical industry and regulatory authorities and to set a new GMP plant for the production of the quantities of the nanoformulation needed for future clinical trials.

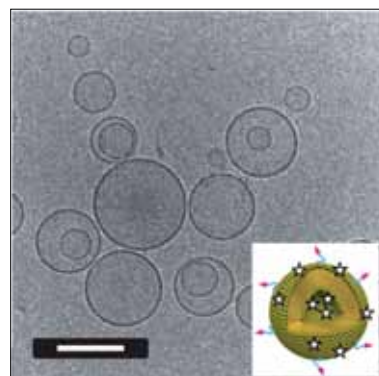


Figure 1: Image and Scheme of GLA Nanovesicles

### ACKNOWLEDGEMENTS

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## ENTRY OF NANOPARTICLES INTO CELLS: MECHANISMS AND CONSEQUENCES

KIRSTEN SANDVIG

Nanoparticles can be used to deliver drugs or other substances both *in vivo* and *in vitro* (1-3). To enter cells the particles exploit the endocytic machinery, and they have been demonstrated to induce changes in cellular uptake and intracellular transport (4,5). Crosslinking of cell surface molecules may cause signaling in cells (6), and nanoparticles have been found to induce macropinocytosis that facilitates uptake of particles. In several instances this process has been shown to be dependent on the large GTP-binding protein dynamin. To optimize nanoparticle delivery into cells one needs to understand the cellular mechanisms involved in their uptake. Such information may help in deciding the type of particle to use, the size of the particle as well as which components to include at the particle surface. Today we know that cells have different types of endocytic mechanisms (7,8), some giving rise to small vesicles (60-200 nm diameter), whereas other mechanisms such as macropinocytosis are required for uptake of larger particles. One should be aware of that cells growing in a polarized manner are likely to have endocytic mechanisms which are under differential influence of signaling substances at the two poles (7,8), and studies of nanoparticle uptake in nonpolarized cells may not give the same results as if uptake in polarized cells is investigated. It may be an advantage to study different cell types as their response to a given nanoparticle may vary, and one should be aware of that even apparently small differences in particle composition may contribute to different types of cell death. Furthermore, increased cell density may induce changes in membrane lipids and intracellular transport (9), and modification of membrane lipids may change the mechanisms of uptake (10). Clearly, well controlled conditions for the cell experiments performed and correct interpretation of the results obtained from cellular studies are essential. For instance, cholesterol is often mistaken for only being important for caveolar uptake, but is involved in several endocytic processes including macropinocytosis (1,7,8). Also, robust methods to determine whether a particle is internalized or only at the cell surface are important to provide the investigator with correct data about uptake efficiency, and a challenge is that the drug in the nanoparticle often has to reach the cytosol or the nucleus to exert its action.

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## MULTIFUNCTIONAL NANOMEDICINES FOR TARGETED DRUG DELIVERY AND IMAGING FOR ISCHEMIC MYOCARDIAL INJURY

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## 1. BACKGROUND AND HYPOTHESIS

Ischemic heart disease is the number one cause of death in the worldwide. A severe myocardial ischemic event is characterized by massive loss of cardiomyocytes and replaced by fibrotic scar tissue, which leads to progressive loss of contractile function and pathological remodelling of the ventricular walls<sup>1</sup>. Particularly, natriuretic peptides with paracrine actions are highly produced in the endocardial layer of the ventricular wall, binding to specific natriuretic peptide receptors<sup>2</sup>. Current therapeutic lines provide only small prolongations in survival of patients with heart failure<sup>3</sup>. Thus, novel therapeutic approaches are needed to significantly reduce mortality and morbidity. We hypothesized that targeting NPR receptors with a drug delivery system offers a unique opportunity for the treatment of ischemic heart disease.

## 2. EXPERIMENTAL

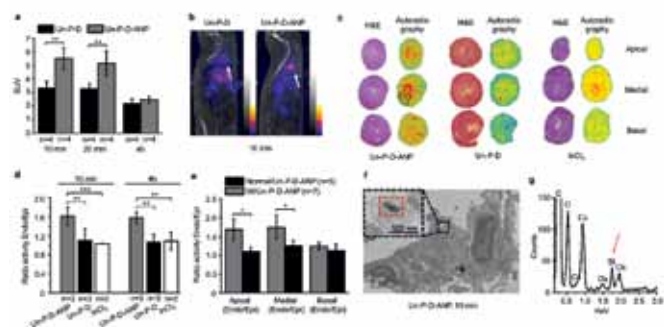
As a continuation of our previous work<sup>4</sup>, we use porous silicon nanoparticles (PSi NPs), which we surface functionalize with polyethylene glycol, a metal chelator for radiolabelling and a targeting peptide using EDC/NHS chemistry. The nanoparticles' physicochemical characterization was done by DLS, TEM, ATR-FTIR, EDX spectroscopy and elemental analysis. *In vitro* cell viability and interaction studies were done with primary cardiac cells. The NPs heart accumulation was evaluated *in vivo* by SPECT/CT and autoradiography. Biological activity of loaded NPs was assessed with Western Blot.

## 3. RESULTS AND DISCUSSION

The developed NPs presented successful biofunctionalization with imaging and targeting ligands. The peptide-modified PSi NPs showed good cytocompatibility and enhanced cellular interaction with primary cardiomyocytes and non-myocyte cells. *In vivo*, enhanced heart accumulation was observed for peptide-PSi NPs



administered i.v., specifically in the endocardial region of the left ventricle. Single PSi NPs were found in the same region (Figure 1). A lead compound was loaded into the peptide-PSi NPs, delivered i.v. to ischemic heart rats, having an effect on the ERK hypertrophic signalling pathway of the heart, showing cardioprotective potential.



**Figure 1.** *In vivo* assessment of the accumulation, specificity and regional location of the nanoparticles in the infarcted myocardium after systemic administration in rats. **a**, SPECT/CT image quantification of the standardized uptake values (SUVs) in the rat heart at 10 min, 20 min, and 4 h after i.v. administration of nanoparticles. **b**, Representative sagittal SPECT/CT images showing the biodistribution of the nanoparticles at 10 min after i.v. administration. Arrows indicate the location of the heart. **c**, Representative H&E stainings and autoradiograms of apical, basal and medial rat heart sections (from a single heart for each treatment: Un-P-D-ANP, Un-P-D and  $^{111}\text{InCl}_3$  control) showing the localization of radioactivity 10 min after the injection of nanoparticles or  $^{111}\text{InCl}_3$ . Delineated regions 1 and 2 represent examples of the regions of interest (ROI) used for activity quantifications. **d**, Autoradiographic quantification of radioactivity in the endocardium (Endo) and epicardium (Epi) for Un-P-D-ANP and Un-P-D nanoparticles and  $^{111}\text{InCl}_3$  presented as a ratio of Endo to Epi. The results are shown for the whole heart 10 min and 4 h after the injection of nanoparticles into rats with isoprenaline-induced myocardial ischemia (MI). **e**, Autoradiographic quantification of the radioactivity in the Endo and Epi regions of the heart in the apical, medial and basal sections in isoprenaline-induced MI and normal rat groups, presented as a ratio of Endo to Epi. **f**, Representative TEM image of a Un-P-D-ANP nanoparticle in the endocardial region of a heart section. **g**, Elemental composition of the selected area (f, red box) by EDX analysis showing the presence of the Si element (red arrow).

#### 4. CONCLUSIONS

Our results show that precisely engineered heart-homing biodegradable PSi NPs can enhance the cellular interactions with cardiac cells, improve cardiac accumulation into the damaged heart after i.v. administration, and deliver a drug to exert biological effect on the heart *in vivo*.

#### 5. ACKNOWLEDGEMENTS

Drug Research Doctoral Programme of University of Helsinki, Academy of Finland, Sigrid Juselius Foundation, University of Helsinki Research Funds, the HiLIFE Research Funds, Tekes large strategic research opening project no. 40395/13, and European Research Council (FP/2007–2013, grant no. 310892) are acknowledged for financial support.

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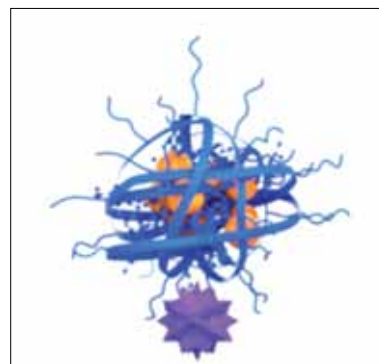
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## SELECTION CRITERIA DURING PRECLINICAL EVALUATION AND RESULTS FROM INTEGRIN TARGETING RS POLYMER DRUG CONJUGATES FOR SOLID TUMORS

**RANA SANYAL**<sup>1,2</sup>, RS Research, Teknopark Istanbul, TURKEY  
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According to the latest reports, cancers figure among the leading causes of morbidity and mortality worldwide, with approximately 14 million new cases and 8.2 million cancer related deaths in 2012. Quality of life for cancer patients is drastically reduced due to deleterious side effects of chemotherapy. As the chemotherapy agents, which are the forefront tools to fight against cancer, are extensively toxic to the whole body, these conventional cancer treatment drugs attack cancer cells as well as healthy cells, leaving the patient vulnerable to other diseases.

Our solution offer to this problem is to package the chemotherapy agent in a manner to protect the agent from the body and the body from the agent till it reaches its destination: the tumor. The packaging, grandiosely termed as the nanomedicine, will be referring to the polymer therapeutic, more precisely polymer-drug conjugate (Figure 1). Polymer therapeutics share many properties of the biologics (proteins, antibodies, oligonucleotides) with a bonus of synthetic chemistry utilities: tailoring of molecular weight and addition of biomimetic and bioresponsive features to the man-made construct. The successful polymer-drug conjugate has many advantages including improved water solubility and pharmacokinetic properties, better toxicity profile, less frequent administration in the clinic and a patent application as a new entity patent, indulging the pharmaceutical industry.



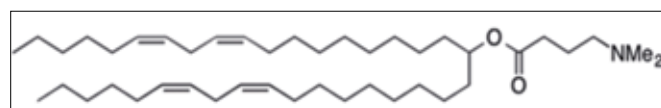
**Figure 1.** RS Research polymer therapeutics (RS-0139)

This presentation will explain the novel class of polymer therapeutics we have prepared in RS Research Inc. and developed through preclinical studies. The talk will emphasize how we selected our lead molecule RS-0139 to move forward to the clinic, with a Phase I short term goal.

## COMPARING EXTRACELLULAR VESICLES TO SYNTHETIC LIPID-BASED DELIVERY SYSTEMS FOR RNA

**RAYMOND M. SCHIFFELERS**, Laboratory Clinical Chemistry & Hematology, University Medical Center Utrecht, Utrecht, The Netherlands

The current state of the art in delivery of RNA appears to be the stable nucleic acid lipid particles (SNALPs)<sup>(1)</sup>. A critical component of these particles is the ionizable lipid, such as heptatriacontan-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate, known as DLin-MC3-DMA. This lipid:



- provides cationic charge during production of the particles at low pH to mediate effective encapsulation.
- Is without net charge at physiological pH, reducing clearance rate and determining a neutral surface for protein corona formation *in vivo*
- Becomes charged again upon endosomal internalization to engage with the organelle's membrane and mediating endosomal escape.

The other critical component is the PEGylated C14 lipid that:

- Is responsible for temporary steric stabilization of the nanoparticle surface upon initial contact with blood, but subsequently is relatively rapidly lost and exchanged for a protein corona featuring apolipoprotein E.

These lipid based systems have shown excellent transfection capabilities, especially of the liver. However, to reach other tissues is not straightforward. Recently, we have also been looking into the delivery of RNA by extracellular vesicles. These systems appear to be Nature's choice for delivery of RNA and have shown surprising activities *in vivo*, for example in tumor transfection<sup>(2)</sup>. In this presentation I will highlight the similarities and differences between extracellular vesicles and SNALPs and focus on the challenges in comparing the delivery efficiency of both systems.

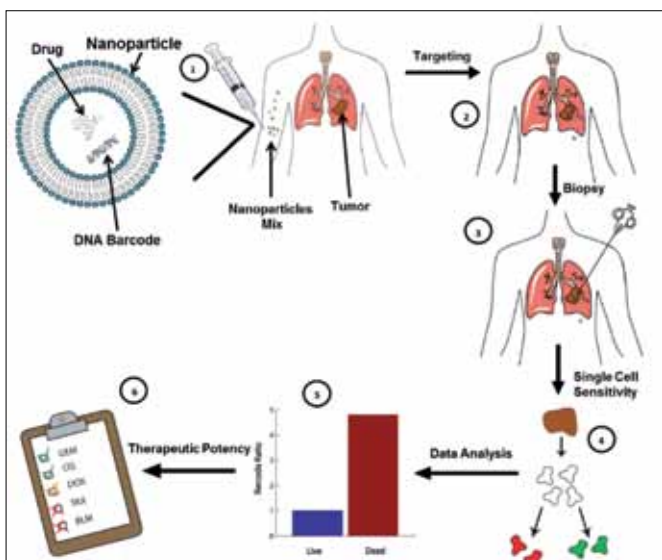
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## PRECISION NANOMEDICINES AND NANO-IMMUNOTHERAPIES FOR TREATING BREAST CANCER

**AVI SCHROEDER**, PhD, Assistant Professor of Chemical Engineering, Technion – Israel Institute of Technology, Haifa 32000, Israel [avids@technion.ac.il](mailto:avids@technion.ac.il)

Medicine is taking its first steps towards patient-specific care. Nanoparticles have many potential benefits for treating cancer, including the ability to transport complex molecular cargoes including siRNA and protein, as well as targeting to specific cell populations.



The talk will discuss 'barcoded nanoparticles' that target sites of cancer where they perform a programmed therapeutic task. Specifically, particles that inform the physician regarding patient-specific drug potency in the primary tumor and metastasis.

The evolution of drug delivery systems into synthetic cells, programmed nanoparticles that have an autonomous capacity to synthesize diagnostic and therapeutic proteins inside the body, and their immunotherapy promise for recruiting T-cells to the tumor and inducing an anti-cancer response, will be discussed.

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## POLYMERIC MATERIALS FOR BIOMEDICAL APPLICATIONS

**ULRICH S. SCHUBERT**, Biomaterials Science Center, Department of Biomedical Engineering, University of Basel, 4123 Allschwil, Switzerland;

For the analysis and evaluation of reconstructed peripheral nerves without cutting and staining processes a three-dimensional (3D), non-destructive imaging method is of high interest. Taking the advantage of true micrometer spatial resolution and suitable contrast for paraffin embedded soft tissues, laboratory-based X-ray microtomography was used to achieve 3D data sets, which were analysed by custom analysis protocol. Using this method, the quantitative assessment of 3D tissue structures, i.e., surface morphology, nerve fascicles, nerve tissue volume, geometry, and vascular re-growth was performed. A significant difference between operated animals and non-operated controls could be detected. In order to provide findings with statistical significance, the results were validated with a sufficient number of specimens. The main advantage of the method is that it avoids the sampling error associated with conventional 2D visualization approaches and allows for automatic analysis of large series of data sets.

**Keywords:** X-ray microtomography, non-destructive, nerve conduits, nerve reconstruction

## NON-DESTRUCTIVE THREE-DIMENSIONAL ANALYSIS OF REPAIRED PERIPHERAL NERVES USING X-RAY MICROTOMOGRAPHY

**GEORG SCHULZ**, Laboratory of Organic and Macromolecular Chemistry (IOMC); Jena Center for Soft Matter (JCSM); Friedrich Schiller University Jena, Germany

Polymers feature a great potential for the delivery of various active pharmaceutical ingredients. An optimum carrier material should be non-toxic, bind and protect its cargo from degradation, be invisible to the immune system and direct cargo to its desired place of action. Although promising polymers exist to fulfill one or two of the requirements, only few are currently exploited for these purposes. The lecture provides an overview about how traditional polymers can be modified, coupled to relevant building blocks, or be replaced by more tailor-made alternatives. In particular the solution self-assembly behavior of the (block co) polymers has to be studied in detail (e.g. by analytical ultracentrifugation, AF4, (cryo)TEM or others).

**Selected references:** Angew. Chem. Int. Ed. 2018, 57, 2479; Biomacromolecules 2018, 19, 748; Biomacromolecules 2017, 18, 3280; Polym. Chem. 2017, 8, 6086; Anal. Chem. 2017, 89, 1185.

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## UNMET NEEDS IN DEVELOPING NANOPARTICLES FOR PRECISION MEDICINE

SIMO SCHWARTZ JR

The use of nanomedicine as therapeutic option in Precision Medicine strategies is gathering more attention every day. However, several issues have still to be solved in order to improve the impact of nanomedicine in prompting forward precision nanomedicine as a discipline. Among them, the need to ensure reproducibility and GMP compliance in regulatory preclinical studies, biological interactions with blood proteins and cell membranes, active targeting of therapeutic nanoconjugates, crossing of biological barriers and accurate biodistribution and circulation times to ensure efficacy and reduce toxicity, are often, unsolved questions. We will discuss on these and other topics and about current strategies intended to overpass some of these unmet needs.

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## BUILDING AN INTEROPERABLE DATA ECOSYSTEM FOR PERSONALIZED HEALTH RESEARCH IN SWITZERLAND

TORSTEN SCHWEDE

Personalized health aims to provide the right treatment at the right time for each individual. By mining real-world health data, researchers can formulate or refine new hypothesis for disease mechanisms and offer novel intervention strategies, while clinicians derive effective treatment plans that comprehend the unique characteristics of their patients. Tailored, predictive interventions have the potential to change from a reactive to a prevention approach, thereby significantly extending the duration of health.

The Swiss Personalized Health Network (SPHN) is a national initiative designed to promote the development of personalized health and medicine in Switzerland. By establishing a dynamic collaborative network of distributed interoperable and sharable resources, such as data, platforms, workflows, and competences, SPHN aims to support internationally competitive personalized health research.

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## CELLULAR MATERIALS FOR ORGAN ON CHIP RESEARCH

GIACINTO SCOLES, (Nanotec-CNR, Lecce, Italy); Daniela Cesselli (UniUD, Udine, Italy); Denis Scaini (SISSA, Trieste, Italy)

Micro- and nano-composite materials will be considered from the point of view of the realization of organoids for applications to *in vitro* diagnostics for neuro-degenerative diseases. Unfortunately, right where the realization of these devices would be more beneficial, there the difficulties are larger because the connection with the real situation is fraught with danger and practical difficulties. An application will be described to the diagnostics of neuro-degenerative diseases choosing the area where the methods would be more beneficial (i.e. the area of rare diseases). We will consider a class of TAUopathies, known as Progressive Supranuclear Palsy (PSP), both in their main phenotype and in the rare PAGF phenotype i.e. Pure Akinesia with Gait Freezing that has an "abundance" of about 1 in a million individuals. Our approach involves the pro-

duction of pluri-potent stem cells from the adipose tissue of a well, clinically, diagnosed individual and their subsequent differentiation to neurons. If the latter result to be diseased then the road to the cure is possible and well planned. The main difficulty is in the complexity of the brain that sees, for example, the localization of the disease in the brain stem. The method has been, however, already proven to be effective in genetic diseases but our interest involves now the more difficult case of sporadic diseases.

First, of course, we will need to measure the diseased portion of the neurons in AVERAGE which will determine the precision of the proteomics measurements that are meant to establish the diseased level of the neurons. Our goal is to establish the level of production of protein TAU and compare it to the cells of a healthy individual from the adipose tissue of which the neurons will be produced following a similar protocol. Two complementary approaches will be followed: (i) if the cellular dilution factor (diseased neurons over the total number of neurons obtained) will not prove to be too limiting, we will be able to proceed directly to search for the molecules that may "wash" out the extra amount of TAU. Otherwise (ii), we will need to go to the second level looking to establish the presence of disease molecular fingerprint at the single cell level establishing, in such way, the heterogeneity of the disease. At this second level of investigation we plan to use a nanopipette to be inserted into the cell and used to suck some of the fluid contained into the cell. By coating the interior of the nanopipette with nanobodies that recognize the PROTEIN of interest the latter will become trapped inside the channel of the pipette changing its value of the ionic conductivity. Next to the nanopipette there will be a reference (uncoated) pipette to provide a reference level for the ionic conductivity.

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## THE ZEBRAFISH: A PRECLINICAL SCREENING MODEL FOR THE OPTIMIZATION OF NANOMEDICINE FORMULATIONS

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Nanomedicines have gained much attention for the delivery of small molecules or nucleic acids as treatment options for many diseases. However, the preclinical development of novel nanomedicines is a very cumbersome and often unsuccessful process. Among other things, this is due to the fact that formulation design and optimization is mainly based on *in vitro* studies which are not able to fully mimic complex biological conditions. Moreover, only a selected number of formulations can subsequently be assessed in rodent *in vivo* experiments, since such studies are expensive, time consuming and require regulatory approval at an experimental level. Obviously, there is a huge gap between *in vitro* cell culture and rodent *in vivo* studies. This does not allow a thorough formulation design and optimization under realistic biological conditions and hampers a detailed understanding of nanomedicine interactions with biological environments at a macromolecular level.

In this talk, the set-up, validation, and application of the zebrafish model during nanomedicine formulation design and optimization are presented. In a first study, described liposome formulation effects on liposome pharmacokinetic properties in rodents were reproduced in zebrafish embryos and suitable experimental protocols were established. A special focus was put on fundamental liposome formulation aspects such as lipid transition temperature, amount of cholesterol, and PEGylation<sup>[1]</sup> (separate PEGylation manuscript submitted to Journal of Controlled Release). Selected findings of zebrafish studies have been verified in rodent *in vivo*



studies in order to check the model's predictive power. Importantly, experimental data obtained in zebrafish and rodents were consistent. This initial findings already indicate the promising value of the zebrafish model making it worth to perform additional studies, a fact which has been highlighted in an editorial comment by Park [2].

In a detailed follow-up study, scavenger receptor mediated nanomedicine clearing mechanisms in the zebrafish were identified and investigated. As a result, it was possible to demonstrate that specific nanomedicine accumulation patterns in zebrafish correspond to nanomedicine clearance in the mammalian liver [3]. This study shows the potential of the zebrafish model to investigate nanomedicine interactions with a biologic environment under live *in vivo* conditions at a macromolecular level, as appreciated by Yin et al. [4].

Based on the above mentioned findings, it has been possible to apply the zebrafish model for various purposes regarding the design of nanoparticulate delivery systems [5,6]. Especially the determination of the optimal ligand density for an actively targeted nanoparticle underlined the additional value of the zebrafish model. Initial *in vitro* studies followed by zebrafish xenograft studies revealed different optimal ligand densities when assessing the systems targeting efficiencies (Manuscript submitted to ACS Nano). Importantly, the findings of the zebrafish experiment were confirmed in a later rodent biodistribution study, highlighting the superiority of live *in vivo* conditions compared to artificial *in vitro* set-ups.

Our findings highlight that the zebrafish model is a useful vertebrate screening tool to predict the *in vivo* performance of nanoparticulate drug delivery systems. First, this facilitates the selection of potentially successful formulations prior to subsequent rodent *in vivo* studies. Second, transparent zebrafish embryos allow the investigation of nano-bio interactions at a macromolecular level. Altogether, this will broaden our understanding of basic nanomedicine formulation effects resulting in an increased translation of novel nanomedicines from bench to bedside.

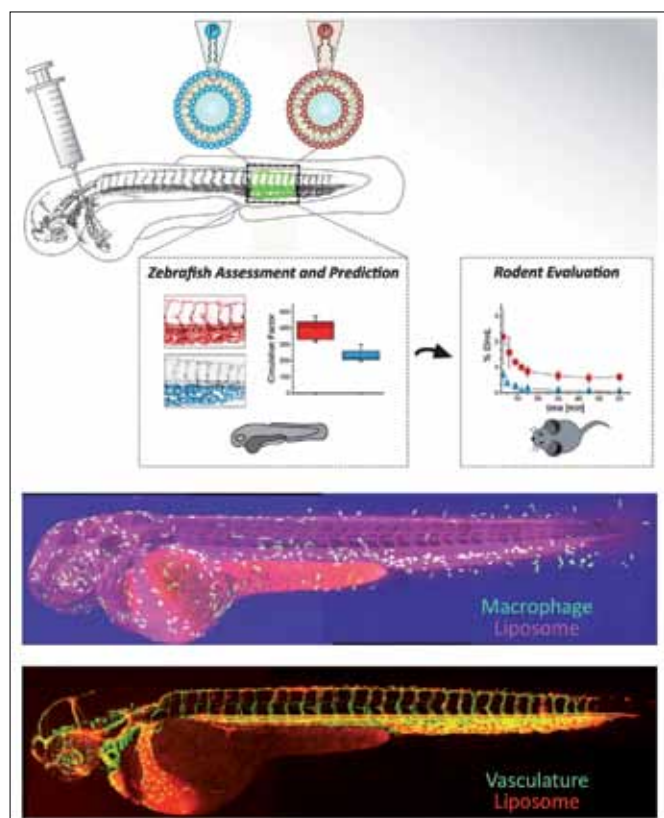


Figure 1. The zebrafish as an *in vivo* model for nanomedicine design and optimization. Fluorescently labelled nanoparticles are injected into 2 days post fertilization zebrafish embryos expressing fluorescent proteins specifically in their macrophages or vasculature. By

*confocal microscopy, different predictive zebrafish factors such as the extend of macrophage phagocytosis or nanoparticles blood circulation behavior can be assessed. Findings of zebrafish experiments have shown to be predictive for outcomes of subsequent rodent in vivo studies.*

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## ROLE OF NATURAL ANTIBODIES AND PROTEIN CORONA IN VARIABILITY AND EFFICIENCY OF COMPLEMENT C3 DEPOSITION ON PRECLINICAL AND CLINICAL NANOMEDICINES

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Activation of complement cascade by nanomedicines may trigger immune clearance and proinflammatory responses. The levels of complement activation differ between human subjects, but mechanisms underlying the variability are poorly understood. We previously demonstrated that opsonization of superparamagnetic iron oxide nanoworms (SPIO NWs) by the third complement protein (C3) was dependent on the protein corona. Here we show a predominant role for serum IgG deposition on SPIO NWs in cohort of healthy subjects (n=12) in efficiency of C3 deposition. Depletion of IgG from normal sera reduces C3 deposition by 70-95%, and reconstitution with purified polyclonal IgG, but not with polyclonal IgM or a monoclonal antibody (trastuzumab), increases C3 opso-

nization. The C3 dependency in IgG binding is universal, and observable with clinically approved nanopharmaceuticals (e.g., iron oxide Feraheme and liposomal irinotecan Onivyde and PEGylated liposomal doxorubicin Doxil) in both healthy sera and plasma from breast cancer patients, regardless of the activation pathway. The evidence suggests that few surface-bound IgG molecules trigger complement activation. While the total amount of absorbed protein does not determine the efficiency of complement deposition, the corona is necessary for efficient IgG binding to all nanoparticle types. These findings suggest that natural antibodies serve as a link between protein corona and C3 opsonization, and might be used to predict interindividual immune responses to nanomedicines.

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## NANOPARTICLES FOR CLINICAL USE: IMPORTANCE OF DEGRADATION AND EXCRETION

**TORRE SKOTLAND**, Department of Molecular Cell Biology, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital, Norway

There are huge expectations for the use of nanoparticles (NPs) to deliver therapeutics and for imaging of different diseases, such as cancer. Carefully designed experiments, both *in vitro* and *in vivo*, are essential in order to fully explore this technology. Despite many promising NPs being made during recent years, the biological studies performed with such NPs very often do not have the quality needed to support the conclusions drawn<sup>(1,2,3)</sup>. More interdisciplinary collaboration to improve the quality of such studies is required.

With a long experience from pharmaceutical R&D, I will discuss improvements that should be made in biological studies with NPs. The design of animal studies, including which time points to take samples and which parameters to analyze, is critical when aiming at developing drugs for clinical use<sup>(1)</sup>. Biodistribution, metabolism and excretion studies are extremely important not only to generate such data (e.g. for an imaging agent), but also to evaluate safety and to predict whether it is likely that the NPs studied ever can receive market approval for clinical use<sup>(3)</sup>.

It is of utmost importance that NPs made of non-endogenous substances are degraded and excreted. The impact of having biodegradable versus non-degradable NPs on toxicity studies, cost of development and the risk/benefit analyses one can expect pharmaceutical companies to perform will be discussed<sup>(3)</sup>.

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## REGULATORY SCIENCE TO ADVANCE PRECISION MEDICINE

**SKOTT STEELE**

Regulatory Science is defined by the U.S. Food and Drug Administration (FDA) as “the science of developing new tools, standards, and approaches to assess the safety, efficacy, quality and performance of FDA regulated products.” Advancements in regulatory science improve the overall translational research process and

enhance the development of safe and effective medical products. Improving regulatory science is particularly important for anticipating the needs of emerging technologies that can advance precision medicine, such as nanotechnology, genomic sequencing, gene editing, data science and digital technologies and bio-manufacturing.

Precision medicine holds significant promise to more accurately tailor treatments to individuals most likely to have a positive response. However, there are a number of regulatory science gaps to ultimately develop and implement personalized medicine technologies. This presentation outlines the initial results of a new Regulatory Science to Advance Precision Medicine Forum, developed to evaluate specific precision medicine technologies and approaches with the goal to: identify regulatory science gaps and specific regulatory considerations, recommend potential approaches to address these regulatory science gaps, and provide suggestions for the development of educational resources. The Forum brings together leaders from academia, industry, FDA, the U.S National Institutes of Health (NIH), the U.S National Institutes of Standards and Technology (NIST), scientific organizations and foundations.

One area for consideration at the fall 2017 Forum was technologies and approaches that integrate and analyze genomic, proteomic, metabolomic, and/or epigenetic data for precision medicine. The second topic focused on considerations for 3D printing of medical products. In addition to identifying several regulatory science priorities related to these areas, educational needs were also considered and will be presented. The 2018 Forum scheduled for later this fall will address digital health, focusing on the emerging role of software (particularly algorithms and data analytics approaches) and sensors in precision medicine. The Forum will examine areas of emerging science, regulatory considerations, ethics and privacy, data storage and use, including use as real world data and patient reported outcomes.

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## NANOENGINEERING GONE VIRAL: PLANT VIRUS CANCER IMMUNOTHERAPEUTICS

**NICOLE STEINMETZ**

Nanoscale engineering is revolutionizing the way we prevent, detect and treat diseases. Viruses are playing a special role in these developments because they can be regarded as prefabricated, naturally occurring nanoparticles. We have developed a library of plant virus-based nanoparticles and through structure-function studies we are beginning to understand how to tailor these materials appropriately for biomedical applications. A particular exciting avenue is the development of plant virus-like nanoparticle platforms for cancer immunotherapy. The idea pursued is an ‘in situ vaccination’ to stimulate local and systemic anti-tumor immune response to treat established disease, and most importantly to induce immune memory to protect patients from outgrowth of metastasis and recurrence of the disease. Our data demonstrate potent efficacy of plant VLP in situ vaccines in mouse models of cancer and canine patients with spontaneous tumors. In this presentation I will discuss the underlying engineering design space for effective VLP in situ vaccination, provide insights into the mechanism triggering innate immunity resulting in adaptive anti-tumor responses, and discuss the next-generation VLP in situ vaccination technologies.

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## NANOPARTICLES AS ANTIVIRALS

**FRANCESCO STELLACCI**, Supramolecular Nanomaterials and Interfaces Laboratory, Constellium Chair, EPFL, Lausanne (CH)

“Nanoparticles and Viruses” abstract “ In this talk novel approaches to develop antiviral drugs will be presented. Specifically, nanoparticles capable of irreversibly damage viruses will be presented. It will be argued that that the mechanism of action is that of exerting a pressure on the viral shell. This property, combined with the fact that the particles have minimal toxicity to mammalian cells, renders the particles a potential candidate to the first virucidal drug to get approval for medical use. In particular the case of herpes simplex 2 will be discussed. Other effects of nanoparticles on virus stability and infectivity will be presented. “

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## ADVANCES IN NANOPARTICLES AND SOLID-LIQUID INTERFACES CHARACTERIZATION

**FRANCESCO STELLACCI**, Institute of Materials, EPFL, Lausanne, Switzerland

A bird eye view of any folded protein shows a complex surface composed of hydrophobic and hydrophilic patches closely packed. To date little is known on the fundamental properties that such packing determines. In this talk I will present my group’s endeavours into the synthesis, characterization, and understanding of a family of nanomaterials (mixed monolayer protected nanoparticles) that possess a surface coexistence of patches of opposite hydrophilicity resembling that present on folded protein. Attention will be placed in describing approach we have developed to characterise such surfaces and possible extension of these approached to characterise similar surfaces. I will show that these materials are ideal model compound to uncover the basic properties that such coexistence determines at the solid liquid interface, and will conclude with example of application of these nanoparticles when used as mimic of biological entities .

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## NANOMEDICINES TARGETING THE MULTIPLE MYELOMA – STROMA ALLIANCE

**GERT STORM**<sup>1,3</sup>, Anil Deshantri<sup>1,2</sup>, Raymond Schifflers<sup>2</sup>, Marcel Fens<sup>1,2</sup>, Tuna Mutis<sup>5</sup>, Richard Groen<sup>5</sup>, Bart Metselaar<sup>3,4</sup> and Gert Storm<sup>1,3</sup>

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Although the prognosis of multiple myeloma (MM) has considerably improved in the past few years due to the introduction of immunomodulatory drugs and proteasome inhibitors, a majority of patients ultimately relapse or experience disease progression even after achieving complete remission. As a prelude to the clinical evaluation of PEG-liposomal dexamethasone phosphate in patients with progressive multiple myeloma, we evaluated the circulation kinetics, biodistribution and efficacy of PEG-liposomal dexamethasone in a mouse model of human multiple myeloma. This human-

mouse hybrid animal model closely resembles the actual disease situation. Multiple myeloma MM.1S cells are grown in an artificially developed human bone scaffold to mimic the bone marrow micro-environment which is of crucial importance in survival of myeloma cells and progression of the disease and therefore more accurately mimics the response to therapy. The outcome of these studies will be presented and discussed.

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## MULTIFUNCTIONAL NANOENGINEERED CAPSULES AND CHAMBERS FOR TIME AND SITE SPECIFIC DRUG DELIVERY, IN VIVO APPLICATION AND USE AS CELL – BASED DELIVERY VEHICLES.

**GLEB SUKHORUKOV**<sup>a,b\*</sup>, Meiyu Gai<sup>a</sup>, Olga Sindeeva<sup>a,b</sup>, Alexander Timin<sup>c</sup>, Albert Muslimov<sup>c</sup>, Maxim Kurochkin<sup>a,b</sup>, Oksana Mayorova<sup>b</sup>, Nikolay Pyataev<sup>b</sup>, Johannes Frueh<sup>c</sup>, Alexey Ermakov<sup>b</sup>, Irina Goryacheva<sup>b</sup>

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b Remote Controlled Theranostic Systems Lab, Saratov State University, Saratov, Russia

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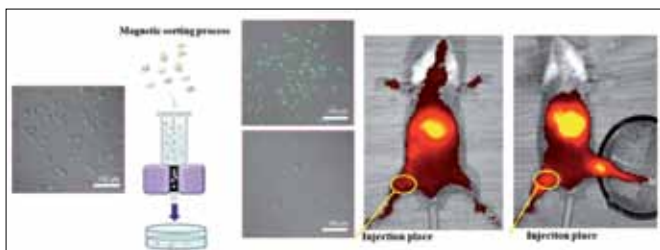
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The presentation highlights recent advances in area of multifunctional nanoengineered systems for drug storage, delivery and release. Particular attention is given to polyelectrolyte multilayer structures on patterned surfaces and *in vivo* application of multilayer microcapsules. Polyelectrolyte nanoengineered capsules had been introduced a decade ago, however, due to complexity and rather tedious procedure for fabrication their potential for multilayer capsules has not been explored in full. Today, the envisaged application of these capsules is their use as cages with cargo to be impregnated into biological cells. Recently various cell lines have been examined to their ability to internalize multilayer capsules. The most promising example is internalization of mesenchymal stem cells (MSC) and their ability to carry polyelectrolyte capsules<sup>[1]</sup>. The data demonstrate the MSCs can impregnate up to 30 capsules and this does not alter their ability to move, migrate toward chemical gradient and also cells viability is not affected much. Also the ability of MSCs to differentiate is not changed if the cells contain microcapsules. Since multilayer capsules can carry multiple functionalities the delivery of genetic materials such as RNA or DNA could be combined with magnetic properties<sup>[2]</sup>. This facilitates magnetic sorting of genetically altered cells which have incorporated capsules with magnetic nanoparticles and hence being altered from these cells which do not have impregnated capsules. Cells containing microcapsules with magnetic nanoparticles are responsive to applied magnetic field and can be easily separated (Figure 1). Results showed that even one few micron sized capsule containing several hundreds of magnetite super paramagnetic iron oxide nanoparticles can serve as magnetic anchor for one cell. Thus, it makes possible to realize the scenario of MSCs delivery with magnetic field in designated location. Conceptually, the own cells might be extracted from organism and impregnated with magnetic capsules which could contain therapeutic cargo and being reinjected. Magnetic field assisted delivery is supposed to accumulate MSCs in particular organs to deploy the cargo with remote physical influence such as ultrasound or electromagnetic irradiation.

*Figure 1. Left - Magnetic sorting of MSCs with impregnated magnetic capsules. Right – injection of fluorescent magnetic capsules (control and with applied magnet – far right). Luminescent spot near magnet indicates accumulation of capsules at leg opposite to that where the capsules were injected. Bright spot indicates liver.*





Although, *in vivo* delivery of magnetized MCSs is not yet shown, the capsules themselves have demonstrated their potential to be addressed *in vivo* with external signaling [3]. Magnetic capsules made of biodegradable polymers such as polypeptides and polysaccharides with magnetite nanoparticles sandwiched between layers have been injected in mice and rats and their distribution was monitored among organs using *in vivo* bioluminescence and MPT imaging and post-mortem histology and capsule marker analysis. Generally, the capsules distribution among the organs changes with time and they get degraded almost during first days and no traces seen after one week. There was no toxicological effect found. Using magnetic field one can accumulate the capsules on particular sites as demonstrated in Fig.1(right) where the capsules labelled with marker were injected in one leg but substantial part of capsules have been collected on another leg where the magnet was applied. Despite if relatively high accumulation in liver the efficiency of magnetic delivery is reasonable as compared with ligand targeting approaches for delivery.

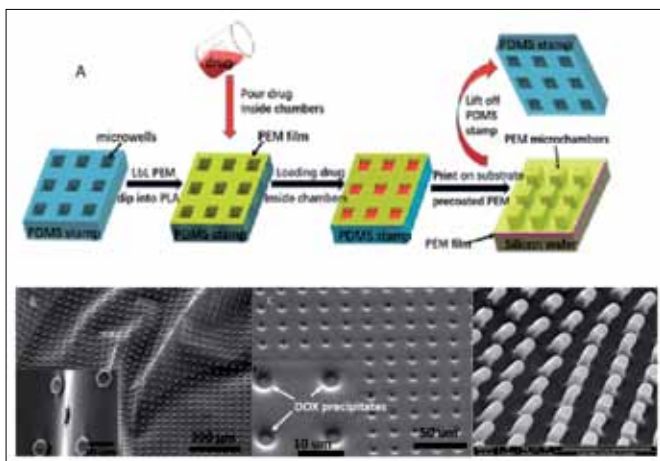


Figure 2. A –Scheeme of fabrication of chambers arrays and loading with water soluble precipitates. B – SEM images of chambers and free standing film of chambers. C- doxycycline precipitates in wells before sealing chambers, D – Nanosized chambers made of PLA

Apart of capsule fabrication what envisages their injection in circulation the polymer multilayer technology can be applied to form so-called chambers arrays which can be filled with drug. These films can be deposited on implants and use for controlled drug supply. The technology is rather simply and can be scaled-up. Polyelectrolyte multilayers can be deposited onto widely used PDMS stamps forming chamber arrays structures enabled to accommodate various biologically active molecules. These chambers can be sealed over with another film made of polymeric layers. The resulted structure represents sealed chambers (Figure 2). Such structures can be pulled off from PDMS stamp to form free standing film with arranged chambers (Fig. 2b). Obviously, the geometry and positions of these chambers can be varied using various design of masks made in silica using conventional lithography approaches. Entrapment of water soluble molecules into sealed chambers is performed by depositing of layer of hydrophobic polymer such as polylactic acid (PLA) or poly(lactic-co-glycolic acid) (PLGA) or polycaprolactone (PCL) as terminating layer in multilayer structure. Thus, while aqueous solution with active substances is evaporating the water drop beads down within chambers and precipitates of water soluble molecules are formed inside wells upon drying as shown on Figure 2c with precipitates of doxycycline. Practically one can get tune the amount of active substances in single chamber by

concentration of the solution. Size of the chamber can go down as much as modern nanoimprinting approach allows, although it has to be reasonable for drug loading and application. Sealing these loaded chambers with another layer of hydrophobic polymer results on formation of air-bubble what can keep water soluble molecules inside the chamber until it released. Release of entrapped precipitates can be done either by gradual degradation of polymer shell or by external stimuli such as light or ultrasound [4]. Carbon nanoparticles are sought as light harvesting centers incorporated in the polymer shells to produce chamber opening. Use of carbon nanoparticles seems to have advances over other light adsorbing nanoparticles due to their biodegradability

In the presentation the perspectives of biomedical applications of these containers based on capsules and use of cell assisted delivery are discussed with particular attention to advantages of remote physical activation and *in vivo* study.

The work was supported by EU project OYSTER 760827-2 and RSF project 16-13-10195.

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## ROADMAP AND STRATEGIES FOR OVERCOMING INFUSION REACTIONS TO NANOMEDICINES

JÁNOS SZEBENI, Nanomedicine Research and Education Center, Dept. Pathophysiology, Semmelweis University, and SeroScience Ltd., Budapest, Hungary

Infusion, or hypersensitivity reactions (HSRs) are complex, immune-mediated side effects that mainly occur within minutes to hours at a therapeutic dose of intravenously administered pharmaceutical products. These products are diverse and include both traditional (e.g., biologics and small molecules) and novel (e.g., nanotechnology-based) pharmaceuticals. Although HSRs are not unique to nanomedicines, they represent a significant hurdle for the translation of nanotechnology-based drug products. This presentation will outline our current understanding of the mechanisms responsible for HSRs, addressing some gaps in knowledge and open questions. The latter will include the values of currently available *in vitro* and *in vivo* assays to predict HSRs, the role of complement activation versus macrophage phagocytosis in the reactions, the use of pigs as a model of complement activation-related HSRs, called CARPA, and the lack of specific methods to prevent these reactions. A roadmap will be proposed to advance basic and clinical research in this field, along with a decision tree for preclinical prediction of the safety of nanomedicines.

## NANOSTRUCTURE AND THE FORMATION MECHANISM OF EXTRACELLULAR VESICLES (EVS) STUDIED BY STATE-OF-THE-ART CRYO-SEM AND CRYO-TEM

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Nanostructural characterization of liquid and semi-liquid systems is performed by imaging techniques, such as electron and light microscopy, and by non-imaging techniques, such as x-ray or neutron small-angle scattering. Electron microscopy provides the needed high-resolution direct-images, but proper specimen preparation

methods are required to make liquid or semi-liquid material systems compatible with the microscopes. Electron microscopy methodologies that are used to directly image nanostructured liquid systems involve ultra-fast cooling of the specimen to cryogenic temperature, and imaging the specimen at the cryogenic state by transmission electron microscopy ('cryo-TEM'), or by scanning electron microscopy ('cryo-SEM'). We have applied these advanced direct imaging techniques for the nanostructural characterization of extracellular vesicles (EVs). We imaged stimulated and unstimulated cells, as they were undergoing shedding, by cryogenic scanning electron microscopy (cryo-SEM), while isolated EVs were imaged by cryogenic transmission electron microscopy (cryo-TEM).

In my talk I will describe in brief the electron microscopy methodologies that are used to directly image nanostructured liquid systems, with emphasis how they are applied in the study of EVs. I will show the effect of stimulation on cells, and how budding of EVs can be captured by cryo-SEM. Finally, I will describe briefly cryo-SEM characterization of EVs released by blood platelets.

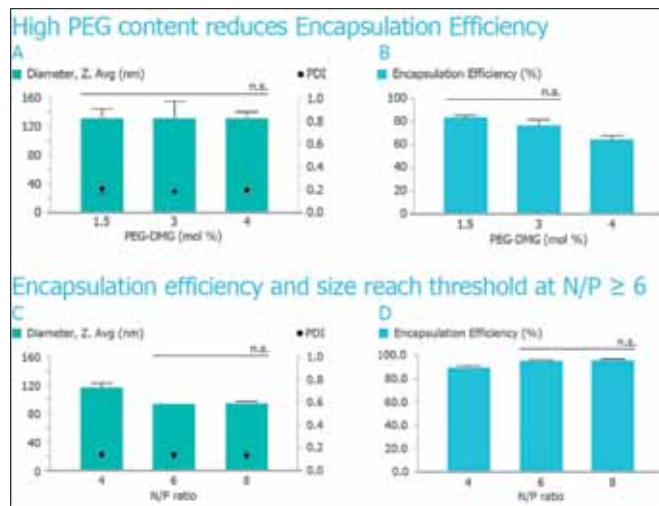
## RAPID DEVELOPMENT AND SCALE-UP OF DRUG DELIVERY NANOPARTICLES USING A MICROFLUIDIC PLATFORM

**R. JAMES TAYLOR**, Chelsea Cayabyab, Andrew Brown, Jagbir Singh, Shyam M. Garg, Anitha Thomas, Keara Marshall, Maria Kerin, Mark Ma, Ben Versteeg, Kevin Ou, Grace Tharmarajah, Richard Broadhead, Shell Ip, Samuel Clarke, Tim J. Leaver, Andre W. Wild, Euan C. Ramsay  
Precision NanoSystems Inc, Vancouver, Canada, V6P 6T7

Microfluidic devices have been broadly used to produce nanoparticles for genetic medicine, vaccines, and drug delivery systems for small molecules, proteins, and peptides. Compared to conventional methods, microfluidic production offers superior control, reproducibility and scalability of the nanoparticle production process that promises to overcome significant challenges in the translation of these therapeutics: Fine control of process parameters afforded by microfluidics, allows optimization of nanoparticle quality and encapsulation efficiency. Automation improves the reproducibility and optimization of formulations. Finally, the continuous nature of the microfluidic process is inherently scalable, allowing optimization at low volumes to conserve precious materials, and seamless scale up of optimized formulations to suit the stage of drug development. Clinical scale manufacturing can be achieved by employing multiple microfluidic mixers performing identical unit operations in parallel. This session will cover examples from literature highlighting how users of this technology are revolutionizing medicines, specifically developing drug delivery systems for small molecules to improve chemotherapy as well as delivering genetic medicines such as for Crispr/Cas9 gene editing. Additionally, original data will be presented to demonstrate how the technology is used to accelerate all stages of nanomedicine development from discovery to manufacturing.

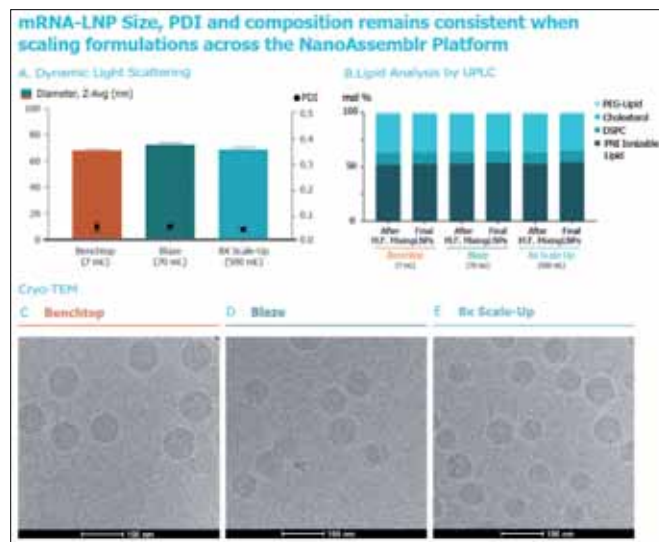
First, microliter formulations encapsulating just tens of micrograms of mRNA per formulation were used to rapidly screen formulations for physico-chemical properties. Microfluidic mixing was ideally suited for rapidly and reproducibly producing of a large number of formulations with minimal consumption of valuable mRNA to better understand the impact of formulation parameters at discovery and early development stages (Figure 1).

*Figure 1: Rapid, reproducible microfluidic production of mRNA-LNPs at microliter volumes and microgram RNA quantities is well suited for screening formulation parameters in early discovery and development.*



Secondly, seamless scaling of mRNA-LNP formulations was demonstrated on three microfluidic systems designed to suit different stages of nanomedicine development. First, formulations were optimized at bench scale, and because of conserved microfluidic geometry, optimized formulations were transferred directly to a large scale pre-clinical system as well as a GMP-ready system employing parallel microfluidic mixers and capable of producing 25L of formulation in ~4h. Size, polydispersity index, lipid composition and particle morphology were all maintained with remarkable fidelity across all scales and systems of production (Figure 2).

*Figure 2: Optimized mRNA-LNP formulations were scaled from bench-scale system using a single microfluidic device to a GMP-ready system with 8 parallel microfluidic devices capable of 25L in ~4h. Particle size, PDI, composition and morphology remained remarkably consistent across all scales.*



In all, microfluidic production offers fast and consistent nanomedicine formulation while maintaining high particle quality at all relevant scales. This approach is well-suited for rapid advancement of nanomedicine development from discovery to the clinic.

## PRECISION CANCER NANOMEDICINE WITH TUMOR HOMING PEPTIDES AND PEPTIDOMIMETICS

**TAMBERT TEESALU**

Our laboratory uses *in vivo* phage display screens to identify homing peptides that bind to specific targets in the vasculature. Corresponding synthetic peptides are used to target drugs, biologicals,

and nanoparticles into tumors to increase their therapeutic index. Our scope includes understanding the molecular interactions involved to drive specificity and activity, focusing on tumor penetrating peptides that trigger bulk extravascular transport in the tumor tissue.

I will discuss our approaches to mapping of vascular heterogeneity using *in vivo* peptide phage display, characterization and validation of candidate vascular homing peptides, and application of the peptides for precision guided delivery of therapeutic and diagnostic nanocarriers. I will provide examples of application of peptides and peptidomimetic compounds with affinity towards malignancy-associated cell surface receptors (such as angiogenic integrins and p32) and extracellular matrix components (such as Tenascin-C and Fibronectin EDB) for precision-guided delivery of nanoparticles loaded with anticancer compounds and contrast agents.

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## THE ROLE OF DIGITAL TRANSFORMATION IN THE QUEST FOR AFFORDABLE INNOVATION

STELIYAN TINKOV

It is a widely known secret that healthcare payers and researching pharmaceutical companies are increasingly involved in an argument about the question “How much should an innovative drug cost?”, and – more importantly – “Why?”.

The R&D costs for developing and launching a new pharmaceutical product to the market currently approximate the low billion range. Like in a vicious circle, progressively more sophisticated research and the pursuit of precision medicine have kept them on the rise by about 3-folds during the past two decades.

Although digital practices in the pharmaceutical sector are still in their infancy, several researching companies have recently made meaningful moves towards digitalization of their drug development. Does the digital transformation hold the promise for more affordable, personalized, and better medicine?

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## PRECISION NANOMEDICINE BY DESIGN: THE FIRST PHASE III FDA CLINICAL APPROVAL FOR DENDRIMER BASED NANOMEDICINE GUIDED BY CNDP ENGINEERING

**DONALD A. TOMALIA**, Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104 USA  
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National Dendrimer & Nanotechnology Center, NanoSynthons LLC, Mt. Pleasant, MI 48858

The use of dendrimers as nanoscale vectors for the delivery of pharmaceuticals, nucleic acids and agricultural chemicals was first proposed over two decades ago.<sup>[1]</sup> In fact, the first documented example of active targeted nanoparticle drug delivery in animal models occurred in 1996 and involved poly(amidoamine) (PAMAM) dendrimers.<sup>[1]</sup> Since that time, interest in these applications has grown exponentially.<sup>[2-3]</sup> A recent survey has revealed >53,000 citations (Google Scholar: 6-15-18), wherein, > 60% of all dendrimer-based patents in the past decade have focused on drug delivery applications<sup>[3-4]</sup> During the past 15 years, extensive dendrimer-based drug/chemical delivery activities have been noted in the USA,<sup>[5]</sup> Europe<sup>[6]</sup> and Australia.<sup>[7]</sup> Although one preclinical failure was noted in the USA,<sup>[5]</sup> a historical milestone was attained in 2017 by Starpharma (Melbourne, Australia) when they announced the first official FDA Phase III clinical approval of their poly(lysine) dendrimers (i.e., VivaGel® BV) for use as topical antimicrobial agents<sup>[7]</sup> against HIV, herpes

simplex virus and bacterial vaginosis (i.e., global market size >1.75 billion /yr.). More recently, Starpharma reported the advancement of other related dendrimer-based injectable candidates, designated as DEP® Drug Delivery, in collaboration with AstraZeneca have progressed to Phase II clinical stages as monoclonal antibody targeted delivery agents for the treatment of major cancers.<sup>[7]</sup> This lecture will review the history of these seminal events, as well as the important role that systematic CNDP engineering<sup>[8-9]</sup> played in dendrimer property optimizations leading to these successes.

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## REGULATORY CONSIDERATIONS FOR DRUG PRODUCTS CONTAINING NANOMATERIALS: US FDA PERSPECTIVE

KATHERINE TYNER

In recent years there has been an increased focus on developing drug products containing nanomaterials. With this increased focus, there has been a corresponding increase in applications for drug products containing nanomaterials to the United States Food and Drug Administration (FDA) submitted for Agency review. With this increase in applications also comes an increase in product complexity. In 2017, the FDA published a draft guidance for industry entitled “Drug Products, Including Biological Products, that Contain Nanomaterials.” This guidance has been developed to provide industry with the Agency’s current thinking for the development of human drug products, including those that are biological products, that contain nanomaterials. This guidance also includes recommendations for applicants and sponsors of investigational, premarket, and postmarket submissions for these products.

CDER does not define or categorize drug products containing nanomaterials and follows the same regulatory processes as for drug products not containing nanomaterials. Adequate characterization of the nanomaterial, understanding of its intended use and application, and how it relates to the product quality, patient safety, and efficacy is considered by CDER to be a suitable framework for evaluating nanomaterials in pharmaceutical products.

Although subject to the same rigorous regulatory standards as any other drug product, unique properties that arise from the small size and large surface area of nanomaterials may lead to additional scientific considerations when following current FDA guidelines and practices. Such considerations may extend to determining the correct analytical techniques to characterize and control the drug product. This presentation will discuss how these considerations are addressed by regulatory science and present current regulatory perspectives for drug products containing nanomaterials.



# SOLID LIPID EMULSIONS FOR DELIVERY OF WATER-INSOLUBLE DRUG CANDIDATES AGAINST LEISHMANIASIS

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Leishmaniasis is a widespread disease, affecting 12 million people around the world with about 1–2 million estimated new cases occurring every year. Chemotherapy is the most efficient strategy in fight against leishmaniasis, however treatment remains insufficient as the current anti-leishmanial agents have several limitations including low efficacy, toxicity, adverse side effects, drug-resistance, length of treatment and cost lines [1]. In leishmanial therapy, pentavalent antimonial drugs stand as the most frequently prescribed treatment. Amphotericin B, pentamidine and miltefosine are second-choice drugs [2]. Although recently introduced, nanotechnology-based anti-leishmanial drug delivery systems find themselves place as a preferred therapy option. AmBisome® and Fungizone®, commercial liposome and micelle formulations of Amphotericin B (AmB), respectively, are currently available for treatment of leishmaniasis. Despite their higher expense, in the western world these formulations already became the first-line treatment as they enable an increase in concentration of the agent at the site of action and an enhancement in the efficacy of the therapy, while the toxic side effects are reduced significantly.

Delivery of the anti-leishmanial drug to the target cell selectively is highly regarded as the prominent strategy. Combined with the discovery of new anti-leishmanial drug candidates with improved efficacy, drug delivery systems are expected to serve the WHO's aim of eliminating Leishmaniasis on many regions around the world by year 2020. However, the need for better anti-leishmanial agents and alternative, low-expense drug delivery systems is still present. Addressing this need, our laboratory focuses on discovery of new anti-leishmaniasis drug formulations to enable alternative, new treatment strategies against leishmaniasis. Due to their advantageous character solid lipid emulsions, or so-named emulsomes, are preferred as the drug delivery system.

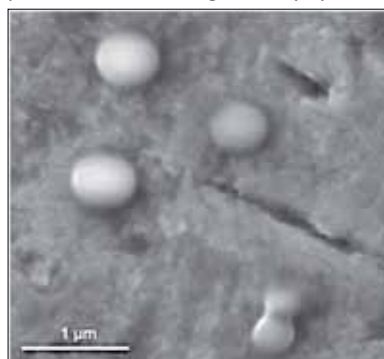


Figure 1. SEM image of BNIP-emulsome formulation. Size of the scale bar corresponds to 1  $\mu$ m.

Emulsomes are lipoidal vesicular systems composed of an internal solid fat core surrounded by phospholipid (PL) multilayers. [3] Emulsomes are preferred mainly because of its four major features.

Firstly, owing a solid lipid core like the solid lipid nanoparticles, emulsome may offer high loading capacities for hydrophobic substances [3,4]. Secondly, composed of only lipids and in the absence of any surfactants, emulsome is highly biocompatible [4]. Thirdly, the solid character of the nanocarrier provides a prolonged drug

release profile, which can be controlled, or tuned, by the selection of the lipid composition as well as by surface modifications [5]. Lastly, but most importantly, the natural feature of lipids allows emulsome to accumulate in the organs of the reticuloendothelial system (RES) instead of the kidney, which will not only largely reduce toxicity, but will also improve the anti-leishmaniasis efficacy of the loaded drug, as parasites are also located in the organs of RES.

Among alternative drug candidates, bisnaphthalimidopropyl (BNIP) derivatives have been recently shown to have promising anti-leishmanial activities, which even surpass the standard Amphotericin B therapy [6,7]. For instance, against *Leishmania infantum* promastigotes Bisnaphthalimidopropyl octane (BNIPdaoct) molecule has shown an activity with IC50 value around  $0.78 \pm 0.049 \mu\text{M}$  (7). BNIPdaoct and other active BNIP derivatives have some drawbacks including low aqueous solubility and toxicity.

Our studies focus on both (i) design of novel BNIP derivatives with improved efficacy and bioavailability, and (ii) development of solid lipid emulsion formulations to deliver the drug specifically to the parasite, and decrease the side effects of the chemotherapy, in particular on macrophages. In our studies we have confirmed the anti-leishmanial activity of BNIPdaoct, obtaining IC50 around  $0.81 \pm 0.08 \mu\text{M}$  against *L. infantum* promastigotes (Table 1). The synthesis of the compound was achieved through a one-step less chemical synthesis achieving higher yield. The encapsulation of the BNIPdaoct into solid lipid emulsions (Figure 1) has resulted in formation of emulsomes with an average size of 361 nm and an average zeta potential  $-10.1 \pm 9.76$  mV. BNIPdaoct concentrations up to 0.49 mg/ml could be achieved. The cell culture studies suggested that the activity of BNIPdaoct has been further enhanced through its emulsome formulation as designated with a declined IC50 value corresponding to  $0.59 \pm 0.02 \mu\text{M}$  (Table 1). The blank emulsome formulation, as control, did not show any cytotoxicity.

Table 1. Cytotoxicity of BNIPdaoct against *Leishmania infantum* promastigotes in its both free and emulsome formulations

	IC <sub>50</sub> ( $\mu\text{M}$ ) 72 h
BNIPDaoct	$0.81 \pm 0.08$
BNIPDaoct-Emulsome	$0.59 \pm 0.02$

Due to on-going patent applications, the data of the novel compounds indicating a high level activity against Leishmaniasis parasites could not be included within this abstract; however, planned to be presented in the conference talk. To sum up, the findings indicate that BNIP derivatives are promising anti-leishmaniasis drug candidates and their emulsome formulations developed in our laboratory may further serve their potential applications in Leishmaniasis.

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## NANOMEDICINE SAFETY TESTING IN A PORCINE MODEL: QUESTIONS REGARDING VALIDITY FOR PRECLINICAL TESTING AND MECHANISM

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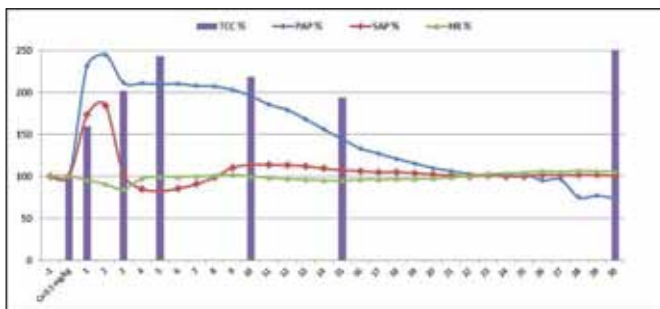
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One of the major risks of administering nanoparticle-based complex medicines (nanomedicines) that they can evoke acute anaphylactic reactions manifested in cardiovascular and laboratory abnormalities. The symptoms may only be light, e.g., flushing or shortness of breath, but in some patients it can be life threatening. Typically these reactions arise at the first application, without earlier sensitization. This makes these responses especially dangerous requiring strictly prescribed premedication. To improve the safe application of the otherwise very promising nanomedicines and find solution to predict the reactions it is necessary to understand the basic causes of these reactions and for that valid *in vitro* and *in vivo* tests, models are required.

The usual laboratory animals, mice and rats are insensitive to i.v. nanomedicine applications. Majority of nanomedicines evoked symptoms in sensitive human beings (2-7%) are reproduced in pigs. The most characteristic and reproducible signs are the pulmonary arterial pressure (PAP), the systemic arterial pressure (SAP) and the blood cell changes, especially the WBC and PLT changes. Biochemical alterations include complement (C) activation, liberation of thromboxane (TxA<sub>2</sub>). The early activation of the innate immune system and liberation of C3a and C5a anaphylatoxins as well as the direct and indirect activation of pulmonary intravascular macrophages (PIM cells) is crucial in the cardiopulmonary distress that are all present in the pig model. The basic difference is that the reaction is much more frequent in pigs (~ 99%). It means that the pig model is not reflecting the reactions in the human population but it is the model of the hypersensitive man. The pig model is sensitive and reproducible with low false negative results. In spite of these features recently some authors questioned the validity of the pig model. In our view a sensitive safety model has to represent the most sensitive patient population not the average, warning to the possible adverse reactions even if it can be occurring relatively rarely.



The other difficulty with the model is that pigs are not a usual laboratory species for preclinical toxicity studies. A recent debate arose about the involvement of the innate immune system, the role of complement activation in the hypersensitivity reaction evoked by complex nanomedicines. The involvement of C activation in these reactions is plausible, since virus-size foreign particles entering to the vascular system will activate this ancient defense mechanism, and the causal role was suggested already in 1980s. The C-Activation Related Pseudoallergy - CARPA - hypothesis was suggested according experimental and clinical observations but until recently only limited direct evidence could be presented about the involvement of the C system in the reaction due to the

lack of specific tests for pig C split-products or end-products. The lecture will provide direct evidence about the involvement of C system in the pseudoallergic reaction in the pig model, measured by pig-specific sC5b-9 ELISA. The serum sC5b-9 or terminal complement complex (TCC) serum concentration following Caelyx bolus injection closely correlated with the cardiovascular reactions (see figure), the PAP, SAP changes. In another series following polystyrene beads signs of complement activation were also detected. Regarding the involvement of PIM cells, which in pigs are abundant in the pulmonary vascular bed - but generally not in humans - due to their location are the first that can be activated following i.v. nanocomplex injection. In a recent study it was suggested that this activation is occurring only by phagocytosis without C activation. We recommended the "double-hit" theory that take in account the direct phagocytic activity of the macrophages, but at the same time due to their anaphylatoxin receptors recognize also the C3a and C5a split factors that presents the foreign material to the macrophages that release numerous vasoactive components orchestrating the HSR.

To better understand the exact mechanism of the complex immune responses to different nano-medicines and nano-diagnostics further assay development and fine-tuning of the model is needed.

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## SINGLE PARTICLE MEASUREMENT OF NUMBER, SIZE AND CHARGE IS REQUIRED FOR CONFIDENCE IN NANOMEDICINE ENGINEERING AND DEVELOPMENT

HANS VAN DER VOORN

In nanomedicine development size matters, the number of particles matters and the particle surface properties matter. As nanomedicine products evolve out of university research groups to detailed development, clinical testing and clinical use, the quality and disci-

pline around measurement and analysis becomes increasingly critical. Measurement technology has developed to meet this need and extremely accurate measurements can now be done quickly and easily. One important aspect is the required resolution of measurement. It has been clear for some time that DLS for instance, lacks the resolution necessary to make nanoparticle measurements in the real world. It is expected that regulators will soon require a minimum level of resolution for nanoparticle measurement, which will likely rule out DLS data. That would be a welcome and profound change for the nanomedicine field.

Repeatability of the measurements by third parties is also an essential component. That logically means that the measurements need to provide the real dimensions and not artificial numbers. Precise single particle measurements are required for this to occur. TRPS offers the level of detail and certainty that the medical world requires. The importance of showing size and number is now well understood. Current developments are aimed at increasing the accuracy and resolution of TRPS single particle charge measurements by a factor of 10.

The extracellular vesicle research community is beginning to make advances using extracellular vesicles, mainly exosomes as a new class of therapeutics. There a number of different configurations including gene therapy, drug delivery and stem cell derived exosomes. These are biological particles, typically more heterogeneous than synthetic particles and typically more adapted for survival in the body than synthetics such as liposomes. The need for precise isolation and measurement of these nano-bio structures provides some additional challenges which TRPS is being developed to meet.

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## SUCCESSFUL DESIGN OF LIPOSOMAL DRUG CARRIERS BY UNDERSTANDING THE INFLUENCE OF THE (PHOSPHO)LIPID COMPOSITION ON THE DRUG RELEASE RATE

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Successful targeting with nanomedicines is dependent on the properties of the used nanomaterials. Liposomes, comprising phospholipids as nanomaterials, are in that respect ideal nano-carriers. Besides their lack of toxicity and biodegradability, the (phospho) lipid composition of the liposomal membrane can be used to design and manipulate the degree of association of drugs with liposomes and the drug release kinetics. In this seminar, these possibilities like e.g. pH sensitive release<sup>(1)</sup> and temperature sensitive release<sup>(2)</sup> are shortly reviewed. As a further example latest findings and general principles on especially the release kinetics of lipophilic drugs from liposomes containing either unsaturated or saturated phospholipids<sup>(3,4)</sup>, in comparison to the release kinetics of hydrophilic drugs are presented. The presented variety of properties of liposomes underscore once again that phospholipids are nanomaterials which deserve utmost attention in the design of superior nano-carriers.

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## BACTERIAL NANOGLUES TARGETING WOUND SITES TO THERANOSTIC APPLICATIONS TO READ OUT TISSUE FIBER TENSIONS IN HEALTH AND DISEASE

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Major transformation of extracellular matrix (ECM) composition, architecture and of its mechanical properties accompany inflammatory disease and cancer progression, yet little is known how this affects ECM imposed outside-in cell signaling. As the ECM acts as reservoir for a plethora of growth factors and cytokines some of which are regulated by changing ECM fiber strain, gaining knowledge on the mechanical strain of ECM protein fibers in health and disease is urgently needed. Nothing is known so far about ECM fiber tension in healthy organs and during pathological transformations due to the previous lack of probes that can sense the tension of ECM fibers. We will discuss novel insights that we obtained using our recently developed mechanosensitive peptide probe (FnBPA5) which specifically binds to relaxed, but not to stretched fibronectin (Fn) fibers<sup>(1)</sup>, and how they can be utilized for theranostic applications.

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## IS NANOMATERIALS-INDUCED PROTEIN CITRULLINATION A COMMON PATHOGENETIC LINK TO DIVERSE AUTOIMMUNE CONDITIONS?

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Increasing and continuous human environmental and occupational exposure to nanomaterials can have a profound impact on the cells, tissues and whole living organism functioning. Several studies demonstrated that nanoparticles of different physical and chemical nature interacting with human cells can induce a specific post-translational modification of proteins called citrullination, a result of enzymatic conversion of amino acid arginine into citrulline possessing a neutral charge thereby leading to impaired protein assembly and causing the production of antibodies to citrullinated proteins (ACP), which may constitute an early step in autoimmune disease development. For instance, the presence of ACP is well known as clinical marker for rheumatoid arthritis (RA). The production of ACP is almost 100% specific for patients with RA, indicating an important role of protein citrullination in the pathogenesis of this disease. Certain genetic predisposition has been established, such as HLA-DRB1, but studies on external triggers that ultimately lead to RA clinical manifestation have been inconclusive. Furthermore, despite possible links between RA and common environmentally imposed factors including cigarette smoke, diesel and petrol exhaust air pollution and occupational exposure to nanomaterials such as silicon dioxide particles, the exact cellular events involved remain unclear. In this talk, we provide a brief analysis of most recent research findings focused on understanding of the dynamics and extent of citrullination phenomenon in human cells following the exposure to nanomaterials with diverse physicochemical properties, aiming to provide a wider insight into crucial pathogenetic mechanisms of the onset and progression of autoimmune diseases.

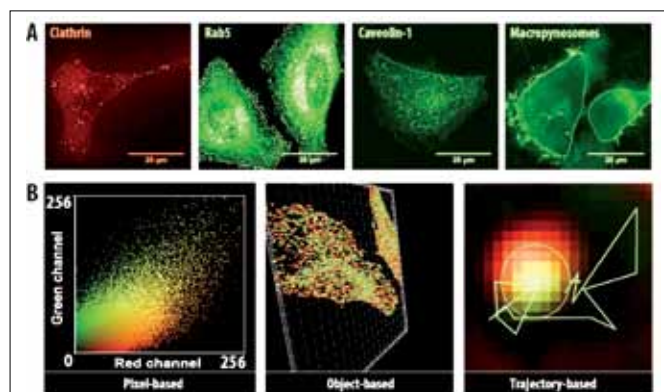


## A LIVE-CELL FLUORESCENCE IMAGING PLATFORM TO CHARACTERIZE INTERNALIZATION OF NANO-MEDICINES BY HUMAN CELLS

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Nanosized materials have received increased interest as potential drug carriers for targeted therapies in nanomedicine<sup>1,2</sup>. They have unique capacity of interacting with the cellular machinery by entering the cells using active cellular pathways, as opposed to many common small drugs that simply diffuse and partition inside cells according to their solubility<sup>3</sup>. For these reasons, nanomaterials have an incredible potential in nanomedicine to deliver compounds to diseases in a targeted way maximizing clinical benefits while limiting unwanted side effects<sup>4</sup>. However, despite of significant efforts and great expectations, only few nanomedicines so far have advanced to clinical practice. Furthermore, a recent review has highlighted that on average only 0.7% of the administered nanomedicine dose is delivered to solid tumors<sup>5</sup>. This suggests that the efficient delivery of drugs to the targeted disease is still a major challenge. A growing realization is that in many cases still little is known about the mechanisms governing the interactions of nanomaterials with cells. A better understanding of the mechanisms by which cells process nanosized materials could allow us to optimize nanomedicine design and achieve higher targeting efficiency with minimal side effects.

Within this context, characterizing the pathways involved in mediating the uptake and intracellular trafficking of nanosized materials is a first crucial step<sup>6</sup>. To this aim, in this work, we employ fluorescence microscopy methods to quantitatively measure nanoparticle (NP) uptake into cells. Live-cell imaging is used to monitor dynamics and kinetics of key endocytic vehicles and intracellular organelles in cells expressing a representative panel of fluorescent proteins involved in different endocytic pathways (Fig. 1A). We then use this panel of labeled cells to determine the involvement of the different targets in NP uptake.



*Fig.1. Live-cell imaging as a tool to characterize uptake and trafficking of nanomedicines into cells. (A) A panel of cells expressing fluorescently labeled key components of the major endocytic pathways used to determine their involvement in the uptake of nanosized materials. From left to right: clathrin, Rab<sup>5</sup>, caveolin-1 and macropinosomes. (B) Different methods are combined and compared for robust co-localization analysis. From left to right: scatterplot of pixel intensities of two fluorescent probes from pixel-based analysis; segmented cellular compartments for object-based analysis in 3D; trajectory-based analysis.*

In order to do this, we carry out robust co-localization analysis using three different complementary methods: a pixel-based approach, an object-based approach, as well as trajectory-based approach, where we follow the movements of the NPs and cellular structures in the live cells (Fig. 1B).

While in other methods of studying cellular uptake of NPs, such as the use of chemical inhibitors that block major endocytic pathways, our method of live-cell imaging allows us to characterize these pathways in unperturbed cells. In this work, the results obtained using the panel of labeled cells are presented and the different methods of analysis are compared. Overall, characterization of the pathways that cells use to internalize and process NPs will greatly enhance targeted drug delivery and accelerate the clinical translation of nanomedicine.

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## PRODUCT AND PROCESS OPTIMIZATION TO IMPROVE LIPOSOMES/LNP CHARACTERISTICS

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Over the past few years liposomal drug preparations have been increasingly used in clinical trials. Until now, several liposomal products have reached the market, many other formulations are still in the pipeline. For all these products, simple, economic and GMP-conform production techniques and facilities are necessary. Here, several points to consider already at the stage of process and product transfer to the CMO should be listed. Product development at early stage should implement the use of high quality raw materials, robust and stable product and process conditions and robust analytical methods. The whole system should be implemented in a robust QA system. Furthermore, the production system should be designed to allow scalable and sterile manufacturing. In addition, it should meet several requirements, such as simplicity, robustness and easy handling of sterilisation procedures. Furthermore, the modified ethanol injection technique itself is distinguished by mild preparation conditions and the avoidance of hazardous solvents and forces, which may disrupt lipids as well as entrapped substances.

Data will be presented, which describe impact of process conditions on the generated particle size and homogeneity. A few examples of drug products and related processes will be shown, where special focus will be set on influencing particle size and size distribution by varying the process parameters of the Polymun liposome technology.

## A NANOSTRATEGY FOR DYNAMIC MONITORING PATIENT THERAPY RESPONSE

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Targeted therapies have been proved to be effective in cancer treatment, but they are limited by the rapid acquisition of drug resistance (within months). A rapid and non-invasive method to monitor drug response would promote precision medicine and improve treatment efficacy. Circulating tumour cell (CTC) analysis has emerged as a useful monitoring tool, but its routine usage is restricted by either limited multiplexing capability or sensitivity. Here we demonstrate the use of antibody-conjugated and Raman reporter-coated gold nanoparticles for simultaneous labelling and monitoring of multiple CTC surface markers (named as 'cell signature'), without the need for isolating individual CTCs.<sup>1</sup> We observe cell heterogeneity and phenotypic changes of melanoma cell lines during molecular targeted treatment. Furthermore, we follow the CTC phenotypic changes of 10 stage-IV melanoma patients receiving immunological or molecular targeted therapies (Figure 1). Our technique maps the phenotypic evolution of patient CTCs sensitively and rapidly and shows drug-resistant clones having different CTC signatures of potential clinical value. We believe our proposed method is of general interest in the CTC relevant research and translation fields.

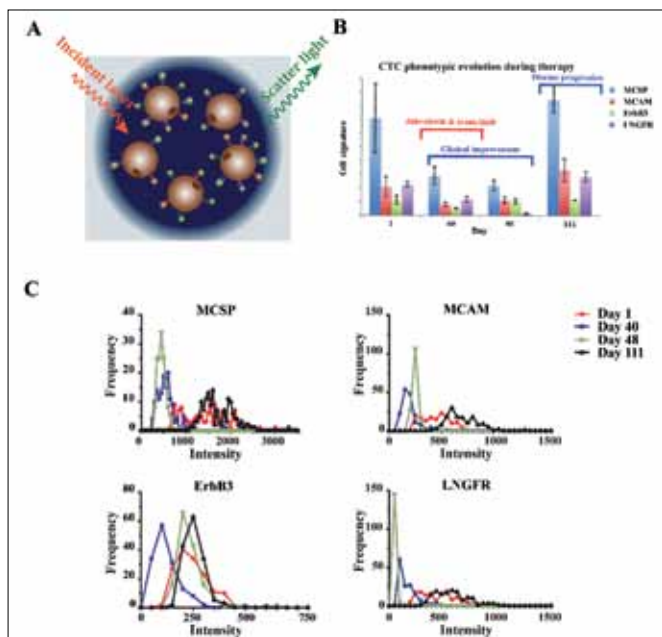


Figure 1. (A) CTC phenotype characterisation achieved by simultaneous labelling with antibody-conjugated and Raman reporter-coated gold nanoparticles; (B) Phenotypic evolution and (C) receptor expression diversity of CTCs from patient #1 before, during and after treatment.

## TARGETED DELIVERY OF BERBERINE VIA LIPOSOMES PROTECTS HEART FUNCTION IN MYOCARDIAL INFARCTION

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Heart failure following myocardial infarction is one of the most devastating diseases with a mortality rate worse than that of many cancers. Pharmacological treatment of myocardial infarction is often limited by insufficient drug concentrations in the damaged myocardium while also systemic side effects can occur. Our group recently found that liposomes could selectively accumulate in the ischemic myocardium likely via the enhanced permeability of locally inflamed blood vessels. Inflammation plays a critical role in the pathological progress from myocardial infarction to heart failure. Berberine, a natural compound extracted from barberry that has a long history in Chinese traditional medicine, is known for its anti-inflammatory and anti-oxidant activities. Long-term administration of high doses of berberine has shown beneficial effects in both experimental animal models and clinical trials in patients with congestive heart failure.

However, its poor solubility and the short half-life in the circulation have impeded its clinical use. We aimed to address these problems by encapsulation of berberine into long-circulating liposomes to achieve targeted delivery of this compound to the damaged myocardium. Intriguingly, most of the liposomes were taken up by infiltrating macrophages and retained in the infarct zone up to one week in a murine myocardial infarction model. Our drug delivery approach significantly improved the therapeutic availability of berberine and therefore enhanced its treatment efficacy in salvaging heart function. These findings point to the possibility of a better clinical usage of berberine in myocardial infarction and highlight the potential of long circulating liposomes for improving pharmacological treatment in cardiovascular disease.

## IMAGING CARBON NANOTUBES: JOURNEY FROM BASIC UNDERSTANDING OF THE CARRIER BIODISTRIBUTION TO ALZHEIMER'S DISEASE MONITORING

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Over the past few years, we have designed and developed various functionalised carbon nanotubes CNTs (f-CNTs) as nanocarriers to deliver a wide range of therapeutics and imaging probes (e.g. drug, genes and radionuclides) for theranostics applications, particularly for cancer and brain delivery (Figure 1). Application of f-CNTs for imaging are discussed here.

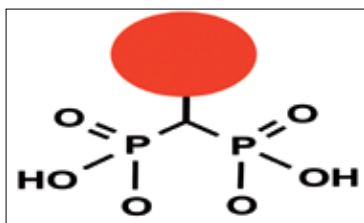
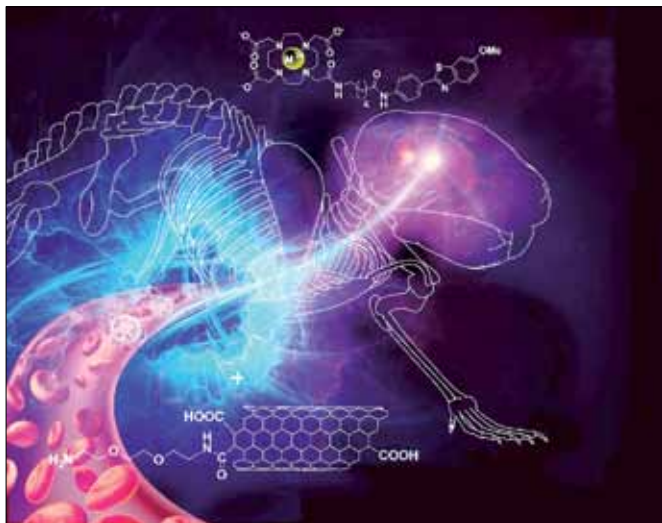


Figure 1: Functionalized carbon nanotubes incorporating diverse biological molecules for various biomedical theranostic applications.

Figure 2: Illustration of brain-targeting theranostic f-CNTs.



Hybrid superparamagnetic iron oxide nanoparticles (SPION) coated f-CNT were prepared for dual nuclear SPECT/CT and MR imaging [1].

We have recently developed with collaborators a novel approach to construct therapeutically active carbon nanocapsules by neutron irradiation of CNTs filled with non-radioactive samarium-152 [2]. The resulting  $^{153}\text{Sm}$ -filled CNTs yielded a single nanocapsule, capable of SPECT/CT imaging and radiotherapy against a lung cancer model *in vivo*.

The interaction of f-CNT with tissues, including brain, using state-of-art spectroscopic imaging techniques such as multi-photon luminescence imaging, fluorescence lifetime microscopy and Raman spectroscopy could also be assessed thanks to their unique optical properties [3-4]. Taking advantages of the f-CNTs' ability to cross the blood-brain barrier (BBB) after intravenous injection, conjugation to a brain targeting peptide, angiopep-2, further increased the brain uptake to reach glioma [5]. A recent follow up study utilised f-CNTs as carriers to deliver amyloid-targeting ligand, Pittsburgh Compound B, in mouse brain which have a potential in theranostic applications for Alzheimer's disease (Figure 2) [6].

In the course of our past few years' endeavor, we have demonstrated that f-CNTs are versatile nanocarriers to deliver a wide arrange of theranostics. The brain targeting feature potentiate their enormous applications in brain disease. Imaging f-CNTs provides a useful tool to understand their biological behaviors which will benefit the design of their applications towards brain disease monitoring and treatments.

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## CLINICAL NEEDS FOR NANOMEDICINE: CHARACTERIZATION AS KEY FOR PRODUCT DEVELOPMENT

FRANK F. WEICHOLD

For the past decade, FDA's public health mission has included measures to foster innovation and bring better therapies to patients sooner, as reflected in the Critical Path Initiative. Technology advances and globalization of development efforts (as well as markets) require collaborative and consortial approaches in translational science, with focus on regulatory science, where FDA scientists are engaged in problem solutions of public interest.

Medical product development costs continue to rise and clinical trial innovation is the lead strategy to curb resource requirements in the most-expensive late phase, whereby drug development tools, such as biomarker, *in vitro* diagnostics, early clinical endpoints and surrogate outcome measures, use of patient information and preference, opportunity for modeling and simulation, new statistical approaches and adaptive trial design, as well as the consideration for *in-silico* clinical trial components represent key elements to make favorable changes.

These proposed advances critically depend on the medical data quality, as well as interoperability and access for all stakeholders. The US Office of the National Coordinator (ONC) has long recognized the importance of interoperability of health data for the public and the expansion of "real world evidence", historically limited to coding and billing information for insurers, to include patient-level electronic health records (eHR) are logical and necessary steps to health data liberation and exchange that will benefit the entire ecosystem, and most-importantly patients and consumers. Despite the mandatory validation of clinical lab tests, the concept of semantic interoperable and standardized laboratory test derived data is not sufficiently implemented yet. This results in problems with source data traceability, data transformation errors and re-coding into different LOINC (logical observation identifiers codes and names) in regulatory submissions. The advantages and opportunities derived from LOINC when developed into a progressive consensus "learning" standard will be discussed, and alliance is sought in this presentation.

## NANOMATERIAL – CELL INTERACTION: A MECHANISTIC PERSPECTIVE

PETER WICK, Head Particles-Biology Interactions, Empa Swiss Laboratories for Materials Science and Technology, St. Gallen, Switzerland

Due to their interesting physicochemical properties, gold nanoparticles (Au-NPs) are the focus of increasing attention in the field of biomedicine and are under consideration for use in drug delivery, bioimaging, radiosensitizers or as nano-based vaccines. Safe and sustainable development of gold enabled technologies or products requires close attention to undesired side effects on human health. Therefore, safety assessment is an integral part of the innovation



process. If we acknowledge that nano-sized materials provide new and useful properties, we must also accept that such 'new' material could pose unanticipated risk. Over the years, some common pathways of cellular or organ damage such as inflammation, oxidative stress, or necrosis were identified as particle related, but it is nevertheless needed to understand how these pathways were triggered and what the underlying structure-activity relationship is.

Here, we examined the influence of three surface modified 3–4 nm Au-NPs on human A549 cells, according to the reactive oxygen species (ROS) paradigm. After 24 h of Au-NP treatment, nanoparticles were taken up by cells as agglomerates; however, no influence on cell viability or inflammation was detected. No increase in ROS production was observed by H<sub>2</sub>-DCF assay; however, intracellular glutathione levels reduced over time, indicating oxidative stress. All three types of Au-NPs induced DNA damage, as detected by alkaline comet assay. The strongest genotoxic effect was observed for positively charged Au-NP I. Further analysis of Au-NP I by neutral comet assay, fluorimetric detection of alkaline DNA unwinding assay, and  $\gamma$ H2AX staining, revealed that the induced DNA lesions were predominantly at alkali-labile sites. As highly controlled repair mechanisms have evolved to remove a wide range of DNA lesions with great efficiency, it is important to focus on both acute cyto- and genotoxicity, alongside post-treatment effects and DNA repair. We demonstrate that Au-NP-induced DNA damage is largely repaired over time, indicating that the observed damage is of transient nature.

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## NANOPARTICLE AND BIOMOLECULE DETECTION IN TISSUE SECTIONS: A COMBINED-METHODS APPROACH FROM THE NANOBIODETECT PROJECT

MARTIN WIEMANN<sup>1</sup>, Uwe Karst<sup>2</sup>, Birgit Hagenhoff<sup>3</sup>, and Members of the NanoBioDetect Project

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Detection of Nanoparticles in tissues usually requires high resolution techniques such as electron microscopy. However, the technique is laborious, hardly quantitative and provides no overview of complete organ sections. During the last decade, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has evolved in such a way that single nanoparticles (NP) can be detected and, under ideal condition, also be quantified in complete tissue sections. Also, time-of-flight secondary ion mass spectrometry (ToF-SIMS) can be used to analyse nanoparticles together with biomolecules at sub-micrometer resolution in tissues, at least in pre-selected regions of interest. Finally, matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) can identify complete ionized molecules, although at lower resolution. Considering sample fixation and preparation requirements, all these techniques may be combined with light microscopic techniques such as conventional histological staining, immunocytochemistry, darkfield microscopy (DFM) or hyperspectral imaging (HSI).

The presentation will give an overview about recent results and technical achievements of the BMBF-sponsored project NanoBioDetect (2014-2017). Emphasis will be laid upon the detection of gold and silver NP in blood and various organs, such as lung, lymph nodes, spleen, kidney, and intestine. While showing the transfer of single NP from the lung via blood to certain cells within remote organs, suitable combinations of various detection techniques will be demonstrated. ToF-SIMS studies on silica NP will show that metal oxide nanoparticles can be detected together with mass signals of phospholipids at high resolution. Finally, a MALDI-MS study will show that silica NP change the complex local lipid composition of the lung and that these effects were abrogated by phosphonate

coating of silica particles. In all these approaches, light microscopic imaging techniques were necessary to deliver complementary information.

In summary, as no single technique is able to provide all the information needed to create a complete set of information, necessary e.g. for safety or regulatory issues of a medical NP, major benefit comes from the experienced combination of different techniques. In ideal cases, quantitative ion distribution images at cellular resolution allow for an estimation of the NP concentration in single cells in situ. This is an exciting possibility to elaborate further on a meaningful *in vitro* dosimetry.

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## A VIRTUAL PATIENT MODEL FOR PERSONALIZED DRUG RESPONSE PREDICTIONS IN ONCOLOGY

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Despite major breakthroughs in cancer research, cancer remains a major cause of death worldwide associated with enormous socio-economic costs. Identification of successful therapies for complex diseases such as cancer is difficult and large fractions of patients remain refractory to treatment with even the most effective targeted drugs. Technological developments and scientific progress over the last years have provided us with new and powerful tools for extracting information from massive amounts of data. In particular, molecular profiling tools such as genomics, proteomics and transcriptomics are generating expansive datasets on cancer cells. They demonstrate in great detail how heterogeneous cancer cells are. Due to the enormous complexity of changes and genetic heterogeneity typically observed in cancer, mathematical models are particularly relevant to understand the underlying complexity of the disease.

Novel powerful computational modelling tools and methods allow us, today, to build computational models of cellular and molecular systems. Such models can help to understand the underlying disease mechanisms and to identify potential therapeutic strategies. Building on current knowledge, we have developed a large mathematical model of cancer-related signalling pathways called ModCellTM using the modelling software PyBioS. ModCellTM integrates information on functional consequences of genetic variants and mechanistic drug action. However, parameterization of such detailed mathematical models is hampered by limited availability of data on reaction kinetics and their respective kinetic parameters. To overcome this bottleneck, we have developed a Monte Carlo strategy that allows us to study the qualitative effects of mutations and drugs on the molecular network. The integration of large-scale molecular data such as genomics, transcriptomics and (phospho-)proteomics can help to refine such complex models.

The multi-national EU Horizon2020 project CanPathPro ([www.can-pathpro.eu](http://www.can-pathpro.eu))

pathpro.eu) is addressing the challenge of translating highly complex and heterogeneous omics data into predictive modelling of cancer signalling. Using ModCellTM as a basis, the project is building and validating a combined experimental and systems biology platform, to be utilised in testing cancer signalling hypotheses, in biomedical research. Parameter estimation methods are applied using comprehensive omics data from mouse models of breast and lung cancer. The temporal changes occurring during cancer development are followed, integrating quantitative histopathological tumor characterization, genome and transcriptome data as well as (phospho-)proteome data obtained by SWATH technology. The *in silico* model is optimised in an iterative fashion, via perturbation experiments of tumor-derived cell lines and organoids, thus enabling the validation of pathway and parameter information. Overall, CanPathPro takes a unique approach, combining classic cancer research with omics data and systems biology tools, to develop and validate a combined systems and experimental biology platform, for generating and testing cancer signalling hypotheses.

The tools, algorithms and models that have been integrated into the ModCellTM modelling platform establish the foundation for the application of systems biology strategies in medical and pharmaceutical research and, based on omics data, enable the development of personalised medicine. Further extensions of the current model to allow for simulation of multi-cellular systems, tissues and organs will pave the way for a comprehensive virtual patient model to achieve a true personalisation of therapy in the future.

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## ENGINEERED EXTRACELLULAR VESICLES FOR DRUG DELIVERY TO THE BRAIN

**JOY WOLFRAM**, Assistant Professor, Mayo Clinic, Jacksonville, Florida (USA); Assistant Affiliate Member, Houston Methodist Research Institute, Houston, Texas (USA).

The blood-brain barrier represents a major transport obstacle for delivery of drugs to the central nervous system. Accordingly, low drug accumulation in the brain is one of the main reasons for the poor prognosis of primary and metastatic brain cancers. The goal of this project is to exploit intrinsic mechanisms of entry into the central nervous system by utilizing engineered brain-tropic extracellular vesicles (EVs) for drug delivery. Specifically, EVs derived from cancer cell lines that have undergone *in vivo* selection rounds to enrich for membrane and secretory components that facilitate BBB crossing have been utilized. Tangential flow filtration was employed as an effective method for EV isolation and comparative analysis of the physicochemical properties of EVs from originating and metastatic breast cancer cells was performed. The functional properties of EVs were also evaluated using *in vitro* blood-brain barrier models. Studies are underway to determine the biodistribution and therapeutic efficacy of drug-loaded EVs in murine models. Moreover, potential toxic and tumor-promoting properties of cancer cell-derived EVs will also be assessed.

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## OPTICAL TRACKING OF SIRNA ENCAPSULATED EXOSOMES IN CANCER MODELS IN VITRO AND IN VIVO

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Extracellular vesicles, in particular exosomes (30-150 nm), are a type of extracellular vesicles currently investigated as potential diagnostic and therapeutic tools for cancer. RNA interference (RNAi) has high specificity, minor side effects and ease of synthesis. However, it has delivery limitations due to its large molecular weight and polyanionic nature. Exosome has a hollow structure with ability to encapsulate siRNA and protect it from nucleases and promotes better cellular uptake. In this study, pancreatic cancer cell-derived exosomes were engineered to deliver siRNA to their parent cells in preparation for the development of homologous cancer therapeutics.

## METHODS

Exosomes were isolated from PANC-1 (human pancreatic adenocarcinoma) cultured in CELLLine AD1000 bioreactor flasks by ultracentrifugation onto sucrose cushion. PANC-1 cell-derived exosomes (PANC-1 Exo) were characterised for size, yield, purity, exosomal markers and morphology using Nanoparticle Tracking Analysis (NTA), microBCA protein measurements, flow cytometry, transmission electron microscopies (TEM) and scanning electron microscopies (SEM). PANC-1 Exo were chemically conjugated with optical probes, Alexa488 dye for *in vitro* study and Cy7.5 dye for *in vivo* study. Labelling efficiency was assessed using ultrafiltration and gel filtration. Atto655-siRNANeg was loaded into PANC-1 Exo via electroporation. The *in vitro* uptake of PANC-1 Exo and encapsulated Atto-siRNA in PANC-1 cells was visually tracked by confocal microscopy, and quantitatively analysed by flow cytometry and imaging flow cytometry. Encapsulation efficiency of siRNA in exosomes was determined using size-exclusion chromatography (Sepharose CL-2B column). Subcutaneous human pancreatic cancer-bearing immunodeficient (NSG) mice were injected intravenously with Cy7.5<sup>\*</sup>-labelled PANC-1 Exo (3x10<sup>11</sup> particles) followed by whole body IVIS imaging and *ex vivo* tumour uptake analysis at 1, 4 and 24 h post-injection.

## RESULTS AND DISCUSSION

PANC-1 Exo yield (from 15 ml culture supernatant) and size were found to be  $7.34 \pm 0.33 \times 10^{12}$  particle/mL and  $101.0 \pm 5.4$  nm in diameter, respectively. The PANC-1 exosomes were of high purity, with particle to protein ratio  $> 2 \times 10^{10}$  particles/ $\mu$ g protein. The isolated exosomes were positive for CD81+, CD63+ and CD9+ when analysed by flow cytometry. No significant differences in size, concentration and zeta potential were observed between naïve and freshly labelled exosomes. Fluorescence intensity, size, zeta potential and concentration remained unchanged when the exosomes were stored at 4°C for up to 3 months, suggesting good physical and chemical stability of the modified fluorescent exosomes. PANC-1 Exo showed time- and dose-dependent uptake by PANC-1 cells *in vitro*. Confocal images 24 h post-treatment showed prominent cellular internalisation of exosomes in acidic organelles such as lysosomes and late endosomes. The encapsulation efficiency of siRNA in exosomes was 10-20%. About 17.7% of the PANC-1 cells showed positive siRNA uptake 24 h post-incubation with siRNA-loaded exosomes at 37°C. Furthermore, the PANC-1 Exo can be efficiently tracked *in vivo* by fluorescence labelling with a near infrared dye (Cy7.5 in this study), followed by IVIS imaging. The biodistribution profile of Cy7.5-exosomes was significantly different from that of dye only control pancreatic cancer model, confirming successful and stable labelling. The PANC-1 Exo *in vivo* accumulation is prominently seen in liver and spleen. Tumour to liver uptake ratio was found to be 0.037. Interestingly, the PANC-1 Exo was found excellent brain tumour accumulation (Brain to liver uptake ratio of 0.053) in mouse glioma (GL261 cells)-bearing NSG mice.

## CONCLUSIONS AND ONGOING WORK

High purity exosomes can be isolated by ultracentrifugation onto sucrose cushion. Chemical modification provides a simple, safe, and stable exosomal surface fluorescence labelling approach without engineering parent cells. Fluorescently engineered exosomes can be stably and effectively tracked *in vitro* and *in vivo*. Current results suggest a good potential of siRNA-loaded PANC-1 exosomes in targeting pancreatic cancer cells as well as great potential to

cross the blood–brain barrier (BBB) for targeting glioma cells, paving the way for promising cancer therapies. Ongoing work focuses on investigating the *in vivo* knockdown efficacy of functional siRNA carrying PANC-1 Exo in both pancreatic cancer model and brain tumour model.

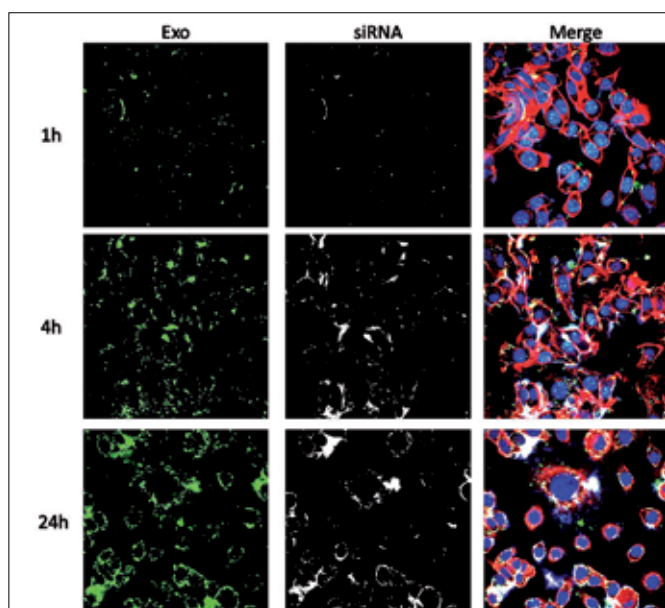
## ACKNOWLEDGEMENT

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*Figure 1. Dual tracking of PANC-1 exosome and siRNA taken up by PANC-1 cells, obtained by Confocal microscopy. The exosomes were fluorescently labelled with Alexa Fluor® 488 dye (green). The siRNA was labelled with Atto-655 (white). Nuclei were counter-stained with DAPI (blue). The F-actin were stained with Alexa Fluor® 568 phalloidin (red). Exosomes were internalised in PANC-1 cells. Atto655-siRNA was encapsulated in PANC-1 exosome via electroporation. As seen from images, the green signal and white signal overlaid each other, both of which were taken up by recipient cells PANC-1.*



## NEXT GENERATION SPECIES SPECIFIC ECO-FRIENDLY ANTIBIOTICS AND THOUGHTS ABOUT ORIGIN OF LIFE

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Resistance to antibiotics is a severe problem in contemporary medicine. Many antibiotics inhibit protein biosynthesis by hampering the ribosome function. Structures of bacterial ribosomes in complex with these antibiotics illuminated common pathways of antibiotics inhibitory action, but not the species-specific diversity in infectious-diseases susceptibility. However, although pathogenicity is not linked to ribosomes, recent structures of ribosome from a multi-resistant pathogenic bacterium revealed novel structural motifs, essential to cellular protein biosynthesis but are not located in the primary ribosomal active sites, hence no mechanism for

mutations leading to resistance of these sites is currently known. These findings led to the design of antibiotics that can be optimized in terms of their chemical properties, toxicity, penetration, species-specificity, thus preserving the microbiome and bio degradability, thus reducing the ecological hazards caused by the spread of the current antibiotics' non-degradable metabolites.

The internal ribosome active site, an RNA pocket where peptide bonds are being formed, is highly conserve, hence it seems to be a remnant of a prebiotic bonding apparatus, called by us the “proto ribosome”. The catalytic capabilities of a lab construct proto ribosome were recently proven by the formation of peptide bonds. This breakthrough result led to the suggestion that the ribosomes, the genetic code and the proteins evolved and optimized together.

## BREAKING NEW GROUND FOR THE FUTURE OF CANCER IMMUNOTHERAPY: TUMOR-ASSOCIATED MACROPHAGES, NANOMEDICINE AND IMAGING

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The success of any given cancer immunotherapy relies on several key factors. In particular, success hinges on the ability to stimulate the immune system in a controlled and precise fashion, select the best treatment options and appropriate therapeutic agents, and use highly effective tools to accurately and efficiently assess the outcome of the immunotherapeutic intervention. Furthermore, a deep understanding and effective utilization of tumor associated macrophages (TAMs), nanomedicine, and biomedical imaging must be harmonized to improve treatment efficacy. Additionally, a keen appreciation of the dynamic interplay that occurs between immune cells and the tumor microenvironment (TME) is also essential. New advances towards the modulation of the immune TME have led to many novel translational research approaches focusing on the targeting of TAMs, enhanced drug and nucleic acid delivery, and the development of theranostic probes and nanoparticles for clinical trials. In this presentation, I discuss the key cogitations that influence TME, TAM modulations and immunotherapy in solid tumors as well as the methods and resources of tracking the tumor response. The vast array of current nanomedicine technologies can be readily modified to modulate immune function, target specific cell types, deliver therapeutic payloads, and be monitored using several different imaging modalities. This allows for the development of more effective treatments, which can be specifically designed for particular types of cancer or on an individual basis. Our current capacities have allowed for greater use of theranostic probes and multimodal imaging strategies which have led to better image contrast, real-time imaging capabilities leveraging targeting moieties, tracer kinetics, and enabling more detailed response profiles at the cellular and molecular levels. These novel capabilities along with new discoveries in cancer biology should drive innovation for improved biomarkers for efficient and individualized cancer therapy.

## IS THERE MORE THAN TUMOR TARGETING? NANOPARTICLES AND THE IMMUNE SYSTEM

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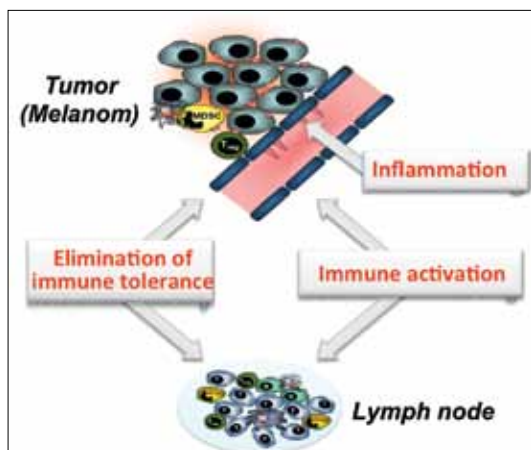
During the last three decades, nanomedicines have provided novel opportunities to improve the delivery of chemotherapeutics in cancer therapy effectively. Now, many principles learnt therefrom have the potential to be transferred to other diseases. This presentation will, on the one hand, critically reflect the limitations of nanomedicines in tumor therapy and, on the other hand, provide



alternative examples of nanomedicinal applications focusing on immunotherapy ([www.crc1066.uni-mainz.de](http://www.crc1066.uni-mainz.de))<sup>[1]</sup>.

Nanotherapeutics have so far mostly concentrated on tumor treatment. Their success is thereby related to the EPR-effect, which leads to an accumulation of nano-sized objects in tissue with a leaky vasculature like e.g. the tumor. Despite the advantages of nanoparticulate carriers, they still have to combat the general problem of any anticancer drug. Tackling the tumor directly requires depletion of literally all malignant cells, as even a small number of surviving tumor cells can induce recurrence. Therefore, any traditional anticancer treatment needs to reach billions of cells localized in each part of the tumor. In this context, alternative concepts (including small molecular or nanoparticulate drugs) become very attractive, e.g. the involvement of the immune system. Based on the potential of the immune system to identify malignant cells first concepts for tumor-immune therapy evolved already at the end of the 19th century [2]. Moreover, in tumor-immunotherapy the activation of several thousands of leukocytes can be sufficient to induce potent responses. Concerning delivery of drugs to modify the immune system this appears to be more realistic task<sup>[3,4]</sup>.

In recent years, it has been recognized that systemic non-specific immune activation, e.g. by administering immune checkpoint inhibitors, can reactivate natural immunity that apparently disappeared during cancer progression. Checkpoint inhibitors can result in impressive and durable remissions in some cancer entities. However, such therapies are often accompanied by significant immune-related side effects and clinical benefit is only found in a fraction of patients, leaving significant medical need to develop more cancer-specific immunotherapies that are both highly effective and have fewer side effects. This might be achievable either by eliminating the immune tolerance selectively in the region of the tumor tissue or by selective activation of the immune system (Fig. 1, [www.crc1066.uni-mainz.de](http://www.crc1066.uni-mainz.de))<sup>[1]</sup>. Specific immune activation against tumor relies thereby on a successful vaccination, meaning that a tumor-associated antigen needs to be presented to the immune system, likely antigen presenting cells, and combined with an immune activator to induce antigen processing and effective induction of T cell mediated immunity<sup>[5]</sup>.



In this respect, nanoparticles are especially attractive since they can combine required functionalities onto one single particle being in the same size range (nm) as viruses and fragments of bacteria, for which our immune system evolved naturally.

In addition, nanoparticles can effectively protect and shield biologically sensitive molecules (e.g. antigen-bearing peptides, DNA or mRNA encoding for antigens or immunostimulatory oligonucleotides) from degradation, increase their half-life in the body and minimize their systemic toxicity<sup>[6-7]</sup>. This enables novel therapeutic approaches, which provide -besides local applications- the possibility for systemic activation of the immune system. In this context, a lot can be learned from efforts to optimize nanoparticles for tumor treatment<sup>[9]</sup>, which requires also prolonged circulation and specific delivery. Interestingly, some sorts-of "passive" accumulation of

nanoparticles also exists within the immune system. It has been recently demonstrated that after intradermal injection nanoparticles accumulate in the lymph nodes<sup>[10]</sup>, whenever the interstitial flow is able to transport them through the lymphatic capillaries into the draining lymph nodes, where these particles can be taken up by antigen-presenting immune cells without further targeting ligands<sup>[11,12]</sup>. Secondly, depending on size and surface charge, nanoparticles may selectively accumulate in certain organs such as spleen or liver, which can be beneficial for systemic immune therapies since these organs have microenvironments that favor the generation of immunity or tolerance, respectively.

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## DELIVERY OF SIRNA IN VIVO TO THE LIVER USING REDOX SENSITIVE AND IONIZABLE LIPIDS

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## FROM PAPILLOMAVIRUS DISCOVERY TO PREVENTIVE ANTI-CANCER VACCINES

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The identification of the high risk human papillomavirus types 16 and 18 in 1983/1984 and the establishment of their causal role in cervical cancer paved the way for the development of specific preventive vaccines. Unfortunately, it took approximately 22 years before these vaccines have been licensed. Presently, close to ~20% of the global incidence of human cancers have been linked to predominantly viral infections. Epidemiological pattern, however, also point to a possible role of additional infections in the etiology of other common human cancers<sup>(1)</sup>. This accounts in particular for colon, breast, and prostate cancers. We specifically suspected infectious nutritional factors, originating from the consumption of Eurasian cattle meat and dairy products<sup>(2, 3)</sup>. We isolated close to 100 novel single-stranded circular DNA genomes (1200-3000 nucleotides) from bovine sera and dairy products, many of them closely related to bacterial plasmids.

Upon transfection into specific human cells, those tested were transcriptionally active, replicated independently their DNA and produced specific proteins. We tested their possible role by analyzing in particular sections of colon cancer biopsies for the presence of their DNA and for the expression of a group-specific replication protein (rep) protein.

The results permit the development of a novel model for the involvement of specific infectious factors in the etiology and pathogenesis of colon cancers, potentially also applicable to other common human cancers. They raise the hope for additional preventive anti-cancer vaccines.

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A lipid nanoparticle (LNP) composed of a series of SS-cleavable and pH-activated lipid-like materials (COATSOME® SS-E-P4C2) was developed as a platform of a gene delivery system. The LNP shows good stability in the serum. The tertiary amine and disulfide bonding of COATSOME® SS-E-P4C2 lead to destabilization of the endosomal membrane and for intracellular collapse. We would like to present our development of a hepatocyte-targeting siRNA carrier by the molecular tuning of the hydrophobic scaffold, and tertiary amine structures. The gene knockdown activity against a hepatocyte-specific marker (factor VII; FVII) was improved when a more fat-soluble vitamin (vitamin E) was employed as a hydrophobic scaffold. Moreover, to allow the tertiary amines to accept protons by sensing a slight change in endosomal acidification, its structural flexibility was minimized by fixing it in a piperidine structure, and the distance between the surface of the particle to the tertiary amine was increased. As a result, the  $pK_a$  value was increased to the approximately 6.18 depending on its distance, while the  $pK_a$  reached plateau when the tertiary amine was linked by an excess number of linear carbon chains. The pH-dependent membrane destabilization activity, as assessed by a hemolysis assay, was increased in parallel with the  $pK_a$  value. Moreover, the gene knockdown activity was improved in parallel with hemolytic activity. Finally, further optimization of the lipid/siRNA ratio, and the use of chemically (2'-fluoro) modified siRNA synergistically improved the gene knockdown efficacy to an effective dose (ED50) of 35 mg/g. The developed COATSOME® SS-E-P4C2 represents a promising platform for use as a hepatocyte-targeting siRNA carrier.

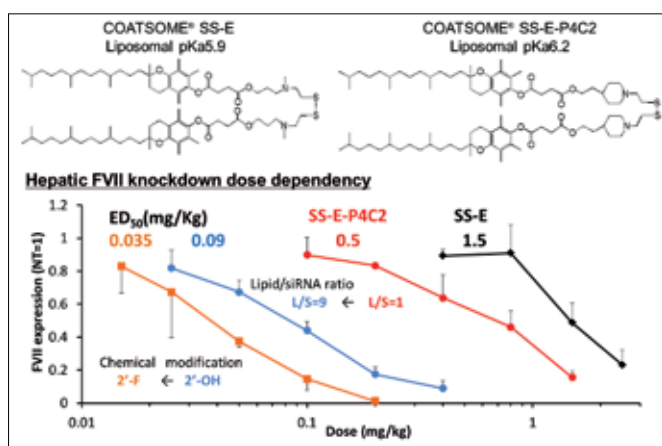


Figure 1. In-vivo gene knockdown of Factor VII protein at 24 h after the administration of the LNPs at indicated siRNA dose. The LNP prepared with an original lipid/siRNA ratio ( $L/S = 1$ ) and the optimized one ( $L/S = 9$ ) that encapsulated native siRNA (2'-OH), or the chemically modified (2'-F) one was i.v. injected at the indicated dose. Data points are presented as the mean  $\pm$  SD of experiments ( $n = 3-4$ ), normalized to those obtained with nontreated group. Statistical analyses were performed by oneway ANOVA followed by the Student's t-test vs nontreated group in each day (\*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ).

### REFERENCES

- H. Akita et. al., Molecular tuning of a Vitamin E-scaffold pH-Sensitive and Reductive Cleavable Lipid-like Material for Accelerated in Vivo Hepatic siRNA Delivery *ACS Biomater. Sci. Eng.*, 1 (9), p. 834-844 (2015).
- K. Tange et. al., Cationic lipid having improved intracellular kinetics, Patent No. US 9,708,628 B2 (2017).

CLINAM

European Foundation for Clinical Nanomedicine

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# CURRICULA VITAE POSTERS





## Mostak Ahmed

Centre for Personalized Nanomedicine, Australian Institute for Bioengineering and Nanotechnology (AIBN), University of Queensland (UQ), Australia  
Phone: +61 414 633 302  
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### PERSONAL HISTORY

Since 2015: PhD student (AIBN, UQ)

2013-2014: Scientific Officer (Bangladesh Council of Scientific and Industrial Research)

2011-2013: Research Fellow (Bangladesh Atomic Energy Commission)

2003-2010: BSc Hons and MSc student (Shahjalal University of Science and Technology, Bangladesh)

### RESEARCH INTERESTS

Protein phosphorylation, Protein folding and misfolding, Electrochemistry, Biosensors

### INTERNATIONAL PEER-REVIEWED PUBLICATIONS

- Alain Wuethrich, Abu Ali Ibn Sina, Mostak Ahmed, Ting-Yun Lin, Laura G Carrascosa, and Matt Trau, Interfacial Nano-Mixing in a Miniaturised Platform Enables Signal Enhancement and in Situ Detection of Cancer Biomarkers, *Nanoscale* (2018).
- Mostak Ahmed, Laura G Carrascosa, Abu Ali Ibn Sina, Ester Marina Zarate, Darren Korbie, Ke-lin Ru, Muhammad JA Shiddiky, Paul Mainwaring, and Matt Trau, Detection of Aberrant Protein Phosphorylation in Cancer Using Direct Gold-Protein Affinity Interactions, *Biosensors and Bioelectronics*, 91 (2017) 8-14.
- Mostak Ahmed, Mohammad B. Khan, Mubarak A. Khan, Syed S. Alam, Md. Abdul Halim, M. Anwar H. Khan, Characterization of polyethyleneterephthalate (PET) based proton exchange membranes prepared by UV radiation induced graft copolymerization of Styrene, *Journal of Power Sources*, 196 (2011) 614-619.
- Mohammad B. Khan, Mostak Ahmed, Mubarak A. Khan, Syed S. Alam, M. Anwar H. Khan, Preparation of polyethyleneterephthalate based proton exchange membranes by UV-induced graft copolymerization of allylmethacrylate for application in fuel cells, *Journal of Applied Polymer Science*, 121 (2011) 2442-2449.
- Mostak Ahmed, Mubarak A. Khan, M. R. Miah, S. A. Monim, M. Anwar H. Khan, Gamma radiation-induced graft copolymerization of styrene onto polyethyleneterephthalate films: Application in fuel cell technology as a proton exchange membrane, *Journal of Macromolecular Science, Part A: Pure and Applied Chemistry*, 48 (2011) 927-936.



## María Julia Altube

María Julia Altube graduated with a bachelor's degree in Biotechnology (2012) and received a PhD in the field of Nanomedicine (2018) at the National University of Quilmes, Buenos Aires, Argentina. She is currently doing a postdoctoral research at the Nanomedicine Research and Development Center (NaRDC). Her work focuses on the design of inhalable nanovesicles

for the administration of antibiotics to lungs infected with bacterial biofilm. She is working as Assistant Professor of Nanobiotechnology at the National University of Quilmes. She was awarded research grants from National Scientific and Technical Research Council (CONICET) for her doctorate and postdoctoral studies. Her works have been published in *Nanomedicine*, *Journal of Material Chemistry B*, *International Journal of Nanomedicine and Colloids and Surfaces B: Biointerfaces*.



## Leila Arabi

Pharm.D. Ph.D.  
Assistant Professor of Pharmaceutical Nanotechnology  
Department of Pharmaceutical Nanotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran  
Email: arabil@mums.ac.ir, leilaa.arabi@gmail.com

### EDUCATION:

- Doctor of Philosophy (Ph.D.) in Pharmaceutical Nanotechnology Oct 2009- Jan 2016, School of Pharmacy, Mashhad University of Medical Sciences, Iran
- Doctor of Pharmacy (Pharm.D.), School of Pharmacy, Mashhad University of Medical Sciences, Iran., Oct 2003- Sep 2009
- Research Scientist, Molecular Pathology department of the Institute for Pathology, Aug 2012-Aug 2013, University Hospital Basel-Switzerland.

### RESEARCH INTERESTS AND EXPERIENCE:

Targeted Drug Delivery, Liposomes, Cancer Stem Cells, Targeting Tumor Cells and Tumor Microenvironment, No-Viral Vectors for Gene Delivery, Non-coding RNAs, Cancer Immunotherapy

### SELECTED PUBLICATIONS:

- Arabi L, Badiee A, Mosaffa F, Jaafari MR. "Targeting CD44 expressing cancer cells with anti-CD44 monoclonal antibody improves cellular uptake and antitumor efficacy of liposomal doxorubicin", *Journal of Controlled Release*. 2015 Dec 28;220(Pt A):275-86
- Quagliata L, Matter M, Piscuoglio S, Arabi L, Ruiz C, Procino A, Kovac M, Moretti F, Makowska Z, Tornillo L, Diederich S, Heim M.H, Cillo C and Terracciano L, "IncRNA HOTTIP/HOXA13 expression is associated with disease progression and predicts clinical outcome in HCC patients", *Hepatology*. 2014 Mar;59(3):911-23
- Ruiz C, Kustermann S, Pietilae E, Vlajnic T, Baschiera B, Arabi L, Lorber T, Oeggerli M, Savic S, Obermann E, Dirnhofer S, Singer T, Rothschild S, Zippelius A, Roth A, Bubendorf L. "Culture and drug profiling of patient derived malignant pleural effusions for personalized cancer medicine", *PLoS One*. 2016 Aug 22;11(8)
- Ruiz C, Vlajnic T, Arabi L, Wyttenbach S, Baschiera B, Rothschild S, Kustermann S, Zippelius A, Roth A, Bubendorf L, "Abstract 4681: The use of patient derived malignant effusions as *in vitro* models for a personalized healthcare-approach in anticancer therapy", *Cancer Research, AACR Annual Meeting*; April 5-9, 2014; San Diego. Published Oct 2014.
- Arabi L, Gsponer J, Smida J, Nathrath M, Jundt G, Ruiz C, Quagliata L and Baumhoer D, "Upregulation of the miR-17-92 cluster and its two paralogs in osteosarcoma – reasons and consequences", *Genes Cancer*. 2014 Apr;5(1-2):56-63.



## Nerea Argarate

R&D Project Manager  
International projects and Initiatives programme, Biomedical Research Networking Center (CIBER), Bioengineering, Biomaterials & Nanomedicine Research Area (CIBER-BBN)  
Tel.: +34 628 915836  
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She obtained her PhD in Pharmacy (University of Basque Country, 2010). Her research is focused on the materials for Health. She has participated in autonomic, national and European projects.

From 2002 to 2008 she worked between the laboratories of AZTI (Basque Country) and CSIC (group of Nanobiotechnology, Nb4D group, IQAC-CSIC). She worked in the research of novel diagnostic methods for Food Safety and Quality within the context of Spanish and European Consortium Projects. Moreover, in 2008, she was enrolled as Researcher of the CIBER-BBN at INASMET (since 2011 Tecnalia Research and Innovation, Basque Country) where she worked on new challenging research objectives related to Biomaterials for health. Her main areas of interest were nanodiagnostic methods based on easy-to-handle Point-of-Care (PoC) devices and therapeutic nanoconjugates for controlled release systems. Since 2016, she works for the Management Area of CIBER-BBN as a Project Manager.



## Nayab Batool

Nayab has studied Veterinary Medicine at the Bahauddin Zakariya University Multan, Pakistan. She received a doctoral degree from Bahauddin Zakariya University in 2014 and was granted affiliation with Pakistan Veterinary Medical Council (PVMC). She was then enrolled in M. Phil for further studies in Institute of Microbiology, University of Agriculture Faisalabad on scholarship.

During her M. Phil, she qualified for the faculty development project Scholarship announced by University of Agriculture under the slot of nanotechnology and was recruited as lecturer at Institute of Microbiology, University of Agriculture Faisalabad and joined Sungkyunkwan University, South Korea in 2016 for commencement of her PhD. She is now working as a graduate student in Structural Biology lab in School of Medicine and working to develop nanomedicines.

She has submitted abstract related to her research titled "Nanobiorobotics based antibacterial approach for targeted Eradication of Multiple Drug resistant Staphylococcus aureus (2017) in The 2nd Annual symposium of Federation of Korean Societies for Molecular and Biomedical Sciences as first author and G-quadruplex mediated regulation of virulence and antibiotic resistance in UPEC through a novel host offensive-defense transcription factor (2017) in the same conference as 3rd author.

She has also participated in an article "Identification of 2',4'-Dihydroxychalcone as an Antivirulence Agent Targeting HlyU, a master Virulence Regulator in Vibrio vulnificus" (accepted, 2018) as second author in "Molecules



## Brahamdutt Arya

Brahamdutt Arya presently pursuing his doctoral degree under the supervision of Dr Surinder P. Singh at CSIR-National Physical Laboratory, New Delhi, India. He received his Bachelor degree in Chemistry honors From M. D. U. Rohtak (2012) and Master degree with specialization in Organic Chemistry from University of Delhi, New Delhi, India (2014). He has submitted

a project report on the topic "Suzuki- Miyura coupling reactions- A Novel approach towards natural products synthesis" as a part of his master's degree. He has also worked as assistant professor of Chemistry at Pt. N. R. S. Government College, Rohtak and taught the stereochemistry, basic organic chemistry, organometallic chemistry and bioorganic chemistry subjects (2014-15). Presently, he is working on the synthesis of Gold and Graphene oxide based multifunctional nanomaterials and exploring their applications in bioimaging, drug delivery, and photothermal therapy with ultimate objective of targeted and personalized nanomedicine. He is presently developing the Indian National Standard for the Gold Nanoparticles. As a research fellow, he is currently working on Graphene oxide-Chloroquine nanoconjugate and studied its antiproliferative mechanism on A549 lung cancer cell lines, along with target application of this novel nanoconjugate.



## Roberta Bartucci

Roberta Bartucci was born on August 19th 1989 in Rogliano, Cosenza, Italy. After finishing the classical gymnasium "B. Telesio" in Cosenza with the highest grade (100/100), she was admitted to the quota course of degree, Chemical and Pharmaceutical Technology, at the University of Calabria. In 2015 she graduated (with 105/110). During her studies Roberta

showed her interest for research therefore, after a 6 months of internship in Pharmacy, she decided to perform 1 year and half of research in the Department of Biochemistry and Cell Biology, Pharmacy faculty, University of Calabria (IT). Moreover during her last year of study, Roberta went to the Utrecht University to do a second internship thanks to the scholarship she won. She performed a 3 months research project in the Department of Biochemistry and Cell Biology, at the Faculty of Veterinary Medicine. Immediately after her graduation Roberta obtained the license as pharmacist. Meanwhile she started her PhD at the Groningen Research Institute of Pharmacy (GRIP), University of Groningen, The Netherlands. Currently she is still PhD student candidate at her 3rd year.



## Idan Biran

Idan Biran received his bachelor's degree (magna cum laude) from the Chemical Engineering Department, Technion – Israel Institute of Technology. During his last year of studies, he focused on research in the field of nanoparticle tracking analysis of cancerous cell lines.

After a short period of time in the material science industry, he returned to study towards an M.Sc. degree at the Nanotechnology and Nanoscience program at the Technion in Professor Yeshayahu Talmon's research group. Biran's research is in the field of Immuno-gold labeling in the liquid phase of extracellular vesicles for cryogenic-temperature electron microscopy.

### PRESENTATIONS AND PUBLICATIONS

- Koifman, N., Biran, I., Aharon, A., Brenner B., and Talmon, Y., A Direct-Imaging Cryo-EM Study of Shedding Extracellular Vesicles from Leukemic Monocytes, J Structural Bio. (Online).
- Ben-Barak, I., Biran, I., and Talmon, Y., Formation of High Internal-Phase Microemulsions with a Purely Ionic Surfactant System (in preparation).
- Biran, I. (May 2017), Nanoparticle tracking analysis (NTA) method and its application in the study of extracellular vesicles (EVs). Presented at the Czech - Israeli seminar - Modern electronic methods of study of polymers – Electron Paramagnetic Resonance (EPR) and Cryo-Electron Transmission Microscopy (Cryo-TEM).



## Nils Bohmer

Dr. Nils Bohmer studied biology at the Freie Universität Berlin, Germany, and finished his diploma in 2008 with a focus on biochemistry/molecular biology, microbiology and genetics. He performed his work for the diploma thesis with the title "Functional Characterization of a 3-D Liver Cell Culture System for Pharmaceutical Testing *in vitro*" at the Charité University Hospital

in the group of Prof. Gerlach (Department Experimental Surgery), where he built and developed novel 3D bioreactors for the early screening of drug candidates.

For his PhD he moved to the group of Dr. Andreas Jordan at the MagForce AG / Charité University hospital (Center for Tumor Medicine, Department of Radiation Oncology and Radiotherapy), where he investigated the uptake mechanisms of nanoparticles by tumour cells. At the MagForce AG he was also involved in several R&D activities regarding targeting of nanoparticles and drug delivery systems. He graduated in 2014 with the thesis "Studies on the Internalization of Iron Oxide Nanoparticles by Tumor Cells and the Underlying Uptake Mechanisms *in vitro*".

After a short period as a postdoc at the Institute of Veterinary Biochemistry at the Freie Universität Berlin he moved for a postdoc position to the Empa in St. Gallen, Switzerland, in the group of Dr. Peter Wick (Particles-Biology Interactions Lab) where he focussed for two years on several aspects of nanoparticle-cell interactions (magnetic blood cleaning, reliability of *in vitro* tests etc.).

Currently Dr. Nils Bohmer is a scientist and project manager at DECHEMA (German Association for Chemical Engineering and Biotechnology) where he, amongst others, represents the German Platform Nanomedicine. He is also a member of the core team of the project DaNa2.0.

### SELECTED PUBLICATIONS:

- H.F. Krug, N. Bohmer, D. Kühnel, C. Marquardt, K. Nau and C. Steinbach: "The DaNa2.0 Knowledge Base Nanomaterials—An Important Measure Accompanying Nanomaterials Development", *Nanomaterials* 2018, 8, 204; DOI:10.3390/nano8040204 [www.mdpi.com/journal/nanomaterials](http://www.mdpi.com/journal/nanomaterials)
- N. Bohmer, N. Demarmels, E. Tsolaki, L. Gerken, K. Keevend, S. Bertazzo, M. Lattuada and I.K. Herrmann: "Removal of Cells from Body Fluids by Magnetic Separation in Batch and Continuous Mode: Influence of Bead Size, Concentration, and Contact Time", *ACS Appl. Mater. Interfaces* 9, 35, 29571-29579
- M. Kucki, L. Diener, N. Bohmer, C. Hirsch, H.F. Krug, V. Palermo and P. Wick: "Uptake of label-free graphene oxide by Caco-2 cells is dependent on the cell differentiation status", *Journal of Nanobiotechnology* 2017 15:46, <https://doi.org/10.1186/s12951-017-0280-7>
- M. Lattuada, Q. Ren, F. Zuber, M. Galli, N. Bohmer, M. T. Matter, A. Wichser, S. Bertazzo, G. B. Pier and I. K. Herrmann: "Theranostic body fluid cleansing: rationally designed magnetic particles enable capturing and detection of bacterial pathogens", *J. Mater. Chem. B*, 2016, 4, 7080-7086, DOI: 10.1039/C6TB01272H
- N. Bohmer, A. Jordan: "Caveolin-1 and CDC42 mediated Endocytosis of silica-coated Iron-Oxide Nanoparticles in HeLa Cells" (*Beilstein J. Nanotechnol.* 2015, 6, 167-176, DOI: 10.3762/bjnano.6.16, PMID: 25671161)



## Hassan Chaddad

Hassan Chaddad has studied pharmacy at the Lebanese International University, Lebanon. He had his master degree in Pharmacology at the Université saint Esprit de KASLIK and currently he is doing his PhD in pharmacology and molecular and cellular biology speciality cancerology at the university of Strasbourg, France.



## Marketa Charousova

Marketa Charousova got her master degree in Animal biotechnology at Mendel University in Brno, Czech Republic. She continued on her bachelor degree and in her diploma thesis examined influence of EPA and DHA on pro-inflammatory and anti-inflammatory genes in live animals. Now she is in 1 year of her Ph.D. study at Department of Chemistry and Biochemistry, Mendel University in Brno, Czech Republic. Her main focus is on elimination of negative side-effects of cancer treatment and combination of chemotherapeutic drug treatment with gene silencing.

She also actively attended conferences in Czech republic (MendelNet2017 in Brno, DPPEO 2017 in Olomouc and BOD 2018 in Brno). In December she became one of the winners in competition for starting Ph.D. students "Brno Ph.D. Talent 2018".

She also actively attended conferences in Czech republic (MendelNet2017 in Brno, DPPEO 2017 in Olomouc and BOD 2018 in Brno). In December she became one of the winners in competition for starting Ph.D. students "Brno Ph.D. Talent 2018".



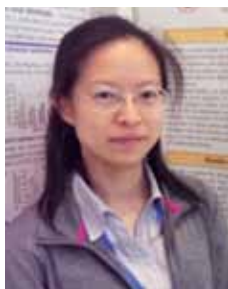
## Calvin Cheung Chun Long

I am Calvin Cheung Chun Long from Hong Kong. I received my BEng. in Mechanical Engineering in 2015 at The University of Hong Kong, Hong Kong. I then submitted my Master dissertation entitled "Coaxial Electrospray of Polymer Microspheres for Delivering Drug-carrying Theranostics", completing my MSc in Mechanical Engineering with distinction in 2016 at The

University of Hong Kong, Hong Kong

I have always been eager to figure out the working principle behind everything. Applying my knowledge to improve the quality of life and to assist other people are very rewarding, which leads me to the path of engineering. Despite studying in mechanical engineering, my interest in physics, biology and chemistry have never faded. With the opportunity to come across biomedical-related courses and projects during my studies, I have developed a passion and wished to contribute my multidisciplinary knowledge in this field. Since 2017, I started my PhD in Pharmacy with the supervision of Dr. Wafa T. Al-Jamal and is currently in my second year of study at Queen's University Belfast, UK. My PhD project aims to evaluate the versatility of preparing multifunctional nanoparticles using microfluidics.





## Er-Chieh Cho

Dr. Er-Chieh Cho studied and received a BSc in Department of Pharmacy at Taipei Medical University, Taiwan. Years later, she received a DPhil degree at University of Oxford, UK, with thesis work based on molecular cancer biology. Then, she moved on to work on translational cancer research as postdoctoral fellow at UCLA and

also City of Hope National Medical Centre at LA, USA. Dr. Cho has done intensive studies on the functional regulation between protein methylation and tumorigenesis, the molecular mechanisms of CREB in leukemogenesis, and biomarker development. Apart from molecular cancer biology, Dr Cho's Lab has also established integrative collaborations with material science fields in recent years, and in which the undergoing projects include apply nanomaterials for the development of cancer drug delivery platform and nanomaterials in anti-oxidation applications.

- Factors controlling the carbon isotope fractionation of tetra- and trichloroethene during reductive dechlorination by *Sulfurospirillum* ssp. and *Desulfotribacterium* sp. str.; D Cichocka, M Siegert, G Imfeld, J Andert, K Beck, G Diekert, HH Richnow, FEMS microbiology ecology 62 (1), 98-107 65 2007
- Accelerated methanogenesis from aliphatic and aromatic hydrocarbons under iron- and sulfate-reducing conditions; M Siegert, D Cichocka, S Herrmann, F Gründger, S Feisthauer, FEMS microbiology letters 315 (1), 6-16 43 2011
- Ipso-hydroxylation and subsequent fragmentation: a novel microbial strategy to eliminate sulfonamide antibiotics; B Ricken, PFX Corvini, D Cichocka, M Parisi, M Lenz, D Wyss, Applied and environmental microbiology 79 (18), 5550-5558 41 2013
- Tetrachloroethene conversion to ethene by a Dehalococcoides-containing enrichment culture from Bitterfeld; D Cichocka, M Nikolausz, PJ Haest, I Nijenhuis; FEMS microbiology ecology 72 (2), 297-310



## Iwona Cicha

Iwona Cicha studied Biology at the Jagiellonian University, Cracow, Poland. After obtaining her PhD in medical sciences at the Ehime Medical School, Ehime University, Japan, she moved to University of Erlangen. She was a postdoctoral fellow in the Department of Nephrology in 2003, before joining the Department of Cardiol-

ogy, where she obtained her habilitation in Experimental Medicine in 2012. She has an extensive research experience in the field of atherosclerosis, with focus on the role of inflammation and blood flow dynamics in plaque development and destabilization. Since July 2013, she has been leading the Cardiovascular Nanomedicine Unit at the Section of Experimental Oncology and Nanomedicine (SEON), University Hospital Erlangen, focusing on the projects involving the application of nanomedical strategies for the diagnosis and treatment of cardiovascular diseases.



## Paul Anthony Curley

Department of Molecular and Clinical Pharmacology, University of Liverpool, The Materials Innovation Factory

I am currently employed as a postdoctoral research associate in the Department of Molecular and Clinical Pharmacology. My research interests currently focus on the

development and pharmacological assessment of novel long-acting antiretrovirals. I have gained substantial experience in *in vitro*, *in vivo* and *in silico* assessment of nanoparticle and small molecule drug delivery.

In addition to research undertaken in my PDRA position, my research interests also focus on the disposition and interactions of drugs within the CNS. This includes investigating drug uptake and toxicity utilising *in vitro* cell systems, screening of genetic markers for increased disposition of drug induced CNS toxicity and *in vivo* disposition of small molecule and nanoformulated antiretrovirals. I have expertise in physiologically-based pharmacokinetic (PBPK) modelling and have utilised this skill to predict plasma and tissue distribution of standard and long-acting antiretroviral formulations prior to preclinical and ultimately clinical *in vivo* studies. I hold a home office personal licence and in recent years have developed strategic protocols and executed numerous preclinical studies for assessing single-dose, steady-state, and long-acting pharmacokinetic behaviour. In 2017 I was competitively awarded a University of Liverpool Early Career Researcher and Returners Fund Grant, and will conduct a sabbatical at the University of Nebraska Medical Centre in April 2018 to establish expertise with humanised mouse models.

### EDUCATION:

**2011 – 2014:** PhD: Understanding Disposition of Efavirenz and Application in Solid Drug Nanoparticle Development, University of Liverpool, Liverpool, UK

**2010 – 2011:** MRes Biomedical Science, (Distinction) University of Liverpool, Liverpool, UK

**2007 – 2010:** BSc (Hons) Pharmacology (First Class) University of Liverpool, Liverpool, UK

### RESEARCH EXPERIENCE:

**2015 – present:** Postdoctoral Research Associate, Department of Molecular and Clinical Pharmacology, University of Liverpool, Nanoengineering of NRTI Sustained Release Formulations

**May – June 2018:** Visiting Researcher, University of Nebraska Medical Centre, Centre for Humanised Mice

**2014 – 2015:** Research Assistant, Department of Molecular and Clinical Pharmacology, University of Liverpool, Nanoengineering of NRTI Sustained Release Formulations



## Danuta Cichocka

CEO, CO-FOUNDER  
RESISTELL AG, Basel, Switzerland

I am a microbiologist driven by a passion for life science, innovation and self-development. With over 10 years of professional experience in microbiology research, research management and biotechnol-

ogy policy development at European level, along with an executive EPFL EMBA and CAS in Management of Biotech, Medtech & Pharma Ventures, my key strength is being able to bridge the gap between research and business. This is borne out in my ability to translate complex problems into manageable challenges. Not only do I see the bigger picture, but I am able to communicate it across the board. A skill sharpened by living and working in six different countries, developing cultural sensibility and understanding of the needs and motivations of others.

### RECENT PUBLICATIONS

- Variability in microbial carbon isotope fractionation of tetra- and trichloroethene upon reductive dechlorination; D Cichocka, G Imfeld, HH Richnow, I Nijenhuis Chemosphere 71 (4), 639-648 73 2008



## Christopher David

Advanced Pharmacological Strategies (APS) Group, Materials Innovation Factory, Department of Molecular & Clinical Pharmacology, The University of Liverpool, 51 Oxford Street, Liverpool, L7 3NY Tel: +44 (0) 151 795 57146, e-mail: cdavid@liverpool.ac.uk

Dr David has a background in biochemistry, regenerative medicine, and nanotoxicology. He has been a member of highly interdisciplinary research groups which has afforded him a holistic approach which he applies to his current research investigating biocompatibility and immunological safety of conventional and nanotechnology-enabled medicines as well as cellular therapies. The intention of this current work is to better inform the assessment methodologies used for biologically applied nanotechnologies.

### RESEARCH

2018– present: Postdoctoral Research Associate - Department of Molecular and Clinical Pharmacology, University of Liverpool. Biocompatibility, nanotoxicology and immunopharmacology.

2017–2018: Postdoctoral Research Associate - Department of Molecular and Clinical Pharmacology, University of Liverpool. Investigating the immunological impact of novel HIV antiretroviral nanoformulations.

### EDUCATION

2013–2017: PhD, University of Liverpool, Department of Molecular and Clinical Pharmacology: Assessing the relationship between nanoparticle physicochemical characteristics and biological interactions; optimisation of *in vitro* techniques and protocols.

2011–2012: MSc Nanomedicine, Swansea University, UK.

2008–2011: BSc Medical Biochemistry, Swansea University, UK.



## Luca Digiacomo

Department of Molecular Medicine, "Sapienza" University, Rome, Italy

I am a postdoctoral researcher who specializes in Biophysics and Medical Physics. After completing my education at the University of Pisa (where I obtained my master's degree in Matter's Physics), I worked

as a Ph.D. student at the University of Camerino. There, I studied the interactions among nanoparticles and biological media, aiming to exploit the biomolecular corona of liposomes for the development of novel targeted therapeutics. Under the mentorship of prof. Giulio Caracciolo, I had the opportunity to conjugate mathematical and physical approaches to the biotechnological and medical research.

I enthusiastically joined that multidisciplinary activity and provided original contributions by combining my expertise and scientific interests, which include fluorescence microscopy and spectroscopy, nanoparticle-protein interactions, bioinformatics, nanomedicine and data science. So far, results have been published in sixteen scientific articles and presented in oral or poster sessions in national and international conferences. Furthermore, I have been actively involved in the supervision of undergraduate students and the teaching activity of Medical Physics for the degree courses in Medicine, Nursing and Bioinformatics at "Sapienza" University (Rome). I am currently working as a postdoctoral researcher at the Department of Molecular Medicine, 'Sapienza' University (Rome). Our project focuses on the development of a nanoparticle-enabled blood test for pancreatic cancer detection. Starting from promising

preliminary results, we aim to design and implement a cheap, non-invasive and accurate tool for the early cancer diagnosis.

### RECENT PUBLICATIONS

- Caputo, D., Papi, M., Coppola, R., Palchetti, S., Digiacomo, L., Caracciolo, G., Pozzi, D. A protein corona-enabled blood test for early cancer detection (2017) *Nanoscale*, 9 (1), pp. 349-354.
- Colapicchioni, V., Tilio, M., Digiacomo, L., Gambini, V., Palchetti, S., Marchini, C., Pozzi, D., Occhipinti, S., Amici, A., Caracciolo, G. Personalized liposome-protein corona in the blood of breast, gastric and pancreatic cancer patients (2016) *International Journal of Biochemistry and Cell Biology*, 75, pp. 180-187.
- Digiacomo, L., Cardarelli, F., Pozzi, D., Palchetti, S., Digman, M.A., Gratton, E., Capriotti, A.L., Mahmoudi, M., Caracciolo, G. An apolipoprotein-enriched biomolecular corona switches the cellular uptake mechanism and trafficking pathway of lipid nanoparticles (2017) *Nanoscale*, 9 (44), pp. 17254-17262.
- Palchetti, S., Digiacomo, L., Pozzi, D., Peruzzi, G., Micarelli, E., Mahmoudi, M., Caracciolo, G. Nanoparticles-cell association predicted by protein corona fingerprints (2016) *Nanoscale*, 8 (25), pp. 12755-12763.
- Pozzi, D., Caracciolo, G., Digiacomo, L., Colapicchioni, V., Palchetti, S., Capriotti, A.L., Cavaliere, C., Zenezini Chiozzi, R., Puglisi, A., Laganà, A. The biomolecular corona of nanoparticles in circulating biological media (2015) *Nanoscale*, 7 (33), pp. 13958-13966.



## Simona Dostálová

Simona Dostalova is a postdoc in Laboratory of Nanomedicine, a part of Research Group for Molecular Biology and Nanomedicine led by Dr. Zbynek Heger at Department of Chemistry and Biochemistry, Mendel University in Brno, Czech Republic. The Department is led by ERC Starting Grant Holder, Prof. Vojtech Adam. She is

currently enjoying a 6-month internship at Centre for BioNano Interactions, University College Dublin, Ireland under the mentorship of Prof. Kenneth A. Dawson, where she focuses on better understanding of the functional impact of nanoparticles on cells.

Her research is focused on the use of nanocarrier based on ubiquitous protein ferritin for targeted therapy of cancer. The aim of her research is to find a nanocarrier that can provide efficient and reliable targeting as well as high degree of safety and biocompatibility. Simona holds a bachelor's and master's degree in Biomedical Engineering from Brno University of Technology and PhD in Chemistry from Mendel University in Brno. She has become the receiver of many awards, including 2017 award by Purkyne foundation for publications in journal with highest IF published in 2016 by Czech researchers; 1st instead at The Conference competition XVI. Workshop of Physical Chemists and Electrochemists in Young Scientists session in 2016; The PhD student competition MendelINET 2013; or Rector's award for outstanding academic and research results at Brno University of Technology in 2013. She has also authored and co-authored 42 original scientific papers in ISI-indexed journals with a total of 181 citations and h-index H=9 according to Web of Science.



## Tomaž Einfalt

Tomaž Einfalt has studied Pharmaceutical Sciences at the University of Ljubljana, Slovenia. During his MSc studies, he participated in the Erasmus research exchange programme at the Universities of Aston (UK) and University of Regensburg (DE), where his research work focused on preparing nano-drug delivery systems and lyophilisation. He received his Pharmacists diploma and MSc based on developing new amorphous forms of antibiotics. After receiving his degree he moved to Basel, Switzerland to pursue his Doctoral studies in Biomimetic Engineering at the Swiss Nanoscience Institute (SNI), University Basel, where his work focused on developing sustainable cellular implants. He received his doctoral degree with summa Cum Laude in 2017, and continued as a postdoctoral researcher at the Division of Pharmaceutical Technology, Department of Pharmaceutical Sciences, University of Basel.



## Linda Elberskirch

Preclinical Nanomedicine  
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My name is Linda Elberskirch and I am working as a PhD student at the Fraunhofer Institute for Biomedical Engineering (IBMT).

I studied „Applied Life Science“ at the university of Kaiserslautern and finished my Master thesis with the title “Photodynamic Therapy of Cholangiocarcinoma – A Novel Nanoparticle-Based Approach” in 2015 at the Fraunhofer IBMT.

Since 2015 I am working on my PhD thesis at the Fraunhofer IBMT with the working title “Development of Novel Preclinical Test Systems for Small Intestine Cancer” at the working group “Preclinical Nanomedicine”. I am focused on the development of specific pre-clinical test systems like microfluidic test systems and vascularized tumour models, which allow to simulate the drug treatment of a tumour in the gastrointestinal tract. In addition to the specific test systems, another important aspect of my work is the development and validation of analytical methods to quantify the characteristics of potential new drugs for the treatment of intestine cancer.



## Farid Faruqu

Farid graduated from the University of Cambridge with both a BA and MSci in Biochemistry in 2014. During his MSci studies, he looked into the involvement of the Rho-family of small G-proteins in tumourigenesis and metastasis of cancer. Upon graduation, he did an internship with Cancer Research Initiative Foundation (CARIF), a non-profit cancer research institute in Malaysia. There, he was involved in the screening of locally sourced natural compounds as potential cancer therapeutics to target cytidine deaminases. He was awarded a scholarship by the Malaysian Government to pursue his PhD at the Institute of Pharmaceutical

Sciences, King’s College London in 2015. He is now working on developing exosomes as delivery systems to target brain cancers.



## Patrícia Figueiredo

Patrícia Figueiredo graduated in Biochemistry in 2012 and obtained her Master’s Degree in Molecular Oncology at the University of Porto (Portugal) in 2014. In 2015, she joined the Faculty of Pharmacy, University of Helsinki (Finland) as a PhD student under the supervision of Associate Prof. Hélder A. Santos. Currently, her research focuses on the developments of

lignin-based nanoparticles for biomedical applications. She has been published in journals like Biomaterials, Advanced Functional Materials and Progress in Materials Sciences. The main publications are:

- P. Figueiredo\*, K. Lintinen, J. T. Hirvonen, M. A. Kostianen, H. A. Santos\*, “Properties and Chemical Modifications of Lignin: Towards Lignin-Based Nanomaterials for Biomedical Applications”, Prog. Mater. Sci. 2018, 93, 233-269.
- P. Figueiredo\*, C. Ferro, M. Kemell, Z. Liu, A. Kiriazis, K. Lintinen, H.F. Florindo, J. Yli-Kauhaluoma, J. Hirvonen, M. A. Kostianen, H. A. Santos\*, “Functionalization of Carboxylated Lignin Nanoparticles for Targeted and pH-Responsive Delivery of Anticancer Drugs”, Nanomedicine 2017, 12 (21), 2581-2596.
- T. Bauleth-Ramos, M.-A. Shahbazi\*, D. Liu, F. Fontana, A. Correia, P. Figueiredo, H. Zhang, J. P. Martins, J. T. Hirvonen, P. Granja, B. Sarmento\*, H. A. Santos\*, “Nutlin-3a and Cytokine co-loaded Spermine-modified Acetalated Dextran Nanoparticles for Cancer Chemo-immunotherapy”, Adv. Funct. Mater. 2017, 27, 1703303.
- P. Figueiredo\*, K. Lintinen, A. Kiriazis, V. Hynninen, Z. Liu, T. Bauleth-Ramos, A. Rahikkala, A. Correia, T. Kohout, B. Sarmento, J. Yli-Kauhaluoma, J. Hirvonen, O. Ikkala, M. A. Kostianen, H. A. Santos\*, “In Vitro Evaluation of Biodegradable Lignin-based Nanoparticles for Drug Delivery and Enhanced Antiproliferation Effect in Cancer Cells”, Biomaterials 2017, 121, 97–108.
- P. Figueiredo, V. Balasubramanian\*, M.-A. Shahbazi, A. Correia, D. Wu, C. G. Palivan, J. T. Hirvonen, H. A. Santos\*, “Angiopep2-Functionalized Polymersomes for Targeted Doxorubicin Delivery to Glioblastoma Cells”, Int. J. Pharm. 2016, 511(2), 794–803.



## Bertalan Fodor

Bertalan Fodor MSc, PhD, Dr. habil., EuSpLM, Professor and Head of Department, at the University of Miskolc, Faculty of Health Care, Department of Nanobiotechnology and Regenerative Medicine and Scientific Advisor at Department of Laboratory Medicine at Central University Teaching Hospital, Miskolc, Hungary. He graduated as microbiologist at the Eötvös

Lorand University in Budapest, Hungary in 1993. He took his PhD degree in clinical immunology at the Medical School of Debrecen, University of Debrecen in 2003. His main research interesting field is the preparation and haemocompatible-, immunotoxicological characterization of different nanoparticles. In parallel he is interested in precision diagnostics of molecular allergy procedures.

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## Valentina Francia

Valentina has studied Molecular Biology at the University of Milan, Italy. After one year of internship at the Institute of Molecular Oncology in Milan (IFOM) she obtain her master degree in 2013 cum laude. In 2014 she joined the group of Anna Salvati as a PhD student at the Groningen Research Institute of Pharmacy, where she is currently studying the endocytic mechanisms involved in the uptake of nanomaterials. She will defend her PhD in September 2018 and continue her work at the University of Groningen as a postdoctoral fellow..

She will defend her PhD in September 2018 and continue her work at the University of Groningen as a postdoctoral fellow..



## Tamás Fülöp

Gyula Tamás Fülöp is a PhD student at the Nanomedicine Research and Education Center at Semmelweis Medical University of Budapest, Hungary. He got his bachelor and also master degree on the medical and pharmaceutical biotechnology program at the University of applied sciences Krems, Austria. He had a training semester in 2010 at the Academic Medical Center (AMC)

Amsterdam, the Netherlands; and later in 2012 another training semester at Centro de Investigaciones Biológicas (CSIC) Madrid, Spain. He will finish his PhD program this year in Budapest, where he is currently studying complement related infusion reactions of liposomal and other nanomedicine products.



## Eduard Gatin

Lecturer, Ph.D  
University of Bucharest, Faculty of Physics, Department Science Materials, P.O.Box, MG 11, Bucharest-Magurele, Romania; University of Medicine 'Carol Davila', Faculty of Dentistry, Dental Materials Department, Calea Plevnei 19, Sect.5, Bucharest, Romania.

Me Eduard Gatin, 1980–1985 Physicist Education, area of research: polymer and materials science, dental materials, calcified tissues and bone tissue regeneration. University of Bucharest, Faculty of Physics. 1990 started as Assist Professor at Faculty of Physics, University of Bucharest. 1994– 2000 Doctor in Biology (Ph. D degree) and present lecturer. Area of interest, polymer membranes for blood filtration. I continued with research in material science - polymers, advanced Nano materials, ceramics, dental metal alloys, corrosion, dental materials and tissue regeneration. I was integrated for post graduated studies regarding this field, University of Bucharest, Faculty of Physics. Beginning with 2008 I am dedicated to material science related to Medical Field (dentistry, as: dental restoration materials, corrosion, dental enamel quality, tissue regeneration – dentine). From 2010 – present, Lecturer and Associate Professor – Biophysics Department and Dental Materials from University of Medicine "Carol Davila", Faculty of Dentistry. Teaching classes: Seminars, classes and Biophysics Laboratories, Dental Materials Lab. Research activity: Materials structure, physical / chemical properties, dental enamel, bacteria activity, polymer resin composites, dental ceramics, metal alloys and corrosion studies. Techniques skills: RAMAN spectroscopy (improved by SERS), SEM, EDX. Between 2010 – 2013, I was postdoctoral student in EU Program PostDoc (to improve research work, EU finance support).

In 2013 it was proposed a method for quality evaluation of dental enamel by Raman method, to be applied "in vivo". On 30th October 2015, patent registration certificate was issued under Nr RO 2013 00043.

On Scopus is available the complete list of publications with Impact Factor, containing 16 publications, largest part of them is focussed on medical dentistry and Raman applications. Regarding international meetings, I must list EMRS 2012, 2013, 2014, 2015 (invited presentation to Symposium V Bioinspired and biointegrated materials) and CLINAM 2014, 2015, 2016; EMRS 2016, Warsaw Meeting (invited presentation).

Is in progress the study "Introducing of RAMAN technique to Periodontology", according bioethical approval dated January 2016, with Semmelweis University Budapest – Faculty of Dentistry, Hungary; the study is improved, with a new step: in vivo evaluation, started up spring 2017. Two important papers based on Raman applications are in press for year 2018.



## Sabrina Gioria

EU-NCL CORE EXPERT TEAM,  
JRC Scientific Project Assistant .

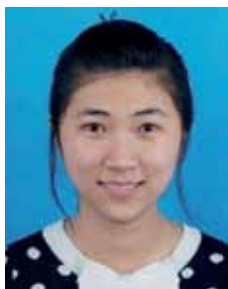
Sabrina Gioria is a scientific project assistant at the Consumer Products Safety Unit of the European Commission's Joint Research Centre.

Her main research interest lies in investigating the in vitro toxicity of engineered nanomaterials (NMs) covering the development and optimization of test methods, the study of nanoparticles (NPs) uptake, as well as investigating the mechanisms involved in NPs toxicity by combining OMICs techniques.

Graduated in Pharmaceutical Chemistry and Technology at the University of Turin, she has been working among others at the European Institute of Oncology in Milano (Italy), the University College of London (UCL) UK, in the Testing Methods Group of the European Chemicals Bureau (ECB, JRC Ispra) and in the Institute for Reference Materials and Methods (IRMM, JRC Geel, Belgium). She joined the European Commission as official in 2002. She is part of the Core Expert Team (CET) of the European Nanomedicine Characterisation laboratory (EU-NCL). She participates in EU competitive research projects and inter-laboratory comparison studies.

### RECENT PUBLICATION

- D. Mehn, F. Caputo, M. Rösslein, L. Calzolari, F. Saint-Antoninc, T. Courant, P. Wick and D. Gilliland (2017), Nanoparticle characterisation methods for recognition of dimers. RSC Advances. 2017; 7:27747-54.
- 2. D. Mehn, P. Iavicoli, N. Cabaleiro, SE. Borgos, F. Caputo, O. Geiss, L. Calzolari, F. Rossi, D. Gilliland (2017), Analytical ultracentrifugation for analysis of doxorubicin loaded liposomes. International journal of pharmaceutics. 2017; 523:320-6.
- 3. S. Gioria, F. Caputo, P. Urban, S. Bremer-Hoffmann, A. Prina-Mello, L. Calzolari, D. Mehn. (2018), Are existing standard methods suitable for the evaluation of nanomedicines: some case studies. Nanomedicine 13 (4), pp 539-554



## Shanyue Guan

Shanyue Guan has obtained her doctor degree in applied chemistry at the Beijing University of Chemistry Technology in 2016 and started her post-doctoral in Technical Institute of Physics and Chemistry CAS. After 21 months, she become an assistant professor in Technical Institute of Physics and Chemistry CAS.

Research area: The biomaterials based on Layered Double Hydroxide, including inorganic-organic nanocomposites. The materials we synthesized have been to simultaneous dual-modal imaging and targeted therapy.

### PUBLICATIONS:

- Shanyue Guan, Di Yang, Yangziwan Weng, Heng Lu, Xiangmin Meng, Xiaozhong Qu,\* Shuyun Zhou.\* Excitation-dependent theranostic nanosheet for cancer treatment. *Adv. Healthcare Mater.*, 2018, 1701123. (IF=5.1) (Front Cover)
- Jun Wang, Di Liu, Yongfa Zhu,\* Shuyun Zhou and Shanyue Guan.\* Supramolecular packing dominant photocatalytic oxidation and anticancer performance of PDI. *Appl. Catal. B Environ.*, 2018, 231, 251–261. (IF=9.6)
- Di Yang, Shanyue Guan,\* Yuefang Niu, Zheng Xie,\* Xiaozhong Qu\* and Shuyun Zhou. Construction of Hypoxia Responsive Upconversion Nanosensor for Tumor Imaging by Fluorescence Resonance Energy Transfer from Carbon Dots to Ruthenium Complex. *J. Mater. Chem. B*, 2018, DOI: 10.1039/C8TB00278A. (IF=4.5)
- Shanyue Guan, Yangziwan Weng, Mengnan Li, Ruizheng Liang,\* Chenghua Sun,\* Xiaozhong Qu\* and Shuyun Zhou. An NIR-sensitive layered supramolecular nanovehicle for combined dual-modal imaging and synergistic therapy. *Nanoscale*, 2017, 9, 10367–1037. (IF=7.4)

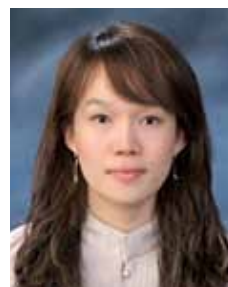
search interest transferred to biomaterials.

**Research area:** Multimodal bioimaging based on gold nanorod and carbon dot.

**Reason to attend:** The CLINAM Summit is focused on the targeted medicine and precision medicine, which is similar with my study area. Therefore, I would like to attend the conference and communicate with the specialist.

### PUBLICATIONS:

- Rui Pang,ab Xiujie Hu,\* Shuyun Zhou, Chenghua Sun, Jun Yan, Xingming Sun, Shizhuo Xiaoa and Ping Chen, Preparation of multi-shelled conductive polymer hollow microspheres by using Fe<sub>3</sub>O<sub>4</sub> hollow spheres as sacrificial templates, *Chem. Commun.*, 2014, 50, 12493-12496.
- Yuxin Li, Xiujie Hu\*, Shuyun Zhou,\* Li Yang, Jun Yan, Chenghua Sun and Ping Chen, A facile process to produce highly conductive poly(3,4-ethylenedioxythiophene) films for ITO-free flexible OLED devices, *J. Mater. Chem. C*, 2014, 2, 916-924.
- Xiaojing Liu<sup>1</sup>, Luting Liu, Xiujie Hu, Shuyun Zhou, Rinat Ankri, Dror Fixler Multimodal bioimaging based on gold nanorod and carbon dot nanohybrids as a novel tool for atherosclerosis detection *Nano Research* 2018, 11(3), 1262–1273.



## Eun Ju Jeong

Eun-ju Jeong received his BS and MS degree in biochemistry from Gyeongsang National University, Korea. She joined ETRI, Korea and is currently a senior researcher of Bio-Medical IT Research Department. Her research experiences are in the area of biomedical optics, diagnostics device and biosensor. Her current research focus are microfluidic system, and diagnostic devices.



## Sonja Horvat

I am a PhD student working at Department of Functional Materials in Medicine and Dentistry (FMZ) in University of Wuerzburg, Germany. After completing studies of pharmaceutical engineering in Serbia, I joined the NanoBioTechnology group of prof. dr. Jurgen Groll to work with nanoparticles for medical applications. My

research is mainly based on design of nanogels suitable for treatment of fungal diseases caused by opportunistic filamentous fungi. Besides that, my interests also include optimization of iron oxide cores and coatings for cancer diagnostics using magnetic particle imaging.



## Xiujie Hu

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Mobile: +86-13521531692  
Institution Address: 29 Zhongguancun East Road, Haidian District, Beijing, 100190, PR China.

Xiujie Hu obtained her master degree in chemistry at the Institute of Changchun

Applied Chemistry, Chinese Academy of Sciences in 1990 and started research in photographic chemistry and conducting polymers in Technical Institute of Physics and Chemistry CAS. Early years, she has focused conducting polymers nanomaterials. Recently, her re-



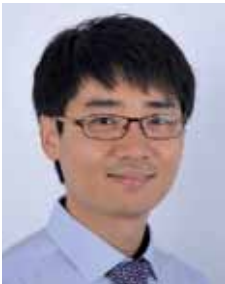
## Igor Khalin

Dr. Khalin Igor has studied Medicine the Kharkiv National Medical University, Ukraine. He received a doctoral decree based on thesis work in chronic irradiated wounds from the Academy of Medical Sciences of Ukraine and did further research in experimental pharmacology at Kharkiv National Medical University. Then he moved to Malaysia and worked as Associate of Professor and Head of Pharmacology Department at National Defence University of Malaysia. There he switched his interest towards nanomedicine. In 2017 he was awarded to Humboldt Fellowship for experienced researches and started working as Research Fellow at Institute of Stroke and Dementia Research, Ludwig-Maximilian University of Munich, Germany. His research is associated with neurodegeneration, blood-brain barrier and development of theranostics. In 2018 he was awarded to Marie Curie Individual Fellowship.

### MAIN PUBLICATIONS

- Igor Khalin, Renad Alyautdin, Tin Wui Wong, Justin Gnanou, Ganna Kocherga & Jörg Kreuter Brain-derived neurotrophic factor delivered to the brain using poly (lactide-co-glycolide) nanoparticles improves neurological and cognitive outcome in mice with traumatic brain injury. // *Drug Deliv.* 2016 Nov;23(9):3520-3528. Epub 2016 Jul 16
- Khalin I, Alyautdin R, Kocherga G, Bakar MA. Targeted delivery of brain-derived neurotrophic factor for the treatment of blindness and deafness // *International journal of nanomedicine.* – 2015,

- Renad Alyautdin, Igor Khalin, Nafeeza Mohd Ismail, Muhammad Huzaimi Haron, Dmitry Kuznetsov. Nanoscale drug delivery systems and the blood-brain barrier //International journal of nanomedicine. – 2014. – T. 9. – P. 795-811.



## Yong-Gun Kim

Yong-Gun Kim has studied School of Dentistry, the University of Gangneung-Wonju, South Korea. I received a doctoral decree based on thesis work in periodontal bone metabolism and from the University of Gangneung-Wonju and did further research in periodontics at Gangneung-Wonju University Dental Hospital and Kyungpook National Dental Hospital, South Korea. I earned specialist degrees in Periodontology. As a fellow the Chung-Ang University Hospital, Seoul, South Korea, worked on clinical and research studies. In 2012, I became professor for department of periodontology at the Kyungpook National University of Daegu, South Korea. Since then, I have been studying about dental biomaterials for periodontal regeneration through experimental animal models and clinical studies.



## Gergely Tibor Kozma

Gergely Tibor Kozma, MSc, PhD, immunologist, senior research fellow at the Nanomedicine Research and Education Center at Semmelweis University, Budapest, Hungary. He received his MSc degree in bioengineering at Technical University Budapest in 2000; thereafter he obtained PhD in immunology and molecular biology at Semmelweis University in 2004. He was working at Semmelweis University and at several companies as a researcher studying mainly the mechanisms of allergy by immunological and genome research methods. He spent one and half a year in Rome (Italy) as a postdoctoral researcher sponsored by the Marie Curie Research Training Network to investigate the antigen presenting processes of dendritic cells. His current field of research is the immunological study of nano-drug induced hypersensitivity reactions including mainly the complement activation related processes and immunogenicity. Besides research he was also involved at scientific developments including protein engineering in *E. coli* and monoclonal antibody production. He has co-authored 22 original papers with about 500 citations.



## Nitzan Krinsky

I was born in October of 1986, the oldest of four children, and live in Kiryat-Ata, a town near Haifa. When I graduated from high school I was offered by the IDF to study biotechnology and food engineering and then served in the IDF in this field. I chose to attend the Technion - Israel Institute of Technology. While completing my bachelor degree in Biotechnology and Food Engineering, I conducted a final research project regarding functional bioactive health foods; at the end of that year I knew I wanted to continue this path and become a scientist after my army service. After my Bachelor degree

(Summa Cum Laude) I was recruited to the IDF as a research engineer and was part of different research projects which were further published 1-3. Alongside my army service I acquired my M.B.A in Bar-Ilan University (Israel).

In October 2014, I was able to pursue my dream to become a scientist by beginning my PhD studies in Professor Avi Schroeder's research group in the Chemical Engineering Department, Technion. My current research is motivated by the need to find novel approaches to treat cancer through the interface of nanotechnology and medicine.

During my PhD research I developed a new cell-free protein synthesis system which contains all the transcription and translation machines and molecules required for protein production 4. This system was used to prepare liposomes (lipid particles) that act as artificial cells, capable of producing protein-based medicine only where it is needed. The produced protein can be tuned to the patient's needs based on a predetermined DNA code which is incorporated inside these synthetic cells. We recently demonstrated the injection of the synthetic cells *in vivo* into a 4T1-induced tumor, which was resulted in high cytotoxicity due to the effective production of the therapeutic protein inside the vesicles 5.

During my PhD studies I was honored to be a "Baroness Ariane de Rothschild Women Doctoral Program" Fellowship scholar, and was selected as the 2018 'Nanotechnology in Medicine' Young Scientist Award and the 2017 'Gordon Research Conference – Cancer Nanotechnology' best poster presenter.

## REFERENCES

- 1 Eynan, M., Mullokandov, M., Krinsky, N., Biram, A. & Arieli, Y. Alteration of blood glucose levels in the rat following exposure to hyperbaric oxygen. *Journal of applied physiology* 119, 463-467, doi:10.1152/jappphysiol.00154.2015 (2015).
- 2 Eynan, M., Krinsky, N., Biram, A., Arieli, Y. & Arieli, R. A comparison of factors involved in the development of central nervous system and pulmonary oxygen toxicity in the rat. *Brain research* 1574, 77-83, doi:10.1016/j.brainres.2014.05.051 (2014).
- 3 Domachevsky, L. et al. Do hyperbaric oxygen-induced seizures cause brain damage? *Epilepsy research* 100, 37-41 (2012).
- 4 Krinsky, N. et al. A Simple and Rapid Method for Preparing a Cell-Free Bacterial Lysate for Protein Synthesis. *Plos One* 11, e0165137, doi:10.1371/journal.pone.0165137 (2016).
- 5 Krinsky, N. et al. Synthetic Cells Synthesize Therapeutic Proteins inside Tumors. *Adv Healthc Mater* 7, e1701163, doi:10.1002/adhm.201701163 (2018).



## Haluk Küçük

Haluk Küçük received B.S. degree in Mechanical Engineering and M.S. degree in Management Engineering from Istanbul Technical University, Istanbul, Turkey, in 1993 and 1995, respectively, he received M.S. degree in Mechanical Engineering from Boğaziçi University, Istanbul, Turkey in 1995, and Ph.D. degree in Mechanical

Engineering – Engineering Mechanics from Michigan Technological University, Houghton, USA in 1999. He is currently a Professor, with Engineering Faculty, Department of Electrical & Electronics Engineering, Marmara University, Istanbul, Turkey. His research interests are robotics and biomechanics.

He has been involved in Development of a Neurostimulator for Drop-Foot and a Robotic System for Orthopaedic Surgery projects. He obtained a patent for Automatic Speaking Valve for Tracheostomy Patients.

X-Ray Image Processing for Quantification of Bone Age, Robust and Smooth Color Tracking by a Tendon Driven Humanoid Neck, A device to measure joint angles and foot force for lower extremity force distribution computations, Biomechanical Analysis of Cervical Spine Sagittal Stiffness Characteristics, Development of a Phren-



ic Nerve Stimulator (Diaphragm Pacemaker) to Treat Respiratory Insufficiency, constitute some recent publications co-authored by Haluk Küçük.



## Fredrik Kullenberg

Fredrik Kullenberg has his background in Engineering Nanoscience, which he studied at the Faculty of Engineering at Lund University. He started there in 2011 and received his Master of Science in 2016.

The education is interdisciplinary and includes many subjects, from quantum physics to immunology and neurobiology.

During his studies he has, besides the subjects mentioned above, also studied physiology, pharmaceutical chemistry, cell biology and programming.

His master's thesis, Formulation and Characterization of a Liposomal Spray Dried Powder Intended for Inhalation, concerned the development and analysis of a model protein drug. The project was a collaboration between a pharmaceutical company and the University, which gave Fredrik an insight into how research is performed in both academia and in the pharmaceutical industry.

After receiving his master's degree, Fredrik also studied Pharmaceutical Bioinformatics at Uppsala University, which he passed with distinction.

After this, Fredrik spent a year working as a Drug Safety Associate at Sobi, a Swedish pharmaceutical company which specializes in rare diseases and protein pharmaceuticals.

Fredrik's current position is as a PhD student in the Lennernäs group at the Department of Pharmacy, Uppsala University, where he started in October 2017. His PhD project has the preliminary title Functional liposome nanoparticle drug carriers as a theranostic for hepatocellular carcinoma: the role of locoregional targeted drug delivery for interventional therapy. As can be seen in the preliminary title, the project concerns the innovation and development of novel nanoparticle formulations to be used in the treatment of primary liver cancer. So far, the project has mainly consisted of testing currently existing formulations, such as Caelyx<sup>®</sup>, as a first step to determine the impact of various parameters in different formulations.



## Dhadhang Wahyu Kurniawan

Dhadhang Wahyu Kurniawan passed bachelor and pharmacist (Apotheker) from School of Pharmacy Institut Teknologi Bandung (ITB), Indonesia. He graduated master of pharmacy from Faculty of Pharmacy Universitas Gadjah Mada (UGM) Yogyakarta, Indonesia. Since February 2016,

he was taking PhD studies at the Department of Biomaterials Science and Technology section Targeted Therapeutics, TechMed Centre University of Twente The Netherlands. His research project about nano-therapeutics for the targeted modulation of inflammatory macrophages in liver disease. He was working with Syk (spleen tyrosine kinase) inhibitor and Src family kinase inhibitor as new approach for the treatment of inflammatory liver disease. He had succeeded made PLGA nanoparticles to deliver Syk inhibitor for the treatment of non-alcoholic steatohepatitis (NASH). He had attended as a poster presentation at FIGON Dutch Medicine Conference 2017, ESCDD (European Symposium on Controlled Drug Delivery) 2018, and he had presented as an oral presentation at Dutch Liver Retreat (DLR) 2018.



## Kuen-Chan Lee

Associate Professor  
National Taipei University of Education,  
Taiwan

Dr. Lee's laboratory focuses on studying materials chemistry in biomedical related research on nanomaterials. Dr. Lee has done intensive study on how to explore the

fundamentals of carbon nanotubes and the interaction between carbon nanotubes and biopolymers. We first synthesized a novel dispersing agent 4-(pyren-1-yl)butanoylated polylysine (PBPL) as a potential platform for further chemical modification. Later our lab found a new kind of polypeptidic dispersing agent which was a mixture of oppositely charged polypeptides such as sodium poly-L-glutamate (SPG) / poly-L-lysine hydrobromide (PL) or SPG / poly-L-arginine hydrochloride (PA). These dispersing agents showed very good dispersion and redispersion properties indicating high stabilities of the polypeptide / CNT conjugates. This could provide a very good basis for synthesis of nanocomposite materials for biomedical uses. We have also developed a novel charge complementarity methodology using soft organic moieties to stabilize and protect arginase via the formation of arginase-AuNPs-PBPL/SWCNT conjugates. The results suggest that the activity of the immobilized arginase is comparable to that of the native arginase, suggesting the entrance of arginine to the active site of the immobilized enzyme is unhindered by our rational design of the single point of immobilization at the enzyme.

### PUBLICATIONS

- Interfacial engineering of melamine sponges using hydrophobic TiO<sub>2</sub> nanoparticles for effective oil/water separation, J. Taiwan Inst. Chem. E., 67, 476-483, 2016.
- Carboxyfullerene decorated titanium dioxide nanomaterials for reactive oxygen species scavenging activities, RSC Adv., 6, 53025-53033, 2016.
- Few-layer Graphene Based Sponge as a Highly Efficient, Recyclable and Selective Sorbents for Organic Solvents and Oils, RSC Adv., 5, 53741-53748, 2015.



## Sharon Wei Ling Lee

PhD Candidate  
Singapore MIT Alliance for Research and  
Technology

Sharon received her Bachelor's Degree in Biomedical Engineering (BME) from the National University of Singapore (NUS) and is currently in her 3rd year of PhD Candidature under a fellowship from the

Singapore-MIT Alliance for Research and Technology (SMART). She holds research positions in both the BioSystems and Micromechanics (BioSyM) interdisciplinary research group at SMART, and the Singapore Immunology Network, Agency for Science, Technology and Research (SigN, A\*STAR). Her research is focused on the development and application of 3D *in vitro* tumour environments for screening and optimizing cancer therapy. During her PhD, she was awarded with the Lee Foundation Travel Fellowship to attend the 250th ACS National Meeting and Exposition, Boston (October 2015), won the best poster presentation award at the Singaporean Society for Immunology 8th Anniversary Symposium (May 2016) and was selected to present her work at the World Preclinical Congress Europe, Lisbon (November 2017). Sharon has acquired experience in cell culture techniques, microfluidics, confocal microscopy, immunocytochemistry and flow cytometry.

Prior to her PhD, Sharon gained research experience both in local and overseas labs. Locally in Singapore, she was involved in developing and characterizing a novel temperature-responsive hydrogel at the Institute of Materials Research and Engineering (IMRE), A\*STAR. This work was later published in the *Journal of Applied Polymer Science* in 2013. In 2014, she served as a student research assistant at the University of California, Davis (UCD) (Davis, California) where she studied the effect of flow-induced stress on the inflammation of endothelial cells in a microfluidic atherosclerosis setting. Later that year, she embarked on a summer attachment in the Johns Hopkins University (Baltimore, Maryland) where she performed a high-throughput quality screen of MRI brain scans for the development of a cloud-based platform for neuroimaging analysis. She then returned to Singapore to conduct research with the NUS Department of BME where she co-authored a paper on the mechanics of nanoparticle migration through blood vessels that was published in *Langmuir* in 2018. That same year, she was first author of a paper published in *Frontiers in Immunology*, which established a 3D tumour microenvironment to study the role of monocytes on engineered T cells. Outside of lab-based experience, Sharon also served as an administrative assistant in the A\*STAR headquarters where she was involved in the review of scientific grant applications, as well as the organization of nation-wide scientific symposiums.

Sharon has received several awards pertaining to innovation in health sciences and technology. In 2013, she led a team effort to develop an automated gait rehabilitation device. Her team was awarded the Best Design Award from the NUS Department of BME and the Bronze Award for Best presentation at the Biomedical Engineering Society (BMES) Singapore, 7th Scientific Meeting. In 2014, she led a different team to develop a novel glaucoma drainage device that had clinched the Merit Award for Innovation & Research from the NUS Faculty of Engineering, and the Merit Award at the BMES Singapore, 8th Scientific Meeting. For their exemplary teamwork and overall performance, her team was further awarded with the Prestigious Director Award from the NUS Department of BME. In 2017, Sharon led yet a different team that sought to develop a diagnostic platform for Diabetic Retinopathy. Their proposal had impressed the reviewing committee who later granted them the Innovation Graduate Programme Seed Fund. Academically, she was on the NUS Faculty of Engineering Dean's list (top 5% of cohort) in 2012 and 2014, and was also on the Dean's list of the UCD School of Engineering when she was an exchange student there during the winter quarter of 2014.

#### SELECTED PUBLICATIONS

- Lee SWL, Adriani G, Ceccarello E, Pavesi A, Tan AT, Bertolotti A, Kamm RD and Wong SC (2018) Characterizing the Role of Monocytes in T Cell Cancer Immunotherapy Using a 3D Microfluidic Model. *Front. Immunol.* 9:416. doi: 10.3389/fimmu.2018.00416
- Ho, Y. T., Lee, S. W. L., Azman, N. A., Loh, F. W. Y., Phan-Thien, N., & Kah, J. C. Y. (2018). Quantifying Vascular Distribution and Adhesion of Nanoparticles with Protein Corona in Microflow. *Langmuir*.



### Jasna Lojk

I'm a postdoc researcher in the fields of nanotechnology and nanomedicine in the Group for nano- and biotechnological applications at the Faculty of Electrical Engineering (University of Ljubljana, Slovenia). In 2015, I finished my PhD in the field of nanoparticle-cell interactions and continued working on analysis of toxicity of different relevant biomedical and industrial nanoparticles, now focusing on the responses of innate immune system to nanoparticle exposure *in vitro*, with some results already published in peer reviewed journals. We also focus on nanoparticle neurotoxicity and the ability of

nanoparticles to affect neurodegeneration *in vitro*.

#### SELECTED PUBLICATION:

- Jasna Lojk, Sonja Prpar Mihevc, Vladimir B. Bregar, Mojca Pavlin, Boris Rogelj. The effect of different types of nanoparticles on FUS and TDP-43 solubility and subcellular localization. *Neurotoxicity research*, 32 (2017): 325-339.
- Jasna Lojk, Klemen Strojjan, Katarina Miš, Vladimir B. Bregar, Iva Hafner Bratkovič, Maruša Bizjak, Sergej Pirkmajer, Mojca Pavlin. Cell stress response to two different types of polymer coated cobalt ferrite nanoparticles. *Toxicology letters* 270 (2017): 108-118
- Jasna Lojk, Vladimir Boštjan Bregar, Maruša Rajh, Katarina Miš, Mateja Erdani Kreft, Sergej Pirkmajer, Peter Veranic, Mojca Pavlin. Cell Type Specific Response to High Intracellular Loading of Polyacrylic Acid Coated Magnetic Nanoparticles. *International Journal of Nanomedicine*, 10 (2015):1449-62.
- Vladimir B. Bregar, Jasna Lojk, Vid Suštar, Peter Veranič, Mojca Pavlin. Visualization of Internalization of Functionalized Cobalt Ferrite Nanoparticles and Their Intracellular Fate. *International Journal of Nanomedicine* 8 (2013): 919-31.



### Bonny Gaby Lui

Bonny Gaby Lui has a degree in bachelor of science in biotechnology. Afterwards, she studied technical biology in TU Darmstadt, Germany, and successfully graduated as a master of science. In late 2014, she started her doctoral thesis in Mainz with the main focus on process development for the early phase of a cystine-knot miniprotein discovery platform with the ultimate goal to engineer tumor imaging agents.

The engineered cystine-knot miniproteins for tumor imaging purpose is currently under patent application.



### Johanna Lutz

Johanna Lutz studied chemistry at the University of Wuerzburg (Germany) where she receives her master of science degree in 2016 concentrating on the synthesis of functionalized poly(glycidol) for click-reaction. She is currently working on her Ph.D. under the supervision of Prof. Groll at the department for functional materials in medicine and dentistry (FMZ). Her research focuses on thiol- and thioether polymeric as well as biological coating systems for gold and silver nanoparticles. Besides that, she is also working with hydrophobic functionalized gold nanoparticles based on thioether poly(glycidol) for controlled protein adsorption.



### Tariq Mahmood

Tariq Mahmood was born in a small village near Bahawalpur, Pakistan, in 1984. Bahawalpur is an under presented district of Southern Punjab and facilities are nominal to date. It was much hard time for him as well as for his parents to provide him opportunities to complete his Science education. He completed his college education in

2001 and moved to Bahawalpur City in order to receive his Pharm.D degree in Pharmacy from the Islamia University of Bahawalpur, Bahawalpur, Pakistan, in 2008, and the M. Phil. and Ph.D. degrees in Pharmaceutics from the Islamia University of Bahawalpur, Bahawalpur, Pakistan, in 2010 and 2013, respectively.

In 2013, he joined the Department of Pharmacy, The University of Faisalabad, as an Assistant Professor, and in 2014 joined University of Central Punjab as an Assistant Professor and Deputy Director Research. Dr. Tariq has several years of experience as a faculty member at Faculty of Pharmacy and has authored several peer-reviewed publications, oral presentations, and has delivered invited professional presentations. Dr. Tariq's research interests are centered on outcomes of drug development and nanocarriers. He is HEC approved PhD supervisor and to date, has supervised more than fifteen postgraduate students in discipline of Pharmaceutics. Additionally, he is reviewer / editorial board member of several internationally recognized peer-reviewed journals. He was short-listed for prestigious LUSH prize award, UK in 2013. In Young Researcher Award category, he was the only one short-listed from all over the Asia.



## Julia Mantaj

From April 2004 to November 2009 I have studied Pharmacy at the University of Braunschweig in Germany which I have completed a final grade of 1.5 (equivalent to 1st Class Honours). After my graduation as a pharmacist, I worked in a community pharmacy in Holzminden (Germany) where I was the deputy to the pharmacy manager. My responsibilities included dispensing

and customer care, staff management, emergency service, full consultation of clients and interaction with physicians, customer presentations and training of staff in the fields of nursing care for the elderly.

In January 2012 I have started my PhD at King's College London in Cancer Drug Discovery supervised by Dr Khondaker Miraz Rahman & Professor David Thurston. The title of my thesis was "Interaction of SJG-136 with cognate sequences of oncogenic transcription factors". I have completed my PhD in February 2016. I have published the following publications during my PhD: 1) "Crispene E, a cisclerodane diterpene inhibits STAT3 dimerization in breast cancer cells" (Mantaj, J., Rahman, S. M. A., Bokshi, B., Hasan, C., Jackson, P., Parsons, R. & Rahman, K. 2015. *Org. Biomol. Chem.* 13, p. 3882-3886); 2) "Covalent Bonding of Pyrrolbenzodiazepines (PBDs) to Terminal Guanine Residues within Duplex and Hairpin DNA fragments" (Mantaj J., Jackson P., Karu K., Rahman K.M., Thurston D.E., 2016, *PLOS ONE* 11(4):e0152303); 3) "From Anthramycin to Pyrrolbenzodiazepine (PBD)-Containing Antibody-Drug Conjugates (ADCs)" (Mantaj, J., Jackson, P. J., Rahman, K. M., Thurston, D. E., 2016, *Angew Chem Int Ed Engl* 56(2):462-488. DOI: 10.1002/anie.201510610). In addition, I have attended several national and international conferences including the Annual Meetings of the American Association for Cancer Research in 2013, 2014, 2015 and 2016.

Following my PhD, I have joined the biotech company Femtogenix Ltd (Welwyn Garden City, United Kingdom) as a postdoctoral research associate where I was responsible for the analytical and biological evaluation of molecules that are capable of binding reversibly and/or irreversibly to DNA, in a sequence-interactive manner, leading to exquisite cytotoxicity toward tumour cells. In April 2018, I have started a postdoctoral research associate position at King's College London within the Institute of Pharmaceutical Sciences with research interests in nanomedicine, particularly nanomedicine interaction with mucosal tissue including absorption/non-invasive delivery. During my research career, I have acquired various skills. My experience in biophysical techniques includes, HPLC, RP-HPLC, TLC, HPTLC, MALDI-TOF, ESI, UV, FRET, CD, gel electrophoresis (aga-

rose, PAGE). My skills in molecular biology cover cloning, plasmid transformation, DNA extraction and purification, enzymatic probing (DNaseI), radiolabelling of DNA with  $\alpha$ -<sup>32</sup>P-dATP, cell culture (primary and immortalised cell lines), cytotoxicity assays, ELISA, apoptosis assays, qPCR, Western blotting, DNA footprinting.

Furthermore, I am a registered pharmacist in Germany as well as United Kingdom and practice frequently as a locum pharmacist in community pharmacies in London.



## Jan-Niklas May

I am a cell biologist by training who is fascinated since childhood by the complexity of life itself. At the end of my undergraduate years during my Bachelor thesis in the lab of Prof. Dr. Ralph Panstruga at the department of Plant Molecular Cell Biology at RWTH Aachen University, I got in touch with the power of confocal microscopy which delighted myself for imaging as a

tool to make biological processes visible. During my Master thesis at the Institute for Experimental Molecular Imaging at Uniklinik RWTH Aachen, I applied several imaging techniques to visualize the accumulation of nanomedicine formulations. I was more than happy when my supervisor, Prof. Dr. Dr. Twan Lammers, offered me a PhD position in his lab to proceed with nanomedicine imaging. In a recently started, international project, we will investigate the combination of nanomedicine, chemotherapy and sonoporation (the combination of ultrasound and microbubbles to open the blood-brain barrier) as a treatment option for brain tumor patients suffering from DIPG (EuroNanoMed III - NSC4DIPG). Furthermore, I am a founding member and the treasurer of the German young Molecular Imaging Community (GyMIC), which is a group of young imaging scientists forming a network that is integrated into the European Society for Molecular Imaging (ESMI).



## Jitkasem Meewan

Strathclyde Institute of Pharmacy and Biomedical Sciences  
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Since I graduated B.Pharm. with second class honors from Chiang Mai University (Thailand) in 2006, my first job as a pharmacist at Boots was an opportunity for me to improve my leadership and communication skills. I matured in my business sense and ability to manage a team. My responsibilities in the drug dispensary enriched my knowledge of the pharmaceutical industry from the perspective of pharmacists and doctors on the ground.

After having experience as a community pharmacist, I returned to academic life and obtained an MSc in Pharmaceutical Technology from Chulalongkorn University (Thailand, 2013). My thesis aimed to investigate the effects of PEGylation on cellular uptake of phospholipid-based liposomes in the human intestinal epithelial Caco-2 cells. My research also centered on P-glycoprotein (P-gp), one of the significant efflux transporters, and how to enhance the delivery of P-gp substrates into the cells. During my study here, I obtained a partial financial support for Master's research from Higher Education Research Promotion and National Research University Project of Thailand and had an opportunity to present my work at the 38th Congress on Science and Technology of Thailand, 2012.



After graduation, I was offered a position as a Clinical Research Associate at Quintiles, which is now IQVIA. In this role, I coordinated with doctors and project teams to ensure quality and integrity of study practices as well as monitored protocol compliance of study sites to protect the rights and safety of clinical subjects. I was a team member in conducting Phase III clinical trials in areas of vaccines, infectious diseases and systemic lupus erythematosus (SLE). I received an award from a sponsor for high quality standard work in vaccine study and achieved 100% satisfaction on customer loyalty survey for SLE study.

I moved to the UK in 2015 and received my second MSc in Cancer Sciences, with merit from the University of Glasgow in 2016. My project was "Characterising circulating tumour cells". The "bench to bedside" approach of this program, which was run by Cancer Research UK, Glasgow, enabled me to learn the latest challenges in cancer research from groups of world-renowned scientists and cancer specialists. I learned about all aspects of cancer research from the fundamental principles of cancer cell biology to its application in clinics.

In 2017, I commenced my PhD journey at Strathclyde Institute of Pharmacy and Biomedical Sciences in Dr Christine Dufès Lab. I am currently working on the development of zein nanoparticles for biomedical applications.



## Tamás Mészáros

Tamás Mészáros, MSc, research fellow at Nanomedicine Research and Education Center, Semmelweis University and SeroScience Ltd., Budapest, Hungary. He received his MSc degree as an Immunologist from Eötvös Lóránd University in 2008, Budapest, Hungary. He is currently pursuing his PhD degree at Semmelweis University.

His research interest is complement system, liposomes and nanomedicine. His special skills include *in vitro* assays and techniques.



## Ana Milosevic

Ana Milosevic obtained her MSc degree in Biochemistry at Faculty of Chemistry, University of Belgrade. In 2014 she started her PhD research at the Adolphe Merkle Institute, University of Fribourg, in the group of Prof. Barbara Rothen Rutishauser and Prof. Alke Fink where she worked with fluorescently labelled nanoparticles and investigated their interaction with cell and ultimate fate. Currently she is a project manager for the newly established national contactpointnano.ch for the safe handling of nanomaterials, regulation and knowledge transfer.



## Nura Adam Mohamed

Nura Adam Mohamed is a Postdoctoral fellow at Qatar University who is working on implementing the advanced made in the nanotechnology field in medicine (nanomedicine) to improve the treatment and the detection strategies of a devastating cardiovascular disease called Pulmonary Arterial Hypertension (PAH). Dr. Mohamed

has obtained her BSc in biomedical science from Qatar University in 2009. Then she worked as a research assistant in the Safallah Medical Genetic Centre, Doha, Qatar gaining experience in in recombinant DNA, before joining the Qatar science leadership program (QSLP) in 2011 from Qatar Foundation and successfully getting a scholarship to Imperial College of London to pursue her studies. She finished her postgraduate studies (both MRes and PhD) in biomedical research from Imperial College of London. Following which she was awarded the Postdoctoral Research Award (PDRA) in 2017 which she started this year. She is a member of the British Pharmaceutical Society, the British Nanomedicine Society and holds an honorary research officer position at Imperial College of London. Her main focus is to apply nanotechnology and stem cells applications to improve cardiovascular disease treatments. Her disease of interest is PAH a devastating/incurable disease with available treatments limited by their short-half life, in addition to the systemic side effects in circulation. Using nanoparticles to trap PAH drugs will not only aid in limiting the systemic side effects but also allow a slow drug release which can increase the drug half-life in circulation. Some nanoparticles such as Metal organic frameworks (MOF); offer more attractive criteria's such as being flexible allowing more room for modifications to accommodate different PAH drugs, their pore sizes can be adjusted to control the rate of drug release and most importantly some MOFs can be traceable using MRI. By using this cutting edge technology we might be lucky enough to not only overcome the current drugs limitations indeed we might be able to convert PAH from a fast progressing disease in to a controllable chronic disease until a cure is developed.



## Daphne Montizaan

Daphne Montizaan was born on 26th of September 1992 in Geldrop, the Netherlands. After finishing high school, she did a Bachelor and Master degree in 'Molecular Life Sciences' at the University of Wageningen. Her educational choice already shows her interest in science, since these studies focus on research. During the Masters she did two internships in the field of Immunology. During her last internship at the University of Queensland in Brisbane, Australia, her interest was awakened for nanomedicine. After graduating cum laude, she started her PhD project at the University of Groningen. Currently, she is in the third year of her PhD where she studies the mechanism(s) by which nanoparticles enter cells.



## Marina Muehlberger

In 2011, I started my academic career with the study programme pharmacy at the Friedrich-Alexander-University Erlangen-Nuremberg (Erlangen, Germany) and graduated in second state examination in 2015. After completing my practical year in the pharmacy of the Klinikum Fuerth and a public pharmacy in Fuerth and passing my

third state examination, I was licensed as a pharmacist at the end of 2016. Since 2017, I am a PhD student at the Section of Experimental Oncology and Nanomedicine (SEON) in cooperation with the Division of Pharmaceutics, under the supervision of Prof. Dr. Christoph Alexiou and Prof. Dr. Geoffrey Lee. My research topic is the functionalisation of T lymphocytes for magnetically controlled immune therapy.



## Erik Örfi

I joined the Nanomedicine Research Center in 2013 through Scientific Students' Associations as a pharmacy student. I acquired my MSc. and Pharm.D degrees at Semmelweis University Faculty of Pharmacy in 2015. During my industrial practice at Servier Pharmaceuticals I investigated NCE (new chemical entity) structure confirmations by NMR, UV/IR, Raman spectroscopy and X-ray crystallography. My further experiences include pharmacy management, GLP and quality control. I'm currently a PhD student at Nanomedicine Research and Education Center at Semmelweis University. My topic is „Pathophysiology of nanomedicines, especially the cardiovascular effects”. I'm performing *in vivo* CARPA (Complement activation related pseudoallergy) experiments on pigs, rats and mice. I'm focusing now on the mice model, where I'm researching novel parameters of CARPA to characterize this phenomenon more precisely.

### MY RECENT PUBLICATIONS:

- Cardiovascular Manifestations of Complement Activation-Related Pseudoallergy Following Administration of Liposomal Nanomedicines. László Dézsi, Rudolf Urbanics, Tamás Mészáros, Csenge Vázsonyi, Tamás Fülöp, Erik Örfi, László Rosivall, János Szebeni, Gábor Szénási. *Acta Physiologica*. 2014 Aug Volume: 211. Pages: 94-94. Meeting Abstract: P4.27. IF: 4.066
- Features of complement activation-related pseudoallergy to liposomes with different surface charge and PEGylation: comparison of the porcine and rat responses. Dézsi L, Fülöp T, Mészáros T, Szénási G, Urbanics R, Vázsonyi C, Örfi E, Rosivall L, Nemes R, Kok RJ, Metselaar JM, Storm G, Szebeni J. *J Control Release*. 2014 Dec 10;195:2-10. doi: 10.1016/j.jconrel.2014.08.009. , IF: 7.633
- The immune system of the gut and potential adverse effects of oral nanocarriers on its function. Örfi E, Szebeni J. *Advanced Drug Delivery Reviews*. 2016 Nov 15;106(Pt B):402-409. doi: 10.1016/j.addr.2016.09.009. Review. IF: 17.214
- Non-Immunogenic Dextran-Coated Superparamagnetic Iron Oxide Nanoparticles: A Biocompatible, Size-Tunable Contrast Agent for Magnetic Resonance Imaging H Unterweger , C Janko , M Schwarz , L Dézsi , R Urbanics , J Matuszak , E Örfi , T Fülöp , T Bäuerle , J Szebeni , C Journé , AR Boccaccini , C Alexiou , Stefan Lyer , I Cicha *International Journal of Nanomedicine* 12: pp. 5223-5238. 2017 doi: <https://doi.org/10.2147/IJN.S138108>, IF: 5,008



## Stefanie Pektor

Since I graduated in Biology at the University of Mainz in 2006 with major focus on Zoology, Genetics and Immunology, I started my professional career in the group of Prof. Markus Neurath (Dept. of Internal Medicine I) as a research associate analyzing the role of the humoral immune system for the pathogenesis of inflammatory colorectal cancer. At the end of 2007 I decided to change the lab and started my PhD thesis in the group of Prof. Stephan Grabbe (Dept. of Dermatology). My main project was the functional analysis of the adhesion molecule LFA-1 and the intracellular Rho-GTPase Myosin9b (Myo9b) for dendritic cells (DC). I could find out that constitutively active LFA-1 on DC and loss of Myo9b in DC leads to reduced immune responses *in vitro* and *in vivo* but can be functionally restored *in vitro* by adding pharmacological inhibitors. After my PhD certificate in February 2013 I moved to the group of PD Dr. Matthias Miederer (Dept. of Nuclear Medicine) as a postdoctoral research fellow. Here, I could effectively bring in my professional immunologic knowledge and set up *in vitro* and *in vivo* assays for

the analysis of sugar metabolism after pharmacological treatment with TLR-ligands. Furthermore, I coordinate projects to evaluate new multifunctional nanoparticles in close cooperation with the multidisciplinary SFB1066. In line with that I develop pharmacokinetic PET/MRI studies analyzing the *in vivo* behavior of nanoparticles in different animal models (rodents). Furthermore, I monitor the biodistribution and toxicology of antibody therapy against cancer in external research collaborations and follow up therapy outcome by analyzing sugar metabolism using 18F-FDG-PET. One major focus of our SFB project is the evaluation of the EPR-effect of nanoparticles in different tumor entities. The knowledge of the strength of this effect without any specific targeting will help to improve therapy outcome, since nanoparticles with strong EPR-effect will be selected for further improvements like drug loading and unloading into the tumor lumen.

### RECENT PUBLICATIONS:

- K. Wagener, M. Worm, S. Pektor, M. Schinnerer, R. Thiermann, M. Miederer, H. Frey, F. Rösch. Comparison of Linear and Hyperbranched Polyether Lipids for Liposome Shielding by (18)F-Radiolabeling and Positron Emission Tomography. *Biomacromolecules*. 2018 Apr 27. doi: 10.1021/acs.biomac.8b00115.
- E. Eppard, A. de la Fuente, N. Mohr, M. Allmeroth, R. Zentel, M. Miederer, S. Pektor\*, F. Rösch\* (2018) Labeling of DOTA-conjugated HPMA-based polymers with trivalent metallic radionuclides for molecular imaging. \* equal contribution last authorship. *EJNMMI Res* (2018) 8: 16. doi:10.1186/s13550-018-0372-x
- M. Diken, S. Pektor, M. Miederer. Harnessing the potential of noninvasive *in vivo* preclinical imaging of the immune system: challenges and prospects. *Nanomedicine (Lond)*. 2016 Oct;11(20):2711-2722.
- S. Pektor, N. Bausbacher, O. Georg, S. Grabbe, M. Schreckenberger, M. Miederer. Toll Like Receptor Mediated Immune Stimulation can be Visualized in Vivo by FDG-PET. *Nucl Med Biol*. 2016 Jul 14;43(11):651-660. doi: 10.1016/j.nucmedbio.2016.07.004.
- Y. Xu\*, S. Pektor\*, S. Balkow, K. Grobe, P. J. Hanley, L. Shen, M. Bros, M. Bähler and S. Grabbe. The Rho-GTPase-activating protein myosin IXb regulates dendritic cell migration and interaction with T cells. \* equal contribution. *J Immunol*. 2014 Apr 15;192(8):3559-68.



## Elzbieta Pietrzykowska

MSc Eng.

Laboratory of Nanostructures, Institute of High-Pressure Physics, Polish Academy of Sciences, Sokolowska 29/37, 01-142 Warsaw, Poland

Email: [e.pietrzykowska@labnano.pl](mailto:e.pietrzykowska@labnano.pl)

Elzbieta is a materials science engineer. Graduated from the Faculty of Materials Science and Engineering, Warsaw University of Technology, with the specialization in materials design. Has worked at the UNIPRESS Institute of High Pressure Physics, Polish Academy of Sciences, at The Laboratory of Nanostructures since October 2011. She began her doctoral programme at the Faculty of Materials Science and Engineering, Warsaw University of Technology. Intends to address the topic of resorbable biomaterials for orthopaedics, methods of their production, moulding and characterisation in her doctoral dissertation. The interest and exceptional inventiveness accompanying the performance of this research resulted in the formulation of the title of the doctoral dissertation: "New methods of production of composites from nano-hydroxyapatite and polymers for application in orthopaedics."

### PARTICIPATED IN THREE RESEARCH PROJECTS EXECUTED AT THE LABORATORY OF NANOSTRUCTURES:

- BIO-IMPLANT – "Bioimplants for the treatment of bone tissue le-

- sions in oncological patients,
- SONOSCA –“Sonochemical technology for bioactive bone regeneration scaffold production”,
  - GoIMPLANT –“Tough, Strong and Resorbable Orthopaedic Implants”
  - iTE –Method of treatment of large bone defects in oncological patients using *in vivo* tissue engineering approach
- The focus is on forming, under high pressure, composites, nanoceramic powders and the characterisation of the obtained materials (structure, morphology and mechanical properties).

#### RECENT PUBLICATIONS:

- J.Wojnarowicz, A.Opalinska, T.Chudoba, S.Gierlotka, R.Mukhovskiy, E.Pietrzykowska, K.Sobczak, and W.Lojkowski, “Effect of Water Content in Ethylene Glycol Solvent on the Size of ZnO Nanoparticles Prepared Using Microwave Solvothermal Synthesis”, *Journal of Nanomaterials*, Volume 2016, pp. 1-15
- Pajchel L, Kowalska V, Smolen D, Kedzierska A, Pietrzykowska E, Lojkowski W, Kolodziejcki W, “Comprehensive structural studies of ultra-fine nanocrystalline calcium hydroxyapatite using MAS NMR and FT-IR spectroscopic methods”, 2013, “*Materials Research Bulletin*”,48,11,4818,4825
- Kędzierska V, Ostrowska B, Smoleń D, Pietrzykowska E, Świążkowski W, Gedanken A, Łojkowski W, “Ultrasonic coating technique of a polymer scaffolds for bone implant applications” “*European Cells and Materials*”, 2013,17 str, Vol. 26 Suppl.



### Chandrashekar Prasad

Chandrashekar Prasad completed his B.Sc. in biotechnology from B N College, Patna, India in 2011. Then he joined Indian Institute of Technology Bombay for M.Sc.-Ph.D. integrated programme. He is currently pursuing his doctoral research under the guidance of Prof. Rinti Banerjee on “Ultrasound triggered nanocarriers for drug delivery in cancer”. He has been awarded with DBT-JRF 2013 by the Department of Biotechnology, Ministry of Science and Technology, India.

#### LIST OF PUBLICATION /SEMINAR/CONFERENCES

1. Seminar on “Mechanism of action of antibiotics” organized by IIT Bombay on August 11, 2012
2. Seminar on “Biotechnology and its expanding horizon” at B. N. College, Patna, Bihar on 28th February 2009.
3. Review paper published: Shahdeep Kaur , Chandrashekar Prasad , Biji Balakrishnan and Rinti Banerjee. Trigger responsive polymeric nanocarriers for cancer therapy, *Biomater. Sci.*, 2015, 3, 955-987.
4. Presented poster: Ultrasound enhanced cellular uptake and Cytotoxicity of topotecan HCl and curcumin encapsulated chitosan nanocapsule microbubble conjugates, ISNACT 2015, 19-21st Feb 2015, Organised at: Indian Institute of technology, Bombay, Mumbai.

#### PATENT

1. Curcumin Formulation (Patent Application No. 201621028126 )



### Sayoni Ray

Ray is pursuing Ph.D. in the department of Chemistry and Biochemistry at the University of California, Los Angeles (UCLA) since September 2013 and advanced to candidacy in August 2015. She has been working with her advisor Prof. Yung-Ya Lin to develop an innovative method to detect tumors at a very early stage using magnetic

resonance imaging (MRI) and treat them by targeted drug delivery with controlled drug release and online monitoring. Her dissertation thesis is on “High Contrast Magnetic Resonance Imaging for Early Tumor Detection and Cancer Nano-Theranostics”. Her research interest includes cancer nano-theranostics, nanomedicine, MRI pulse sequence designing for early tumor detection, magnetic hyperthermia, theranostic nanomaterial synthesis, targeted drug delivery and controlled drug release.

Previously she presented posters and gave talks at the International Cancer Imaging Society (ICIS) – 2015, London, UK (title: Contrast Enhancement for Early Cancer Imaging by Gd-Nanoparticles and Active Feedback MRI) and International Society of Magnetic Resonance in Medicine (ISMRM) – 2017, Honolulu, US (title: Early Cancer Detection Using Paramagnetic Liposome by a Novel Contrast Mechanism with Active-feedback Magnetic Resonance Imaging”). National Science Foundation (NSF-EAPSI), USA awarded her a summer fellowship to visit the National Taiwan University in the summer of 2016 to set up the protocols of this new procedure of MRI in their *in vivo* laboratory to perform experiments on mice. She was awarded a special departmental fellowship (2013-2016) by Chemistry department of UCLA and she received Pauley Fellowship (2016-2017) from UCLA.

In the graduate school, she has gained experience working with *in vivo* and *in vitro* magnetic resonance imaging (MRI), nuclear magnetic resonance (NMR) spectroscopy, nanomaterial synthesis, transmission electron imaging (TEM) , cryo-TEM, dynamic light scattering (DLS), UV-Vis Spectroscopy, fluorescence spectroscopy, inductively coupled plasma optical emission spectrometry (ICP-OES), cell culture and magnetic heating (hyperthermia) experiments. She has experience in MATLAB programming for data acquisition, analysis and theoretical modelling of spin dynamics, and writing manuscripts for publication.

Before entering the field of magnetic resonance imaging for cancer detection, she studied undergraduate Chemistry in India and received the Innovation in Science Pursuit for Inspired Research (INSPIRE) scholarship from the Department of Science & Technology (DST), Govt. of India, for her academic excellence. She obtained first class B.Sc. (Hons) degree in Chemistry from the University of Calcutta, India with distinction and M.Sc. from the Indian Institute of Technology (IIT), Kanpur, India. During her stay in India, she was associated with a couple of research projects, where she learnt different experimental techniques like bacteria culture, protein extraction and purification, atomic force microscopy etc. and applied them to biological problems like protein folding. One of her undergraduate summer projects was on single molecule atomic force microscopy on protein folding mechanism. This work was published in the *Journal of American Chemical Society* (“Multiple unfolding pathways of leucine binding protein (LBP) probed by single-molecule force spectroscopy (SMFS)” *J. Am. Chem. Soc.*, 2013, 135 (39), pp 14768–14774) and highlighted in later issues.

Ms. Sayoni Ray has extensive teaching experience at the undergraduate level at UCLA and is very passionate about her teaching. She received Hanson-Dow Distinguished Teaching Assistant award in 2015 from the Department of Chemistry and Biochemistry, University of California, Los Angeles for excellence in teaching.





## Susanne Resch

Mag. pharm. Susanne Resch, MSc (female): studied Pharmaceutical Sciences at University of Graz; focused on pharmaceutical technologies, including novel drug delivery systems like various nanoparticles/nanocarriers. Worked for her thesis on the intestinal uptake of nanoparticulate systems, including the interactions of nano-

particles with cell surfaces in different cell culture models. Within BioNanoNet she is working as scientist addressing the key topics nano-safety & safe-by-design (i.e., EU H2020 pilot line projects Hi-Response, INSPIRED, R2R Biofluidics; national project SbD-AT), regulations and standardisation in the field of nanotechnologies, as well as modeling nanomaterials toxicity (i.e., EU H2020 MSCA-RISE NANOENTOOLS). In addition, she is responsible for dissemination actions and coordination of training schools (i.e., EU H2020 BIORIMA). She is part of national and international working groups (e.g. "Task Force Safety" for High Level Group of EU Member States and Associated Countries on Nanosciences, Nanotechnologies and Advanced Materials; EU-U.S. Building Bridges NanoEHS; COST-Action AMiCi) and co-coordinates the platform NanoMedicine-Austria.



## Julia Rogowska-Tylman

MSc Eng. Research Associate at Laboratory of Nanostructures, Institute of High-Pressure Physics, Polish Academy of Sciences, Sokolowska 29/37, 01-142 Warsaw, Poland Email: juliaRT@labnano.pl

Graduated from the Faculty of Medicine of the Medical University of Warsaw in the field of dental materials in 2011 and the Faculty of Materials Science and Engineering of the Warsaw University of Technology with a field of biomaterials in 2014. In 2015 became the founding member of the Polish Nanomedicine Association. Currently, PhD student at Warsaw University of Technology and postgraduate student at MBA – Innovation and Data Analysis studies at IPI PAS (Poland)/ Woodbury School of Business, Utah Valley University (USA). Her research work mainly focuses on surface modification of metallic, ceramic and polymeric implants with bioactive nanoparticles by ultrasonic coating and fabrication and pre-clinical studies of fibrous polymer implants for maxillo-facial and oral surgery and implantology.

### Experience:

- 2013 Internship student at National Institute for Materials Science, International Center for Materials Nanoarchitectonics (MANA), Biomaterials Unit, Biomaterials Group, Tsukuba, JAPAN
- Since 2014 Research Associate at Laboratory of Nanostructures for Photonics and Nanomedicine, Institute of High Pressure Physics, Polish Academy of Sciences
- Since 2014 PhD studies at Faculty of Materials Science and Engineering Warsaw University of Technology. PhD thesis studies conducted at Laboratory of Nanostructures IHPP PAS.
- Since 2015 Secretary of the Board at Polish Nanomedicine Association
- 01.2016 - 11.2016 Guest PhD student – HAPfibers project at Swiss Federal Laboratories for Materials Science and Technology (EMPA), St.Gallen, SWITZERLAND

### RECENT PUBLICATIONS:

- M.S. Enayati, T. Behzad, P. Sajkiewicz, R. Bagheri, L. Ghasemi-Mobarakeh, S. Kuśnieruk, J. Rogowska-Tylman, Z. Pahlevanneshan, E. Choińska and W. Świążkowski Fabrication and characterization of electrospun bionanocomposites of poly (vinyl alcohol)/nanohydroxyapatite/cellulose nanofibers INTERNATIONAL JOUR-

NAL OF POLYMERIC MATERIALS AND POLYMERIC BIOMATERIALS, 2016, VOL. 65, NO. 13, 660–674.

- J.Rogowska-Tylman, N. Saha, A. Chodara, O. Zandraa, W. Łojkowski, P. Saha Monograph, Nanotechnological approach to bacterial cellulose/nanohydroxyapatite biocomposites fabrication for bone regeneration, Faculty of Chemical and Process Engineering, Warsaw University of Technology, 2018. ISBN: 978-83-936575-5-1.



## Intouch Sakpakdeejaroen

Strathclyde Institute of Pharmacy and Biomedical Sciences  
University of Strathclyde  
161 Cathedral Street  
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I obtained my B.Pharm from the Faculty of Pharmaceutical Science, Prince of Songkla University (Thailand) in 2006 and MSc in Medical Sciences at the Faculty of Medicine, Thammasat University (Thailand) in 2009, where I was awarded "Best Thesis" award. I became a lecturer at the Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University (Thailand) in 2009.

I decided to pursue a PhD at Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde (Glasgow, UK) in Dr Christine Dufès Lab in 2015. I am currently working on the development of novel drug delivery systems for tumour targeting.

### PUBLICATIONS

- Ratanavalachai, T., Thitiorul, S., Tanuchit, S., Itharat, A. and Sakpakdeejaroen, I. 2015. In Vitro Enhancement of Doxorubicin Genotoxic Activities and Interference with Cell Cycle Delay by Plumbago indica Root Ethanolic Extract in Human Lymphocytes. Journal of the Medical Association of Thailand, 98(Suppl.2): 38-44.
- Rattarom, R., Sakpakdeejaroen, I., Hansakul, P. and Itharat, A. 2014. Cytotoxic activity against small cell lung cancer cell line and chromatographic fingerprint of six isolated compounds from the ethanolic extract of Benjakul. Journal of the Medical Association of Thailand, 97(Suppl.8): 70-75.
- Thitiorul S., Ratanavalachai T., Tanuchit S., Itharat A. and Sakpakdeejaroen I. 2013. Genotoxicity and Interference with Cell Cycle Activities by an Ethanolic Extract from Thai Plumbago indica Roots in Human Lymphocytes in Vitro. Asian Pacific Organization for Cancer Prevention, 14(4): 2015-2018
- Ratanavalachai, T., Thitiorul, S., Tanuchit, S., Sakpakdeejaroen, I. and Itharat A. 2012. In vitro Cytotoxic and Genotoxic Effects of Ethanolic Extract of Pikutbenjakul on Human Lymphocytes. Thammasat Medical Journal, 12(2): 262-268
- Ruangnoo S., Itharat A., Sakpakdeejaroen I., Rattarom R., Tappayutpijarn P., Kumarn Pawa K. 2012. In Vitro Cytotoxic Activity of Benjakul Herbal Preparation and Its Active Compounds against Human Lung, Cervical and Liver Cancer Cells. Journal of the Medical Association of Thailand, 95(Suppl.1): 127-134



## Oana G. Schramm

Dr. Oana G. Schramm obtained her Bachelor of Science degree in organic chemistry from Technical University "Gh. Asachi" Iasi, Romania, in 1998 and her Master of Science in organic chemistry from Technical University of Bucharest, Romania, in 1999. She earned her Ph.D. in chemistry from the University of Heidelberg, Organic

Chemistry Institute (OCI), Germany, where she worked on the one-pot synthesis of novel and naturally occurring heterocycles using metal-catalyzed cross-coupling reactions. She then pursued post-doctoral research at the University of Eindhoven, Department of Macromolecular Chemistry and Nano Science (SMN) developing new biocompatible polymeric nanocontainers as drug delivery systems.

As project coleader at Fraunhofer Institute for Molecular Biology and Applied Ecology in Aachen, she worked on functionalization and detection of nanomaterials in complex biological matrices. Currently, she is working on synthesis and characterization of novel polymeric nanomaterials for oral drug delivery, at TH Köln, Department of Macromolecular Chemistry & Polymer Technology, in Leverkusen, Germany.

Main research Interests: Synthesis, surface functionalization, characterization and detection of smart nanomaterials for biological applications.

#### SELECTED PUBLICATIONS:

- Rapid One-pot Synthesis of Anti-parasitic Quinolines Based upon the Microwave-Assisted Coupling-Isomerization Reaction (MACIR), Oana G. Schramm, Thomas Oeser, Marcel Kaiser, Reto Brun, Thomas J. J. Müller, Synlett. 2008, 3, 359-362.
- Polymeric Nanocontainers with High Loading Capacity of Hydrophobic Drugs, Oana G. Schramm, Michael A. R. Meier, Richard Hoogenboom, Hannes H. P. van Erp, Jean François Gohy, Ulrich S. Schubert, Soft Matter 2009, 5, 1662-1667.
- A Versatile Approach to Unimolecular Water-Soluble Carriers: ATRP of PEGMA with Hydrophobic Star-Shaped Polymeric Core Molecules as An Alternative for PEGylation, Oana G. Schramm, Georges M. Pavlov, Michael A. R. Meier, Richard Hoogenboom, Ulrich S. Schubert, Macromolecules 2009, 42, 1808-1816.
- pH-Dependent Nano-Capturing of Tartaric Acid Using Dendrimers, Oana G. Schramm, Xaviera López-Cortés, Leonardo S. Santos, V. Felipe Laurie, Fernando Danilo González Nilo, Michael Krolik, Rainer Fischer, Stefano Di Fiore, Soft Matter, 2014, 10, 600-608.
- Methods for Providing Stable Isoindole Derivatives, Oana G. Schramm, Stefano Di Fiore, Rainer Fischer U.S. Pat. Appl. Publ. 2016, US 20160137601 A1 20160519.



### Floriane Séquier

Senior Formulation Scientist, AstraZeneca, UK

Floriane Séquier is a Senior Formulation Scientist in AstraZeneca, in Pharmaceutical Technology and Development (based at Macclesfield, UK). She is a pharmacist by trainings and joined AstraZeneca in 2014 after completing a PhD in drug delivery at the Institut Galien Paris Sud (UMR CNRS 8612). Floriane worked on the development of oral solid dosage forms during 3 years before joining the parenteral area where her work is now focused on the development of nanomedicine.

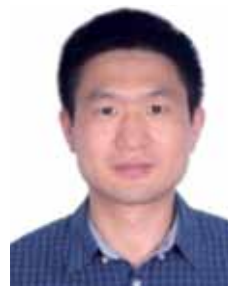


### Maja Severic

Queen's University Belfast, School of Pharmacy  
E-mail: mseveric01@qub.ac.uk

My name is Maja Severic and I obtained my BSc Degree in Biology from Faculty of Science, University of Zagreb, Croatia in 2013. After graduation, I enrolled double master

programme of bio-industrial techniques between the University of Orleans, France and the University of Zagreb. During my master study, I spent six months at the University of Montpellier, France working on my master project "Synthesis and functionalization of mesoporous silica nanoparticles for nucleic acid delivery", and in 2015 I graduated with a Master Degree in Experimental Biology. In September 2016, I joined the School of Pharmacy at the University of East Anglia, UK to undertake my PhD study in the development of novel drug delivery systems by engineering PSMA-targeted exosome-like vesicles for advanced prostate cancer treatment. Since August 2017, I moved to the Queen's University Belfast together with my research group to continue my PhD project.



### Yang Shi

Yang Shi joined Prof. Wim Hennink's group (Utrecht University, the Netherlands) in 2010, where he completed his PhD research in 2014, focusing on chemical synthesis and *in vivo* studies of polymeric micelles for tumor-targeted drug delivery and imaging. After PhD, he was appointed as an Associate Professor at South China

University of Technology, and subsequently as a Group Leader at RWTH Aachen University Clinic in Germany. His research group "Polymeric Nanomedicines" is working on nanomedicines based on synthetic polymers for tumor-targeted drug delivery. He has published >25 articles in peer-reviewed journals and serves as a reviewer for numerous journals including ACS Nano, Angewandte Chemie International Edition, Advanced Functional Materials, Journal of Controlled Release, etc. In 2016, he was elected as a "Rising Star" by the 4th Symposium on Innovative Polymers for Controlled Delivery (SIPCD).



### Oscar Ferreira Silvestre

Oscar F. Silvestre is a postdoctoral fellow at INL - International Iberian Nanotechnology Laboratory, Braga, Portugal. In 2016 he was awarded a Marie Curie COFUND and joined the Ultrafast Bio- and Nanophotonics group under the supervision of Jana B. Nieder. His main research topics are related with the development of mitochondria nanoplatfoms for drug delivery and advanced imaging to track the therapeutics delivery, including sensing the organelle dynamics/metabolism in live cells.

Oscar F. Silvestre holds a degree in Biotechnology Engineering from University of Algarve, Portugal. He was awarded a Richard Whipp interdisciplinary studentship and received his PhD in 2011 from the School of Medicine/School of Physics, Cardiff University, Wales, UK in the field of cellular imaging and systems cytometry. His graduate research include innovative techniques to study tumor cells drug response inside 3D hollow fiber implants 1. Concretely single cell analysis with the use of quantum dots to track live cells proliferation by fluorescent microscopy 2 and flow cytometry 3.

As an AXA Research Fund postdoctoral fellow he joined in 2012 the Laboratory of Molecular Imaging and Nanomedicine, NIBIB-National Institutes of Health, USA, where he worked on nanoparticle platforms for gene/drug therapy and pre-clinical models studies. His research contribution was crucial for the development and optimization of a nanoparticle platform that was able to incorporate both RNA and anticancer drugs for combined cancer therapy, resulting in two high impact publications 4,5. Additional, gained experience in working with several types of nanoparticles (gold, carbon nano-

tubes, graphene and polymer nanoparticles) for both *in vitro* and *in vivo* biomedical research.

Overall, Oscar F. Silvestre has a vast international and multidisciplinary experience, ranging from the development of new methodologies for cell tracking using flow cytometry and optical imaging, whole genome expression profiling, to nanomedicine platforms for RNA/drug delivery and sensing. His main research goals are centered on the development of “precision tools” to reverse engineer and modulate biological systems towards translatable diagnostic/therapeutic solutions.

## SELECTED PUBLICATIONS

- Smith PJ, Griesdoorn V, Silvestre OF, Errington RJ. (2017) Microenvironment Cytometry. In Single Cell Analysis: Contemporary Research and Clinical Applications; Robinson JP, Cossarizza A, Eds, Springer Singapore: 1–38.
- Holton MD, Silvestre O, Errington RJ, Smith PJ, Matthews DR, Rees P, Summers HD. (2009) Stroboscopic fluorescence lifetime imaging. *Optics Express* 17(7): 5205-5216.
- Errington RJ, Brown MR, Silvestre OF, Njoh KL, Chappell SC, Khan IA, Rees P, Wilks SP, Smith PJ, Summers HD. (2010) Single cell nanoparticle tracking to model cell cycle dynamics and compartmental inheritance. *Cell Cycle* 9(1): 121-130.
- Choi KY#, Silvestre OF#, Huang X#, Hida N, Liu G, Ho DN, Lee S, Lee SW, Hong JI, Chen X. (2014) A nanoparticle formula for delivering siRNA or miRNAs to tumor cells in cell culture and *in vivo*. *Nature Protocols* 9 (8): 1900-1915.
- Choi KY#, Silvestre OF#, Huang X, Ming KH, Howard GP, Hida N, Jin AJ, Carvajal N, Lee SW, Hong JI, Chen X. (2014). Versatile RNA Interference Nanoplatfor for Systemic Delivery of RNAs. *ACS Nano* 8 (5): 4559-4570.

# co-first authors



## Sofie Snipstad

Sofie Snipstad holds a MSc in Nanotechnology and a PhD in Biophysics from the Norwegian University of Science and Technology (NTNU). She is currently employed as a Postdoc at the Department of Physics, NTNU, and as a Research Scientist at Department of Biotechnology and Nanomedicine, SINTEF Industry, both located

in Trondheim, Norway. Her research is focused on ultrasound-mediated delivery of nanomedicine across biological barriers, for improved treatment of cancer and diseases in the brain.

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## RECENT PUBLICATIONS INCLUDE:

- Sulheim E, Kim J, van Wamel A, Kim E, Snipstad S, Vidic I, Hovde Grimstad I, Wideroe M, Torp SH, Lundgren S, Waxman DJ, Davies C. Multi-modal characterization of vasculature and nanoparticle accumulation in five tumor xenograft models. *Journal of Controlled Release* (2018)
- Baghirov H, Snipstad S, Sulheim E, Berg S, Hansen R, Thorsen F, Mørch Y, Davies C, Åslund AK. Ultrasound-mediated delivery and distribution of polymeric nanoparticles in the normal brain parenchyma and melanoma metastasis. *PLoS ONE* 13(1): e0191102 (2018)
- Snipstad S, Berg S, Mørch Y, Bjørkøy A, Sulheim E, Hansen R, van Wamel A, Maaland AF, Torp SH, Davies C. Ultrasound improves the delivery and therapeutic effect of nanoparticle-stabilized microbubbles in breast cancer xenografts. *Ultrasound in Medicine and Biology* 43, 2651-2669 (2017)
- Åslund A, Snipstad S, Healey A, Kvåle S, Torp SH, Sontum PC, Davies C, van Wamel A. Efficient Enhancement of Blood–Brain-Barrier Permeability Using Acoustic Cluster Therapy (ACT). *Theranostics* 7, 23-30 (2017)

- Mørch Y, Hansen R, Berg S, Åslund A, Glomm W, Eggen S, Schmid R, Johnsen H, Kubowicz S, Snipstad S, Sulheim E, Hak S, Singh G, McDonagh B, Blom H, Davies C, Stenstad P. Nanoparticle-stabilized microbubbles for multimodal imaging and drug delivery. *Contrast Media & Molecular Imaging* 10, 356-366 (2015)



## Sukrut Somani

Sukrut Somani studied Bachelor of Pharmacy at the Institute of Pharmacy, Nirma University, India (2010). He was awarded an overseas scholarship (part tuition fee) from The School of Pharmacy, University of London (now UCL School of Pharmacy) to undertake MSc in Drug Delivery. During his MSc, he undertook a research project entitled “In vitro Cytotoxicity of carbon nanomaterials on Erythrocytes” under the supervision of Professor Kostas Kostarelos (2011) ([www.nanomedicinelab.com](http://www.nanomedicinelab.com)). In 2012, he joined a PhD programme at University of Strathclyde under the supervision of Dr Christine Dufès ([www.dufeslab.com](http://www.dufeslab.com)) on a project entitled “Novel gene delivery systems for brain targeting”. He was awarded the “Best Poster” prize at Nanomedicine conference in 2014 (Edinburgh, UK) and selected for a “Research Highlight talk” at the 42nd Annual meeting and Exposition of the Controlled Release Society (Edinburgh, UK). He has published 10 peer-reviewed articles in the field of nanomedicine. His 5 most recent research publications are as follows:

## RECENT PUBLICATIONS:

- S. Somani, P. Laskar, N. Altwaijry, P. Kewcharoenpong, C. Irving, G. Robb, B. Pickard, C. Dufès. “PEGylation of polypropylenimine dendrimers: effects on cytotoxicity, DNA condensation, gene delivery and expression in cancer cells. *Scientific Reports*. 2018 Jun 20;8(1):9410 (Impact Factor 2018: 4.2)
- N. Altwaijry, S. Somani, J. Parkinson, R. J. Tate, P. Keating, M. Warzecha, G. R. Mackenzie, H. Y. Leung, C. Dufès. Regression of prostate tumors after intravenous administration of lactoferrin-bearing polypropylenimine dendriplexes encoding TNF- $\alpha$ , TRAIL and Interleukin-12. *Drug delivery*, 25(1): 679-689 (Impact factor 2016: 6.4)
- R. Karim, S. Somani, M. Al Robaian, M. Mullin, R. Amor, G. McConnell, C. Dufès (2017). Tumor regression after intravenous administration of targeted vesicles entrapping the Vitamin E  $\alpha$ -tocotrienol, *Journal of Controlled Release*, 246, 79-87 (Impact factor 2017: 7.6)
- S. Somani, G. Robb, B. Pickard, C. Dufès (2015), Enhanced gene expression in the brain following intravenous administration of lactoferrin-bearing polypropylenimine dendriplex, *Journal of Controlled Release*, 217, 235-242 (Impact factor 2015: 7.7)
- L.Y. Lim, P.Y. Koh, S. Somani, M. Al Robaian, R. Karim, Y.L. Yean, J. Mitchell, R.J. Tate, R. Edrada-Ebel, D.R. Blatchford, M. Mullin, C. Dufès (2015), Tumor regression following intravenous administration of lactoferrin- and lactoferricin-bearing dendriplexes, *Nanomedicine: Nanotechnology, Biology and Medicine*, 11, 1445-1454 (Impact factor 2015: 5.9)



## Einar Sulheim

PhD student NTNU Biophysics/Researcher SINTEF Biotechnology and Nanomedicine

Einar Sulheim is a phd candidate at the Norwegian University of Science and Technology department of physics and MSc at SINTEF Biotechnology and Nanomedicine. He has a master degree in bionanotechnol-



ogy from NTNU in 2014. In his phd Einar is studying how nanoparticles and ultrasound can improve the therapy of tumor xenografts with different vascular properties. His project is supervised by professor Catharina de Lange Davies and is in collaboration with the medical faculty at St.Olavs hospital and with SINTEF Materials and Chemistry.

At SINTEF Einar is working on the development of polymeric nanoparticles, specifically on poly(alkyl cyanoacrylates) (PACAs), particle-loaded micro bubbles and on different characterization methods.

#### RECENT PUBLICATIONS:

1. Sulheim et al. 2018. Multi-modal characterization of vasculature and nanoparticle accumulation in five tumor xenograft models. *J Control Release*, 279, 292-305.
2. Sulheim et al 2017. Cytotoxicity of Poly(Alkyl Cyanoacrylate) Nanoparticles. *International Journal of Molecular Sciences*, 18.
3. Åslund, Sulheim et al. 2017. Quantification and Qualitative Effects of Different PEGylations on Poly(butyl cyanoacrylate) Nanoparticles. *Mol Pharm*.
4. Sulheim et al. 2016. Cellular uptake and intracellular degradation of poly(alkyl cyanoacrylate) nanoparticles. *J Nanobiotechnology*, 14, 1.



### Gábor Szénási

Gábor Szénási, is biologist researcher, currently a senior research fellow at the Institute of Pathophysiology, Semmelweis University in Budapest, Hungary. He is a native of Budapest, born in 1950. He obtained his M.Sc. degree in biology at the Eötvös Loránd University (1975, Budapest). He started to work at the 2nd Department

of Medicine, Semmelweis University. At that time his research focused on the neural regulation of kidney function and hemodynamic characteristics of rats with bilaterally denervated kidneys. He obtained his Candidates Degree (PhD) in medical sciences from the Hungarian Academy of Sciences in 1991. From 1991 he spent two years at the Baker Medical Research Institute, Melbourne, Australia. During this period he studied renovascular hypertension in conscious dogs, and the role of the kidneys in blood pressure regulation.

Returning home he joined EGIS Pharmaceuticals and worked as a pharmacologist in drug discovery. He soon he became the head of the Cardiovascular Pharmacology Laboratory. His research tasks focused on cardioprotective and antiarrhythmic agents. Later his interest turned to stroke studies, which yielded several publications on the effects of neuroprotective agents. After conclusion of the stroke project his duty was to perform hemodynamic safety pharmacology tests in compliance with GLP regulations, and he was also responsible for testing pharmacokinetic and metabolic characteristics of drug candidates. During this period he also led a project aiming to test chronobiological actions of drug candidates. He also collaborated in theoretical and clinical human arrhythmia research, primarily as an expert of statistical processing of data.

From 2012 he joined Institute of Pathophysiology, Semmelweis University. His research interest returned to nephrology, primarily to the mechanism leading to chronic kidney disease, and he also started to study complement activation-related pseudoallergy (CARPA) focusing on the hemodynamic effects of liposomes in rodents. He is a tutor of two PhD students, and also some students involved in research work under the patronage of Students' Scientific Association of Semmelweis University.

Gábor Szénási published 93 papers and 6 book chapters as a co-author and filed more than 30 patents as a co-inventor. The cumulative impact factor of his papers is more than 220, and his works have been cited more than 1700 times (Google Scholar) excluding books and book chapters. He has never applied for any prize, award

or medal. He has refereed countless number of scientific papers for various journals such as *Neurochemical Research*, *European Journal of Pharmacology*, *Human and Experimental Toxicology*, *British Journal of Pharmacology*, etc., and national grant applications. He is the co-editor of *Physiology International*. He is the Member of the Presidency of the Hungarian Physiological Society, and the member of the Hungarian Hypertension, Nephrology and Cardiology Societies.



### Mohammad Taleb

Ph.D. student

Mohammad is a Ph.D student at the National Center for Nanoscience and Technology, China. He received his B.S. degree from Shahid Beheshti University in 2015, followed by an M.S. degree from University of Chinese Academy of Sciences (NCNST) in 2017. In 2017, he accepted in Ph.D qualification exam from NCNST, Chinese Academy of Sciences. He was ranked in the upper 5 % among around 500,000 participants in the nationwide undergraduate entrance exam of universities in Iran 2011. He has been research member at two bilateral project and symposium between Chinese Academy of Sciences and Vice Presidency for science and technology of Iran. His main interests are design of bio-inspired materials to overcome the barriers in tumor therapy and nanobiomedicine. He would like to working toward the controlling the chemical properties of multi-functional nanoparticles in order to allow specific targeting and regulation of tumor cells and their microenvironment.

**His research interests include:** Targeting and regulation of tumors and their microenvironment mediated by intelligent functional nanomaterials for diagnostic and therapeutic applications, especially Breast cancers.

#### RECENT PUBLICATION:

- Nasrollahi, Parisa; khajeh, khosro; tamjid, elnaz; taleb, mohammad; Soleimani, Masoud; Nie, Guangjun. Sustained release of sodium deoxycholate from PLGA-PEG-PLGA thermosensitive polymer. *Artificial Cells, Nanomedicine and Biotechnology* 2018
- Bin Wang, Yanping Ding, Xiaozheng Zhao, Xuexiang Han, Na Yang, Yinlong Zhang, Ying Zhao, Xiao Zhao, Mohammad Taleb, Qing Robert Miao, Guangjun Nie. Delivery of small interfering RNA against Nogo-B receptor via tumor-acidity responsive nanoparticles for tumor vessel normalization and metastasis suppression. *Biomaterials* 2018

submitted:

- Yang, Na ; Ding, Yanping; Zhang, Yinlong; Wang, Bin; Zhao, Xiao; Cheng, Keman; Huang, Yixin; Taleb, Mohammad; Zhao, Jing; Dong, Wen-Fei; Nie, Guangjun. Surface Functionalization of Polymeric Nanoparticles with Umbilical Cord-Derived Mesenchymal Stem Cell Membrane for Tumor-Targeted Therapy. *ACS Applied Materials & Interfaces* 2018

**Book:** Translate book 'essential laboratory skills for bioscience' (English to Persian) 2013



### Barbora Tesarova

Barbora Tesarova is currently in the first year of her PhD studies at Department of Chemistry and Biochemistry, Mendel University in Brno, Czech Republic. She is a member of a Research group for Molecular Biology and Nanomedicine, which is led by Dr. Zbynek Heger.

Her research is focused on the investiga-

tion of nanoparticle surface modifications to control their interactions with internal milieu of multicellular organisms. The aim of this research is to find a surface modification, which is suitable for use in drug delivery systems. Appropriate surface modification should eliminate protein corona formation, prolong half-time of encapsulated drug, avoid aggregation of nanoparticles, etc. She became Brno Ph.D. Talent scholarship holder with this topic in 2017. She was also awarded 2nd place at the conference competition MendelNET 2017.

Barbora holds a Bachelor and a Master's degree in Chemistry for medical applications from the Faculty of Chemistry, Brno University of Technology, Czech Republic. Her bachelor and master thesis dealt with preparation of polyelectrolyte gels as potential drug carriers. During her studies, she also spent one semester at Instituto Superior Técnico in Lisbon, Portugal by joining the Erasmus+ program.



## Kanika Thakur

M.Pharm., Ph.D pursuing  
(DST INSPIRE-SRF) Commonwealth PhD  
Research Scholar  
E-mail id: kanikathakur95@yahoo.com

### EDUCATION AND RESEARCH EXPERIENCE

**Commonwealth PhD Split-Site Scholar (2017-Till date)**- Working on Development of Biomimetic scaffolds for wound healing at Centre for Biomolecular Sciences, University of Nottingham, United Kingdom

**DST INSPIRE PhD Senior Research Fellow (2014-2017)** – Worked on QbD based approaches to develop nanomedicine carriers for anti-infectives at University Institute of Pharmaceutical Sciences, Panjab University, Chnadigarh, India

**Masters in Pharmacy (Pharmaceutics, 2011-2013)** – Worked as a Junior Research Fellow on Modification of polymers for pharmaceutical applications at Guru Jambheshwar University of Science & Technology, Hisar, India

- Bachelors in Pharmacy (2007-2011) – Kurukshetra University, India

### RECENT PUBLICATIONS

- Kanika Thakur, Gajanand Sharma, Bhupinder Singh, Rajeev Tyagi, Sanjay Chhibber and Om Prakash Katare [2018]. Cationic-Bilayered Nanoemulsion of Fusidic Acid: An Investigation on Eradication of MRSA 33591 Infection in Burn Wound. *Nanomedicine (London)* [IF: 4.72].
- Gajanand Sharma, Mandeep Kaur Saini, Kanika Thakur, Namarta Kapil, Neeraj Kumar Garg, Kaiser Raza and OP Katare [2017]. Aceclofenac cocrystal nanoliposomes for rheumatoid arthritis with better dermatokinetic attributes: a preclinical study. *Nanomedicine (London)*. 12(6):615-638. [IF: 4.72].
- Gajanand Sharma, Kanika Thakur, Kaiser Raza and OP Katare [2017]. Nanostructured Lipid Carriers: A New Paradigm in Topical Delivery for Dermal and Transdermal Applications. *Critical Reviews™ in Therapeutic Drug Carrier Systems*. 34(4):355-386. [IF: 5.36].
- Purnima Kataria, Gajanand Sharma, Kanika Thakur, Vikas Bansal, Sunil Dogra and OP Katare [2016]. Emergence of Nail Lacquers as Potential Transungual Delivery System in the Management of Onychomycosis. *Expert Opinion on Drug Delivery*, 13(7), 937-52. [IF: 5.65]
- Munish Ahuja, Kanika Thakur and Ashok Kumar [2014]. Amylopectin-g-poly (N-vinyl-2-pyrrolidone): Synthesis, characterization and *in vitro* release behavior. *Carbohydrate Polymers* 108, 127-34. [IF: 4.81]



## Giulia Torrieri

Giulia Torrieri was born in Giulianova (Italy) on 19th August 1992. She graduated in Pharmaceutical Chemistry and Technology, in University of Chieti – Pescara “G. D’Annunzio” on 20th March 2017, presenting a Master’s thesis entitled “Innovative approaches for the transdermal delivery of Saffron derivatives: synthesis, physico-

chemical characterization, and *in vitro* tests”. Her Master’s thesis score was 110 cum laude (in Italy the final score or voto di laurea can go from 80 to 110, with 110 being the best score). After graduation, she spent four months in Finland, in Prof. Helder Santos’ laboratory, as Erasmus traineeship student. Then, she extended her staying in Prof. Hélder A. Santos group, at the Faculty of Pharmacy, University of Helsinki, working as research assistant from October to December 2017. Finally, she started her Ph.D. in January 2018, working on a project named “Development of multifunctional hybrid cell membrane/exosome-based nanovectors for myocardial reprogramming and repair”. During the last year she contributed in the realization of the following research/review papers:

Mónica P.A. Ferreira, Virpi Talman, Giulia Torrieri, Dongfei Liu, Gonçalo Marques, Karina Moslova, Zehua Liu, João Pinto, Jouni Hirvonen, Heikki Ruskoaho, Hélder A. Santos Dual-drug delivery using dextran-functionalized nanoparticles targeting cardiac fibroblasts for cellular reprogramming, *Adv. Funct. Mater.* 2018

João Pedro Martins, Giulia Torrieri, Jouni Hirvonen, Hélder A. Santos The importance of microfluidics for nanoparticle preparation of advanced drug delivery systems, *Expert Opin Drug Deliv.* 2018

In her research experience she learned how to prepare liposomes, niosomes, ethosomes\*, transferosomes\* and polymeric nanoparticles using different methodologies. She learned also how to use the following instruments to produce and characterize the nanoparticles: Rotavapor, Zetasizer, FT-IR, HPLC, tip- and bath- sonicators, different types of scales, extruder, flow cytometer, spectrophotometer, fluorimeter, CellInsight™ High Content Screening (HCS), 3i Marianas microscope, TEM, freeze-dryer. She is able to work with both continuous and primary cell lines and isolate cell membranes. She is able to use computer programmes like Power Point, Excel, Word, SigmaPlot, Origin and ImageJ.



## Daria Tretiakova

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Daria S. Tretiakova is a PhD student at Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS (Laboratory of Lipid Chemistry), received her specialist degree in Chemistry in 2015 in Lomonosov Moscow State University. Her student specialization was Enzymology. Since graduate thesis during PhD work she is involved in lipid chemistry and liposomal drug discovery research. Research experience and interests:

- Liposomal formulations design
- Interaction of liposomal formulations with blood components, mainly proteins
- Fluorescence analytical procedures

### SELECTED PUBLICATIONS

- D.S. Tretiakova, A.S. Alekseeva, T.R. Galimzyanov, A.M. Boldyrev, A.Yu. Chernyadyev, Yu.A. Ermakov, O.V. Batishchev, E.L. Vodovo-

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## Driton Vllasaliu

I am a registered pharmacist and gained my PhD in Drug Delivery (University of Nottingham, UK). Following my PhD, I spent two years as a postdoctoral research associate working on the development of improved *in vitro* intestinal models. I was then appointed to a lectureship at the University of Lincoln in 2012, where I

contributed towards the establishment of a new Pharmacy department. I joined the Institute of Pharmaceutical Science, King's College London, in 2017. My specific research interests centre around the understanding of the mucosal barrier in health and disease and overcoming this barrier to improve drug delivery. As part of this, I have specific interests in: non-invasive (mucosal) drug delivery of biologics, nanomedicine, mucosal barrier defects in disease and *in vitro* mucosal models. My major achievements lie in the field of understanding and modulation of epithelial barrier to promote drug delivery, epithelial interaction with nanomaterials and basement membrane-epithelial cell interaction.

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- Hashem L, et al. Intestinal uptake and transport of albumin nanoparticles: potential for oral delivery. Nanomedicine. Accepted March 2018.
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## Vivian Vu

Vivian Vu is currently a Professional Research Assistant at the University of Colorado Skaggs School of Pharmacy and Pharmaceutical Sciences in the Translational Bio-Nanosciences Laboratory under Dr. Dmitri Simberg, PhD PharmD. Her current research projects focuses on the therapeutic modulation of innate immune

system pathways in nanoparticle biodistribution and developing a novel prostate tumor perfusion model in mice. Utilizing techniques such as peptide synthesis, protein assays, clinical samples and xenograph models, she aims to design novel therapeutic concepts with the potential to gain clinical use in immune and inflammatory-induced disorders.

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## Adam A. Walters

Adam obtained his BSc degree in Microbiology from University of Nottingham (2008). Identifying his interest in the treatment and prevention of disease he then went on to obtain a PhD in Vaccinology from University of Surrey under the supervision of Dr Simon Graham and Prof. Graham Stewart. The focus of this project was

on development of particulate based systems as novel immune adjuvants as well delivery vehicles for vaccines. This involved the development of targeted nanoparticles as well as rationally designed biomimetic nanoparticles. He then worked at Imperial College London, Department of Mucosal Immunology under Dr John Tregoning in partnership with an industrial collaborator. The project was looking at novel DNA vaccine formulations for induction of humoral and cellular immune responses in numerous models. Following this Adam had a 5 year stint as a Research Fellow at the Jenner Institute, University of Oxford investigating controlled delivery of antigen for induction of spatially and temporally restricted immune responses primarily for malaria working with Dr Anita Milicic and Prof. Adrian Hill. In his current position Adam has moved from infectious disease to the assessment of complex carbon based nanostructures for use in novel prophylactic and therapeutic cancer vaccines. Specifically looking at targeted delivery and adjuvanticity of constructs. Throughout his career the consistent theme of Adam's research has been the development of novel vaccine formulation with special focus on the interaction of biomaterials with the immune system.



Sep. 2010 – Jul. 2015: Diploma of Higher Education (with honors). Major in Biophysics.

## BIOGRAPHY

My PhD research focuses on the application of hybrid nanosized photosensitizer carriers based on liposomes and cyclodextrins in photodynamic therapy. The current abstract describes PhD study results of development and application of drug-in-cyclodextrin-in-liposome nanoparticles to improved delivery of porphyrin photosensitizer to the tumor targets. PhD project found the support of several grant programs as French “Ligue Nationale contre le Cancer”, Belarussian Republican Foundation for Fundamental Research and the Ministry of Education of the Republic of Belarus. The presentation of this PhD project at BioSE Doctoral School (Nancy, France) was awarded by the first prize in 2017.

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## Yilun Wu



Specialises in calcium phosphate nanoparticle preparation, gene delivery, and immunotherapy for melanoma

Yilun Wu is focusing on developing lipid-coated calcium phosphate/carbonate (LCCP) nanoparticles (NPs) for effective siRNA delivery in melanoma immunotherapy by silencing PD-L1 related genes. Queensland State, “the capital of melanoma”, has the highest prevalence of melanoma around the world. Conventional treatments of melanoma, such as chemotherapy and radiotherapy, can cause some severe side effects. So finding an efficient treatment for late stage melanoma is of great importance, where immunotherapy is one of the promising approaches. To overcome tumour tolerance to the immune system, one effective approach is to suppress the programmed cell death ligand-1 (PD-L1) with siRNAs. Herein, LCCP NPs are designed as novel siRNA delivery platforms for knockdown of PD-L1 gene and/or upstream genes. Successfully completing this project may provide a novel nanoparticle-based siRNA delivery approach for melanoma immunotherapy.

- 2015.7- present, the University of Queensland, Australia. PhD candidate. International Postgraduate Research Scholarship (IPRS) and UQ Centennial Scholarship (UQCent) in Australia;
- 09.2012-03.2015, Donghua University, Shanghai, P.R.China. Master of Engineering. Full Scholarship at Donghua University, China Patent CN102784397 B and CN104147608B;
- 09.2008-06.2012, Donghua University, Shanghai, P.R.China. Bachelor of Engineering. Donghua University Scholarship, Outstanding Undergraduate Dissertations

## KEY PUBLICATIONS

- Yilun Wu, Wenyi Gu, et al., Devising New Lipid-coated Calcium Phosphate/Carbonate Hybrid Nanoparticles to Control Release in Endosome for Efficient Gene Delivery *J. Mater. Chem. B*, 2017, 5, 7194-7203
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## Ilya Yakavets



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## EDUCATION

Nancy, France: University of Loraine. CRAN laboratory CNRS UMR 7039  
Minsk, Belarus: Belarussian State University. Laboratory of Biophysics and Biotechnology  
Oct. 2016 – current Co-joint PhD thesis  
Minsk, Belarus: Belarussian State University. Faculty of Physics  
Sep. 2015 – Jun. 2017: Master degree in Physics (eq. Master 2). Major in Biophysics.

## Antonella Zacheo



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Antonella Zacheo, presently working as researcher at the Cancer Institute “Giovanni Paolo II” -IRCSS based in Bari (Italy), obtained her first degree in Biomedical Technician of Laboratory (specialization: Biotechnology and Biomedical Research), at the University of Rome “La Sapienza” in 1997. In 1998 she started to work at the Vascular Pathology Laboratory of the Istituto Dermatologico dell’Immacolata (IDI) in Rome, spending during 1998 itself a six-month research period at the New York Medical College in Valhalla, New York (USA); research carried out was on vascular remodelling in response to ischemia and potentially therapeutic interventions to restore blood flow and promote angiogenesis in ischemic tissues. In the period October 2000 – September 2002 she held a research fellowship at the Cardiovascular Research Institute of the New York Medical College in Valhalla, New York (USA); research focused on cardiomyocytes and cardiac stem cell isolation and characterization. From 2003 to 2006 she worked again at IDI, searching on the therapeutic strategies for myocardial tissue regeneration after infarction, while getting a second degree in Biology (five-year program), at the University of Rome “Roma Tre”. Since 2007 she works in Nanotechnology and Nanomedicine fields. She started her experience as a Research Fellow at the Nanotechnology Institute of Lecce. She received her PhD in Science and Interdisciplinary Tech-

nology (Nanoscience) from the Istituto Superiore Universitario di Alta Formazione Interdisciplinare - ISUFI, University of Salento, in 2011. In October 2016, she started to work at the Cancer Institute "Giovanni Paolo II" -IRCSS of Bari (Italy), dealing with the synthesis of nanopharmaceuticals acting as innovative healthcare solutions for Antiviral and Cancer Therapy. Throughout all of the above-mentioned professional experiences, she acquired multiple competences ranging from cell biology to histology, from cell engineering to microfabrication, from nanocarrier synthesis to microfluidics. Moreover, she has gained excellent ability to work in a multidisciplinary team improving interpersonal and communication skills.



## Zeynep Islek

Zeynep Islek has studied Pharmacy in Yeditepe University, Turkey. She is a PhD student in both Pharmaceutical Technology, Ege University and Biotechnology, Yeditepe University, Turkey and has continued to work on two theses in different research areas including targeted drug delivery to melanoma via monoclonal anti-

body-conjugated solid lipid nanoparticles and the neurological aspects of leishmaniasis. She had also been Research Assistant in Parasitology Laboratory and Molecular Diagnostic Laboratory at Institute of Biotechnology in Yeditepe University. Research efforts are focused on the targeted drug delivery systems to Leishmania parasites and various cancer cell types including melanoma and glioblastoma using solid-lipid based nanocarriers and exosome-based systems. From 2016, she is a scholarship student in COST Action "Targeted chemotherapy towards diseases caused by endoparasites" focused on "Discovery of Novel Drug Molecules and Development of Nanoparticulate Drug Delivery Systems for Treatment of Leishmaniasis" in collaboration with Yeditepe University and Istanbul Medipol University. With the COST Action, she has continued her experimental studies on Leishmania parasites with the contribution of IBMC (Institute of Molecular and Cell Biology, Parasitology) in Porto for two months. She has also participated in courses and workshops relevant to "Therapeutic mAb Engineering and Production" in Dokuz Eylül University, Turkey and, Cell culture and Tissue Engineering Courses in Stem cell and Gene Therapy Research and Application Center in Kocaeli, Turkey. Posters presented at conferences have included "CurcuEmulsomes as a new drug delivery system for anticancer therapy" and "Delivery of anti-leishmanial BNIP Derivatives to Macrophages using Emulsomes" in NanoTR12 in Kocaeli, Turkey; "Co-Delivery of Piperine and Curcumin-loaded Emulsomes Enhanced Anti-cancer Activity on Various Cancer Cell Lines" in Mol-BiyoKon17 in Boğaziçi University, Turkey.



## Marina Pöttler

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Marina Pöttler is a biologist with the main focus in nano-medicine, tissue engineering and cancer research. After she studied biology at the Paris Lodron University in Salzburg (Austria), where she finished her master in zoology/cell biology and physiology with excellent degree, she started her PhD studies at the Medical University Vienna (Austria), in the field of oncology with main research area of molecular signal transduction and malignant diseases. Herby,

she focused on the development of tumor markers in solid tumor as well as in tumor angiogenesis. As a PostDoc she investigated tumor-immunological questions at the Moore Cancer Center at the University of San Diego (CA, USA). Working as a PostDoc at the Section of Experimental Oncology and Nanomedicine, (SEON, University Hospital Erlangen) she started to work on toxicological evaluations of superparamagnetic iron oxide nanoparticles for the use in cancer therapy and diagnosis and is now focusing on tissue engineering using nanotechnology aimed at formation of 3D cell-structures via magnetic tissue engineering (MTE).



## David Strassburger

David started his chemical career with an apprenticeship as lab assistant in 2007 at Boehringer Ingelheim Pharma GmbH & Co KG, Germany. He completed among the top ten of the state Rhineland-Palatinate in 2010 and moved to Austria for a research internship at the Oncology Regional Center Vienna of Boehringer. In

the same year he took up his studies in Biomedical Chemistry at the Johannes Gutenberg-University in Mainz. After achieving his bachelor degree 2013, he spent a semester abroad at the University of Massachusetts (USA), in the Polymer Science & Engineering Department (PSE) where he joined the group of Prof. Dr. G. Tew and gathered more experience in polymer chemistry. He continued his studies in Mainz and did his master thesis in the group of Prof. Dr. H. Kunz on MUC1 glycopeptides for polymer conjugation in the context of antitumor therapy. His thesis was awarded in 2015 with the Adolf Todt prize for excellent scientific work. For his Ph.D. studies he joined the emerging group of Prof. Dr. P. Besenius in Mainz. Currently he is working on the development of a supramolecular, self-assembly driven, modular platform for vaccine design. He especially focuses on tumor immunotherapy through MUC1 derived B cell epitopes in the context of epithelial cancers in close collaboration with Prof. Dr. E. Schmitt from the Institute of Immunology at the University Medical Center Mainz. At present he participates in the research training group of the Collaborative Research Center "Nanodimensional polymer therapeutics for tumor therapy" (SFB 1066) engaging in the development of new nanotherapeutics.

### RECENT PUBLICATIONS:

- Synthetic MUC1 Antitumor Vaccine with Incorporated 2,3-Sialyl-T Carbohydrate Antigen Inducing Strong Immune Responses with Isotype Specificity. D. Straßburger, M. Gläffig, N. Stergiou, S. Biaslas, P. Besenius, E. Schmitt, H. Kunz\*, ChemBioChem 2018, 19, in press. DOI: 10.1002/cbic.201800148.
- Mannose-Decorated Multicomponent Supramolecular Polymers Trigger Effective Uptake into Antigen-Presenting Cells. D. Straßburger, N. Stergiou, M. Urschbach, H. Yurugi, D. Spitzer, D. Schollmeyer, E. Schmitt, P. Besenius, ChemBioChem 2018, 19, in press. DOI: 10.1002/cbic.201800114
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- Kinetic Control in the Temperature-Dependent Sequential Growth of Surface-Confined Supramolecular Copolymers. S. Engel, D. Spitzer, L.L. Rodrigues, E.-C. Fritz, D. Straßburger, M. Schönhoff, B.J. Ravoo\*, P. Besenius\*, Faraday Discuss. 2017, 204, 53-67. DOI: 10.1039/c7fd00100b.
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**ABSTRACTS  
POSTERS**



## A NEW INTERFACIAL BIO-SENSING APPROACH FOR DETECTING ABERRANT PROTEIN PHOSPHORYLATION IN CANCER

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Protein phosphorylation is one of the most prominent post-translational mechanisms for protein regulation, which is frequently impaired in cancer. Through the covalent addition of phosphate groups to certain amino-acids, the interactions of former residues with nearby amino-acids are drastically altered, resulting in major changes of protein conformation that impacts its biological function. Herein, we report that these conformational changes can also disturb the protein's ability to interact with and adsorb onto bare gold surfaces. Based on the direct interaction of proteins with the gold interface, we further developed an extremely simple method for aberrant phosphorylation detection that circumvents the current need for phospho-specific antibodies. The novel interfacial bio-sensing method, which only requires 50 ng of purified protein, was applied to EGFR phosphorylation analysis in several lung cancer cell lines and also enabled monitoring their cell sensitivity to tyrosine kinase inhibitors (TKI) — a drug frequently used in the clinic for lung cancer treatment.

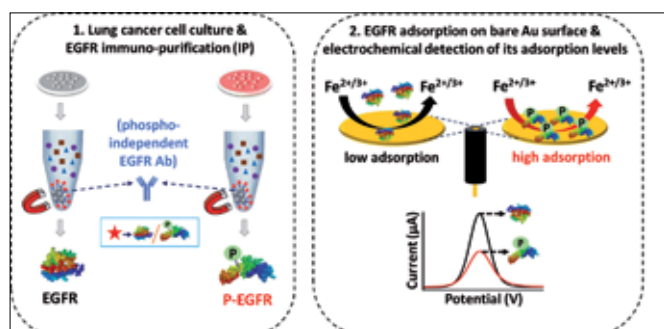


Figure 1. Workflow of label free detection of protein phosphorylation using protein-bare gold interactions. ('P' implies 'phosphorylated')

## REFERENCE

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## NEBULIZABLE AZITHROMYCIN INTO ANTI-BIOFILM SUPERSTABLE NANOVESICLES

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## INTRODUCTION

Azithromycin is a macrolide antibiotic of broad-spectrum of action and immunomodulatory activity that is used for the treatment of chronic pulmonary diseases like cystic fibrosis (CF). CF is a life-limiting autosomal recessive genetic disorder. The disease is caused by mutation of a gene that encodes a chloride-conducting transmembrane channel which regulates anion transport. One of the consequences of this dysfunction is the impediment of the mu-

ciliary clearance in the airways. This allows the development of the pulmonary disease characterized by mucus retention, bacterial infections and airway inflammation. Pulmonary disease is the major cause of mortality and therefore the major focus of clinical attention and research (Elborn, 2016). The first lung infections of CF patients are produced by colonies of *Staphylococcus aureus* and during the development of the infection; there is a co-infection with *Pseudomonas aeruginosa*. The infection with *P. aeruginosa* becomes persistent by adaptive mechanisms that elude the immune system defense and resist antibiotic therapy, like bacterial biofilm formation (Ciofu et al., 2015). Treatment with oral AZ is recommended for patients with CF for up to six months, since one of the main drawbacks to the prolonged use of oral AZ is its tolerability, being the most serious side effect in cardiac toxicity (Altenburg, 2011). The inhaled administration of AZ could be an alternative to minimize the systemic adverse effects and maximize the accumulation of the drug in the target site of the disease. However, due to the chemical characteristics of AZ, it is difficult to achieve an adequate suspension for nebulization (Hickey et al., 2006).

The pharmacokinetics and pharmacodynamics of inhaled antibiotics could be modified using delivery systems to improve tolerability, increase the retention time in the lungs, as well as minimize the premature mucociliary clearance of drugs (Zhou et al., 2015). Currently, there are two formulations of liposomal antibiotics, which are in advanced clinical phases for use in CF: Lipoquin (liposomal ciprofloxacin) and Arikace (liposomal amikacin). In addition, one of the major barriers against efficient CF therapy is the fact that the CF patients have thickened and viscous mucus that adheres to the airway surface. Antibiotic delivery with inhaled nanovesicles capable of penetrating the thick mucus layer, could improve interaction of antibiotics with bacterial cells. For this, nanovesicles must be able to avoid adhesion to mucin fibers and being small enough to avoid significant steric inhibition produced by the mucin dense fiber mesh (Nafee et al. 2014).

Archaeosomes (ARC) are nanovesicles made of total polar archaeolipids (TPA). Archaeolipids are novel biomaterials extracted from a non-conventional source (neither animal and vegetal nor bacterial), the hyperhalophilic archaeobacterial *Halorubrum tebenquichense*. The presence of these archaeolipids, particularly that of the major component 2,3-di-O-phytanyl-sn-glycero-1-phospho-(30-sn-glycerol-10-methylphosphate), renders the nanovesicles resistant to physical, chemical, and enzymatic attacks, including to the shear stress of nebulization (Altube et al., 2016). Previous studies have shown that ARC and related structures are more extensively captured by macrophages than conventional liposomes (Perez et al., 2014). Furthermore, no *in vitro* deleterious effects on pulmonary surfactant activity have been detected for ARC (Altube et al., 2017). Based on the above-mentioned advantages that nanovesicles could provide to lung delivery, in this work we propose, for the first time, the incorporation of AZ on nebulized nanovesicles aiming to achieve a nebulizable formulation, to provide long term-action and reduced systemic adverse effects, which could result from the oral administration. To that aim, AZ was loaded in highly stable nanovesicles (ARC-AZ) and its performance was compared with that of ordinary hydrogenated phosphatidylcholine liposomes (L-AZ). The formulations were tested for stability upon storage, nebulization and mucins contact. In addition, biological aspects were evaluated like safety of epithelial and macrophage cells as well as maintained anti-bacterial activity against *S. aureus* and *P. aeruginosa*.

## RESULTS

Nanovesicles with AZ were prepared by the film hydration method, conventional liposomes were made of HSPC:cholesterol:AZ 7.5:2.5:5:4 w:w (L-AZ) and archaeosomes were made of TPA:AZ 10:4 (ARC-AZ). Our results showed that only ARC-AZ were stable upon 6 months of storage at 4 °C, while L-AZ rendering variable drug load and undergoing aggregation.

Evaluation of the formulation stability during the nebulization process is a key factor to achieve an efficient delivery of an inhalable

formulation system. (Lehofer et al., 2014). Although the vibration mesh nebulizer is a dispositive recommended for nebulization of liposome formulations, it produces mechanical stress due to shears forces during the nebulization process (Cipolla, Gonda, & Chan, 2013). Although very rigid HSPC-cholesterol liposomes can support this process well, the incorporation of AZ drastically changed this behavior, causing the formulation to be retained in the vibration mesh nebulizer and the remainder that managed to be nebulized (~ 50 % LT) had a size in the order of micrometers. On the other hand, the formulation with ARQ-AZ had excellent nebulization efficiency without significant losses of mass and with colloidal stability (Figure 1).

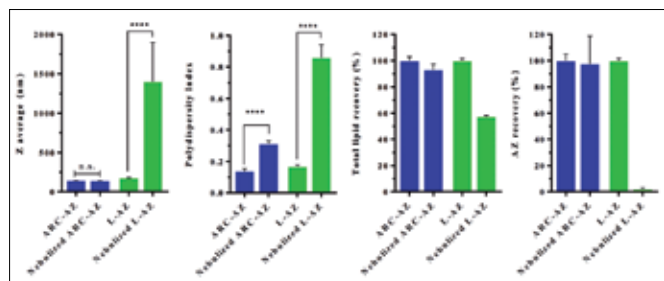


Figure 1. Nebulization efficiency of ARC-AZ and L-AZ after nebulization with OMRON NE-U22 vibration mesh nebulizer.

The incubation of empty nanovesicles or with AZ with mucins during 4 hours at a mucin:nanovesicle 1:1 (w:w) ratio, showed no significant change on the nanovesicles size. ARC were not mucoadhesive: the size distribution of the nanoparticles/mucin mixtures is in the same size range of nanoparticles alone. Instead, we found that ARC were more mucus penetrating than L upon 9 h, ARC diffusing significantly deeper ( $80 \pm 4\%$ ) than L ( $58 \pm 2\%$ ) through a mucin layer.

Cell viability was evaluated on two cell types representative of the alveolar structure. One was the human lung adenocarcinoma cell line A549, which has characteristics of type II alveolar cells and the other was the human macrophage cell line, THP-1. In our work, no decrease in cell viability was recorded by the MTT method by both ARC-AZ and L-AZ after 24 hours incubation up to a concentration of  $45 \mu\text{g/mL}$  of AZ and  $225 \mu\text{g/mL}$  of LT. After 48 hours of incubation, cell viability slightly decreased ( $\sim 15\%$ ) with these concentrations. On the other hand, for THP-1 cells, free AZ induced a significantly decreased of viability ( $\sim 40\%$ ) with  $5 \mu\text{g/mL}$  of drug.

The antibacterial activity of free AZ and incorporated on nanovesicles was evaluated on *S. aureus* ATCC25923 and *P. aeruginosa* PAO1, planktonic and forming biofilms. For *S. aureus*, a MIC of  $4 \mu\text{g/mL}$  was determined for free AZ or in nanovesicles and for *P. aeruginosa* a MIC of  $32 \mu\text{g/mL}$  for free AZ and of  $8 \mu\text{g/mL}$  for ARQ-AZ and  $16 \mu\text{g/mL}$  for L-AZ. In addition, it was evaluated the ability of AZ in nanovesicles to inhibit *S. aureus* biofilm formation. At 1/2 of the MIC, both free drug and on the nanovesicles inhibited  $\sim 50\%$  of planktonic bacteria growth, but only when it was incorporated into nanovesicles the amount of biofilm generated decreased by 40%. Finally, when AZ is incorporated in nanovesicles remained its antibiofilm activity, decreasing 70% of viable biofilm bacteria.

## CONCLUSION

Despite ARC-AZ formulation showed comparable anti-bacterial activities with L-AZ and free AZ, only ARC-AZ remained structurally stable upon nebulization process and storage as well as displaying deeper mucopenetration. Therefore, the advantages of ARC-AZ formulation over free AZ on ordinary AZ liposomal formulations constitute a promising tool for inhalatory treatment of diseases characterized by bacterial biofilm formation.

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## TARGETING THE MUCIN 1 MARKER IN COLON CARCINOMA IMPROVES THE THERAPEUTIC EFFICACY OF LIPOSOMAL DOXORUBICIN

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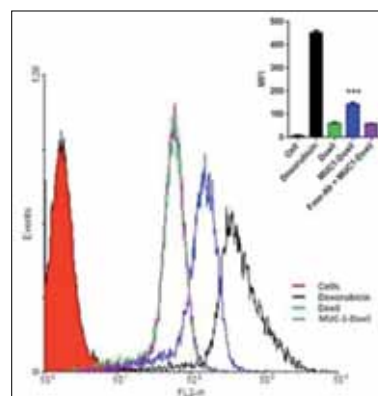
## INTRODUCTION

Among the most important challenges in drug delivery to cancer cells is the entry of drug into the cells and improvement of therapeutic efficacy. This is particularly important in the case of PEGylated liposomes because PEG coating on the surface of the carrier reduces drug uptake by cells. Active targeting by surface ligands can deliver the cargo of PEGylated liposomes into the cancer cells through endocytosis. The purpose of this study was to use monoclonal antibody (mAb) against Mucin 1 (MUC1), one of the one of the most well-known surface markers associated with Cancer Stem Cells (CSCs) which plays a key role in regulating the growth of cancer cells (1), for targeted delivery of liposomal doxorubicin (Doxil) into the cancer cells in order to increase the efficacy of drug and reduce side its effects.

## METHODS

Immunoliposomes were prepared via the post-insertion method. Anti-MUC1 antibody was coupled to the end-functionalized groups in PEG of preformed micelles. Then MUC1-PEG conjugates were transferred in a simple incubation step from the micelles into the outer monolayer of preformed Doxil (2). The measurement of the particles size and polydispersity index and zeta potential of liposomes was performed by dynamic light scattering (Nano-ZS;Malvern, UK). The amount of anti-MUC1 antibody on the liposomes was quantified using BCA protein assay (Pierce). In vitro cytotoxicity of liposomal preparations containing doxorubicin was determined using MTS assay (Promega, USA). The cellular uptakes of formulations by C-26 cells were analysis by flow cytometry (FCM) and visualized with confocal laser scanning microscopy (CSFM) (Nikon Inc., Switzerland). Chemotherapy study and pharmacokinetic and biodistribution evaluations were performed in BALB/c mice bearing C-26 murine carcinoma.

## RESULTS



The size of the immunoliposome was in the desirable size for EPR effect. Based on the FCM analyses and CSFM, MUC1-targeted Doxil showed a higher uptake and *in vitro* cytotoxicity in C-26 cell line. Doxil. In BALB/c mice bearing C-26 murine carcinoma, MUC1-targeted Doxil groups exhibited significantly higher doxorubicin concentration (than Doxil) inside the tumor cells and resulted in superior tumor growth inhibition.

In vitro cell uptake of Doxil, MUC1-Doxil and DXR to C-26 at  $37^\circ\text{C}$  in 3h. MFI results are expressed as Mean  $\pm$  S.E.M for 3 independent experiments. \*\*\* indicate significant difference  $p < 0.001$  of targeted MUC-1-Doxil and Doxil in cellular uptake.

This study showed a simple and commercially feasible modification of Doxil with anti-MUC1 mAb. The results indicated that MUC1-targeted therapy confer promising therapeutic efficacy and antitumor activity and may be a successful approach for targeted delivery to cancer cells.

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**Keywords:** Liposome, MUC1, Antibody, Doxorubicin, Active Targeting, Therapeutic Efficacy



Figure 1. (1) CIBER-BBN's programmes map; (2) Main scientific and technological CIBER-BBN results (2012-2016).

## CIBER-BBN: A SUCCESS STORY OF COLLABORATION BETWEEN DIFFERENT RESEARCH INSTITUTIONS IN THE NANOMEDICINE FIELD IN SPAIN

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Translation to the clinical practice of novel nanomedicines, medical devices and other nanotechnologies and tools for biomedicine is a complex process that requires specific knowledge and experience. Over the last decade, nanomedicine has seen its deployment as a fully-fledged sector within an organised and growing community, supported by national and European policies for funding research and translation. As a result, there exists currently a mature and excellent research community working at cutting-edge technologies. However, translation into clinical applications is still slow.

Within the Biomedical Research Network (CIBER-BBN), forty-six groups of internationally recognized scientific and technological high level collaborate in projects with the purpose of conducting both translational research and industrial transference. CIBER-BBN counts with its own scientific programme that is divided into three different areas: i) Bioengineering, ii) Biomaterials and iii) Nanomedicine. Focused on these three research fields, early stage development projects of new biomedical tools are designed, taking into account from the very beginning new and advanced technologies for manufacturing processes and advanced characterization and regulation aspects to guarantee the success and viability of the developed technologies.

The new Master Plan 2018-2021 has defined five strategic activities: a) International Projects and Initiatives, b) Research and Collaboration, c) Valorisation, d) Industrial Transference and e) Go-to-market. The objective is to guide excellent basic and applied research through the innovation process to develop new nanomedicine products/applications/tools. Moreover, the close collaboration with experts on Nanosafety, regulatory issues, investors and consultancy experts help us in the preparation of business plans for the creation of new spin-offs and start-up companies.

Examples of some of our initiatives are the calls raised within CIBER-BBN, launched in January 2018: a) the Intramural collaborations 2018-2021 call and ii) the Early Stage projects call. As a result, 112 collaborative projects, with direct funding for the groups, and 28 early stage projects, in which young researchers lead their own projects, are today ongoing. Results from previous calls (from 2012 to 2016) have shown a very fruitful collaborative network. The fostering of the translation of early research of excellence in Nanomedicine is always the fixed and common objective of CIBER-BBN and its groups.

## CIBER-BBN: A SUCCESS STORY OF COLLABORATION BETWEEN DIFFERENT RESEARCH INSTITUTIONS IN THE NANOMEDICINE FIELD IN SPAIN

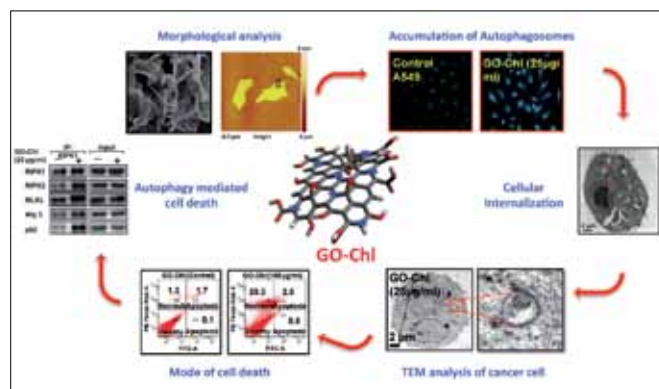
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Cancer is a leading cause of morbidity and mortality with appearance of nearly 14 million new cases every year causing 8-9 million deaths worldwide. Despite of the enormous technology advances in healthcare sector, cancer still triggers the modest impacts on patient survival. Chemotherapy is preferred therapeutic modality for cancer but hydrophobicity of drug, poor target recognition, non-specific accumulation and severe side effects restricts its use. Therefore, efforts are continuing to develop novel therapeutic platforms with enhanced clinical translational efficacy.



Nanomedicine represents an innovative field with immense potential for improving cancer treatment employing state of the art drug delivery nanoplatform for improving drug therapeutic index by increasing efficacy and reducing toxicities, targeted delivery of drugs in tissue or organelle specific manner, by sustained and stimulated drug release and capability to administered systematically in solid tumor. Nanoparticles administration in cancer cell causes modulation in cell signaling cascade upon interaction with tumor microenvironment leading to cell death either through apoptosis, necroptosis, pyroptosis, necrosis and autophagy.

Recently, modulation of autophagy by exposure of nanoparticles has been exclusively explored as novel treatment modalities for cancer. In a conventional process of autophagy, the cytoplasmic material mainly endogenous (e.g., organelles) as well as foreign (e.g., pathogens) materials are sequestered in double-membrane vesicles (i.e., the autophagosomes) and degraded upon fusion of these autophagic vesicles with lysosomes. Exposure of nanoparticles can exploit autophagy process at different stages to regulate



cell death in cancer cells through different cell signaling cascades. Graphene oxide (GO) owing to its unique physicochemical properties can also exhibit autophagy response in cancer cells providing a better therapeutic opportunity for personalized, target specific and translational nanomedicine. GO and its derivatives preliminary internalized into the cells through endocytosis leading to accumulation in lysosomes. This endosomal/ lysosomal transport of GO provides therapeutic window for activation of cell death signaling through alteration in autophagy process. However, the up regulation of autophagy provides therapy resistance to cancer cells; it is therefore argued that the inhibition of autophagy could potentially be used as perspective anticancer therapy. Chloroquine (Chl), an FDA approved antimalarial drug, is a known autophagy inhibitor via lysosomotropic activity has also shown its anticancer potential. Therefore, we endeavored the conjugation of Chl onto GO nanosheets and evaluated the antiproliferative efficacy of novel GO-Chl nanoconjugate on human lung cancer A549 and normal lung BEAS-2B cell lines with detailed investigation on cell death mechanism. Morphological properties of GO and GO-Chl have been analyzed through TEM, FESEM and AFM and formation of nearly monolayer highly exfoliated GO nanosheets has been observed. Further, Raman spectroscopy, FTIR spectroscopy and UV-Visible spectroscopy have been employed for analysis of structural, functional and optical properties respectively. MTT assay has been performed for in-vitro cytotoxicity evaluation of GO-Chl on A549 lung cancer and BEAS-2B normal lung cell lines. The GO-Chl treatment exhibited significant cell death in A549 cancer cell lines, in contrast to an almost 90% cell survival in normal BEAS-2B cells. Flow cytometry based cellular internalization mechanism reveals that GO-Chl nanoconjugate has been internalized through clathrin mediated endocytosis mechanism. The cell permeable DCFDA dye assay demonstrate the significant increase in ROS levels of GO-Chl exposed A549 cells. The fluorescence microscopic analysis (MDC staining and GFP-LC3 plasmid) and TEM observations confirm that GO-Chl induces the accumulation of autophagosomes in the A549 cells. Western blot analysis has been performed to access the expression level of autophagy and necrotic biomarkers. Enhanced level of LC-3 I/II signifies the formation of autophagosomes and elevated expression of p62/SQSTM1 indicates the inhibition of autophagy at later stage playing a role in defining cell death mechanism. Further, interaction between autophagy and necroptosis proteins has been analyzed through co-immunoprecipitation assay. Results showed that p62/SQSTM1 found to be coimmunoprecipitated with RIPK1 causing the accumulation of autophagosomes by disruption of the autophagic flux, which serves as scaffold for necrosome assembly and activates necroptotic cell death in A549 cancer cells. On the basis of results obtained, it is envisioned that GO-Chl nanoconjugate could be used as an effective cancer therapeutic agent, by targeting the autophagy necroptosis axis.

**Keywords:** Graphene oxide, Chloroquine, Autophagy, Necroptosis and lysosomal destabilization.

## PRECISION CUT TISSUE SLICES (PCTS) AS AN EX VIVO MODEL TO STUDY NANOMEDICINE UPTAKE AND DISTRIBUTION IN TISSUE

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Nanomedicine is showing its potential to change the way drugs are delivered to their target. However, for their successful development, detailed understanding of their distribution and behavior at organism, tissue and cellular level is required.

In-vitro and in-vivo studies are both fundamental for understanding nanomedicine behavior at cellular level and for more complex distribution and pharmacodynamics studies respectively. However sometimes it is difficult to bridge the gaps between what observed *in vitro* and *in vivo*. Within this context, Precision-Cut Tissue Slices

(PCTS) could be used as ex-vivo model to gain some important information on nanomedicine behavior within a real tissue, while allowing also obtaining information at single cellular level<sup>1</sup>.

PCTS (Figure 1a) have already been validated and extensively used for toxicological studies and drug metabolism<sup>2</sup>; the maintained cellular complexity of the tissue and the possibility of preparing slices from diverse species, including interestingly also from human tissue, and from different organs, are only few of the big advantages of using this model. Furthermore the use of PCTS can significantly contribute to the reduction of in-vivo studies in accordance with the 3Rs – replacement, reduction and refinement.

Thus here we aim to test whether PCTS can be used for studying nanomedicine behavior in a real tissue. When nanomedicines are delivered in the blood stream, high percentage of the injected dose ends up in the liver and within the organ often are accumulated by Kupffer macrophages. Therefore, as a first step we used rat Precision Cut Liver Slices (PCLS) and a panel of model nano-sized materials of well characterized behavior at cellular level.

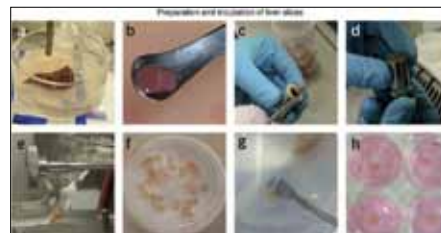


Fig 1. Preparation of Precision Cut liver Slices (PCLS)

Uptake and distribution of fluorescently labeled carboxylate polystyrene (PS-COOH) nanoparticles and amino-modified polystyrene (PS-NH<sub>2</sub>) nanoparticles, known to be toxic to cells<sup>3</sup>, have been studied after exposure to the rat Precision Cut Liver Slices (PCLS) for up to 72h.

The nanoparticles have been added to PCLS in relevant biological media containing serum in order to mimic realistic exposure scenarios<sup>4</sup>.

The behavior of the NPs in the PCLS has been explored by using confocal fluorescence imaging, in order to determine whether they enter cells and in which cell types they accumulate over time. Eventual effects on cells have been assessed by histological analysis, and by measuring ATP content, caspase 3/7 activity and TUNEL assay. Upon increasing doses of PS-NH<sub>2</sub>, toxic effects have been observed in the tissue comparable to what has been shown with the same nanoparticles in in-vitro studies, including sign of apoptosis as determined by activation of Caspase 3/7 and TUNEL assay. Optimization of different steps of NP exposure to the PCLS and sample preparation has been performed to follow eventual uptake and distribution of fluorescently labeled PS-COOH NPs within the tissue. Despite the large adsorption of NPs on the outer cell layer, uptake of NPs was clearly visible inside the section and interestingly, mainly in specific cell types.

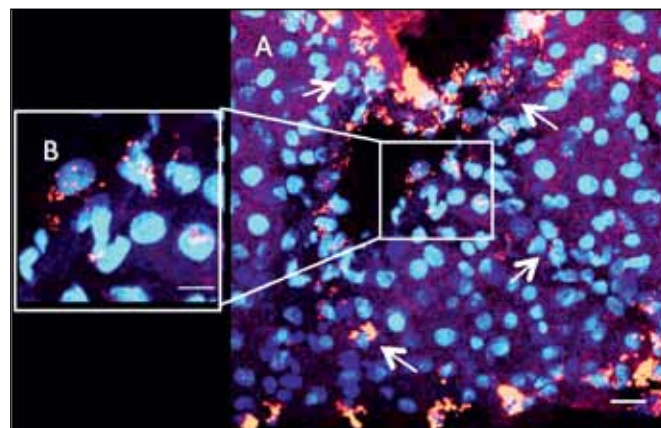


Fig 2. Distribution of fluorescently labeled PS-COOH NPs in a

cross-section of Precision Cut Liver Slice (PCLS). Blue: DAPI for nuclei staining. Red: NPs. (A) Scale bar = 20 μm. (B) Scale bar = 10 μm.

Although more work has to be done to further implement PCTS for this kind of studies, these results suggests that PCLS could be used as *ex vivo* models to resemble several aspects of what observed both *in vitro* at cellular level and *in vivo* within – in this case- the liver.

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## NANOROBOTICS BASED PHYSICAL ANTI-BACTERIAL APPROACH FOR TARGETED ERADICATION OF MULTIPLE DRUG RESISTANT STAPHYLOCOCCUS AUREUS

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Staphylococcus aureus infections which were once treated well by using β-lactam antibiotics (penicillins and, a beta-lactamase insensitive methicillin), quinolones and vancomycin have now acquired resistance against these antimicrobial agents and are evolved as multiple drug resistant bacteria (MDRB). The evolution of MDRB poses serious setback of chemical antibiotics mediated treatments and necessitates to devise an alternative physical antibacterial approach. A physical antibacterial strategy consisting of magnetic nanoparticle (MNP) in combination with electromagnetic waves was proposed and demonstrated for MDR E. coli CFT073 as a proof of concept. However, the reported study was non-specific, where-in the MNP, in general, binds to both the host and pathogens by electrostatic interaction, therefore, the killing effect in such strategy was expected to be common for both the host and pathogen. The targeted binding of MNP followed by specific eradication of bacterial pathogen and risk assessment of the host cells remained to be addressed in the previous study. Here, we constructed a nanobiorobot comprising iron oxide/silica (Fe<sub>2</sub>O<sub>3</sub>@SiO<sub>2</sub>) core-shell nanoparticles which were covalently conjugated to cell-wall binding domain (CBD) of endolysin after carboxylation. This nanobiorobot is programmed for unswerving itineration to detect and bind specifically on the staphylococcal cell surface even in a mixture of bacterial strains and host cells; mimicking the natural infection conditions. The MNP component of nanobiorobot bound to staphylococcal cell surface through CBD of endolysin, receives the radio-frequency (RF) signal. This antibacterial approach involves the targeted binding to Staphylococcus strains but not to the mammalian cells. The resonance responsiveness of RF caused the perturbation of bacterial cell membrane and localized heat generation resulted into dysfunctional membrane complexes and specific eradication of multiple drug resistant S. aureus.

**Keywords:** β-lactamase, Multiple drug resistance bacteria, Endolysin-CBD, Radiofrequency, Nanobiorobot

## IMMUNO-GOLD LABELING IN THE LIQUID PHASE OF EXTRACELLULAR VESICLES FOR CRYOGENIC-TEMPERATURE ELECTRON MICROSCOPY

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Extracellular vesicles (EVs) are the entire range of closed bilayer segments released from cells. First discovered by Wolf et al. in 1967, originating from platelets, they are of great interest, because they play a significant role in cancer, coagulation, stem cell renewal, inflammation, and cell-to-cell communication. EVs are shed from many types of normal and malignant cells. EVs include several different types, two of which are exosomes and microparticles (MPs). MPs, or microvesicles (MVs), are sections of cell membrane ranging from 100 nm to 1 micrometer in diameter that are released by shedding, mostly under conditions related to stress. Exosomes are 40 to 100 nm vesicles released from the cell as a consequence of multivesicular body (MVB) fusion with the plasma membrane. Distinction between those two sub-groups is not yet clear.

Immunogold labeling in transmission electron microscopy (TEM) and scanning electron microscopy (SEM) utilizes the high electron density of gold nanoparticles conjugated to proteins. Cryogenic temperature-TEM (cryo-TEM) allows the examination of individual vesicles of the whole range of EVs, while preserving their native state and nanostructure. Cryogenic temperature-SEM (cryo-SEM) allows imaging of immunogold labeled cells without any chemical fixation.

We have developed a two-step labeling procedure of biotinylated annexin-V in the presence of Ca<sup>2+</sup> ions and gold-conjugated streptavidin. The method has been found to be successful and a large number of EVs were labeled, due to phosphatidylserine (PS) migration from the inner leaflet to the outer leaflet of the membrane in the shedding process, exposing it to annexin-V. Figure 1 shows an unlabeled EV next to a labeled one.

We have studied several methods for stimulating EVs release from the cells, and investigated the immuno-gold labeling from each stimulation type (Figure 2).

Figure 1. Left- Unlabeled THP1 EV after LPS stimulation.

Right- Gold-labeled THP1 EVs after LPS stimulation. Bars = 100 nm

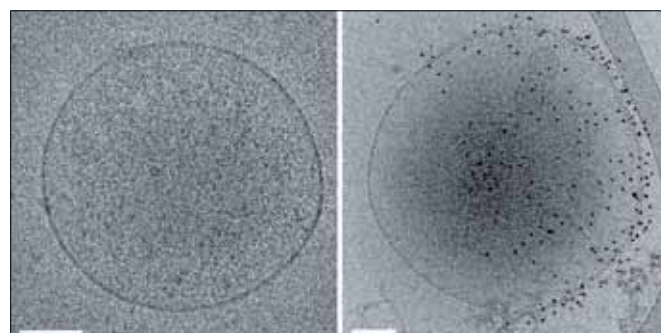
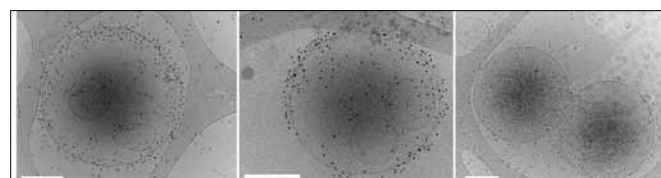


Figure 2. Cryo-TEM micrographs of EVs isolated and labeled with biotinylated annexin-V and gold conjugated streptavidin labeling.

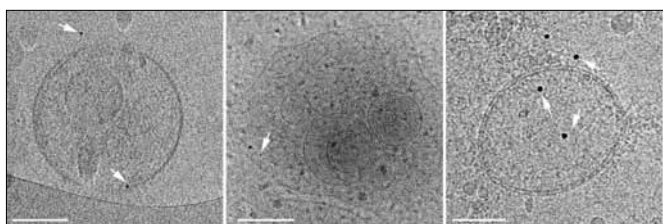
Left- EV from unstimulated THP1 cells; middle- from starvation stimulated THP1 cells; right- from LPS stimulated THP1 cells. Bar = 200 nm.



Another approach was to use anti CD-14 conjugated with biotin for binding the CD-14 that is naturally expressed on THP-1 cells membranes. We also used biotin-N-hydroxysuccinimide (biotin-NHS), to bind peptides on the membrane that can form amide bonds. A one-step procedure has been also tried, in which the gold nanoparticles are directly conjugated with NHS or NHS-antibody (for example NHS-anti CD14).

While for labeling EVs after shedding the results are promising, results from gold-labeling the cells and later distinguishing between MPs and exosomes are not yet conclusive (Figure 3). Although fluorescently labeling cells is a well-known protocol for many methodologies (e.g., light microscopy, flow cytometry), in cryo-SEM the approach is different and labeling protocols and sample preparation must still be improved.

Figure 3. Cryo-TEM of MPs isolated from gold-labeled (black spots marked with white arrows) THP1 cells. Left- MP containing another EV; bar = 100 nm; middle- Multivesicular body; bar = 200 nm; right- MP marked with several gold nanoparticles; bar = 50 nm.



## DANA<sup>2.0</sup> - RELIABLE INFORMATION ON THE SAFETY OF NANOMATERIALS

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The nanosafety aspect is a topic around the world. However, consumers, journalists, politicians as well as regulators often miss reliable and understandable information on nanomaterials and their applications. There is a high demand for answers to questions such as “Are nanomaterials dangerous for human health?” or “Do nanoparticles threaten our environment?”. Even for scientists not familiar with the field it is often hard to decide on the toxicological properties of nanomaterials.

Communication of scientific facts with the public is an ambitious task as complex issues need to be simplified for the broad public whilst ensuring scientific correctness. Due to the multidisciplinary nature of nanotechnology, communication on the related safety aspects is particularly challenging. The DaNa<sup>2.0</sup> project (data and knowledge on nanomaterials) is addressing these challenges by collecting and evaluating scientific results. Alongside, a criteria checklist for quality evaluation and management of scientific publications has been developed. This checklist includes mandatory and desirable criteria ensuring a thorough and comprehensive assessment of the used nanomaterial in any given setting.

These evaluated research findings are presented in a worldwide unique knowledge base, correlating material properties and applications, tailored to interested citizens, students and stakeholders. In more detailed material related sections, in depth information on the properties of certain nanomaterials are provided to address target groups like journalists but also scientists. For this, a tiered approach is chosen; the articles are starting on a simple level and end up with a more scientific explanation including links to the evaluated original literature.

The platform [www.nanoobjects.info](http://www.nanoobjects.info) offers reliable data on the 26 most widely used nanomaterials together with answers to frequent questions, as well as on cross-cutting topics like nanomedicine. DaNa<sup>2.0</sup> is a national project funded by the German Federal Ministry of Education and Research (FKZ O3X0131).

		Level of complexity				
		1	2	3	4	5
		General explanation	One-pager	Short summary	Detailed article	Original references
Example						
	Content	Simple language, not addressing a specific type of nanomaterial	Short and basic summary, covering the most striking information	subdivided into sections on material information, exposure, uptake and behaviour	In-depth information for each section and nanomaterial	List of the literature used to compile the detailed articles

Fig. 1: Graded levels of complexity realised in the DaNa<sup>2.0</sup> knowledge base to address different backgrounds of our visitors (from *Journal of Materials Education* Vol. 38 (3-4): 93-104 (2016))

## MAPPING NANOMEDICINE TERMINOLOGY IN THE REGULATORY LANDSCAPE

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A common terminology is essential in any field of science and technology for a mutual understanding among different communities of experts and regulators, harmonisation of policy actions, standardisation of quality procedures and experimental testing, and the communication to the general public. It also allows effective revision of information for policy making and optimises research fund allocation.

In particular in emerging scientific fields with a high innovation potential, new terms, descriptions and definitions are quickly generated, which are then ambiguously used by stakeholders having diverse interests, coming from different scientific disciplines and/or from various regions. As such the application of nanotechnology in health -often called nanomedicine- is considered an emerging and multidisciplinary field with a growing interest of various communities.

In order to support a better understanding of terms used in the regulatory domain, the “Nanomedicines” Working Group of the International Pharmaceutical Regulators Forum (IPRF) has prioritised the need to map, compile and discuss the currently used terminology of regulatory scientists coming from different geographic areas. We have used automatic web crawling and text mining tools in order to extract terms currently used by regulatory scientists. In total websites of 13 regulatory authorities and clinical trial registries globally involved in regulating nanomedicines have been crawled. The compilation and analysis of extracted terms demonstrated sectorial and geographical differences in the frequency and type of nanomedicine related terms used in a regulatory context. We have compiled, discussed and analysed around 30 relevant and most frequently used terms deriving from various agencies for their similarities and differences. Our analysis will provide the necessary background information to advance the discussion among stakeholders



and strengthen activities aiming to develop harmonised standards in the field of nanomedicine, an essential factor to stimulate innovation and industrial competitiveness.

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## COMBINING 2D ANGIOGENESIS AND 3D OSTEOSARCOMA MICROTISSUES TO IMPROVE VASCULARIZATION

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### INTRODUCTION:

The number of patients suffering from cancers worldwide is increasing, and one of the most challenging issues in oncology continues to be the problem of developing active drugs economically and in a timely manner. Considering the high cost and time-consuming nature of the clinical development of oncology drugs, better pre-clinical platforms for drug screening are urgently required. So, there is need for high-throughput drug screening platforms to mimic the *in vivo* microenvironment. Angiogenesis is now well known for being involved in tumor progression, aggressiveness, emergence of metastases, and also resistance to cancer therapies.

### MATERIALS & METHODS:

In this study, to better mimic tumor angiogenesis encountered *in vivo*, we used 3D culture of osteosarcoma cells (MG-63) that we deposited on 2D endothelial cells (HUVEC) grown in monolayer. Combination 2D HUVEC/3D MG-63 was characterised by Indirect immunofluorescence, Scanning electron microscopy, Optical microscopy and mRNA expression (qPCR).

### RESULTS:

We reported that endothelial cells combined with tumor cells were able to form a well-organized network, and those tubule-like structures corresponding to new vessels infiltrate tumor spheroids. These vessels presented a lumen and expressed specific markers as CD31 and collagen IV. The combination of 2D endothelial cells and 3D microtissues of tumor cells also increased expression of angiogenic factors as VEGF, CXCR4 and ICAM1.

### CONCLUSION:

The cell environment is the key point to develop tumor vascularization *in vitro* and to be closer to tumor encountered *in vivo*.

Keywords: angiogenesis; Osteosarcoma cells; Spheroids; Tubule-like structure; Tumor vascularization

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## USING APOFERRITIN AS SUITABLE NANOCARRIER FOR siRNA TO ACHIEVE ACTIVE TARGETING TO CANCER CELLS

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Small interfering RNA also known as siRNA are a short 21-23 base-pair RNA duplexes. Its leading strand is complementary to a sequence of mRNA. Interaction between mRNA and siRNA leads sequence-specific gene expression suppression, also known as RNA interference (RNAi) [1]. RNAi was first discovered in plants and later

was demonstrated in *Caenorhabditis elegans* [2]. Now it is known, that this mechanism can be naturally found also in mammalian cells [3].

The therapeutic application of siRNA has the potential to treat various difficult-to-treat diseases including cancer and viral infection [4]. Even though the usage of siRNA as therapy sounds very promising, there are still some obstacles that need to be solved. First there needs to be ensure, that siRNA will be delivered into specific tumor or other target cells. Applied siRNA face biological and physiological barriers when it's administered systemically; these barriers include recognition by the immune system, degradation, renal clearance, inability to penetration inside target cells or off-target effect [5, 6]. Many of these barriers could be solved by modification of siRNA or use some suitable nanocarrier.

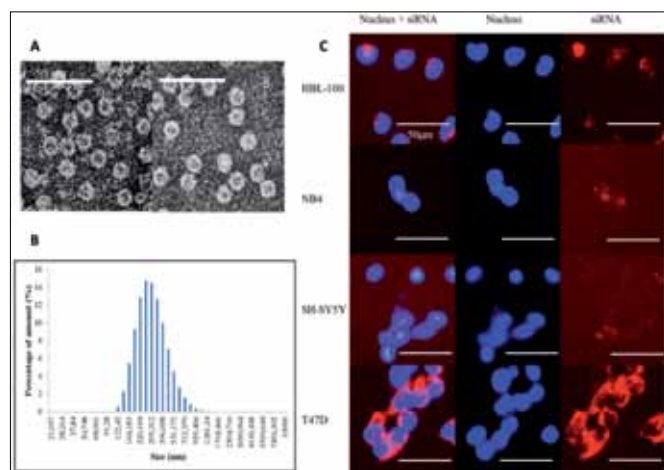
As nanocarrier we choose apoferritin (Apo), a ubiquitous protein cage, with outside diameter of 12 nm. Apo is non-toxic, biodegradable, and stable, it is easy to modify its surface to achieve active targeting and it has a simple disassemble/reassemble protocol. The structure of Apo is stable and active in pH 6-7, below pH 4 the structure starts to disassemble, with returning pH to 6-7 the structure will reassemble itself, by these protocol various of molecules can be loaded inside apoferritin cavity [7]. We used this protocol to encapsulate fluorescence siRNA to manage to prolong its stability, simplify internalization inside cancer cells and also manage to target these molecules to various cancer cell lines. Our first data will be presented in this abstract.

First there was a need to optimize encapsulation protocol for this arrangement. For optimization we used short ssDNA oligonucleotides. The length was 23 bp, which is similar to actual siRNA, concentration of stock solution was 100  $\mu$ M, that was diluted to 10  $\mu$ M. Four different encapsulating protocols were developed. In all protocols Apo was first mixed with water then in two samples 1M HCl was added and the mixture was incubated for 15 min at 600 rpm agitation. Then, solution of short oligonucleotides with 1M NaOH was added, followed by agitation to disassembled Apo. In two other protocols, oligonucleotides were added with 1M HCl to Apo for 15 min at 600 rpm agitation, and then solution was mixed with 1M NaOH. Amount of added 1M HCl was different in samples to achieve pH 4 or 2.7. Amount of 1M NaOH was added to achieve pH 6.5. The best and further used encapsulated protocol was: 20  $\mu$ L Apo+100  $\mu$ L H<sub>2</sub>O+200  $\mu$ L DNA+0.75  $\mu$ L HCl (pH 3.25), 15 min agitation, + 0.6  $\mu$ L NaOH (pH 6.17), 15 min agitation.

This protocol was used to encapsulate fluorescently labelled, non-coding siRNA-Cy3. For further analyses, we took 1.25  $\mu$ L, 2.5  $\mu$ L, 5  $\mu$ L and 10  $\mu$ L of siRNA and added water to final amount of 200  $\mu$ L. The encapsulation efficiency was determined by measuring the absorbance at 260 nm before and after filtration (3x Amicon 50K, 15 min, 6000 rcf). Encapsulation efficiency was above 70% in all samples, however after calculation of biggest yield the best came out from the sample with 10  $\mu$ L siRNA (concentration before filtration 311.2 nM, after filtration 233.4 nM, encapsulation efficiency 75%). Optimized protocol enables for a fast and facile production of Apo with siRNA encapsulated in high amount within the inner cavity of protein. Apo with siRNA was observed under transmission electron microscope (TEM) and no alteration of Apo morphology (degradation or incomplete assembly) was found. On the other hand, dynamic light scattering (DLS) revealed partial clustering. Further, four cell lines from which, two was breast cell lines (HBL-100 and T47D), and two was neuroblastoma cell lines (NB4, SH-SY5Y) were used for analysis of siRNA internalization. The cells were cultivated in 24-well plate, 5,000 cells per well for 12 h. Then, in each well we added 500  $\mu$ L of Apo-siRNA in medium concentration of added siRNA was 191.2 nM. Incubation with Apo-siRNA was for 16 h. Cell nuclei were counterstained by Hoechst 33342. The cells were observed under fluorescent microscope (Fig. 1).

*Fig. 1 Physico-chemical characterization and preliminary data of internalization of fluorescence siRNA inside various cell lines. A micrographs from TEM, A – Apo, B – Apo-siRNA, on both pictures*

are spherical Apo molecules, which are dispersed and not creating any aggregates with size around 15 nm. B picture shows results from DLS, C micrographs showing internalization inside various cell lines.



In conclusion, we performed optimization of encapsulation of siRNA inside Apo. We were able to create stable Apo-siRNA molecules with ability to internalize inside various types of cells with different internalization rates. We expect that these disparities are due to differential expression of receptors that recognize ferritins (i.e. SCARA5 and Tfr1). The micrographs from TEM revealed that there is no degradation of Apo and that there are no aggregates in the solution and the Apo-siRNA retain the hydrodynamic diameter about 15 nm, which is perfect for using in nanomedicine. The future outlooks comprise the testing of a long term stability of nanoparticles, stability against RNAses degradation, testing of internalization using surface-immobilized targeting ligands and finally using anti-Bcl-2 gene siRNA instead of fluorescent, non-coding siRNA. This will be utilized for simultaneous silencing and drug delivery to chemoresistant cells. We anticipate that our optimized protocol would be also helpful for siRNA transfection into hard-to-transfect cells.

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## SINGLE STEP PRODUCTION OF CLINICALLY RELEVANT LOW-TEMPERATURE-SENSITIVE LIPOSOMES USING MICROFLUIDICS

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Liposomes are spherical lipid-based delivery systems that dramatically improve the pharmacokinetics and biodistribution of chemo-

therapeutics after systemic administration. Liposomes encapsulating doxorubicin (Dox), such as Doxil<sup>®</sup>, have been superior to free drug primarily due to their reduced cardiotoxicity<sup>[1]</sup>. Despite their preferential accumulation at the tumor site, the therapeutic efficacy of such liposomal drug formulations has been limited due to the low drug bioavailability within the tumor interstitium. ThermoDox<sup>®</sup> is the most clinically advanced low-temperature-sensitive liposomes (LTSLs). It consists of DPPC, MSPC (lysolipid) and DSPE-PEG2000, and exhibits burst release kinetics under mild hyperthermia (41-42°C), which is clinically achievable. ThermoDox is used in combination with radiofrequency ablation, and is currently in phase III clinical study for the treatment of liver cancer<sup>[2]</sup>.

Despite intensive research efforts, liposomes clinical translation has been hindered by the limitation of conventional preparation methods, such as reproducibility, batch-to-batch variation, and cost-effectiveness. Microfluidics preparation of liposomes is advantageous over conventional methods with its precise and well-defined mixing behaviour. More important, the preparation process is continuous, straight-forward to operate and easy to scale-up. Furthermore, a new generation of microfluidics chip known as staggered herringbone micromixer (SHM) enables higher throughput, greater mixing efficiency, and lower dilutions of the final product.

In the present work, we report for the first time, the microfluidics production of clinically relevant LTSLs using SHM. In contrast to the conventional liposomes, LTSLs were more challenging to produce with microfluidic, due to lack of cholesterol and the presence of MSPC lysolipid. Nevertheless, stable and nano-size LTSLs were successfully and reproducibly produced following investigating the effect of lipid composition, aqueous buffer, flow rate, ethanol content, dialysis conditions, and doxorubicin-remote loading method on the properties of the engineered LTSLs. The prepared vesicles were characterized using different techniques, such as dynamic light scattering (DLS), transmission electron microscopy (TEM), differential scanning calorimetry (DSC) and pyrene fluorescence assay. Doxorubicin loading and release from LTSLs were also determined. The findings of our study will open the door for a straight-forward approach for LTSLs microfluidic production, which will facilitate their scaling up for clinical use

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## PH-INDUCED CONTROLLED RELEASE OF CONDENSED POLYPEPTIDE DRUG BALLS SPLIT FROM NANO-DELIVERY SYSTEM AS AN EFFICIENT STRATEGY FOR CANCER THERAPEUTICS

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Nanotechnology gets more and more popular in many biomedical applications. Peptide self-assembled nanodrug is one of the most promising applications among them because its enhanced permeability and retention effect (EPR). Peptide-originated structures have tremendous advantages including its intrinsic low toxicity, high

translation values, excellent biocompatibility and biodegradability, high tissue permeability, non-immunogenicity, tunable bioactivity and stimuli responsive at tumor areas. (Fan, Yu et al. 2017)

Herein, we report the development of a high drug loading, polypeptide assembled drug delivery nanostructures which can split into smaller carrier responded with the acidic environment at disease sites. The PD nanoparticles is formed by the antitumor drug doxorubicin and pyrene modified polypeptide (PBPL) which was first time applied to be a building block of drug delivery system via non-covalent interaction. The most promising feature of this carrier is the therapeutic itself is considered as one of the building blocks to composite the carriers instead of the conventional ones to be encapsulated in the delivery system. (Li, Xing et al. 2017) The strategy of co-assembly the peptide with drug ensures the carrier super stability, enhanced drug loading efficiency, fully interpenetrated interacting process of both components in a controlled and modulated way. The pH-responsive drug delivery system displayed uniform sphere shape in TEM images (Figure 1) and showed high threat to cancer cells *in vitro* (Figure 2).

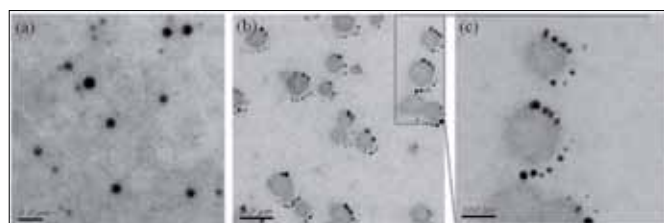


Figure 1. TEM image of assembled PD using polypeptide and drug as building blocks. (a) PD nanospheres with diameter around 80 nm at neutral pH value. (b)(c) Nanospheres became swelling (with diameter around 150 nm) and there are some smaller drug balls born on the border of spheres in an acidic environment for an hour.

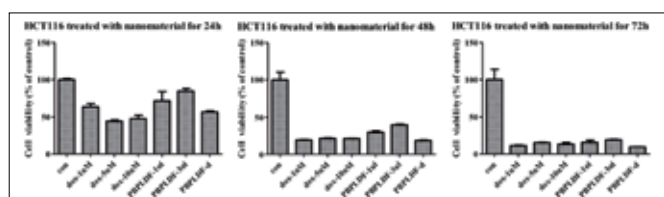


Figure 2. MTT assay results after HCT116 cancer cells treated by PD nanostructures over 24hr, 48hr and 72hr.

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## FEASIBILITY OF MAGNETIC DRUG TARGETING TO VASCULAR INJURY REGIONS: PILOT STUDIES IN A RABBIT MODEL OF ATHEROSCLEROSIS

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**Background:** Magnetic drug targeting (MDT) utilises the properties of superparamagnetic iron oxide nanoparticles (SPIONs) to accumulate particle-bound drugs in specified vasculature regions under an external magnetic field. Liposomal prednisolone was shown to reduce inflammation in a rabbit model of atherosclerosis. In order to address the feasibility of targeted inhibition of proinflammatory processes in early atherogenesis, we produced and tested a novel

nanosystem comprising SPIONs conjugated with dexamethasone phosphate (SPION-DEXA).

**Methods:** Lauric acid/albumin-coated SPIONs were synthesized and conjugated with DEXA, followed by characterization of dexamethasone release properties and cellular effects *in vitro*. A rabbit model of balloon injury- and Western diet-induced atherosclerosis was used to test the MDT efficacy. Following the injury of lower abdominal aorta, an immediate intra-arterial infusion of SPIONs (control or DEXA-conjugated) was performed under external magnetic field. Subsequently, the animals received high-cholesterol diet for 5 weeks, followed by 2 weeks of normal diet. After the angiographic visualization of the plaques, the animals were sacrificed and the aortas dissected for further histochemical analyses (Fig. 1).

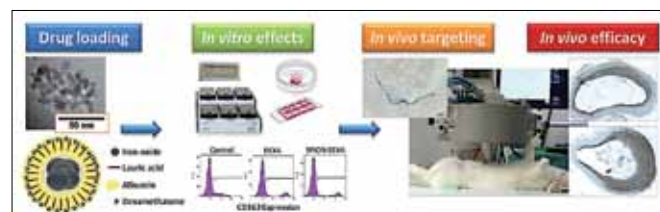


Figure 1. Graphical abstract of the study. The schematic presentation of nanoparticle composition is shown. The effects of SPION-DEXA were evaluated *in vitro* and *in vivo*.

**Results:** The release curve of the dexamethasone from SPION-DEXA showed that nearly 45% of the loaded drug is released during the first 24h of incubation *in vitro*, which should be beneficial to the acute aortic injury scenario. *In vivo*, the feasibility of MDT to abdominal aorta was tested by targeting of control SPIONs to the injured aortic region directly after the ballooning. Prussian blue staining confirmed that it is possible to accumulate intra-arterially administered SPIONs at the arterial wall. To assess an anti-inflammatory effect of local glucocorticoid therapy under the conditions of endothelial injury followed by high-cholesterol diet, animals received intra-arterial administration of SPION-DEXA immediately after the injury, followed by atherogenic diet. Contrary to our hypothesis, the initial results seem to indicate that local administration of SPION-DEXA enhances, rather than reduces the inflammatory burden in the plaques. The histochemical analyses performed on the excised abdominal aortas showed an increased macrophage content (RAM-11 staining), as well as more advanced plaque stages and larger necrotic core sizes (Crossman's trichrome staining) in animals treated with SEON-DEXA as compared with control SPION-administered group. Prussian blue staining was negative in all animals, indicating the complete clearance of iron oxide particles from the aortic wall within 7 weeks post-administration.

**Conclusions:** Our findings indicate that MDT is a feasible approach to target arterial regions of vasculature *in vivo*. However, treatment of vascular injury-related inflammation in the lipid-rich environment using dexamethasone-conjugated SPIONs may lead to enhanced monocyte recruitment and accelerated plaque growth.

**Funding:** This work was supported by the DFG (CI 162/2-1, CI 162/2-2) and by the EU ("NanoAthero" project FP7-NMP-2012-LARGE-6-309820).

## RESISTELL

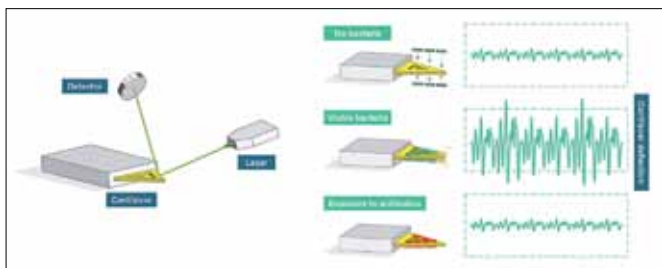
DANUTA CICHOCKA

## THE PROJECT

Resistell proposes an alternative to culture based antibiogram, the current gold standard in antibiotic susceptibility testing. Our offering is a diagnostic device. Because the test is growth independent, we reduce the time taken to get a result from days to minutes.



Resistell provides information on which antibiotic should be used to treat the patient, and the concentration at which it should be administered. Resistell's diagnostic method is based on the detection of movement caused by living bacterial cells. We use cantilevers, also used in Atomic Force Microscopy, as sensors. Our device is much simpler and cheaper than commercial AFM because all optics have been removed. Living bacteria cause the cantilevers' oscillations, which can be detected by our device. When antibiotic is added, bacteria react and the cantilever's vibrations return to the level of an abiotic sample (a sample without bacteria) within minutes. Laser and photodiode are used to detect the cantilever's movement. The custom made software is used to process the signal and classify the strain as susceptible or resistant.



The technology is being developed since 2012 at EPFL Laboratory of the Physics of Living Matter, by the group of Professor Giovanni Dietler. The work on Resistell start-up started in January 2017. Currently we are incorporating Resistell A.G. and started clinical validation of the MVP at CHUV Lausanne.

## THE TEAM

- Dr. Danuta Cichocka, CEO, Co-Founder
- Prof. Giovanni Dietler, Inventor, Co-Founder
- Dr. Sandor Kasas, Inventor, Co-Founder
- Grzegorz Gonciarz, Business operations, Partner
- Andreas Bühler, Product & Market development, Partner
- Wojciech Chomicki, Engineer
- Dr. Petar Stupar, Engineer
- Prof. Gilbert Greub, Scientific Advisory Board Member

## RESISTELL IN NUMBERS

- Start of the project: January 2017
- 7 Team Members (4 full time, 3 part time),
- around 2M invested in technology development over 6 years
- EU market entry planned for 2019

## THE MARKET

Our clients – hospitals, will buy our products, because it saves lives, saves the time spent in hospital and this way makes the treatment effective and much cheaper. The AMR diagnostics market is growing and diagnostics is one of most important tools in the fight against the spreading antimicrobial resistance. Our market entry strategy are sepsis and tuberculosis indications. Target countries for the first 3 years operations are: CH, EU, Norway, US (in total 14000 hospitals).

More Information can be found on [www.resistell.com](http://www.resistell.com)

## ROADMAP AT A GLANCE

2018	2019	2020	2021	2022
Feasibility study (CHUV) Further development	Design freeze "one channel" Clinical trial: sepsis, TB CE mark "one channel" Market entry CH, "one channel" Development of "multichannel"	•EU market entry "one channel" •Market entry CH, "one channel" •Product dev. and clinical "multichannel"	FDA "one channel" Market entry USA "one channel" CE mark "multichannel"	FDA "multichannel" Global expansion

## IMPROVED ORAL IN VIVO PHARMACOKINETICS OF NOVEL OIL IN WATER SOLID DRUG NANOPARTICLE FORMULATIONS OF TENOFOVIR DISOPROXIL FUMARATE FOR THE TREATMENT OF HIV

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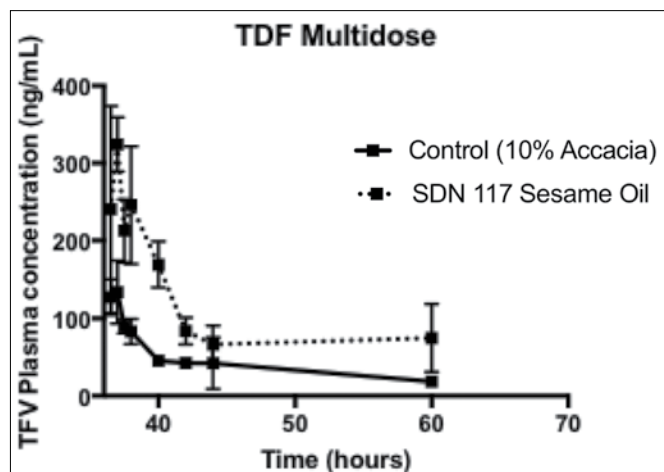
**Introduction:** The prodrug tenofovir disoproxil fumarate (TDF) (and the active form, tenofovir [TFV]) have been used in front line antiretroviral therapy for over a decade. TDF (in combination with emtricitabine, Truvada) is also FDA approved for pre-exposure prophylaxis (PrEP) <sup>(1)</sup>. Additionally, there are ongoing patient studies investigating the utility of single drug TDF preparations for PrEP <sup>(2)</sup>. A disadvantage to the current oral formulation of TDF is poor bio-availability, ~30% in humans <sup>(3)</sup>. This is due to intestinal enzymatic degradation and efflux transporters, which limit absorption. It has been demonstrated that the pharmacokinetics (PK) of TDF may be improved when delivered with a high fat content meal, with a 40% increase in AUC and 14% higher Cmax <sup>(4)</sup>. We describe here the synthesis and *in vivo* pharmacological assessment of novel solid drug nanoparticle (SDN) formulations for improved oral delivery of TDF.

**Methods:** SDNs of TDF were synthesised using a spontaneous methanol in dichloromethane nanoprecipitation method. Nanoprecipitations of up to 80 wt% active loading were generated after addition of an oily dispersal media and freeze-drying to remove volatile organic solvents. The formulations then underwent PK analysis in rodents after administration by oral gavage.

An initial screen of 8 candidate formulations was conducted in Wistar rats. Rats were dosed with SDN test formulation (15 mg/kg based on TDF content) or a conventional preclinical formulation of TDF (delivered in 10% acacia) <sup>(5)</sup>. Plasma samples were taken at 20 min, 40 min, 1h, 1.5h, 2h, 4h, 6h 8h and 24h for analysis via LC-MS/MS. Previous studies have demonstrated beneficial PK exposure of SDN's when examined at steady-state <sup>(6)</sup>. Following single dose PK studies, a single candidate was selected for further investigation. SDN formulation or conventional TDF (delivered in 10% acacia) were administered by oral gavage (15mg/kg based on TDF content, 1mL/kg) every 6 hours for 36 hours to achieve steady state, in a multiple dose study. <sup>(7)</sup> Following administration of the final dose, plasma samples were collected at 30 min, 1h, 1.5h, 2h, 4h, 6h, 8h and 24h for PK analysis via LC-MS. Statistical analysis was performed by unpaired t-test (for normally distributed data) or Mann-Whitney U test (for non-normally distributed data), and significance was defined as  $P < 0.05$  (SPSS v21).

**Results:** The data generated from single dose studies demonstrated improved Cmax (314ng/mL vs 293ng/mL) Cmin (67ng/mL vs 43ng/mL) and AUC (2465ng/h/mL vs 1492ng/h/mL) from the lead formulation dispersed into sesame oil. Following multiple doses, the steady-state PK of this formulation showed significant improvement when compared to the conventional formulation (figure 1). The Cmax, Cmin and AUC following the final dose at 36h demonstrated a 2.4-fold increase (353ng/mL vs 144ng/mL,  $P=0.004$ ), 4.1-fold (74ng/mL vs 18ng/mL,  $P=0.004$ ) and 2.3-fold (2311ng/h/mL vs 969ng/h/mL,  $P=0.004$ ) increase respectively.

Figure 1 PK profiles of the lead SDN-TDF candidate and conventionally formulated TDF control over 24 hours following 7 oral administrations delivered at 6-hour intervals (15mg/kg based on TDF content). Error bars represent standard deviation.



**Discussion:** The *in vivo* data presented here shows the preclinical PK assessment of a novel nanoprecipitated SDN-TDF formulation. The data demonstrate improved oral bioavailability of TDF. Interestingly, the beneficial effect on PK of TDF is only fully observed when at steady-state. This indicates that the nanoformulation is able to exploit time-dependant mechanisms, unavailable to conventional formulations. The application of SDN formulation demonstrates the ability to achieve greater exposure of TDF, which has the potential to facilitate dose reduction and potentially impact the cost of therapy or prevention.

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## INVESTIGATION OF KU-812 CELLS AS AN IN VITRO MODEL FOR BASOPHIL ACTIVATION AND ASSESSMENT OF COMPLEMENT RELATED INTERACTIONS

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Intravenous administration of nanomedicines and biologicals can result in hypersensitivity reactions (HSR) related to the activation of the complement system [1]. A number of reviews have summarised the details of many HSR to various therapeutic compounds [2,3]. The mechanisms behind the activation of complement are diverse and difficult to ascertain due to multiple, linked, characteristics. Complement-mediated HSR are associated with complement activation-related pseudoallergy (CARPA) which can lead to life threatening conditions such as disseminated intravascular coagulopathy (DIC) [4].

Pseudoallergy is characterised by immediate systemic reactions demonstrating similarity to anaphylaxis symptoms, however, the mechanisms involved are mediated by Immunoglobulin E (IgE)-independent release of mediators from basophils and mast cells [5]. Anaphylatoxins are the causative factors that trigger CARPA [6]. Most notably, complement components C3a and C5a are known to recruit basophils, subsequently releasing secondary mediators [6]. The release of histamine is known to augment the anaphylactic potential of a given tissue site [7], leading to the symptomatic presentation of CARPA. Due to the, relatively, limited amounts of circulating basophils in peripheral blood it can be difficult to obtain sufficient cells in order to conduct exposure-response investigations. It may be possible to use cell lines for screening purposes, in order to refine subsequent investigations in primary basophils. The aim of this study was to assess the suitability of KU-812 cells as an *in vitro* model for basophil activation to investigate complement-activated pseudoallergy. KU812 were seeded at a density of 5x10<sup>5</sup> cells/mL and treated with either combined phorbol myristate acetate (PMA) and calcium ionophore (CI) (20 nM + 0.5 μM, 40 nM + 1 μM or 80 nM + 2 μM), C3a (6.25, 12.5, 25 or 50 nM) or C5a (6.25, 12.5, 25 or 50 nM) for 24 hours prior to MTT assay, or 48 hours for flow cytometric analysis of cell surface markers related to basophil phenotype and activation (CD63, CD203c and CD164). Statistical analysis was conducted via one-way ANOVA using Stats Direct software (version 2.7.9) with a P value < 0.05 considered statistically significant.

To assess the potentially proliferative impact of treatment conditions, the MTT assay was chosen. PMA and CI mimic early cellular signalling expressed during inflammatory reaction [8]. Their presence has been shown to suppress the rate of proliferation within white-blood cells such as basophils [9]. Treatments with all three concentrations of this combination led to significantly (p<0.05) less cellular conversion of the MTT reagent compared to the untreated control (Figure 1). C3a and C5a are potent anaphylatoxins and have both shown to be efficient activators of basophils, which results in degranulation [10], leading to the expression of cell surface markers such as CD63 [11]. Significant proliferative effect was observed following treatment with C3a at 12.5 nM (14.1%, p = 0.0179) and 25 nM (10.2%, p = 0.0008), displayed in Figure 1A. Three cell-surface markers were chosen for assessment via flow cytometry. These include CD203c and CD63, both of which are heavily expressed upon activated basophils in anaphylactic pathways, following degranulation [13]. Also included was CD164 which, in addition to cell adhesion, plays a key role in mediating both apoptosis and proliferation [14].

Figure 1. Responses to 24-hour treatment with positive controls and

(A) C3a, or (B) C5a at stated concentrations in MTT assay by KU812 cells. Data displayed as average ( $n = 3$ )  $\pm$  standard deviation. \* denotes  $p < 0.05$

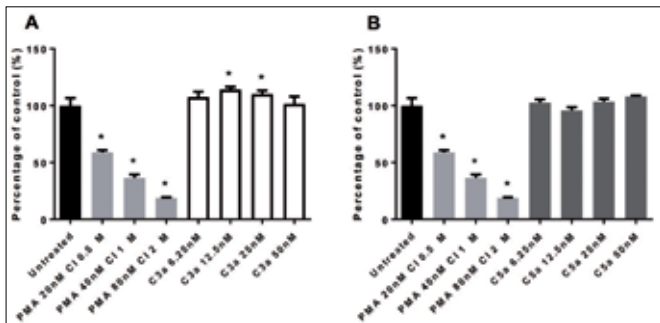
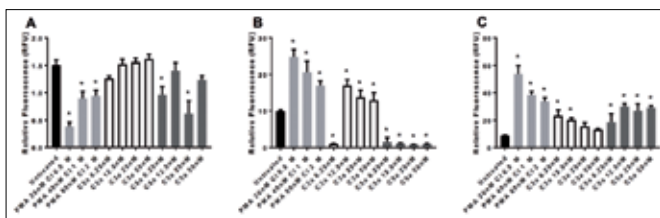


Figure 2. Flow cytometric determination of relative fluorescence generated by KU812 treated for 24 hours under stated conditions labelled for (A) CD63. (B) CD203c. (C) CD164. Data displayed as average ( $n = 3$ )  $\pm$  standard deviation. \* denotes  $p < 0.05$



PMA/CI treatment at 20nM/0.5 $\mu$ M resulted in the largest change in the expression of all the proteins, resulting in less CD63 and more CD164 and CD203c than the untreated control (Figure 2). The PMA/CI treatment was specifically formulated to ensure activation of the basophils (via Fc receptors)<sup>[15]</sup> and therefore act as a positive control, against which we can compare the action of C3a and C5a. The efficiency of the lower concentrations of PMA/CI could be explained by the saturation of Fc receptors for a prolonged period of time leading to de-sensitization. This may lead to the down-regulation of Fc molecules and therefore a reduced amount of downstream signalling occurring in the period prior to staining and analysis. This would therefore result in the less-significant changes in expression at higher concentrations. The lower level of CD63 (Figure 2A) was unexpected as upon activation basophils undergo degranulation. This typically involves the integration of CD63 containing granule membranes, into the cell surface membrane. However, our results show a substantial decrease (PMA/CI values lower by at least 30%). Hancharou et.al showed a significant increase in expression of CD63 as a result of basophil activation<sup>[16]</sup>. However, the difference in results may be a representative of the different incubation periods used with the two investigations as well as possible differences in regulatory mechanisms between primary basophils and cells lines. CD203c, alongside CD63, is established as a basophil activation marker, and are both found expressed upon granular membranes<sup>[16]</sup>. Therefore, it would be expected that CD203c would also show an increase in expression upon activation. This is reflected in the >1.5-fold increase over the positive control (Figure 2B). It would prove useful to assess the expression of C3a and C5a cell-surface receptors to link exposure-response to receptor density, especially when making comparisons between the responses of KU812 and primary basophils. CD164 is a novel basophil marker, which has been shown to have relatively low expression upon the surface of resting basophils<sup>[17]</sup>. This claim was supported by the positive control, resulting in a >350% increase in expression upon activation. (Figure 2C). This evidence suggests that CD164 would be a potential marker of basophil activation, as not only did it produce a response to the positive control but its expression was also altered in the presence of complement proteins C3a and C5a. The responses generated by KU812 under the treatments and assessments described here show the utility of this cell line as a, possible, model of basophil responses in pseudoallergy. The differing responses to known mitogens highlights the importance of assessing multiple markers of activation within samples, as has been included in the present study. Further

investigation is necessary to conduct exposure-response relationships for nanomedicines known to result in complement activation. While KU812 demonstrate some behavioural differences relative to primary basophils, such as the proliferative effect observed here<sup>[12]</sup>, the data presented demonstrates the suitability of KU812 as a model to provide a screening platform from which any notable responses can be further assessed in primary cells

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## NOVEL INSIGHTS ON NANOPARTICLE-BLOOD INTERACTIONS FOR EARLY DIAGNOSIS OF PANCREATIC CANCER

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To date, pancreatic ductal adenocarcinoma (PDAC) carries a poor prognosis, which is related to both tumor biology and advanced stage at the moment of detection (Zhang, Zeng et al. 2016). Thus, PDAC early diagnosis is a crucial aspect and new, cheap, user-friendly techniques for biomarker identification are needed. In this regard, recent advances in nanotechnology have provided promising outcomes that could pave the way to future developments of early diagnostic tools. They are based on the study of the interactions of nanoparticles (NPs) and blood plasma biomolecules. Indeed, upon incubation with human plasma, NPs act as accumulators of proteins, which adsorb on their surface and form an outer biomolecular layer (Fig. 1), or biomolecular corona (BC). Features and composition of the BC depend on NP's physical-chemical properties (size, shape, surface chemistry), environmental factors (temperature, PH) and molecular source, i.e. the biological medium within which the NP is embedded (Verderio, Avvakumova et al. 2014, Mahmoudi 2016, Caracciolo, Farokhzad et al. 2017, Docter, Westmeier et al. 2015). Hence, the detection of specific corona molecules, which are related to pathological conditions, could represent an effective way to exploit NP-blood interactions for diagnostic purposes. As an instance, it has been reported that electrophoretic protein patterns of BC formed on lipid NPs after exposure to PDAC plasma exhibited significant differences with respect to non-cancer samples. (Caputo, Papi et al. 2017) Encouraged by these findings, we carried out mass spectrometry (MS) experiments to identify PDAC protein biomarkers by studying the BC of liposomes.

In this study, we used three liposomal formulations with similar size (about 130 nm) and different surface charge. We prepared liposomes by lipid film hydration method, measured their physical-chemical properties and incubated them with plasma samples both from PDAC patients and healthy subjects, which are employed as a control. Cyto-histologically proved PDAC subjects, clinically staged according to the UICC TNM staging system 8th Edition, that met specific inclusion criteria (involving age, personal medical history, absence of uncontrolled infections) were considered eligible for the analysis. Ethical Committee of University Campus Bio-Medico di Roma approved this study. After blood sample collection, plasma was obtained by centrifugations. Liposomes were exposed to human plasma for one hour and after further characterizations, the protein compositions of the coronas were determined by mass spectrometry experiments. Finally, we compared the relative abundances of the detected proteins to find analogies and differences between control and PDAC samples.

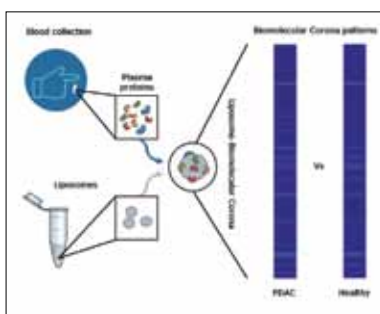


Fig. 1. Upon incubation with human blood plasma, liposomes get covered by a biomolecular corona (BC), whose composition depends on their intrinsic features, environmental factors and molecular source. By mass spectrometry experiments and statistical analysis of the resulting protein patterns,

we detected significant differences between healthy samples and PDAC patients. This approach may help to identify PDAC biomarker and develop novel diagnostic tools for early cancer detection.

To this end, we defined a relative protein abundance (RPA)-based parameter that quantifies a discriminating ability for each of the detected proteins. The discriminating ability measures how differently a given protein contributes to the healthy and cancer coronas. The application of this method on three different liposomal platforms (Fig. 2) allowed us to find patterns that are independent on the employed formulations, but are specific for a few proteins among hundreds populating the coronas. Interestingly, some of the PDAC identified proteins are currently associated to cancer conditions and some of them have never been reported as involved in the tumor biology.

In conclusion, we predict that a systematic investigation of the NP-BC may improve our knowledge of PDAC biology and that the BC technology may offer new opportunities for PDAC biomarkers identification, as well as the development of novel diagnostic tools.

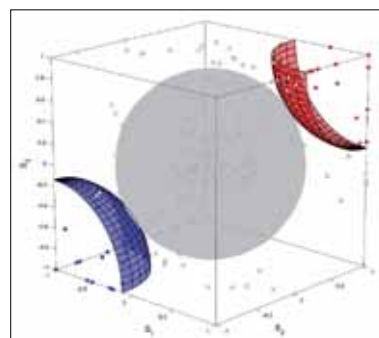


Fig. 2. Scatter plot of the measured discriminating ability. For each identified protein, we calculated the percentage variation of its abundance in the BC of PDAC ( $x_1$ ) vs. its counterpart in that of healthy patients ( $x_2$ ). Percentage variation is given by  $S = (x_1 - x_2) / (x_1 + x_2)$ . By definition,  $S$  varies between -1 (protein

exclusively identified in the BC of healthy people) and +1 (protein uniquely found in the BC of PDAC patients). For each protein,  $S_1$ ,  $S_2$  and  $S_3$  are the values assumed by  $S$  for each liposomal formulation. As a result, each protein can be represented by three coordinates ( $S_1$ ,  $S_2$ ,  $S_3$ ) in a 3D space. Gray dots indicate proteins whose abundance is not significantly different between PDAC and healthy patients. In contrast, red and blue dots indicate proteins that are enriched in the BC of PDAC and healthy patients respectively.

## TAKING ADVANTAGE OF FERRITIN DISASSEMBLY FOR DRUG DELIVERY

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Many highly potent low molecular antitumor drugs suffer from severe off-target toxicity, such as the cardiotoxicity of doxorubicin (DOX) [1] or hematological toxicity of tyrosine kinase inhibitors [2]. Therefore, their encapsulation in nano-sized carriers able to deliver the drug molecules selectively to tumor cells, while avoiding the healthy ones, can prove beneficial [3]. But choosing the correct material for such a nanocarrier can be a quite daunting challenge [4]. It needs to have suitable size, high loading capacity, easy encapsulation protocol, or prolonged stability (both during storage and in organism), while still removable from organism upon delivery of the drug. It should also be easily modified with targeting moieties and not recognizable by the immune system, unless immune system is the target [3-5].

Many different materials have been developed, from gold to liposomes. However, such nano-delivery systems can already be found ubiquitously from bacteria to humans. Ferritins (FRTs), ~12nm hollow cages with high interspecies homology, are formed by self-assembly of 24 protein subunits. Based on the ratio of 2 subunit forms – 19-kDa (light) and 21-kDa (heavy) subunit – FRTs are identified as L-chain or H-chain. This ratio not only influences FRT's molecular weight but also its cellular uptake, as L-chain FRTs predominantly internalize via scavenger receptor class A member 5 (SCARA5), while H-chain FRTs via transferrin receptor 1 (TfR1) [6].

FRT subunits naturally disassemble in acidic environment and reassemble in neutral environment and this property can be used to physically entrap low molecular drugs. Furthermore, the complex protein shell provides various active groups for easy conjugation with actively targeting moieties.

The most well-studied is L-chain FRT isolated from equine spleen [7-10], which we have also previously investigated for actively targeted drug delivery to prostate cancer cells [11, 12]. Its structure and disassembly/reassembly conditions have been described in detail [13]. However, concerns regarding its immunogenicity, as well as an extremely low pH described for its disassembly (<3.4) led us to investigate drug delivery using alternative FRTs, such as H-chain horse and human FRT and FRT from archaea *Pyrococcus furiosus* (PFU), often used for nanoparticle synthesis due to its high thermostability [14].

As can be seen from evolutionary distances (Fig. 1A), H-chain FRTs from different organisms are more closely related than H- and L-chain FRTs from the same organism. X-ray structures (Fig. 1B-E) revealed similar icosahedral structure with various active groups on the outer surface for all tested FRTs.

The encapsulation of low molecular drugs inside FRT cavity was tested using naturally fluorescent low molecular antitumor drug DOX. To disassemble the subunits and load DOX inside, the required pH varied between individual FRTs, as well as the loading efficiency (Fig. 2A). Although the size after DOX loading was increased for all FRTs (probably due to formation of oligomers), none of the FRTs increased their size above 106 nm (Fig. 2B).

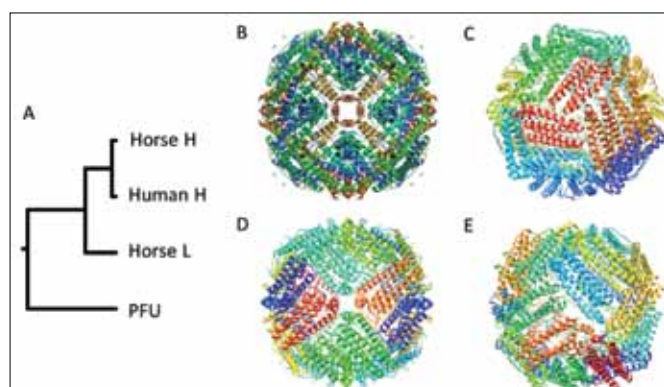


Fig. 1: FRTs from various organisms. A) Phylogenetic tree depicting evolutionary distances between horse L- and H-chain, human H-chain and PFU FRTs. B) Structure of horse L-chain FRT. C) Structure of

horse H-chain FRT. D) Structure of human H-chain FRT. E) Structure of PFU FRT.

The DOX loading was measured by UV/Vis spectroscopy and the structural integrity of DOX-loaded FRTs was investigated using 6% native PAGE (Fig. 2C). Next, cellular uptake into human prostate cancer cell line PC-3 was observed using fluorescent microscopy and 34  $\mu$ M DOX, either free or loaded into FRTs (Fig. 2D). Large differences between the uptake were observed. The highest uptake was of free DOX, determined as 100%. However, DOX-loaded PFU FRT showed uptake only slightly lower (80%). Out of mammal FRTs, highest uptake was observed for human H-chain FRT (34%), while horse L-chain and horse H-chain had uptake only 29 and 28%, respectively.

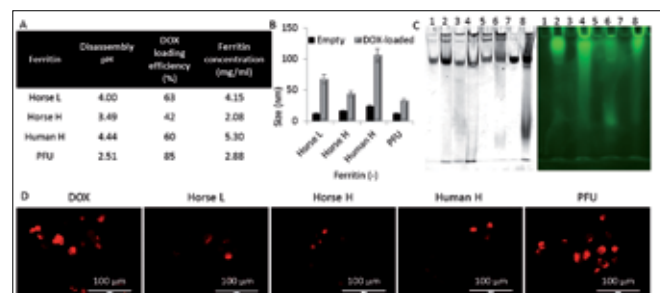


Fig. 2: Low molecular drug delivery employing FRTs from various organisms. A) DOX loading efficiency, FRTs concentration and pH required for disassembly. B) Average particle size of empty FRTs and FRTs loaded with DOX. C) 6% native PAGE showing native FRTs and after DOX loading (left part – coomassie blue staining; right part – DOX fluorescence); 1 – horse L-chain FRT; 2 – DOX-loaded horse L-chain FRT; 3 – horse H-chain FRT; 4 – DOX-loaded horse H-chain FRT; 5 – human H-chain FRT; 6 – DOX-loaded human H-chain FRT; 7 – PFU FRT; 8 – DOX-loaded PFU FRT. D) Cellular uptake of 34  $\mu$ M DOX, either free or loaded into FRTs, in PC-3 cells.

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## BIOINSPIRED MOLECULAR FACTORIES WITH ARCHITECTURE AND IN VIVO FUNCTIONALITIES AS CELL MIMICS

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Despite significant development efforts, to date artificial cells have limited compositions and have not yet been investigated *in vivo* to determine whether they preserve their architecture and functionality. By using an innovative combined approach, we overcome these limitations and achieve a flexible biomimetic strategy to create artificial cells that survive *in vivo* testing in a vertebrate model. We designed these cells as giant plasma membrane vesicles (GPMVs) generated by donor cells that, prior to the vesiculation process, internalized and/or overexpressed the building elements of the molecular machinery. An unprecedented level of complexity is achieved because the intrinsic nature of GPMVs, which mirrors the membrane and cytoplasm of the donor cells, is supplemented with desired functional elements, ranging from small molecular compounds over biomolecules (e.g., enzymes, proteins) up to nanometer sized artificial organelles, to provide cell-like functionality. These artificial cells were evaluated in a zebrafish vertebrate animal model where they retained their structure and function, successfully performing conversion of hydrogen peroxide. The unique advantages of highly varied composition, multi-compartmentalized architecture and preserved functionality *in vivo* open new avenues ranging from the study of bio-relevant processes in a robust cell-like environment up to patient-oriented medical solutions<sup>1,2</sup>.

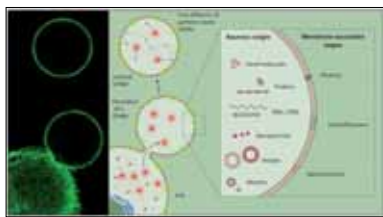


Fig. 1. GPMV-based strategy for creating bio-inspired artificial cells. Representation of molecular units that can be incorporated in the GPMV structure to support cell-like architecture and functionality.

Small molecular weight molecules, biomacromolecules, solid-sphere nanostructures, and hollow-sphere nanostructures are integrated into the cytoplasmic cavities of GPMVs to form complex molecular machinery (bottom-up approach). Loading of GPMV cavities is achieved through transfer of material from the donor cell cytoplasm during GPMV formation, while in addition the membrane associated cargo is transferred from the cell to the plasma membrane of GPMVs during vesicle formation (top-down approach).

Synthetic artificial cell mimics (ACMs) have previously been used in bridging the tissue-culture gap, circumventing use of living cells in the standard array of bio-assays (i.e. permeability and drug screening) used in academic or industrial research. However, ACM remain to this day predominantly synthetic, expensive, poorly biocompatible and lack a biomaterial diversity that is true to Cells. Inspired by Nature, we are introducing a solution that takes reactions and materials out of cells, packs them up in cell derived compartment vesicles and delivers them to researchers as next-to-life models of Cells – Equipped giant plasma membrane vesicles (E-GPMVs) (Patent Filed)<sup>2</sup>. E-GPMV represent the next generation of cell mimics and disrupt the current state of the art in how we perceive artificial cell technologies.

In contrast to established ACMs, E-GPMVs can be produced, isolated and delivered to customers from any cell-type (i.e. immune, cancer or primary cells), with minimum costs in production and a natu-

ral material diversity that is supreme to established ACM. E-GPMVs take »culture« out of tissue-culture by packing up cellular components in cell-sized packets, which can be shipped worldwide and analysed by established biological techniques (i.e. microscopy, flow cytometry) in the same way that cells do, but without the culturing. E-GPMVs directly mirror donor cells they are produced from and inherit both the cellular architecture (plasma membrane and cytosol) in addition to the cellular machinery (i.e. enzymes), making them 100% biodegradable and biocompatible.

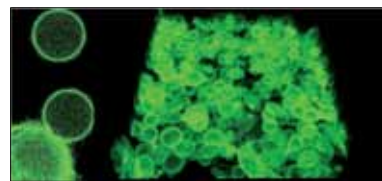


Fig. 2. GPMV-based strategy for creating bio-inspired artificial cells. Representation of molecular units that can be incorporated in the GPMV structure to support cell-

like architecture and functionality. Large scale production of E-GPMVs.

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## A NOVEL FLUIDIC INTESTINE-TUMOR-MODEL AS A TOOL TO STUDY THE ABILITY OF NANOPARTICLES TO ADSORB AND PERMEATE IN A FLUIDIC SYSTEM

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Nanoparticles as a drug formulation are widely used in pharmaceutical research. Nanoparticles have the ability to improve the characteristics of drug substances like changing hydrophobic properties to hydrophilic because of the drug incorporation in a nanoparticle system. The desired properties vary according to the application form as well as the target side. The basic characterizations of nanoparticles are the size, morphology, surface charge and advanced imaging techniques like atomic force microscopy, scanning electron microscopy and transmission microscopy. The second step is the *in vitro* biological testing. Therefore, the common assessment method is the use of cell lines or primary cells as a 2D or 3D cell culture model. The environment of these cell culture models is most commonly a static system like monolayer cell cultures in a microtiterplate. These cell models permit a high throughput screening of potential nanocarrier systems. To get a focused characterization on the desired properties further studies with specifically test systems are needed.

In this work, the focused characteristic of the nanoparticles are mucus adhesion and penetration into a tumour of the gastrointestinal tract. The gastrointestinal tract has different barriers that need to be overcome by the nanoparticles before they reach the tumour. The first barrier is the intestine fluid which has a pH with values



from 5-9 over the gastrointestinal tract. It also consists of enzymes which can degrade the nanoparticles before they reach the target site. Furthermore, the adhesion and the uptake can be reduced because of the fluidic flow and peristaltic movements of the gastrointestinal tract. However, one of the most important barriers is the mucus layer. It acts as a barrier which is hard to overcome for particle systems. To combine all these features into one model, a fluidic chip was built. The chip consists of a fluidic channel in which a tumour spheroid can be added (Figure 1).

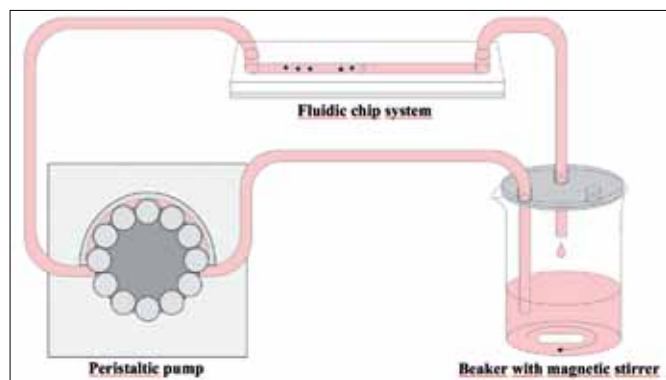


Figure 1 Schematic drawing of the fluidic system

The peristaltic pump circulates the fluid through the system. The fluid is stored in a beaker with constant stirring and the tumour spheroids are introduced into the microfluidic channel.

As a model for the intestine tumour, the cell line HT29-MTX-E12 was used. HT29-MTX-E12 cells are goblet cells which are able to produce mucus and build microvilli on the surface. The fluidic and peristaltic movement is simulated by a tubing system in combination with a peristaltic pump. Various liquids, substances and particles can be introduced into the system, according to the desired conditions. Initial studies with tumour spheroids in the fluidic system have already been carried out. There was no influence of the fluidic system on the vitality of the tumour spheroid in comparison to the static system over a testing period of three hours. Future studies will investigate potential nanocarrier systems. The use of the system makes it possible to qualify the adhesion and uptake of the nanoparticles into the tumour spheroid by using microscopy techniques. The quantification of the permeated amount could be measured by HPLC analysis (high performance liquid chromatography).

The development and the validation of the fluidic system is completed. It allows a focused characterization of nanoparticles based on their adsorptive and penetrative properties in a simulated fluidic intestine-tumour-model. The development and validation of the system as well as the setup for the experiments will be presented.

## MEMBRANE RADIOLABELLING OF EXOSOMES FOR COMPARATIVE BIODISTRIBUTION ANALYSIS IN IMMUNOCOMPETENT AND IMMUNODEFICIENT MICE – A NOVEL AND UNIVERSAL APPROACH

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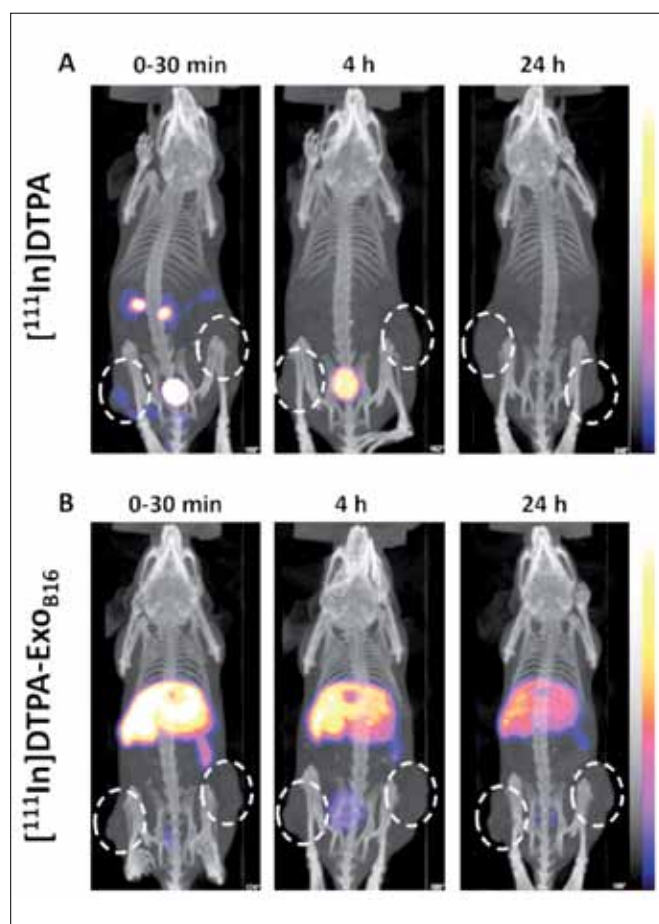
## INTRODUCTION

Extracellular vesicles, in particular exosomes, have recently gained interest as novel drug delivery vectors due to their biological origin, abundance, and hence their low immunogenicity. An in-depth knowledge of their *in vivo* biodistribution is therefore essential. This work aimed to develop a novel, reliable and universal method to radiolabel exosomes to study *in vivo* biodistribution in mice.

## METHODS

Melanoma (B16F10 cells)-derived exosomes (Exo<sub>B16</sub>) were isolated and characterised for size, yield, purity, exosomal markers and morphology using Nanoparticle Tracking Analysis (NTA), protein measurements, flow cytometry and electron microscopy. Exo<sub>B16</sub> were radiolabelled using 2 different approaches – intraluminal labelling (entrapment of <sup>111</sup>Indium via tropolone shuttling); and membrane labelling (chelation of <sup>111</sup>Indium via covalently attached bifunctional chelator DTPA-anhydride). Labelling efficiency and stability was assessed using gel filtration and thin layer chromatography. Melanoma-bearing immunocompetent (C57BL/6) and immunodeficient Exo<sub>B16</sub> (1x10<sup>11</sup> particles) followed by metabolic cages study, whole body SPECT-CT imaging and ex vivo gamma counting at 1, 4 and 24 h post-injection.

Fig. 1 Whole body SPECT/CT imaging of membrane-labelled <sub>B16</sub>F10 exosomes in melanoma-bearing C57BL/6 mice. (A) Animal was injected intravenously with free [<sup>111</sup>In]DTPA complex as control. (B) Animal was injected with [<sup>111</sup>In]DTPA-Exo<sub>B16</sub>. Imaging was done immediately, 4 h, and 24 h post-injection. White circles indicate the position of tumours.



## RESULTS

Membrane-labelled Exo<sub>B16</sub> ([<sup>111</sup>In]DTPA-Exo<sub>B16</sub>) showed superior radiolabelling efficiency and radiochemical stability compared to the intraluminal-labelled exosomes ([<sup>111</sup>In]-Exo<sub>B16</sub>). Exo<sub>B16</sub> showed similar biodistribution profile with both labelling approaches where or-

gan accumulation is prominently seen in liver and spleen (Fig 1). Interestingly, intraluminal-labelled Exo<sub>B16</sub> showed tumour accumulation of 6.7% injected dose per gram of tissue (ID/gT), which did not significantly differ to that of the mice injected with free [<sup>111</sup>In]Trop, whereas its membrane-labelled counterpart showed a significantly lower value of 0.6% ID/gT. The superior radiolabelling efficiency and stability of the membrane-labelling approach rendered its result more reliable and so was selected as the method to compare Exo<sub>B16</sub> biodistribution in melanoma-bearing immunocompromised (NSG) mice. Similar biodistribution profiles were observed in both C57BL/6 and NSG mice, where prominent accumulation was seen in liver and spleen, apart from the lower tumour accumulation observed in the NSG mice.

## CONCLUSION

Membrane labelling of exosomes is a reliable approach that allows for both live imaging and quantitative biodistribution studies to be performed on potentially all exosome types without engineering parent cells.

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## IN VITRO EVALUATION OF LIGNIN-BASED NANOPARTICLES FOR DRUG DELIVERY TO CANCER CELLS

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## SUMMARY

We have developed three lignin nanoparticles (LNPs): pure lignin nanoparticles (pLNPs), iron(III)-complexed lignin nanoparticles (Fe-LNPs), and Fe<sub>3</sub>O<sub>4</sub>-infused lignin nanoparticles (Fe<sub>3</sub>O<sub>4</sub>-LNPs) with round shape, narrow size distribution, reduced polydispersity and good stability at pH 7.4. The LNPs showed low cytotoxicity in all the tested cell lines and hemolytic rates below 12% after 12 h of incubation. Concerning the drug loading, pLNPs showed the capacity to efficiently load poorly water-soluble cytotoxic agents, e.g. benzazulene (BZL), and improve their release profiles at pH 5.5 and 7.4 in a sustained manner. Furthermore, the BZL-pLNPs presented an enhanced antiproliferation effect in different cells compared to the pure BZL and showed a maximal inhibitory concentration ranging from 0.64 to 12.4 mM after 24 h incubation. In addition, the superparamagnetic behavior of Fe<sub>3</sub>O<sub>4</sub>-LNPs makes them promising for cancer therapy and diagnosis, such as magnetic targeting and magnetic resonance imaging.<sup>1</sup> Posteriorly, we synthesized carboxylated lignin towards the functionalization of carboxylated lignin nanoparticles (CLNPs) with a block copolymer made of poly(ethyleneglycol) and poly(histidine) (NH<sub>2</sub>-PEG-PHIS) and a cell-penetrating peptide (CPP). A poorly water-soluble cytotoxic agent was successfully loaded into the CLNPs, improving its release profiles in a pH-sensitive manner and showing an enhanced antiproliferative effect in

the different cancer cells compared with a normal endothelial cell line.<sup>2</sup> Thus, the resulting LNPs are promising candidates for anticancer therapy.

## 1. INTRODUCTION

According to World Health Organization (WHO), cancer is one of the diseases with highest incidence and mortality worldwide, with 14.1 million new cases and 8.2 million deaths in 2012, and this is expected to increase in the future, reaching 20 million new cases per year by 2025.<sup>3</sup> The conventional cytotoxic chemotherapies options present some limitations, including non-specific systemic delivery of anticancer drugs, insufficient drug concentrations reaching the tumor site, intolerable cytotoxicity to the healthy tissues and development of multiple drug resistance. Therefore, the development of novel nanomaterials and nanocarriers are needed for cancer detection, diagnosis and treatment.<sup>4</sup> Being the lignin one of the most abundant biopolymers in nature, combined with the fact that it is naturally biodegradable, biocompatible and present very good stability, all make the lignin an ideal precursor for the development of environmentally friendly nanomaterials, compared to other nanoparticles used for anticancer therapy. Lignin is one of the most abundant aromatic polymer and renewable resource obtained from biomass in the Earth, comprising 20–35% of the composition of lignocellulosic materials.<sup>5</sup> In this work, we aimed to prepare and characterize three lignin-based nanosystems that can be used for different biomedical applications: pLNPs, Fe-LNPs and Fe<sub>3</sub>O<sub>4</sub>-LNPs. For this purpose, LNPs were used to evaluate the in vitro cytotoxicity using several cell lines, and their hemotoxicity was also assessed. BZL is a poorly water-soluble cytotoxic agent that is an inhibitor of the oncogenic Pim kinases that are often overexpressed in some solid tumors (e.g., prostate and colon cancers), promoting the cell survival and increasing the resistance against chemotherapy and radiation therapy.<sup>6</sup> Thus, BZL was used as a model drug to test the drug loading into pLNPs, and the release profile of drug loaded pLNPs were then investigated. Then, in vitro antiproliferation effect of BZL-loaded pLNPs was also studied using different cancer cell lines. In addition, the magnetic property of Fe<sub>3</sub>O<sub>4</sub>-LNPs was also assessed by placing the nanoparticles in a 6-well plate to guide them according to a magnetic field, and also by measuring their hysteresis loop. Posteriorly, we prepared (CLNPs) conjugated with NH<sub>2</sub>-PEG-PHIS and CPP in order to investigate the pH-responsive release of BZL from CLNPs. Also, the chemotherapeutic potential of the resulting BZL-loaded CLNPs was evaluated. Moreover, LNPs were characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM), and their stability in different media was evaluated.

## 2. EXPERIMENTAL RESULTS AND DISCUSSIONS

We have developed three types of LNPs with round shape, narrow size distribution and moderate dispersity (Figure 1). The LNPs showed low cytotoxicity in all the tested cell lines and reduced hemolytic rates after 12 h of incubation. The pLNPs showed the capacity to efficiently load poorly water-soluble drugs and improve their release profiles in a sustained manner. Also, BZL-pLNPs presented an enhanced antiproliferation effect compared to the pure BZL.

To test the magnetic behavior of Fe<sub>3</sub>O<sub>4</sub>-LNPs, their suspension was guided magnetically as shown in Figure 2. The Fe<sub>3</sub>O<sub>4</sub>-LNPs were attracted towards the point where the magnetic field is higher and translocated to another place of the well after moving the magnet, and then redispersed by gently shaking. Since the Fe<sub>3</sub>O<sub>4</sub> NPs are classified as superparamagnetic iron oxide NPs (SPIONs), they exhibit the phenomenon of superparamagnetism, and under application of an external magnetic field, they become magnetized up to their saturation magnetization. In this way, the magnetic properties of Fe<sub>3</sub>O<sub>4</sub> NPs and Fe<sub>3</sub>O<sub>4</sub>-LNPs were also assessed by the hysteresis loop, given by the magnetization versus magnetic field curves (Figure 2). Although the decrease on the saturation magnetization, 31 and 7 A m<sup>2</sup> kg<sup>-1</sup> for Fe<sub>3</sub>O<sub>4</sub> NPs and Fe<sub>3</sub>O<sub>4</sub>-LNPs, respectively, the results showed that the superparamagnetic properties were preserved after the encapsulation of Fe<sub>3</sub>O<sub>4</sub> NPs into LNPs.

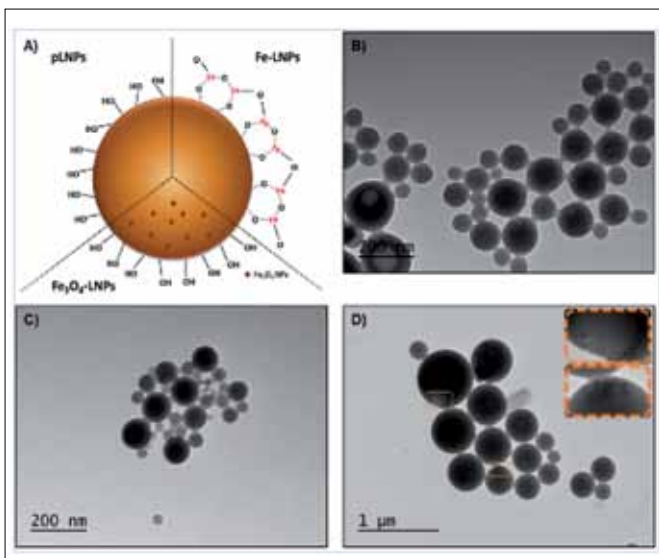
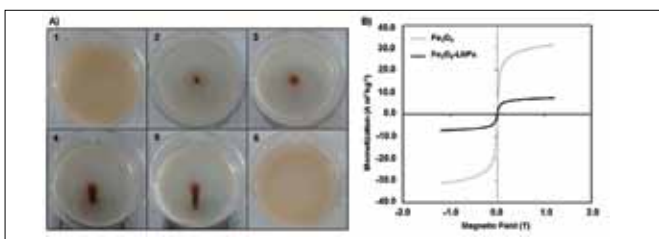


Figure 1 – Schematic representation of the three types of LNPs evaluated in this work (A), and TEM images of (B) pLNPs, (C) Fe-LNPs and (D) Fe<sub>3</sub>O<sub>4</sub>-LNPs.

Figure 2 – Characterization of the magnetic behavior of the Fe<sub>3</sub>O<sub>4</sub>-LNPs. (A) Sequence of images showing the magnetic-guided behavior of Fe<sub>3</sub>O<sub>4</sub>-LNPs in suspension: 1, Fe<sub>3</sub>O<sub>4</sub>-LNPs in suspension; 2 and 3, Fe<sub>3</sub>O<sub>4</sub>-LNPs accumulated around the magnetic field after placing the magnet under the well; 4 and 5, Fe<sub>3</sub>O<sub>4</sub>-LNPs guided by the magnetic field after moving the magnet under the well; 6, Fe<sub>3</sub>O<sub>4</sub>-LNPs redispersed by gently shaking. (B) Hysteresis loop of Fe<sub>3</sub>O<sub>4</sub> NPs and Fe<sub>3</sub>O<sub>4</sub>-LNPs.



Then, after synthesis of CLNPs conjugated with NH<sub>2</sub>-PEG-PHIS and CPP, the prepared CLNPs showed spherical shape and good size distribution, good stability in physiological media and low cytotoxicity in all the tested cell lines. Moreover, BZL was successfully loaded into the CLNPs, improving its release profiles in a pH-sensitive manner and showing an enhanced antiproliferative effect in the different cancer cells compared with a normal endothelial cell line (Figure 3).

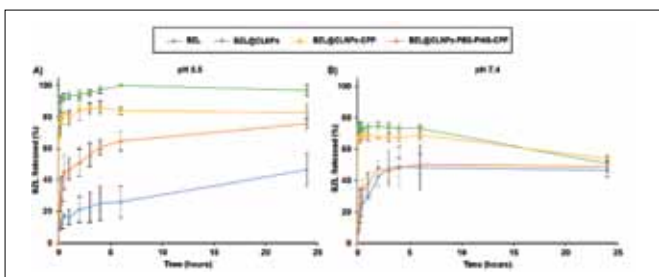


Figure 3 – Release profiles of pure drug and drug-loaded CLNPs using: (A) HBSS–MES (pH 5.5), and (B) HBSS–HEPES (pH 7.4), containing 2% Tween-80, at 150 rpm at 37 °C for 24 h. Errors bars represent mean ± sd (n = 3).

### 3. CONCLUSIONS

Overall, these LNPs showed important features for drug delivery and biomedical applications, including biocompatibility, good colloidal stability, ability to load hydrophobic drugs and sustain their release, and good cellular interaction. Furthermore, the superparamagnetic behavior of Fe<sub>3</sub>O<sub>4</sub>-LNPs makes them promising for cancer therapy and diagnosis.

### ACKNOWLEDGMENT

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### HAEMOCOMPATIBILITY TESTING OF GELATIN COATED MAGNETITE NANOPARTICLES

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Magnetic nanoparticles for biomedical applications are usually formed by a mineral core of a magnetic element, such as iron oxides, and an organic coating. In our study we used magnetite nanoparticles with gelatin coating. The magnetite nanoparticles were synthesized using co-precipitation method and in the coating procedure we used different concentration of gelatin for figuring out the optimal stabilization.

Haemocompatibility testing is a simple, reliable and efficient method to analyze the cytotoxicity activity of magnetite nanoparticles on erythrocytes membrane, evaluated spectrophotometrically by observing the release of oxyhemoglobin. In our preliminary study, the hemolysis was investigated on the red blood cells using magnetite nanoparticles with different concentrations and compositions. The erythrocyte haemocompatibility assay showed no significant hemolytic potential of the samples containing uncoated or gelatin coated magnetite nanoparticles, indicating no cytotoxic activity on human erythrocytes as showed in Figure 1. Preliminary experiments revealed that the gelatinated magnetite nanoparticles are especially efficient for targeted therapies under the influence of magnetic field.

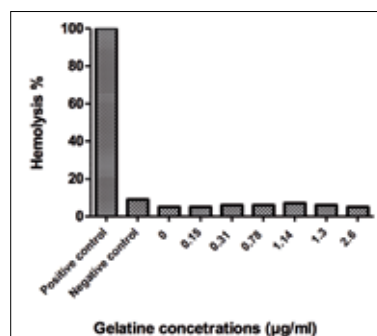


Figure 1: Haemocompatibility testing on human erythrocytes with increasing concentrations (0-2.6  g/ml) of gelatin coated magnetite nanoparticles.



## EFFECT OF THE BIOMOLECULAR CORONA COMPOSITION ON THE MECHANISM OF ENDOCYTOSIS OF NANOSIZED MATERIALS

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Nanosized materials have drawn a lot of interests in the last decades in drug delivery due to their unique physicochemical characteristics and biological properties. In fact, their high cellular uptake efficiency and their ability to interact with the cellular machinery in new ways compared to conventional drugs has made them promising candidates to be used as drug delivery systems. However a clear understanding of the mechanisms of cellular recognition, internalization and processing of these objects is still missing in many cases. Only with this knowledge it will be possible to design truly targeted nanomedicines and control their localization, uptake and fate inside cells<sup>1</sup>.

Within this context, the aim of our study is to characterize the endocytic mechanisms involved in the internalization of nanosized materials. We use model nanomaterials of different sizes, such as fluorescently labelled amorphous silica (50nm SiO<sub>2</sub> NPs), because of their stability and their well-defined properties. Particle uptake is quantified by flow cytometry and fluorescence imaging. Chemical inhibitors together with RNA interference directed toward key proteins involved in endocytosis are used to determine the role of specific pathways or molecules in the uptake. In order to determine the effect of the biomolecular corona on the mechanism of uptake, we performed this study in the presence of relevant biological media, such as human serum, at different concentrations up to *in vivo* conditions. In fact, it is known that the environment in which nanosized materials are dispersed confers them new properties that influence remarkably their cellular interactions<sup>2</sup>.

The results indicate that the proteins adsorbed on the nanoparticle surface have a key role first in triggering cellular recognition and then to direct nanoparticles towards specific endocytic routes (Figure 1). In fact, we found that not only the corona composition depends on the serum content but that the involvement of the LDL receptor in the uptake of these particles, already reported elsewhere<sup>3</sup>, is present just when the particles are incubated in high serum concentrations. Furthermore we show that the serum content and resulting coronas also affect the mechanism by which the particles enter cells. Finally, the results also suggest that multiple endocytic pathways and novel protein targets might be involved in the uptake mechanism of nanomaterials.

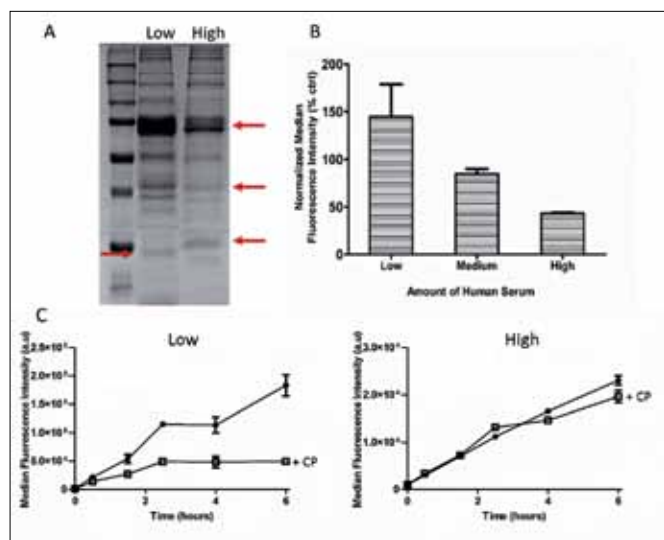


Figure 1 Serum concentration affects the corona composition of 50nm SiO<sub>2</sub> NPs and leads to different uptake mechanisms on cells.

(A) Gel electrophoresis of corona proteins on 50nm SiO<sub>2</sub> NPs incubated with high or low amounts of human serum. The arrows indicate key proteins identified. (B) NP uptake on HeLa cells after silencing of the LDL receptor: the uptake of these NPs is reduced upon LDLR silencing only when they are incubated in the presence of high serum concentrations. (C) The same NPs enter cells by different pathways depending on their corona: an example is shown for the effect of chlorpromazine (CP) on the uptake after corona formation at low (left) or high (right) serum concentration.

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## THE ROLE OF THROMBOXANE A2 IN COMPLEMENT ACTIVATION-RELATED PSEUDOALLERGY

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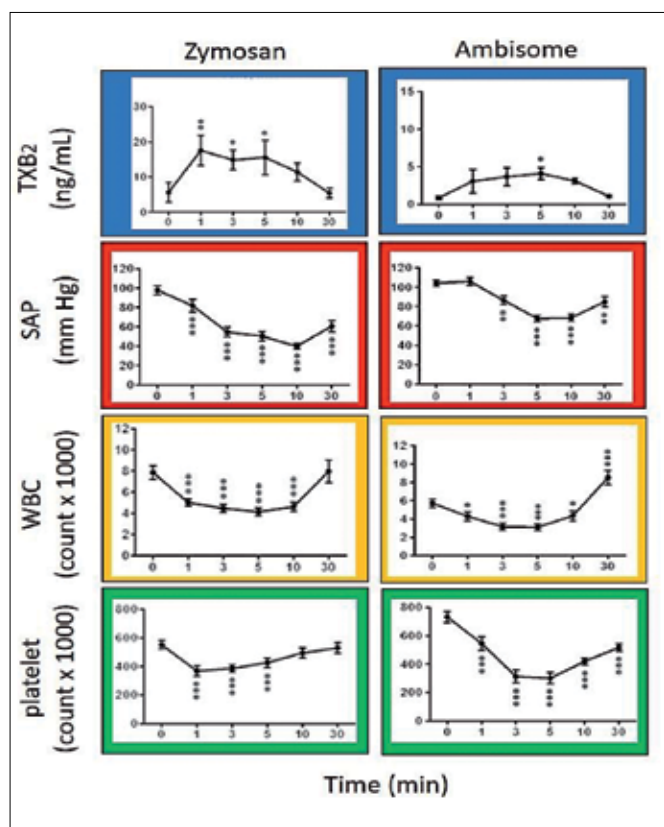
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## INTRODUCTION:

Numerous state-of-art drugs and imaging agents can cause an acute immune reaction known as complement (C) activation-related pseudoallergy (CARPA): an anaphylactoid or infusion reaction whose rise can be associated with activation of C system<sup>(1-6)</sup>. These can include nanoparticles and nanomaterial such as the liposomal drugs Doxil and AmBisome and micellar solvents containing amphiphilic lipids Cremophor EL. It has a complex molecular and cellular mechanism that involves the production, actions and interactions of numerous vasoactive mediators in blood. A CARPA reaction starts with the liberation of numerous vasoactive mediators from a variety of allergy-mediating cells, e.g., mast cells, basophil leukocytes, secretory macrophages, white blood cells and platelets. The mediators, referred to as “allergomedins”, include, among others, thromboxane A 2 (TXA2), tryptase, proteases, histamine, leukotrienes, platelet activating factor (PAF) and slow reacting mediators (such as SRS-A (slow-reacting substance of anaphylaxis) causing anti-histamine resistive prolonged, slow contraction of smooth muscle, or an increase in vascular permeability and mucous secretion by prostaglandins and eosinophil chemotactic factors, etc.)<sup>(1)</sup>. TXA2 and CARPA:

Thromboxane A2 (TXA2), a member of the family of lipids known as eicosanoids, is an oxygenated metabolite of arachidonic acid (AA). It is generated from AA by the actions of cyclooxygenase (COX-1 and 2) and thromboxane-synthase (TXS). It was discovered in the lung perfusate of guinea pigs undergoing anaphylactic shock<sup>(7)</sup>. Anaphylactic shock, ending in cardiac failure (cardiac anaphylaxis) is also the most severe manifestations of rat<sup>(8)</sup> and porcine CARPA<sup>(9)</sup> therefore, it seems logical to connect TXA2 to CARPA not only in guinea pigs but in other animals and man as well. As for its biological activity, TXA2 is a potent vasoconstrictor and pro-coagulant agent, and stimulates platelet activation and aggregation.

Figure 1: Typical manifestations of CARPA in rats injected with AmBisome (22 mg/kg) and Zymosan i.v., (5 mg/kg). The panels with different background colors are different symptoms, specified on the Y axes. The panels in each column are measurements from the same reaction, averages and SE from 6 rats for each test agent <sup>(10)</sup>



The CARPA “syndrome” involves hemodynamic, hematological, skin and laboratory changes. Out of these symptoms, TXB2 is used as the best “laboratory” biomarker, as in all animal models studied to date its rise was significant and highly reproducible, closely paralleling the hemodynamic and blood cell changes. Figure 1 shows the close parallelism of TXB2 changes with the decline of systemic arterial pressure (SAP) and biphasic drop followed by a rise of WBC and platelet counts in rats in response of i.v. injected liposomes (AmBisome) and Zymosan.

Animal experiments and recent clinical observations suggest that the cyclooxygenase blocker indomethacin, may represent an effective new approach to prevent liposome-induced CARPA, lending clinical relevance to better understand the involvement of TXA2 and other eicosanoids in this adverse immune effect. Figure 2A reminds of some basic information on indomethacin, while Figure 2B recapitulates the experiment indicating full inhibition of multilamellar liposome (MLV)-induced CARPA in pigs by 5 mg/kg indomethacin. Importantly, according to anecdotal evidence, indomethacin has also been used successfully to prevent Doxil- and other liposome-induced hypersensitivity reactions in cancer patients <sup>(11)</sup>.

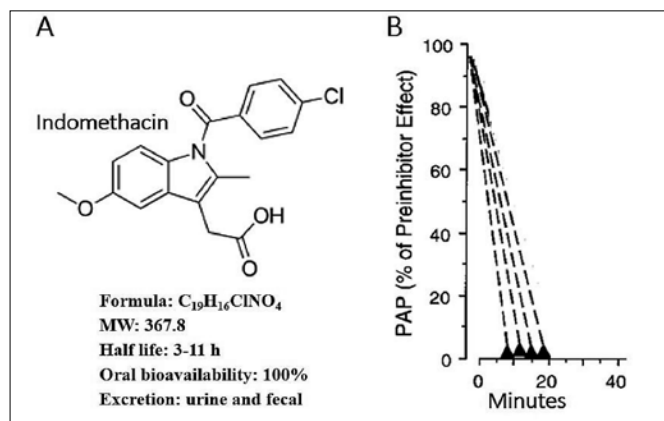


Figure 2. Basic characteristics of indomethacin (A) and its effect on MLV-induced CARPA in pigs (B). In panel B MLV was injected in 4 pigs at time 0 to induce reactions, and the rises of PAP were measured to quantify baseline CARPA. 15 min later, after returning of baseline values, the animals were treated with 5 mg/kg i.v. indomethacin i.v., followed by injection of the same dose of MLV as given at time 0. The maximal rise of PAP at this time was related to the maximal rise of PAP at baseline, and the ratios are given in %.

## DISCUSSION:

The consistent observation in many studies and different animals over the past decade, that TXA2 is a key, rate-limiting mediator of hemodynamic changes in CARPA represents a major step towards solving the CARPA problem. The other practical benefit of focusing on thromboxanes, or other AA products generated during CARPA is that they serve as a quantitative biomarker to indicate the severity of reactions. To our experience to date, none of the blood tests correlate so well with the hemodynamic changes in CARPA than TXB2, providing a valid endpoint for *in vivo* screening assays for regulatory evaluation of the CARPAgenic effect of drugs under R&D.

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## A NEW APPROACH OF BONE EVALUATION IN ORAL REGENERATIVE SURGERY BASED ON RAMAN SPECTROSCOPY. VIABLE ALTERNATIVE TO HISTOLOGY.

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**Resume:**The majority of studies evaluating the effects of different surgical procedures aimed at defect fill with bone grafts and only employed clinical outcome measures, such as probing pocket depth, probing attachment level, radiological analysis and direct visualization, following surgical re-entry procedures. Such approaches did not facilitate the determination of true bone regeneration, an outcome that requires histologic investigation. A non-invasive and quick method for evaluation of chemical compounds from bone tissues is requested. We suggest a new method, based on the Raman spectroscopy. This non-destructive optical method is able to characterize and differentiate initial normal cortical bone, initial augmentation material and final regenerated bone.

Regarding our study, for harvested bone samples were selected 2 patients, before and after maxillary - sinus lift augmentation procedure (cerabone material as bone substitute was used). The healing period was approximatively 8 months for both patients. Bioethical approval was obtained.

Raman Spectroscopy was performed respecting same geometrical conditions for data recording. Corresponding spectra were acquired before and after surgical augmentation procedure. Differences in peaks intensity on raw spectra reflect the differences in the quantities of the chemical components (related to specimens concentration) for investigated specimens. Sensitive information (related to bone tissue nanoscale processes) obtained from the Raman spectra (shape related to fluorescence) using raw data, were compared with the histological results (collagen matrix / quantity / bone substitute integration).



*Figure 1. Histological results. Details: collagen matrix, augmentation material and new bone regenerated tissue.*

For both patients' bone samples, higher PPI peak intensities were obtained before treatment (73.04 % - patient #1 and 81.22 % - patient #2; highest value recorded for patient #2 with previous periodontal problems) and lower values after treatment (48.76% - patient #1 and 38.39% – patient #2). PPI is known

acting as a potent inhibitor of HAP crystals precipitation (biological mineralization, a nanoscale process), aspect that might causes periodontal disease. From histology investigation, morphometric results for ratio areas (bone tissue / implant material) are: 1.6067 - patient #1 and 0.6970 – patient #2. Histological results confirm Raman evaluation of bone samples.

Raman technique is capable to offer a complete bone evaluation (qualitative / quantitative), in the meantime being an independent method.

**Acknowledgment:** Part of financial support was obtained from B&W Tek Company USA, by equipment parts acquisition.

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## ARE EXISTING STANDARD METHODS SUITABLE FOR THE EVALUATION OF NANOMEDICINES? – SOME CASE STUDIES

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The use of nanotechnology in medical products has been demonstrated at laboratory scale, and many resulting nanomedicines are in the translational phase towards clinical applications, with global market trends indicating a strong growth of the sector in coming years.

The translation of nanomedicines towards the clinic and subsequent commercialisation may require the development of new or adaptation of existing standards to ensure the quality, safety, and efficacy of such products.

This work addresses some identified needs, and illustrates the shortcomings of currently used standardised methods when applied to medical-nanoparticles to assess particle size, drug loading, drug release and *in vitro* safety. Alternative physicochemical and *in vitro* toxicology methods, with potential to qualify as future standards supporting the evaluation of nanomedicine, are provided.

### KEYWORDS:

Safety assessment, nanomedicine, regulatory, particle size distribution, drug release, cytotoxicity, nanoparticle tracking analysis, field flow fractionation, analytical ultracentrifugation, high content screening.



## FUNDING

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<http://www.euncl.eu/>

## EXCITATION-DEPENDENT THERANOSTIC NANOSHEET FOR CANCER TREATMENT

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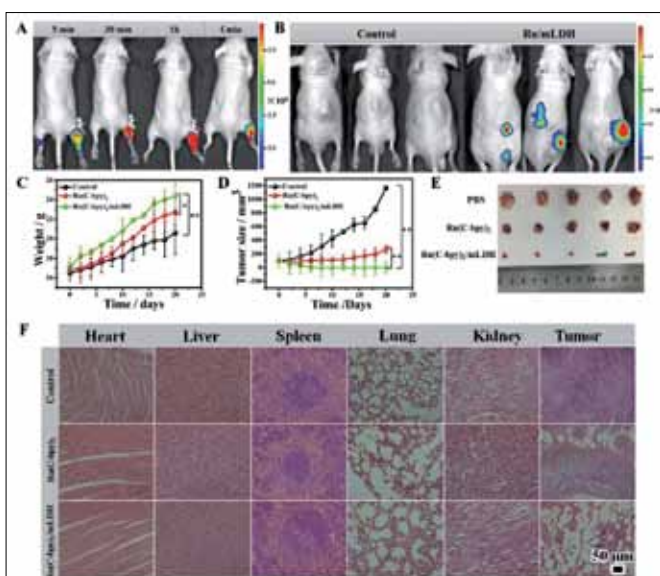
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Figure 1. Schematic illustration of Ru(C-bpy)<sub>2</sub>/mLDH for hypoxia-specific cancer imaging and photodynamic therapy.

Figure 2. *In vivo* imaging of mouse whose right leg was tied and then was administrated by 50  $\mu$ L Ru(C-bpy)<sub>2</sub>/mLDH via i.m. injection to both legs (A). *In vivo* imaging of Hela-bearing mice after the i.v.

injection of PBS and Ru(C-bpy)<sub>2</sub>/mLDH (B). The excitation was at 488 nm. Body weight (C), tumor size (D) and isolated tumor tissue (E) of various groups ( $n = 5$ ) after the cancer therapy by i.t. injection of the formulations and irradiated under 520 nm for 8 min. Statistics analysis, \* $p < 0.05$ , \*\* $p < 0.01$ . Histology staining of heart, liver, spleen, lung, kidney and tumor slices collected from different groups of mice after 20 d treatment (F).



To examine the hypoxia triggered fluorescence imaging *in vivo*, the right leg of nude mice was tied up to induce a low oxygen concentration condition. Then the Ru(C-bpy)<sub>2</sub>/mLDH was dosed to both legs via i.m. injection. As shown in Figure 2A, significant contrast is seen between the two legs throughout the test, i.e. from 5 mins to 1 h post-injection, where the fluorescence on the tied leg gradually enhances, and no fluorescence is viewed on the other leg during the period. After 1 h, we released the tied leg. Figure 2B shows the *in vivo* imaging results which confirm that the luminescence distribution is mainly in the tumor region upon an excitation at 488 nm.

Furthermore, the therapeutic ability of the Ru(C-bpy)<sub>2</sub>/mLDH was tested. As indicated in Figure 2C, there is no body weight lost after the cancer treatments in the Ru(C-bpy)<sub>2</sub>/mLDH group, an indication of the tolerance of the animal upon the administration of the formulation. In addition, although there is an obvious inhibition of tumor growth by the treatment with Ru(C-bpy)<sub>2</sub> when compared with the control, the Ru(C-bpy)<sub>2</sub>/mLDH sample demonstrates significant suppression of tumor volume rather than the other two groups (Figure 2D and 2E). This is attributed to the favorable singlet oxygen production of nanosheets under the 520 nm irradiation even for a short-time. Histopathology assessment using hematoxylin and eosin (H&E) staining demonstrates the serious necrosis in the tumor tissue after treated by the Ru(C-bpy)<sub>2</sub>/mLDH, while there is no obvious damage to the main organs including heart, liver, spleen, lung and kidney (Figure 2F). Therefore, this Ru(C-bpy)<sub>2</sub>/mLDH nanosheet to be an excellent theranostic agent for the cancer treatment, with which the diagnosis and therapy processes.

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## THERMO-RESPONSIVE IRON OXIDE NANOPARTICLES FOR NON-INVASIVE MAGNETIC IMAGING OF CANCER CELLS

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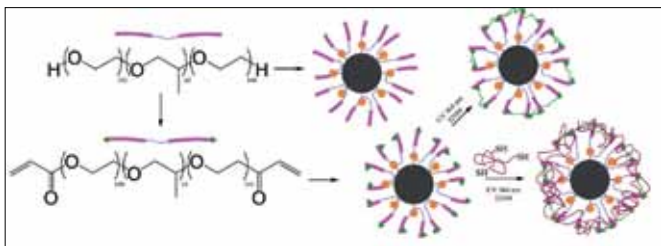
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Non-invasive imaging of tissues, cells and pathogens using magnetic fields has been widely employed in diagnostics. Specifically, magnetic nanoparticles received considerable attention since they enhance the image signal or contrast and decrease the time needed to obtain high-resolution images. In comparison to magnetic resonance imaging (MRI), where signal intensity depends mainly on properties of the tissues along with imaging parameters, magnetic particle imaging (MPI) is a real-time imaging technique that is based on the imaging of superparamagnetic nanoparticles without background noise coming from tissues. Here, we report on thermo-responsive core-shell iron oxide probes for non-invasive imaging of the multiple myeloma cancer cell line MOPC. The polymeric shell of particles is composed of biocompatible amphiphilic polymers, whereas the core is composed from 18 nm iron oxides synthesized by thermal decomposition. Furthermore, we compared how cross-linking of polymeric shell influences thermo-responsiveness, relaxation time and signal in magnetic particle spectroscopy. It is found that synthesized particles are not toxic to MOPC and healthy cells in *in-vitro* tests. In addition, our results indicate that the prepared particles have sufficient magnetic particle spectroscopy performance to be visualized by MPI after cellular uptake. These properties allow them to be used as novel biocompatible tracers for non-invasive MPI of multiple myeloma.

**Keywords:** SPIO, thermal decomposition, cross-linked micellar shell, poloxamers, thermo-responsive coating, MOPC, MRI, MPI, MPS.

## GRAPHICAL ABSTRACT:

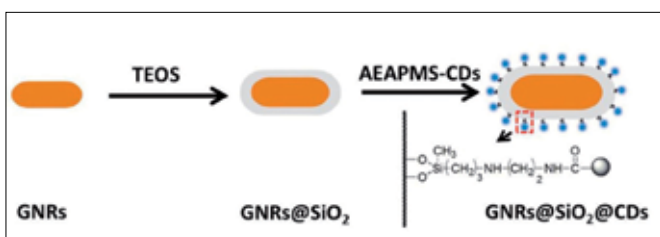


## MULTIMODAL BIOIMAGING BASED ON GOLD NANOROD AND CARBON DOT NANOHYBRIDS AS A NOVEL TOOL FOR ATHEROSCLEROSIS DETECTION

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Advanced biocompatible and robust platforms equipped with diverse properties are highly required in biomedical imaging applications for the early detection of atherosclerotic vascular disease and cancers. Designing nanohybrids composed of noble metals and fluorescent materials is a new way to perform multimodal imaging to overcome the limitations of single-modality counterparts. Herein, we propose the novel design of a multimodal contrast agent; namely, an enhanced nanohybrid comprising gold nanorods (GNRs) and carbon dots (CDs) with silica (SiO<sub>2</sub>) as a bridge. The nanohybrid (GNR@SiO<sub>2</sub>@CD) construction is based on covalent bonding between SiO<sub>2</sub> and the silane-functionalized CDs, which links the GNRs with the CDs to form typical core-shell units. The novel structure not only retains and even highly improves the optical properties of the GNRs and CDs, but also possesses superior imaging performance in both diffusion reflection (DR) and fluorescence lifetime imaging microscopy (FLIM) measurements compared with bare GNRs or fluorescence dyes and CDs. The superior bioimaging properties of the GNR@SiO<sub>2</sub>@CD nanohybrids were successfully exploited for *in vitro* DR and FLIM measurements of macrophages within tissue-like phantoms, paving the way toward a theranostic contrast agent for atherosclerosis and cancer.



## MICROFLUIDIC CONTROL DEVICE USING ORGEL ROLL FOR GENETIC ANALYSES

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An automated microfluidic control system using orgel roll capable of precisely controlling reaction solution and applicable to various lab-on-a-chips and biosensor. There have been developed biosensors for easily and quickly diagnosing and analyzing a biological sample. Upon loading the biological sample (for example, blood, urine, saliva or the like), the orgel roll carries out on film chip for

sample pretreatment and DNA amplification, followed by separation and detection sequentially performed on a single chip. The orgel roll delivers sample/reagents to the film chip for DNA purification from a biological sample. The orgel roll transfers each storage chamber and receiving chamber communicate with each other through the transfer channel directs purified nucleic acid and PCR reagents from the reaction chamber for DNA amplification. Pressurizing protrusion formed on an outer peripheral surface of the roller and rotating the roller to pressurize the storage and reaction chamber with microliter aliquots of PCR reaction mixture allow separation and detection to provide genetic information.

## INTRODUCTION

Recent development trends of biochips include the development of high-performance biochips with high sensitivity, quantification, reproducibility, and multiple simultaneous analyses. In addition, a lab on-a-chip has been developed in which sample pretreatment, analysis and detection are sequentially performed on a single chip. Thus, in order to develop a biochip in the form of a high-performance lab-on-a-chip, a reproducible implementation of a complicated reaction protocol is required, which may be achieved a precise and automated supplying of reaction solutions. Therefore, it is necessary to study the microfluidic control system for genetic analysis. The development of this systems involves the integration of orgel roll type components such as microscale separations reactions into microfluidic control devices. Several polymerase chain reaction (PCR) device that have integrated DNA amplification reactions and electrophoretic separations have been developed. Devices that employ hybridization optical detection have been fabricated to perform sample preparation, various microfluidic operations, and PCR. Integration of multiple steps of genetic analysis on microfluidic control device provides significant advantages in terms of sample/reagent consumption, process automation, analysis speed and efficiency. Fig. 1 shows schematic of the microfluidic control device using orgel roll may move, fix, mix, or dispense the reaction solution in the microfluidic chip. Fig. 2 shows design and fabrication of orgel roll for microfluidic control device.

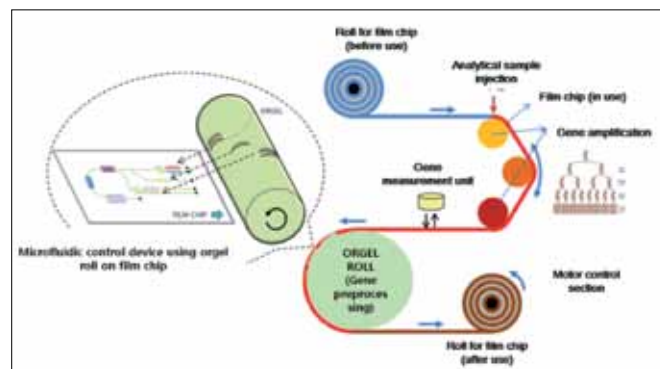


Fig. 1. Schematic of the microfluidic control device using orgel roll on film chip

The operation of the microfluidic control system for controlling the reaction solution. A first roller which is in contact with the microfluidic chip and rotates together with the movement of the microfluidic chip and pressurizing protrusion formed on the outer peripheral surface of the first roller, wherein the pressurizing protrusion has a shape corresponding to the storage chamber. Examples of applications include early detection and control for toxic pathogens (MERS, foot-and-mouth disease, avian influenza, swine flu, etc.). It is important to contain the genetic information in pathogen are required for monitoring and fast diagnostics at the location where diagnosis is needed (point of care) and automation required to perform tests. However, attention for quality improvement aspects regarding the usual diagnostics tests are mainly need for manual sample preparation, amplification products and analysis the user is necessary. The use of orgel roll device will contribute to in diagnostic procedure analysis are qualified for sequentially movement and automation. An automated microfluidic control system for genetic analysis has described the current state of the technique with



respect to automatic system for clinical applications. Research has shown that disease surveillance and monitoring system are using driving mechanism of film chip and orgel type device plays automatic mechanically. The use of orgel type device by toxic pathogen monitoring will increase if they are required in the development of new devices that meet their needs. This way, the benefits of genetic analysis using microfluidic control system will improve quality of easily and quickly diagnosing.

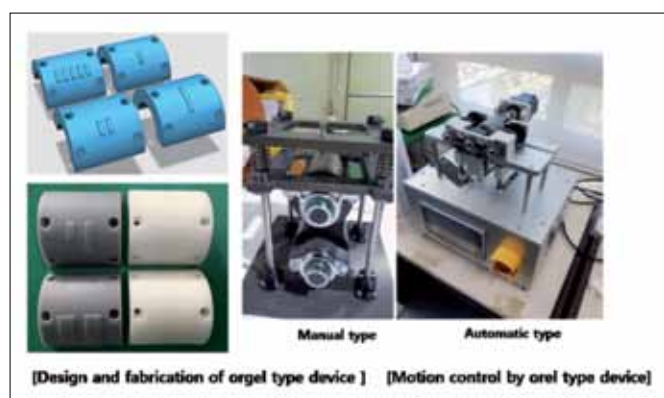


Fig. 2. Design and fabrication of orgel roll for microfluidic control device

## IN VIVO BRAIN IMAGING BY ULTRA-BRIGHT DYE-LOADED FLUORESCENT POLYMERIC NANOPARTICLES

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**Introduction:** In bio-imaging, polymeric nanoparticles (NP) could be a good alternative to the quantum dots, since they are biodegradable, biocompatible and active substance could be loaded inside or adsorbed onto the surface [Reisch, A.; Klymchenko, A.S. Fluorescent Polymer Nanoparticles Based on Dyes: Seeking Brighter Tools for Bioimaging. *Small*, 2016, 12, 1968-1992.]. However, the brightness of such NPs is usually limited by aggregation and self-quenching of the dye molecules. To achieve high brightness of dye-loaded fluorescent polymeric NPs we recently proposed to encapsulate cationic dyes with their bulky hydrophobic counterions [Reisch, A.; Didier, P.; Richert, L.; Oncul, S.; Arntz, Y.; Mély, Y.; Klymchenko, A. S. Collective fluorescence switching of counterion-assembled dyes in polymer nanoparticles. *Nature Commun.* 2014, 5, 4089; Andreiuk, B.; Reisch, A.; Lindecker, M.; Follain, G.; Peyrieras, N.; Goetz, J. G.; Klymchenko, A. S.: Fluorescent Polymer Nanoparticles for Cell Bar-coding In Vitro and In Vivo. *Small* 2017, 13, 1701582]. The aim of the present study is to design fluorescent polymeric NPs characterized by high brightness and sufficiently long circulation time for the brain imaging *in vivo* using two-photon excitation microscopy.

**Materials and methods:** Dye-loaded polymeric NPs based on poly(methyl methacrylate) (PMMA) bearing sulfonate groups and the fluorescent dye R18/F5-TPB, a rhodamine B derivative featuring efficient encapsulation and high brightness (quantum yield and two-photon absorption cross-section), were prepared by nanoprecipitation from organic solution into aqueous buffer [Reisch, A, Runser A, Arntz Y, Mély Y, Klymchenko AS. Charge-controlled nanoprecipitation as a modular approach to ultrasmall polymer nanocarriers: making bright and stable nanoparticles. *ACS Nano* 2015, 9, 5104.]. The final concentration of the dye was 20 wt% relative to the polymer and NP concentration was 0.4g/L. Characterization

of the size, polydispersity and zeta potential of the NPs was performed by dynamic light scattering (Malvern Zetasizer ZSP). Stability of NPs PEGylated by Pluronic amphiphiles in different biological media was characterized by a home-built fluorescence correlation spectroscopy (FCS) setup using Olympus IX70 inverted microscope and Two-photon excitation at 830 nm by an InSight DeepSee laser (Spectra Physics). For multiphoton imaging we used an upright Zeiss LSM710 confocal microscope. All animal experiments were conducted in accordance with institutional guidelines and approved by the Government of Upper Bavaria. 8-week old C56/Bl6J mice were anesthetized and then endotracheally intubated and ventilated in a volume controlled mode (MiniVent 845, Hugo Sachs Elektronik, March-Hungstetten, Germany) with continuous recording of end-tidal pCO<sub>2</sub>. Throughout the experiment, body temperature was monitored and maintained by a rectal probe attached to a feedback-controlled heating pad. A probe was placed in the femoral artery for measurement of mean arteriolar blood pressure and for administration of the fluorescent dye. A rectangular 4x4-mm cranial window was drilled over the right fronto-parietal cortex located 1-mm lateral to the sagittal suture and 1-mm frontal to the coronal suture. Subsequently, an exact fitting rectangular cover glass of 0.175 μm thickness was placed upon the window and fixed onto the skull with dental cement. Animals were administered either 0.1 mL of PMMA nanoparticles or 0.1 mL of PMMA nanoparticles coated by 1% Poloxamer 188 (Sigma-Aldrich, Germany), directly injected into the femoral artery. With the aim to identify the brain vessels mice were then injected with FITC-dextran (0.05 mL). Afterwards, mice were placed on the multiphoton microscope adapted for intravital imaging of small animals, and images were taken immediately after the injection of solution with NPs.

**Results:** Fluorescent NPs of different sizes ranging from 40 to 60 nm were prepared, depending on the buffer and presence of salt in the medium. After coating with Poloxamer the hydrodynamic diameter increased by ~10 nm, corresponding to a monolayer of this amphiphile deposited on the nanoparticle surface. FCS studies suggested that the size of bare fluorescent NPs increased significantly in the presence of fetal bovine serum, probably due to strong adsorption of biomolecules. In sharp contrast, NPs coated with Poloxamer 188 showed nearly no change in the particle size suggesting the efficient protection from the non-specific interaction. Therefore, Poloxamer-coated NPs appeared promising for *in vivo* imaging. *In vivo* study explicitly showed that both solutions: bare PMMA NPs and NPs coated by Poloxamer 188 are clearly detectable in mouse brain vasculature using two-photon microscope (Fig.1). Importantly, Poloxamer-coated NPs showed much longer circulation time than bare ones, in line with much weaker adsorption of protein components of serum in our *in vitro* measurements.

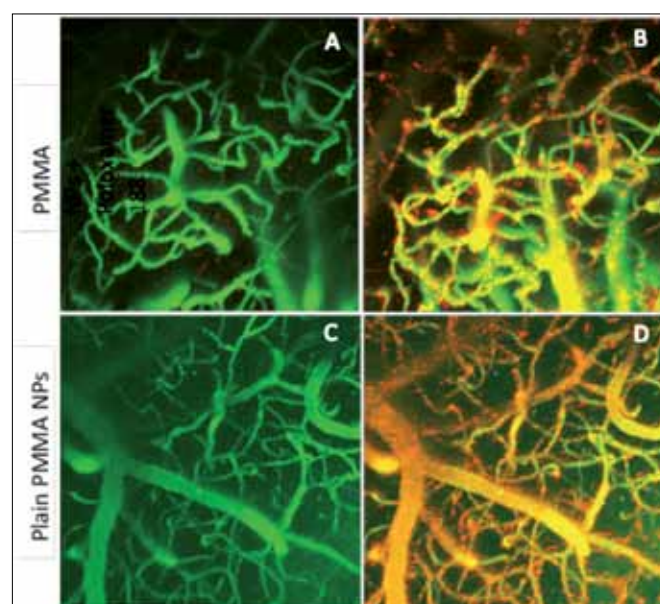


Fig.1 Brain vasculature of C57/Bl6 mice. Z-stack, 200 μm depth, maximum intensity projection. Scanned images immediately after



injection of A, C: FITC-dextran, B: Bare PMMA NPs, D: PMMA NPs coated by Poloxamer 188.

**Conclusion:** We have created robust, super-bright, safe, and bio-compatible polymeric nanoparticles which are clearly detectable both *in vitro* and *in vivo*. Such data can serve as the base for future developments of brain bio-imaging and drug-delivery systems.

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## EFFECTS OF ULTRAVIOLET PHOTO-FUNCTIONALIZATION WITH 5% TiO<sub>2</sub> COATED HA ON BONE REGENERATION IN RABBIT CALVARIAL DEFECTS

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Regeneration of bone defects destroyed by periodontal disease, trauma and inflammation is an important task in dental clinics. Porous hydroxyapatite (HA) is a bone graft material with osteoconductivity. However, the hydrophobic properties of HA can be a disadvantage in the initial healing process. Therefore, TiO<sub>2</sub> can be coated on the HA to improve the hydrophilicity. At this time, when TiO<sub>2</sub> is heat-treated and adhered to HA, the stability of the mixture is improved. Ultraviolet (UV) irradiation is also a method that can further increase the hydrophilicity of the surface by photofunctionalization. Therefore, the aim of this study was designed to evaluate the effect of using HA with 5% TiO<sub>2</sub> on rabbit calvarial defect models as a bone graft material and compare it with a histological and histomorphometric analyses of the effect of photofunctionalization on newly formed bone in early healing period. Wettability test, SEM and XRD analysis were performed to investigate the characteristics of the graft materials. Twelve white New Zealand white rabbits were used *in vivo*. The study groups were divided into the following four groups: untreated negative control group, porous HA group, TiO<sub>2</sub> coated porous HA group, and TiO<sub>2</sub> coated porous HA with UV group. The defects were performed by radiography, histological and histomorphometric analyses after the animals were sacrificed at 2 and 8 weeks postoperatively. At 2 and 8 weeks after surgery, treatment with TiO<sub>2</sub> coated HA with UV group and TiO<sub>2</sub> coated HA group showed significantly higher percentage of new bone formation on the defect area than the control group. There was no significant difference between TiO<sub>2</sub> coated HA group and TiO<sub>2</sub> coated HA with UV group, however, UV irradiation showed more increased extent of new bone formation. Thus, the combination of TiO<sub>2</sub> / HA and UV irradiation in bone regeneration appears to expect a favorable response.

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## INVOLVEMENT OF COMPLEMENT ACTIVATION IN THE PULMONARY VASOACTIVITY OF POLYSTYRENE NANOPARTICLES IN PIGS – PART I

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Complement (C) activation has been associated with hypersensitivity reactions (HSRs) to certain nano-pharmaceuticals, a phenomenon referred to as “C activation-related pseudoallergy” (CARPA). Many symptoms of these infusion reactions can be modeled in pigs, most sensitively the cardiopulmonary distress, with pulmonary hypertension taken as a quantitative measure of the drugs’ reactogenicity. A recent study reported that spherical polystyrene nanoparticles (PS-NPs) cause pulmonary hypertension in pigs without C activation, challenging the CARPA concept. This challenge was recently questioned on theoretical grounds, and here we

sought experimental evidence for C activation playing a role in PS-NP-induced HSRs in pigs.

After suspending different sized PS-NPs in normal pig sera and incubating them together allowing *in vitro* C activation, deposition of C fragments was monitored either by FACS analysis of particles’ surface after staining them with antibodies against C9 neo-epitope or C3b decay fragments, or by Western blot analysis of C3 fragmentation products.

Exposure of 500 and 750 nm PS-NPs to pig serum led within minutes to the deposition of terminal C complex and C3b fragments on the surface of particles with paralleling rise of proteolytic cleavage fragments of C3 corresponding to C3dg and C3d formation.

Our results clearly demonstrate that PS-NPs can effectively activate the C system within minutes in serum, leading to the accumulation of terminal C complex and C3 cleavage products on surface which may be essential for the opsonization of the particles. Similar kinetic of *in vitro* C deposition on PS-NPs and the *in vivo* pulmonary hypertensive effect of these particles suggests further connections between opsonization and CARPA. Our data are consistent with the double (or multiple) hit theory of HSRs, wherein C activation contributes to allergic mediator-secreting phagocyte activation via opsonization of reactogenic NPs.

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## SYNTHETIC CELLS PRODUCE THERAPEUTIC PROTEINS INSIDE TUMORS

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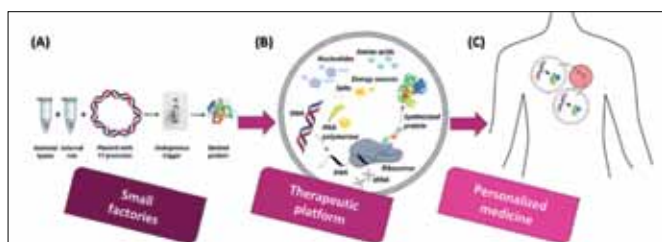
The existing dogma is that protein medicines are produced in large-scale, and then injected to the patient. We propose to produce a protein of interest directly in the disease site, by utilizing nanotechnologies to achieve therapeutic precision and high functionality. The research approach is based on the development of synthetic therapeutic cells – miniature artificial inert “factories” that can be located in the body and latter remotely activated to synthesize a protein-based medicine only where it is needed. The produced protein can be tuned to the patient’s needs based on a predetermined DNA code which is incorporated inside these synthetic cells. We believe that this therapeutic platform (Figure 1) can significantly increase treatment efficiency and reduce adverse effects to healthy tissues.

We developed a new CPFS system which contains all the transcription and translation machines and molecules required for protein production (Krinsky et al., 2016). This system was used to prepare liposomes (lipid particles) that act as artificial cells, capable of producing proteins autonomously in response to a physical trigger. Functional enzymes (luciferase and tyrosinase) and fluorescent proteins (Green fluorescent protein, GFP) were successfully produced using this system inside synthetic cells both *in vitro* and *in vivo*. In addition, the therapeutic capabilities of the synthetic cells were demonstrated by producing Pseudomonas exotoxin A, an extremely potent protein, for treating cancer. Applying the particles on 4T1 cells (a triple-negative breast cancer cell-line) *in vitro* or injecting them into a 4T1-induced tumor *in vivo*, resulted in high cyto-

toxicity due to the effective production of the therapeutic protein inside the vesicles (Krinsky et al., 2017).

Synthetic cells can serve as autonomous, trigger-able, artificial particles that produces a variety of proteins. I believe this platform can be applicative as a protein delivery system addressing the patients' need, as well as to address a wide range of fundamental questions associated with protein synthesis in nature.

*Figure 1. The therapeutic platform. Onsite synthesis of protein medicines inside the body is achieved by encapsulation of all the transcription and translation factors required for protein production inside a liposome. (A) Cell-free protein synthesis (CFPS) work flow. (B) Protein producing particle. (C) Personalized medicine; triggered protein production inside the patient's body at the diseased tissue.*



## DESIGN AND IMPLEMENTATION OF MULTIFUNCTIONAL GRIPPER AND APPARATUS, FOR ROBOTIC-LAPAROSCOPIC SURGICAL AND INTERVENTIONAL RADIOLOGY

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Classical surgical interventions have rapidly been replaced by Robotic surgical interventions equipped with electromechanical systems. The graspers, grippers, retainers and compactors are auxiliary medical devices constitute some of the indispensable parts of such systems. The auxiliary apparatus in which hydraulic-pneumatic systems, hybrid systems, electric motors such as servo, stepper is used in order to perform cutting, holding, parting, burning, sewing during electromechanical surgical interventions. These systems are generally accepted and, proven their reliability but they have a number of disadvantages. Initially, due to electronic circuits, drivers and structural materials, their physical structures pose some complexities. This brings in high costs but more importantly it does not allow volumetric miniaturization. However, grasping, gripping, cutting and sewing operations can be possibly made by nickel titanium alloys which have shape memory effect and pseudoelasticity features. Thus, complicated structures of can be reduced to simpler structures and can be produced in minimal sizes as well as solutions that will allow for precise and milimetric interventions.

The work described here includes the design, manufacture an applicable apparatus for Ultrasound, Computure Tomography (CT), Magnetic Resonance Image (MRI) guided Radiological Interventions and Robotic- Laparoscopic surgeries. The operation is expected to partially or completely robotized by medical arm and a robotic head capable of performing more than one of the operations of gripping, cutting, sewing, burning, sampling at the same time concurrently.

The apparatus of the medical device which is widely used is now can be made dimensionally smaller and lighter in light of this project. Particularly, if radiological interventions which are performed in the presence of ionized radiation may be conducted and controlled by remote access, the doctor and auxiliary personnel who perform these medical radiological examinations could be largely

protected from the negative effects of ionizing radiation.

The multifunctional medical device we propose in this project contains a carbon fiber tube as a body (Figure 1). Four nickel-titanium wires pass through this tube. These wires are supported by a polymer bellow to form the neck. A second small head-tube is connected to the neck holding the gripping apparatus. The head mounted apparatus are millimeter-sized which are designed and manufactured in various shapes from nickel titanium sheets, wires and springs in order to perform holding, gripping, cutting and sewing operations. The diversity of these apparatuses depend on the size of the anatomical region and its size and also which operations can be done together i.e. grasping, cutting, burning or sampling etc. In order to obtain mechanical movements, nickel titanium materials were trained by thermo-mechanical methods. The motion controls are obtained by Pulse-Width Modulation (PWM) method using pulse currents to stimulate functional wires. Due to heating effect of the current flow, treatment of the wires were preformed at high temperature phase (i.e. open position). The return to original shape at low-temperature phase is accomplished by cooling (i.e. closure position). During these interventions, closed circuit isolations can provide a protection to healthy neighboring tissue regions from the adverse effects of electrical current.

Figure 1. Smart Materia Based Micro Manipulator



## LANSOPRAZOLE PRETREATMENT INCREASES CYTOTOXICITY OF NANOFORMULATION IN 2D CELL MODEL

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**Introduction:** In recent years, countless nanoformulations of the cytostatic drug doxorubicin (DOX) have been developed with the aim of treating various recent cancers.<sup>i</sup> Caelyx<sup>®</sup> (named Doxil<sup>®</sup> in the US), a liposomal formulation of DOX, was the first nanoformulation to receive regulatory approval in 1995 and is still the best example of successful clinical nanomedicines.<sup>ii</sup> More research has investigated the synergistic effects observed when giving a proton pump inhibitor (PPI) as a concomitant medication to conventional DOX treatment. Several clinical studies are ongoing and encouraging results have already been seen both in-vitro and in-vivo.<sup>iii</sup> PPI's appear to increase the amount of DOX located in the cell nucleus, by increasing extracellular pH and thereby reducing endosomal sequestration of the drug, resulting in higher cytotoxicity.<sup>iv</sup> However, very little is known about how PPI pre-treatment will affect uptake and distribution of nanoformulations with DOX in tumor cells.

The primary objective of this study (described in figure 1) was to compare the effect of pre-treating cancer cells with different concentrations of a common proton pump inhibitor (PPI) before exposure to liposomal DOX (Caelyx<sup>®</sup>) or DOX in solution. As Caelyx<sup>®</sup> is indicated for breast cancer, common breast cancer cell line MCF7 was chosen, as well as three different hepatocellular carcinoma (HCC) cell lines. Since 2011 our group in Uppsala has worked within the area of HCC and this study can be regarded as the first step towards later development and evaluations of HCC targeted nanoformulations.

## THERAPEUTIC INHIBITION OF SYK KINASE IN MACROPHAGES USING PLGA NANOPARTICLES FOR THE TREATMENT OF NON-ALCOHOLIC STEATOHEPATITIS

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**Introduction:** Non-alcoholic steatohepatitis (NASH) is the leading cause of cirrhosis worldwide and is the most rapidly growing indication for liver transplantation. Macrophages are an important cellular component in the inflammatory milieu in NASH. The inflammatory and pro-fibrotic mediators produced by macrophages causes significant tissue injury leading to initiation and progression of many inflammatory diseases especially NASH. These inflammatory (classically-activated or M1) macrophages also recruit scar-forming fibrogenic myofibroblasts and inflammatory cells further driving liver injury to fibrosis, cirrhosis, and hepatocellular carcinoma. Therefore, inhibition of the inflammatory macrophages would be a promising approach to attenuate liver inflammation.

**Aims:** In this study, (i) we aim to study the implication of SYK signaling pathway in NASH and inflammatory macrophages; (ii) we investigated PLGA nanoparticles based delivery of SYK signaling pathway inhibitor as an highly effective and promising therapeutic approach for the treatment of NASH.

**Methods:** To accomplish the goals of this study, we used R406, a small-molecule Syk kinase inhibitor that blocks Fc receptor signaling pathway and reduces immune complex-mediated inflammation. We tested the therapeutic efficacy of R406 in the differentiated inflammatory RAW macrophages. Thereafter, we synthesized PLGA nanoparticles to deliver SYK kinase inhibitor (R406) to increase the drug pharmacokinetics for the efficient treatment of liver inflammation in NASH. We investigated the efficacy of R406-PLGA nanoparticles in differentiated RAW macrophages and bone marrow derived macrophages. Furthermore, we evaluated the therapeutic effects of R406 and R406-PLGA nanoparticles *in vivo* in Methionine Choline deficient (MCD)-diet NASH model.

### Results

Analysis of livers from NASH patients showed a highly significant induction of SYK expression that correlated with the increasing NAS score as compared to normal livers as determined from transcriptome data analysis (GEO accession number: GSE48452). We observed significant upregulation of SYK expression and activation of SYK signaling pathway (SYK phosphorylation) in inflammatory macrophages. R406 dose-dependently inhibited LPS- and IFN $\gamma$ -induced activation of SYK signaling pathway consequently resulting in inhibition of M1-induced nitric oxide (NO) release (M1 activation) and M1-specific markers (IL-1 $\beta$ , Fc $\gamma$ R1, CCL2, iNOS, and IL-6) in RAW macrophages and bone marrow derived macrophages (BMDMs). R406 loaded PLGA nanoparticles (R406-PLGA) were successfully synthesized and were characterized for the size, charge, stability, and the drug entrapment efficiency. R406-PLGA inhibited NO release and M1-specific markers (IL-1 $\beta$ , Fc $\gamma$ R1, CCL2, iNOS, and IL-6) in RAW macrophages and BMDMs. Finally, we performed an *in vivo* study on a typical murine model of NASH-associated liver inflammation and liver injury i.e. diet-induced (methionine-and-choline-deficient, MCD) model and the result showed that R406-PLGA ameliorates inflammation, fibrosis, and steatosis in MCD-diet-induced

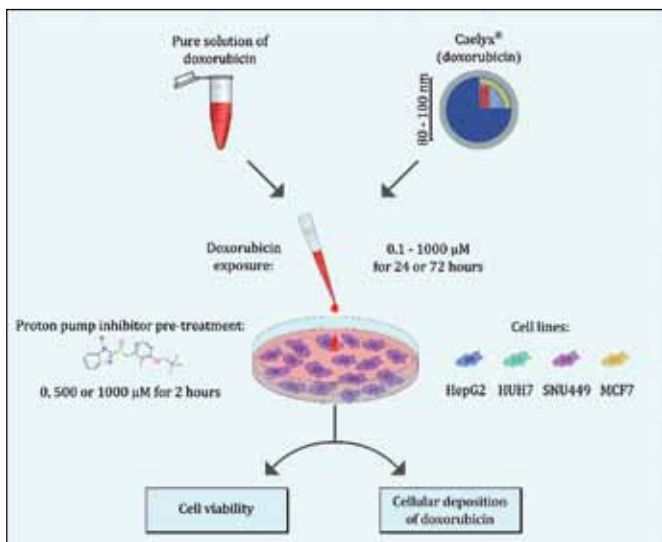


Figure 1. Overview of the study conducted.

**Methods:** Four different cell lines, three HCC (HepG2, Huh7 & SNU449) as well as one breast cancer cell line (MCF7), were grown as monolayers in a 2-D assay. The cell lines were exposed to varying concentrations of Caelyx<sup>®</sup> or DOX in solution with or without pre-treatment of lansoprazole. Cell viability was measured to determine the inhibitory concentrations (IC50) of the treatments at different exposure times. A cell deposition method using UPLC-MS is currently under development in order to quantify and determine the intra- and extracellular location of DOX.

**Results & Conclusions:** Cell viability and IC50 values vary greatly depending on the cell lines, with especially SNU449 having a high resistance to DOX, as expected from literature. Pre-treatment with lansoprazole resulted in lower cell viability for both SNU449 and Huh7, as seen in figure 2. This study is currently still ongoing and a complete set of results will be published in the autumn of 2018.

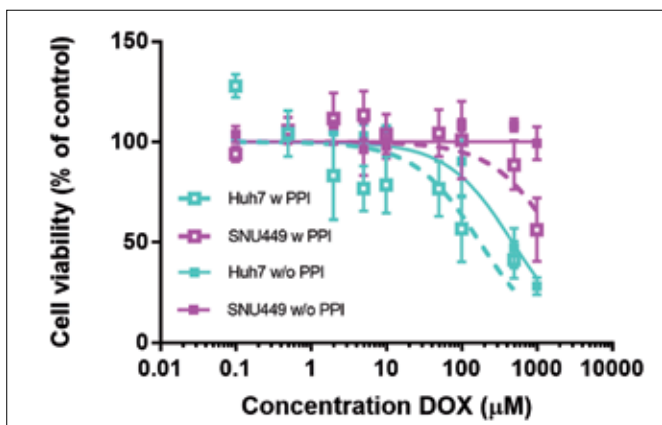


Figure 2. Comparison of cell viability of Caelyx<sup>®</sup>-treated SNU449 and Huh7 cells when pre-treated with (w) or without (w/o) the PPI lansoprazole for two hours.

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NASH liver mouse model.

## Conclusion

Inhibition of SYK signaling pathway using small molecule inhibitor and delivery of SYK inhibitor using PLGA nanoparticles can be a highly effective and potential therapeutic approach for the treatment of Non-alcoholic steatohepatitis (NASH).

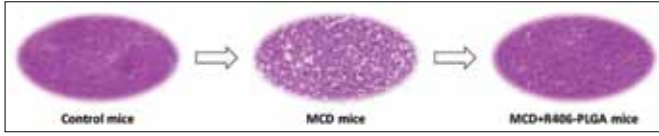


Figure 1. Schematic illustration of the liver tissue from control mice group (without any treatment); MCD mice group (with MCD-fed); and MCD+R406-PLGA mice group (with MCD-fed and treated by R406-PLGA nanoparticles).

## HYALURONIC ACID (HA) ENCAPSULATED GRAPHENE QUANTUM DOT (GQD) POLYMERIC NANOPARTICLES AS POTENTIAL DRUG DELIVERY SYSTEM FOR CANCER THERAPEUTICS

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The combined HA/GQD polymeric nanoparticles drug delivery system has many great properties including specific targeting, bio-imaging, biosafety, dual drug therapy, high drug loading efficiency, controlled release etc. Therefore, this study attempts to combine two kinds of drug delivery vehicles - graphene quantum dots and hyaluronic acid polymeric nanoparticles (HA-NPs) for enhancing more efficiency of killing cancer cells for the all in one drug delivery system. Recently, GQDs have attracted more attentions as drug delivery vehicle for cancer therapeutics due to their versatile photoluminescence, large surface area, high photostability, low cytotoxicity, and excellent biocompatibility (Chen et al., 2018). In addition, HA is widely used in anticancer drug delivery because of their biocompatible, biodegradable, non-toxic, and non-immunogenic properties. Particularly HA-NPs can accumulate into the tumor site by a combination of passive and active targeting mechanisms (Dosio et al., 2016)

This study provided a novel strategy to utilize HA-NPs drug delivery vehicle not only specific targeting cell receptor (CD44) but also encapsulating enormous GQD drug carriers for greatly increasing drug loading for cancer therapeutics. Furthermore, analyze the materials characterizations and *in vitro* cell viability in cancer cell line (HCT-116). At first, pyrene modified HA derivatives which can assemble cancer drugs (T drug) through interaction of hydrophobic moiety and hydrophobic drug (Fig. 1). In addition, PEG (PEG = polyethylene glycol) is well known to have excellent biocompatibility, therefore, PEGylated GQD is able to improve the water solubility and pharmacokinetics. So GQD-PEG could be utilized to load the antitumor drug doxorubicin (DOX) via hydrophobic interaction for cancer therapeutics. Afterwards, two carriers GQD-PEG and HA-NPs could be able to link together through simple physisorption via electrostatic interaction forming GQD-PEG/HA-NPs composite (Fig. 1). The *in vitro* cytotoxicity of HA-NPs alone and GQD-PEG-DOX/HA-NPs composite in colon cancer cell line was determined by growth inhibition assay (MTT) to evaluate the efficiency of the targeted delivery (Fig. 2). The results of MTT for HA-NPs alone and GQD-PEG-DOX/HA-NPs composite were shown significantly difference in 24hr and 48hr. This novel drug delivery system could be a potential platform for improving cancer therapeutics.

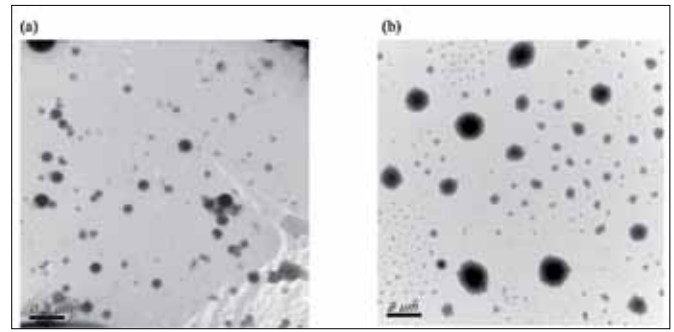
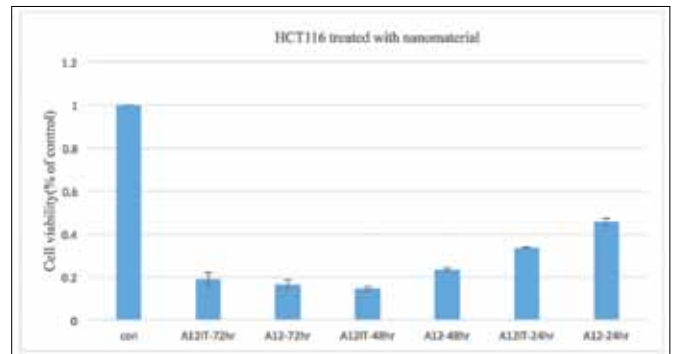


Fig. 1. Transmission electron microscopy (TEM) of hyaluronic acid (HA) encapsulated graphene quantum dot (GQD) polymeric nanoparticles. (a) HA-NPs encapsulate 0.25mg/ml anticancer drug. (b) GQD-PEG-DOX/HA-NPs composite.

Fig. 2. MTT assay results hyaluronic acid (HA) encapsulated graphene quantum dot (GQD) polymeric nanoparticles over 24hr, 48hr and 72hr.



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## 3D CO-CULTURE PLATFORM OF MONOCYTES AND ENGINEERED T CELLS FOR PRE-CLINICAL SCREENING OF CANCER IMMUNOTHERAPY

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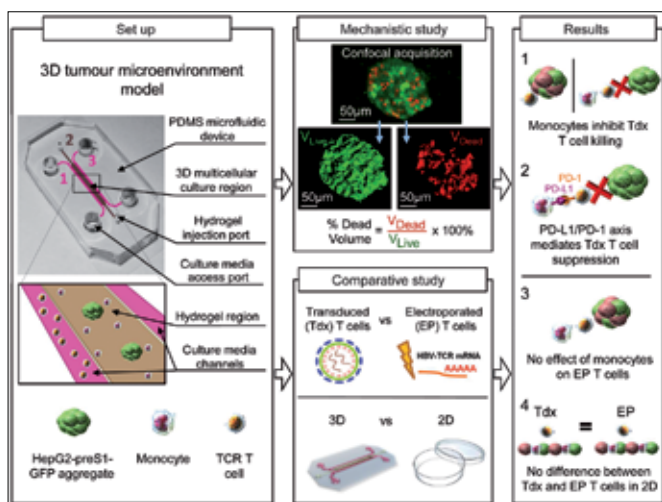
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**Keywords:** immunotherapy, immune checkpoint, PD-L1, tumour microenvironment, monocytes, T cell receptor-redirected T cells, microfluidics

## ABSTRACT

Clinical trials of cancer therapy based on the adoptive cell transfer of patient-derived T cell receptor-redirectioned T cells (TCR T cells) have progressively shown promise.<sup>1,2</sup> In virus-related cancers such as hepatitis virus-associated hepatocellular carcinoma (HBV-HCC), HCC cells present HBV peptides on their surface and patient-derived T cells can be engineered to inherit antigen specificity to these peptides using different strategies.<sup>3,4</sup> Amongst these, transduction results in sustained T cell anti-tumour cytotoxicity while transgene transfection, being transient, confers T cells with self-limiting cytotoxicity.<sup>4</sup> In HBV-HCC, monocytes in the tumour microenvironment (TME) are known to primarily inhibit the function of endogenous T cells through the immune checkpoint Programmed Death-Ligand 1 (PD-L1),<sup>5,6</sup> and the present study demonstrates that the same pathway is responsible for the suppression of TCR T cells. Notably, in tailoring T cell therapy for patient-specific needs, a balance must be sought among therapeutic efficacy, safety, toxicity, and the ability to overcome key immunosuppressive mechanisms in the TME. Therefore, we developed a 3D co-culture microfluidic model of the intrahepatic TME and demonstrate the capability to rapidly assess TCR T cell efficacy across different physiological settings.<sup>7</sup> Specifically in this study, we investigated the immunosuppressive potential of monocytes and PD-L1/PD-1 signalling toward TCR T cells that were engineered by different means.<sup>8</sup>

We observed that, similar to endogenous T cells, retrovirally-transduced (Tdx) TCR T cell cytotoxicity toward cancer cells was inhibited by monocytes and we also observed heightened proportions of PD-L1+ monocytes and PD-1+ T cells. Correspondingly, antibody blockade of either PD-L1 or PD-1 restored TCR T cell cytotoxicity. In contrast, mRNA electroporated (EP) TCR T cells were not inhibited by monocytes despite similarly elevated proportions of PD-L1+ monocytes and PD-1+ TCR T cells (as was seen with the Tdx T cells). Importantly, all observations obtained using our 3D platform were not replicated when assessed using standard 2D *in vitro* assays, highlighting the relevant improvement that 3D systems offer over 2D systems for immunological studies and pre-clinical testing. Figure 1 provides an overview of the methodology and key results of the study.<sup>8</sup>



**Figure 1.** (A) A 3D multicellular tumor microenvironment microfluidic model consisting of a middle hydrogel channel (2) flanked by two media channels (1, 3) for the mechanistic study of the effect of monocytes on T cell receptor-redirectioned T cell (TCR T cell) killing of tumor cell aggregates. Human monocytes were inserted together with target HepG2-preS1-GFP cell aggregates in collagen gel in the central hydrogel region (2), while hepatitis B virus (HBV)-specific TCR T cells were added into one fluidic channel (1) to mimic the intrahepatic carcinoma environment. (B) Representative confocal image of a target cell aggregate (in green), in which the presence of dead target cells is DRAQ7+ (in red). Target cell death is quantified as shown based on the DRAQ7+ volumetric portion in the total volume of each GFP-labelled aggregate. Comparisons were made between co-cultures with transduced T cells and mRNA-electroporated T

cells, or between 3D and 2D assays. (D) Monocytes had differential effects on different TCR T cell types in a 3D setting but not in 2D.

In addition, following what we have done in evaluating different TCR T cells, we propose that this platform carries strong potential for polytherapy screening and toxicity testing of nanoparticle-based therapeutic strategies. We are currently exploring the use of this platform to test the specific targeting of nanoparticles to particular immune cells in a cancer immunotherapy setting. Several parameters will need to be considered in optimizing nanoparticle-based therapies. Researchers would greatly benefit from a 3D co-culture platform that offers a high degree of control over design parameters to mimic physiologically relevant *in vitro* systems for the accelerated bench-to-beside translation of nanomedicine. The research was supported by the Biomedical Research Council, A\*STAR and the National Research Foundation, Prime Minister's Office, Singapore, under its CREATE programme, SMART BioSyM IRG.

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## ASSESSMENT OF ANTIRETROVIRAL IMPACTS ON CELLULAR HEALTH FOLLOWING INCORPORATION INTO SOLID DRUG NANOPARTICLES

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Nanoformulation of antiretrovirals offers the potential for improvements in the pharmacokinetics of the drug which may lead to dose reduction and the possibility of long acting formulations<sup>[1, 2]</sup>. The protease inhibitor (PI) class of antiretrovirals used to treat HIV are known to have a number of clinical side effects that may result in comorbidities resulting in switching from one drug to another<sup>[3, 4]</sup>. Lopinavir (LPV) is a well-established PI for use in highly active antiretroviral therapy, which is boosted with ritonavir for the treatment of HIV. Some serious adverse reactions have been reported for the combination, including haemophilia, hepatotoxicity and hyperlipidaemia<sup>[5]</sup>. The mechanisms behind these clinical

presentations have been linked to the impact of LPV on a number of measures of cellular health such as oxidative stress, autophagy, caspase activation, endoplasmic reticulum stress and apoptosis [6-9]. The aim of this study was to assess measures of cellular health in primary, human, CD4+ and CD14+ cells exposed to a bioequivalent solid drug nanoformulation of lopinavir [10]. CD4+ and CD14+ cells were isolated using Ficoll-Paque followed by magnetic bead separation from whole PBMCs (n=4). Cells were plated at a density of 1x10<sup>5</sup> cells/well and treated with LPV (10 μM), LPV-SDN (10 μM) or positive control (Camptothecin or Menadione; 10 μM) for a period of 24 hours prior to analysis of the respective cellular health markers (reactive oxygen species, glutathione content, apoptosis, mitochondrial membrane polarisation and caspase-1 activation) using commercially available reagents. Impact on cellular health was compared to untreated controls and between LPV and LPV-SDN treated cells.

Treatment with LPV or LPV-SDN had no impact on autophagic flux, apoptosis or mitochondrial membrane polarisation when measured at 24 hours in either CD4+ or CD14+ positive cells. In both CD4 and CD14 cells, lopinavir treatment resulted in a 3-fold (P=0.032) and 1.14 (P=0.047) greater level of reactive oxygen species compared to untreated control. Comparatively, LPV-SDN exposure resulted in only a 2.2-fold greater level of reactive oxygen species in CD4 cells whereas no difference was observed in CD14 cells (figure 1)

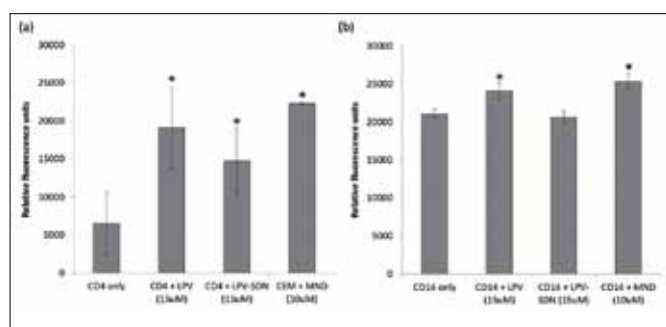


Figure 1. Impact of lopinavir (LPV) and lopinavir solid drug nanoparticles (LPV-SDN) on reactive oxygen species in (a) CD4+ and (b) CD14+ primary cells from healthy volunteers. Measurements taken after 24 hour exposure, menadione (MND) included as a positive control. Data presented as mean ± standard deviation of analysis of 4 healthy volunteers. \* denotes P<0.05

Concomitantly, lower levels of glutathione (figure 2a) were observed in CD4 cells treated with lopinavir (29% less than untreated cell, P=0.028) and LPV-SDN (19% less than untreated cells, P=0.031). Similar results were observed in CD14 cells (figure 2b) following exposure to LPV (31% lower than untreated cells, P=0.032) whereas no difference between untreated and LPV-SDN treated cells was observed.

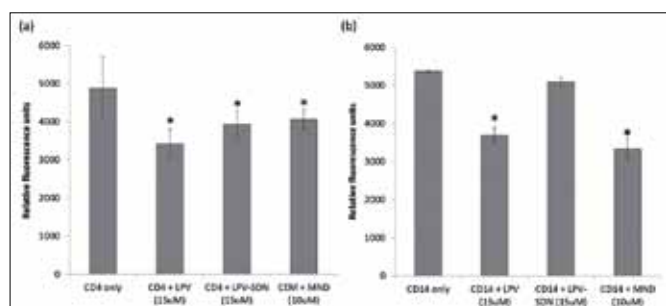


Figure 2. Impact of lopinavir (LPV) and lopinavir solid drug nanoparticles (LPV-SDN) on glutathione levels in (a) CD4+ and (b) CD14+ primary cells from healthy volunteers. Measurements taken after 24 hour exposure, menadione (MND) included as a positive control. Data presented as mean ± standard deviation of analysis of 4 healthy volunteers. \* denotes P<0.05

Treatment of primary immune cells with LPV results in generation

of oxidative stress as evidenced by greater levels of reactive oxygen species in exposed cells, as well as the generation of an antioxidant response demonstrated by lower levels of total glutathione. The differences observed between LPV and LPV-SDN suggest that nanoformulation may mitigate some undesirable effects, possibly due to differential accumulation mechanisms and subsequent cellular localisation of LPV and LPV-SDN in the cells they accumulate. However, it can be seen that LPV-SDN had less of an impact on these systems than LPV. Further work is now in process to investigate the possible benefits of these observation, *in vivo*. Additionally, clinical investigation is now required to assess the possible benefits to cellular health in patients.

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## ASSESSMENT OF ANTIRETROVIRAL IMPACTS ON CELLULAR HEALTH FOLLOWING INCORPORATION INTO SOLID DRUG NANOPARTICLES

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A number of nanomedicines are currently licensed for use clinically with many more in development [1]. However, in order to translate these nanoparticles through to use clinically, careful consideration must be given to their compatibility with immunological and haematological systems [2]. Nanoparticles have been shown to both stimulate and suppress the immune system via a number of



mechanisms [3] which may be of use therapeutically. However, the relationship between particle characteristics and biological effect must be carefully defined. The uptake of nanoparticles by cells of the immune system can affect their biodistribution as well as their biocompatibility [4, 5]. Neutrophils are part of the first line of defence against pathogens and foreign material [6] and, as phagocytic cells, capable of internalising nanoparticles [7, 8]. Previous reports have shown that uptake of nanoparticles into phagocytic cells may cause oxidative stress [9]. Uptake of nanoparticles into neutrophils has been shown to generate a respiratory burst, characterised by the presence of reactive oxygen species (ROS) [10]. The generation of oxidative stress has also been shown to be linked to the generation of Neutrophil Extracellular Traps (NETs) [11, 12], and many nanoparticles are known to generate ROS upon accumulation within cells [13, 14], providing a putative mechanism for their toxicity and/or immunogenic effects [9]. Recent evidence also suggests that increased NET formation or insufficient dismantling of NETs can lead to breaking of tolerance and the promotion of autoimmunity [15].

We investigated the capacity of TiO<sub>2</sub> or SiO<sub>2</sub> nanoparticles to generate oxidative stress in primary human neutrophils by measuring intracellular ROS production with and without N-Formylmethionine-leucyl-phenylalanine (fMLP; 1µM) to induce the respiratory burst or primed with tumour necrosis factor alpha (TNFα; 1µM). Intracellular ROS were measured using dihydrorhodamine 123 (DHR123) and cells analysed by flow cytometry, gating for neutrophils. Additionally, inhibition of ROS production was achieved using Diphenyleneiodonium (DPI) in order to link ROS production to NET formation. NET formation was assessed by confocal microscopy, staining for neutrophil elastase and myeloperoxidase. FlowJo version 10 and GraphPad Prism version 6 was used to collate results. A Kruskal-Wallis Test was used to compare medians and a Dunn's post hoc Tukey test was used to compare significance.

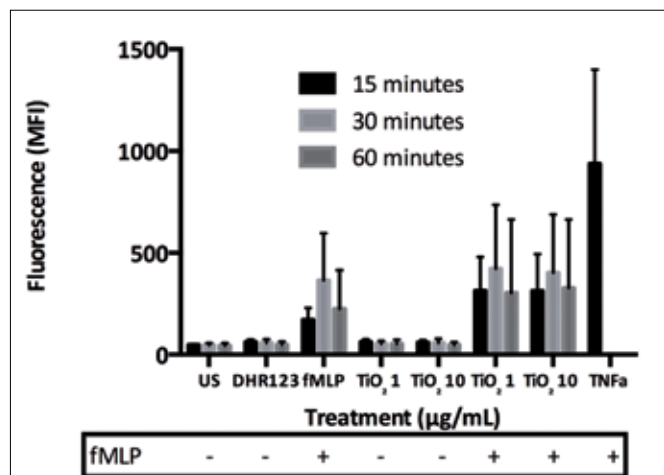


Figure 1. Intracellular ROS production in primary, human, neutrophils exposed to inorganic nanoparticles. Average fluorescence of ROS production when neutrophils are stimulated with TiO<sub>2</sub> nanoparticles. Neutrophils were also treated with and without fMLP. Data expressed as mean ± SD, N=3.

Figure 1 shows when cells are not treated with fMLP there is minimal difference in average fluorescence emitted at both concentrations of TiO<sub>2</sub> over time and compared to unstimulated. Upon the activation of neutrophils with fMLP there is a significant increase of fluorescence with all conditions and each time point. This suggests that TiO<sub>2</sub> themselves are unable to activate ROS production in neutrophils however; they are able to prime the cells in a similar fashion to TNFα as evidenced by the greater ROS production in cells treated with fMLP and TiO<sub>2</sub>.

A marked degree of inter-individual variability was also observed in ROS production between healthy volunteer samples. Figure 2 shows the level of ROS production amongst neutrophils isolated from five healthy volunteers. Whilst all cells responded to treatments, it is possible that the variability in response to TNFα may

be due to variation in the expression of TNFα receptors. Given that there is also variability in the response to nanoparticles, this may be due to differences in accumulation of the nanoparticles within the cells and/or varying levels of expression of intracellular receptors.

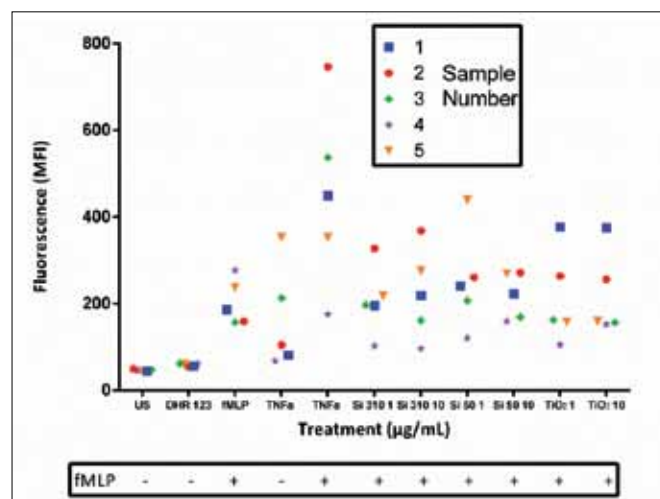


Figure 2. Inter-individual variability in ROS production in primary, human, neutrophils. Data is average mean fluorescence intensity (MFI) of cells treated with combinations of fMLP, TNFα and either TiO<sub>2</sub> or SiO<sub>2</sub>.

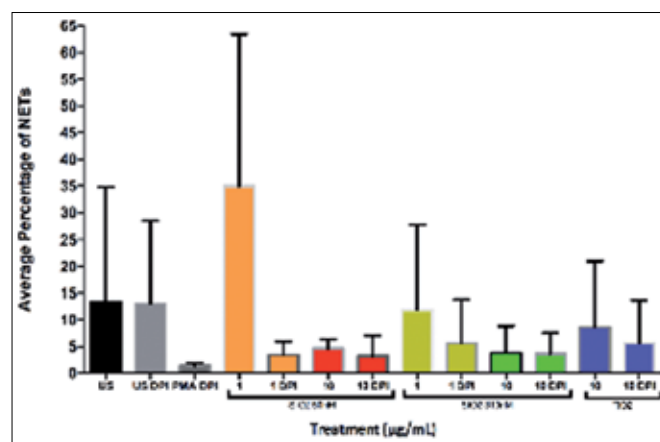


Figure 3. Inhibition of NETosis in primary, human, neutrophils using Diphenyleneiodonium (DPI). NETosis was achieved using phorbol 12-myristate 13-acetate (PMA) as a positive control and nanoparticles at various concentrations. Data expressed as mean ± standard deviation, N=4.

Nanoparticles used were able to trigger NETosis following exposure (figure 3). Additionally, NETosis was markedly reduced when ROS production was inhibited using DPI, suggesting the primary mechanism of NET generation in response to nanoparticles is via ROS induction and signalling.

In summary, we have shown that, in primary human neutrophils, nanoparticles are unable to activate ROS production however; they can significantly increase ROS production in primed neutrophils. Additionally, when oxidative stress is inhibited, NET production is lower. This has a number of impacts for inflammatory diseases and may suggest that therapies aimed at ROS production may serve to mitigate these response. The generation of oxidative stress by nanoparticles, within neutrophils, may contribute to the induction of NETosis. The generation of NETs has been linked to inflammation and autoimmunity, which may serve to impact the biocompatibility of nanomaterials via an indirect mechanism. We are now investigating the impact of organic based nanomaterials that may be used as drug delivery systems on NETosis and if this may serve as a useful marker of nanoparticle biocompatibility. Additionally the consequences of nanoparticle induced NETosis must be determined *in*

*vivo* to fully understand its consequences for translation of novel, engineered, nanoparticles.

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The small size of nanoparticles (NPs) gave new optical, mechanical and reactive properties to these materials compared to the same bulk particles. As such, NPs quickly became important components of novel material formulations, such as paints, coatings, food colourings, cosmetics, cleaning reagents and clothes, providing a chronic source of NP exposure in our everyday life.<sup>1</sup> Industrial NPs come in contact with organisms through inhalation, ingestion or skin absorption<sup>2</sup> and several *in vivo* studies have shown accumulation of such NPs in different organs and induction of related tissue stress and damage, as also indicated by the correlation between ambient/personal exposure to ultrafine particles and adverse health symptoms/biomarkers in different human epidemiological studies.<sup>3,4</sup> Industrial NPs are especially problematic also because they are mostly non-biocompatible and biologically non-degradable and can stay in the body for long time periods. Moreover, the incidence of primary brain tumors, developmental and neurodegenerative diseases has increased in the last decades. The exact etiology of these diseases is unknown, but environmental pollutants, including NPs, could be one of the risk factors.<sup>5</sup> A very recent study reported finding combustion derived magnetite NPs in the brains of Alzheimer's patients,<sup>6</sup> but no such studies have been conducted for industrial NPs. Unfortunately, despite ever increasing daily use of NP containing consumer products, research in the potential health risks of NP exposure lags behind the rapid industrial development and commercialization of nanotechnology.

NPs can also accumulate in the brain, either passing the blood-brain barrier (BBB) or through axonal translocation in the airway epithelia.<sup>7</sup> Brain is a particularly problematic accumulation site due to its restricted immune responses, low regeneration abilities, high lipid content and high energy consumption, which make it highly vulnerable to oxidative stress.<sup>8</sup> *In vitro* studies have shown that NPs can cause cell damage through several common nanotoxicity mechanisms, such as ROS induction, disruption of membrane integrity, genotoxicity, destabilization and permeabilization of lysosomes, induction of mitochondrial and endoplasmic reticulum stress, autophagy and lysosomal dysfunction, cell cycle disruption and ion dissolution.<sup>9</sup> Moreover, studies have emerged in the recent years reporting the ability of NPs to induce changes in the localization and solubility of proteins related to neurodegenerative diseases,<sup>10-12</sup> indicating the potential involvement of increased NP exposure in the increase of developmental and neurodegenerative diseases in the recent decades.

The focus of this study was to analyse the neurotoxicity of different engineered NPs, with which we come in contact in our everyday life; SiO<sub>2</sub> NPs (industrial, designed for use in cleaning products; DLS hydrodynamic radius >3000 nm, 2 mV zeta potential in distilled water), food grade (FG) TiO<sub>2</sub> NPs (used as food colouring in baking, confections and other dry grocery products; DLS hydrodynamic radius 220 nm, -25 mV zeta potential in distilled water), TiO<sub>2</sub> P25 (industrial, photocatalytic, used as reference material; DLS hydrodynamic radius 100 nm, 37 mV zeta potential in distilled water) TiO<sub>2</sub> 21 nm (industrial, photocatalytic; DLS hydrodynamic radius 160 nm, 33 mV zeta potential in distilled water) and TiO<sub>2</sub> N (industrial, designed for use in a coating material; DLS hydrodynamic radius 350 nm, 30 mV zeta potential in distilled water). All experiments were performed on a SH-SY5Y human neural cell model *in vitro*.

To determine the toxicity of NPs, cells were incubated with increasing NP concentrations for 24 h and analysed with Propidium iodide (PI) viability assay. None of the selected NP types induced extensive cell death, however a small concentration dependent decrease in cell number was observed for the highest concentrations of SiO<sub>2</sub>, TiO<sub>2</sub> P25 and TiO<sub>2</sub> 21 nm NPs (Figure 1A-E). Since there is no increase in dead cells, the observed decrease might be due to lower

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## THE EFFECT OF SELECTED INDUSTRIAL NANOPARTICLES ON NEUROTOXICITY AND POSSIBLE NEURODEGENERATIVE CHANGES NANOPARTICLE

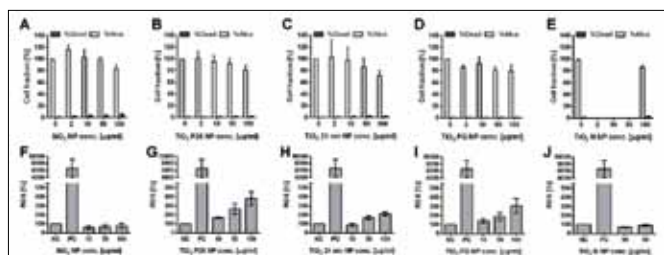
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cell proliferation. On the other hand, TiO<sub>2</sub> P25, TiO<sub>2</sub> 21 nm and TiO<sub>2</sub> FG induced concentration dependent increase in ROS, which was evident already at shorter incubation times (Figure 1G-I). A slight decrease in ROS was observed for SiO<sub>2</sub> NPs (reduced to 60-80%) (Figure 1F), while no changes in ROS were observed for TiO<sub>2</sub> N NPs (Figure 1J). Interestingly, despite changes in ROS, no changes in mitochondrial membrane potential were observed after 24 h incubation with NPs (results not shown). Using TEM, internalization was confirmed for TiO<sub>2</sub> P25, TiO<sub>2</sub> 21 nm and TiO<sub>2</sub> FG NPs (results not shown). In all three cases, NPs were found enclosed in endosomal compartments in the cytosol.



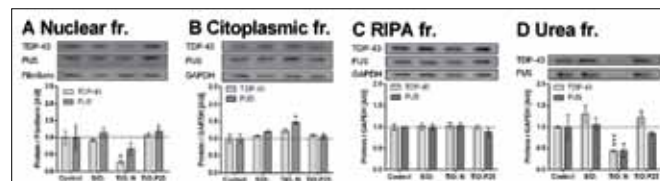
**Figure 1:** The effect of different industrial nanoparticles (NPs) on cell viability (A-E) and oxidative stress (F-J) in SH-SY5Y human neuroblastoma cell line. Increasing concentrations of industrial SiO<sub>2</sub> NPs (A, F), TiO<sub>2</sub> P25 NPs (B, G), TiO<sub>2</sub> 21 nm NPs (C, H), TiO<sub>2</sub> FG NPs (D, I) and TiO<sub>2</sub> N NPs (E, J) were used. Cells were incubated with NPs for 24 h and cell viability was determined using PI viability assay and reactive oxygen species (ROS) generation was analysed using CM-H2DCFH-DA assay. 100 nM H<sub>2</sub>O<sub>2</sub> was used as a positive control (PC) in ROS experiments. Mean and standard error are shown for three independent experiments.

Several of the toxicity mechanisms induced by NPs, such as ROS, inflammation, autophagy and lysosomal dysfunction and others, have also been shown to play a role in the occurrence and progression of neurodegenerative diseases.<sup>13,14</sup> The progress of these diseases is marked by abnormal protein folding, protein aggregation, formation of stress granules and insoluble protein inclusions.<sup>14,15</sup> Fused in Sarcoma (FUS) and TAR-DNA binding protein 43 (TDP-43)<sup>16</sup> were chosen as model proteins, related to amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). TDP-43 and FUS are RNA-binding proteins that shuttle between nucleus and cytoplasm and regulate transcription and post-transcriptional RNA processing, from mRNA splicing to mRNA stabilization and transport.<sup>17,18</sup> To determine if the presence of NPs affects the activity, localization and solubility of FUS and TDP-43 proteins *in vitro*, SH-SY5Y cells were incubated with selected NPs for 24 h and analysed with fluorescent microscopy and Western blot.

Fluorescence microscopy showed normal protein localization and no significant morphological changes. FUS and TDP-43 proteins were predominantly localized to cell nucleus, there was no evident protein aggregation and no formation of stress granules, as indicated by the PABP1 stress granule marker (results not shown). However, cytoplasm/nucleus fractionation and biochemical fractionation, followed by western blot analysis, showed that certain NPs can induce changes both in intracellular localization and in solubility of both proteins. The most pronounced changes were induced by TiO<sub>2</sub> N NPs, which caused a significant decrease in the nuclear concentration of both TDP-43 and FUS proteins (0.28 ± 0.05 and 0.67 ± 0.19 of control, respectively) with a following increase only in the cytoplasmic fraction of FUS (1.47 ± 0.05 of control) (Figure 2A, B). Interestingly, although there were no changes in the more soluble, RIPA fraction, TiO<sub>2</sub> N NPs induced considerable decrease in less soluble urea protein fraction of both FUS and TDP-43 proteins (0.45 ± 0.01 and 0.39 ± 0.20 of control, respectively) (Figure 2B, C), indicating a reduction of the potentially pathognomonic inclusions.<sup>19</sup>

**Figure 2:** Changes in distribution of TDP-43 and FUS between nuclei (A) and cytoplasm (B) and changes in protein solubility (C soluble fraction, D insoluble fraction) following 24 h incubation with silica oxide (SiO<sub>2</sub>) nanoparticles (NPs) (50 µg/ml), industrial grade titanium

oxide (TiO<sub>2</sub> N) NPs (50 µg/ml) and TiO<sub>2</sub> P25 NPs (50 µg/ml). The obtained intensities on western blot were normalized to the quantity of Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) or fibrillarlin, which were used as loading controls. Mean and standard error are shown for three independent experiments performed in three experimental repeats. Representative western blots are shown on top. Statistical significance is displayed as \*P ≤ 0.05 and \*\*\*P ≤ 0.001.



Taken together, in this study we analysed the toxicity mechanisms of several industrial NP types on *in vitro* neuronal cell culture, and the potential ability of these NPs to affect proteins related to neurodegenerative changes on a cellular level. Although we observed an effect on cell number and ROS induction for several NP types, only TiO<sub>2</sub> N NPs affected FUS and TDP-43 proteins. Also, these changes were relatively minor compared to pathological changes and there was no formation of stress granules or aggregates. These results indicate that the selected NPs do not induce changes of FUS or TDP-43 and stress response in short-term *in vitro* conditions, however long term and accumulation studies are required to determine the long-term impact of NP exposure on ALS and FTLD.

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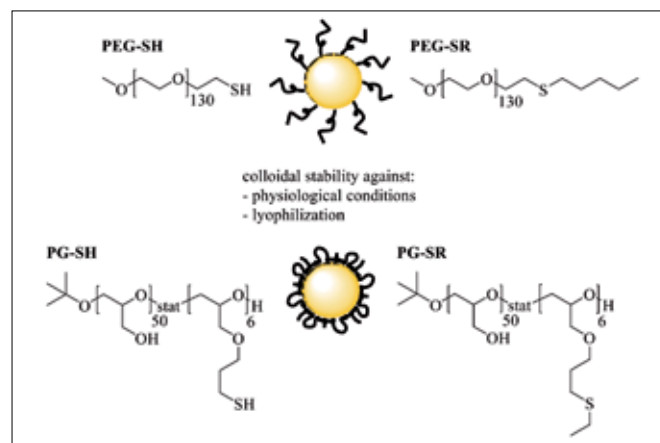


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coating and biofunctionalization of gold nanoparticles (AuNPs). However, the highly nucleophilic and oxidative character of thiols limits the possibilities introducing functional groups and provokes polymeric crosslinking.

We have thus examined whether thioether may be used as alternative to thiols for stabilizing gold colloids. Here we present a systematic comparison of PEG-SH and PEG thioether (PEG-SR) with multifunctional analogs, linear polyglycidol (PG) with multiple thiols (PG-SH) or ethylthioether (PG-SR) as coating system for AuNPs.[1] We show that especially the multivalent PG-SR displays outstanding colloidal stabilization, even under physiological conditions and after lyophilization and resuspension of such coated particles. Furthermore, the non-nucleophilic and non-oxidative character of thioether moieties provides the introduction of any functional mercaptan compound to allyl groups of the PG-SR backbone via thiol-ene click reaction.[2] In this manner a library of multifunctional PG-SR for AuNP coating was generated, featuring functionalities, such as charged moieties[3], biotin and diazirine moieties that can be used as generic tool for covalent immobilization of bioactive molecules.

In addition, we show the applicability of such a coating for silver nanoparticles (AgNPs).



## LITERATURE

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## NANOCARRIERS FOR GENE THERAPY: RECENT APPROACHES

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For gene-associated human diseases, gene therapy has become a promising tool especially against cancers, infectious diseases, cardiovascular disorders, blood disorders, dermatological, ophthalmologic, and neurodegenerative pathologies. In past, gene therapy has been limited to the delivery of DNA only. Currently, other therapeutic nucleic acid materials such as small interfering RNA, antisense oligonucleotides, or microRNA have also been included into the protocols of gene therapy. Another success factor is the correct choice of vector, which is a key factor in the success of gene therapy, where both viral and non-viral vectors are commonly used. Unfortunately, viral vectors are associated with some severe side effects (e.g., immunogenicity and carcinogenicity). On

## THE DEVELOPMENT OF AN ENGINEERED CYSTINE-KNOT MINIPROTEIN FOR TARGETING OF EDB EXPRESSING TUMORS

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In the last decades, targeted tumor imaging has accomplished substantial value for precise cancer diagnosis and therapy. This method requires highly cancer specific target proteins to enable accurate visualization. Fibronectin extra domain B (EDB) is a splice variant exclusively expressed in the tumor-associated vasculature of a wide range of different cancer types, offering a high potential as selective biomarker. Protein scaffolds constitute a new generation of affinity proteins specialized to complement antibodies and antibody derivatives for therapeutic and diagnostic applications. Engineered cystine-knot miniproteins represent an alternative protein scaffold with drug-like properties such as high target affinity and specificity, extraordinary stability, solubility and favorable pharmacokinetic characteristics. Additionally, these small peptidic molecules with a simple architecture facilitate a straight-forward chemical production and the construction of multi-functional fusion molecules.

In this study, we describe the development of an engineered cystine-knot miniprotein for targeting of EDB expressing tumors. For this, a phage library based on trypsin inhibitor II from *Momordica cochinchinensis* (oMCoTI-II) was used to select a cystine-knot miniprotein (MC-FN-010) against recombinant EDB. Engineered cystine-knot miniproteins, MC-FN-010 and a derivate MC-FN-016, feature high EDB specificity as well as reasonable affinities. Chemical oligomerization of the ligands and site-directed fluorescence dye conjugation increased the binding strength enormously while retaining its high specificity and allowed for *in vivo* imaging in a U-87 MG based xenograft glioblastoma mouse model. Both EDB-binding molecules showed strong accumulation in the tumor and low background signals except for the kidneys. Our results demonstrate the high suitability of cystine-knot miniproteins as molecular scaffolds for tumor imaging technologies.

## NOVEL MULTIFUNCTIONAL THIOETHER-POLYGLYCIDOL COATING FOR METAL NANOPARTICLE

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Thiofunctional polymers, especially thiol-terminated terminated poly(ethylene glycol) (PEG-SH) are the established standard for the

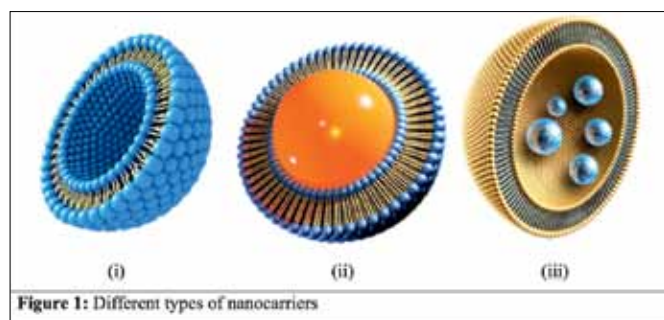
the other hand, poor target cell specificity, unable to transfer large-sized genes and cost are limitations of conventional viral vectors. Nanocarriers as non-viral vectors have become a realistic alternative to viral vectors for achieving better efficacy in gene therapy<sup>[1,2]</sup>. Data on some preliminary clinical trials of nanoparticles for gene delivery revealed promising effects Table 1. However, the development of safe, efficient, and controllable gene delivery nanoparticles for gene delivery is still now a tailback to successful clinical applications. The most important setbacks for nanocarriers are encapsulation efficiency, stability, degradation in blood circulation, endocytosis by target cells, endosomal escape, delivery efficiency, and toxicity of nanocarriers itself.

Table 1: Nanoparticle-based gene therapy under clinical evaluation<sup>[2]</sup>.

Delivery System	Sponsor	Diseases	Administration
PEI-based nanoparticles	BioCancell	BC	Local
		OC	IP
		PN	Local
Lipid-based nanoparticles	National Cancer Institute	HM	IA
	Tekmira Pharmaceuticals	HC	IV
	Tekmira Pharmaceuticals	ACC	IV
	Silence Therapeutics GmbH	ASC	IV
	MD Anderson Cancer Center	LC	IV
	Nitto Denko Corporation	EHF	IV
PLGA-based nanoparticles	Silenseed	PC	Local

Where; ACC, adrenocortical carcinoma; ASC, advanced solid cancer; BC, bladder cancer; EHF, extensive hepatic fibrosis; HC, hepatocellular carcinoma; HM, hepatic metastases; IA, intra-arterial; LC, lung cancer; OC, ovarian cancer; PLGA, poly(lactic-co-glycolic acid); PN, pancreatic neoplasms.

To overcome these obstacles, many types of nanocarriers are proposed; broadly into three categories; (i) lipid-based nanoparticles, (ii) polymer-based nanoparticles, and (iii) inorganic nanoparticles (Figure 1).



Biodegradation is the primary factor for the clinical application of nanoparticle-based gene delivery. Biodegradable polymeric nanoparticles have the potential to be safer alternatives to viruses for gene delivery; however, their use has been limited by poor efficacy *in vivo*. Several approaches are being anticipated for fabrication of polymeric gene delivery nanoparticles and to evaluate their efficacy. Various techniques of synthesizing polymeric nanoparticles are presented in this poster presentation with special focus on anticancer therapeutics. Emerging platform for cancer therapy like block copolymers, cyclodextrins, copolypeptides, charged lipids, and cholesterol-modified small interfering RNA (siRNA) via lipoprotein-based advanced Nanocarriers with an ability to successfully deliver siRNA into target cells, are elaborated. Potential nanocarriers could be based on following materials;

- PLGA
- Polypeptide
- Poly( $\beta$ -amino ester)
- $\beta$ -cyclodextrin-containing polycation
- PAMAM dendrimer
- Polyphosphoester

- Linear and branched PEI
- Liposomal nanoparticles

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## PROBING THE BARRIER PROPERTIES OF THE BASEMENT MEMBRANE TO MUCOSAL DELIVERY OF NANOPARTICLES

JULIA MANTAJ

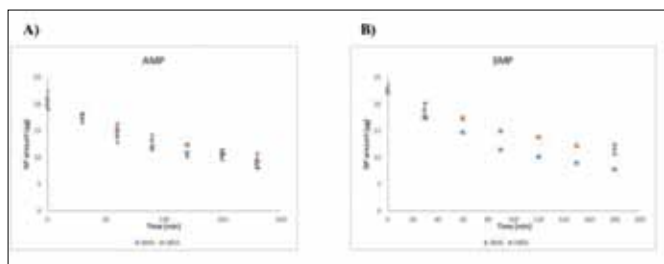
Research into nanomedicine has proliferated in the past two decades. These complex therapeutics are currently almost exclusively intended for administration by injection. Non-invasive administration is preferred over injections due to patient convenience, elimination of injection-associated side effects and, potentially, reduced costs<sup>[1, 2]</sup>. However, mucosal administration is currently not a viable option for systemic delivery of most nanomedicines due to the challenges of overcoming the mucosal barriers.

Mucosal tissues comprise multiple barriers to drug delivery, including mucus, epithelium and the basement membrane (BM). The epithelium is considered to be the principal mucosal barrier component to systemic absorption of complex therapeutics such as nanomedicines. Mucus has also been shown to hinder the diffusion of nanoparticles. However, effective drug delivery strategies now exist to enhance the diffusion of nanomaterials in mucus (e.g. via the 'PEGylation approach')<sup>[3-6]</sup> and also facilitate their translocation across the epithelial barrier (e.g. by exploiting epithelial transcytosis to shuttle material across the epithelium)<sup>[7, 8]</sup>. On the other hand, the BM barrier, as another physical barrier component of the mucosae, has not been fully characterised. With an increasing proliferation of nanomedicines and efforts to achieve non-invasive delivery of these therapeutics, there is a need to study the barrier properties of the BM to systemic delivery of nanomedicines.

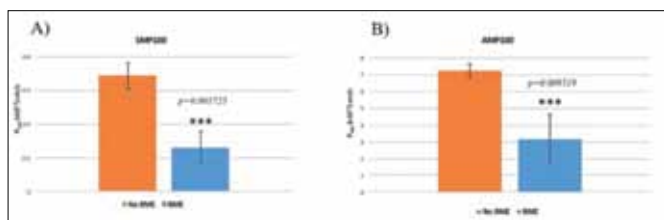
BMs are thin, specialised sheets of extracellular matrices found between epithelia and connective tissue in the human body<sup>[9, 10]</sup>. Collagen is the main protein of the extracellular matrix (ECM) and is linked by multiple bonds, including disulphide and hydrogen bonding, that gives tensile strength to BM<sup>[11, 12]</sup>. Alongside collagen, laminin, which strongly associates to cell surface, provides additional organised structural support to BM<sup>[13]</sup>. BMs have several roles including the regulation of cell adhesion, differentiation and motility<sup>[14]</sup>. BM also serves a filter function due to its selective passage of molecules across its barrier<sup>[15]</sup>.

In this work, we have characterised the barrier property of the BM towards interaction with and permeation of model nanoparticles. Multi-well plates and permeable inserts were coated with the BM extract (BME) derived from murine Engelbreth-Holm-Swarm sarcoma. Alternatively, BM was obtained by filter insert culture, followed by decellularization of BM-producing bronchial epithelial cells, Calu-3. Interaction with and translocation of fluorescently-labelled polystyrene nanoparticles across these BMs was analysed. Figure 1 shows the interaction of 100 nm, positively charged (amine-modified, AMP) (A) and negatively (sulphate-modified, SMP) (B) polystyrene nanoparticles with BME-coated plates. The data shows no difference compared to control in terms of the interaction of AMP with BM-coated plates. On the other hand, the data indicates some interaction of SMP with BM, as suggested by a decreased amount of the nanoparticles at all sampling points compared to the control.

**Figure 1.** Interaction of 100 nm polystyrene nanoparticles with the Basement Membrane. A) Positively-charged (amine-modified, AMP) nanoparticles. B) Negatively-charged (sulphate-modified, SMP) nanoparticles. Nanoparticles were applied at 100 ug/ml concentration in Hank's Balanced Salt Solution. Interaction was characterised by sampling the applied nanoparticle suspension every 30 min for 3 hrs and quantifying nanoparticles via fluorescence measurement.



**Figure 2** shows the permeation of model nanoparticle systems across BM-coated inserts. The data clearly demonstrate that nanoparticle permeability across BM-coated inserts is significantly lower compared to control, hence clearly indicating that the BM barrier impedes the movement of both positively charged and negatively charged nanoparticles.



**Figure 2.** Apparent permeability ( $P_{app}$ ) of A) sulfate-modified (SMP, 100 nm) and amine-modified (AMP, 100 nm) polystyrene nanoparticles across BME-coated Transwell® inserts compared to uncoated inserts acting as control ( $n=3$ ). Nanoparticles were applied at 100 ug/ml concentration in Hank's Balanced Salt Solution. Interaction was characterised by sampling the applied nanoparticle suspension every 30 min for 3 hrs and quantifying nanoparticles via fluorescence measurement. Statistical significance ( $p < 0.001$ ) is represented by \*\*\*.

## DISCUSSION

As the clinical use of nanomedicines becomes more widespread, patient-friendly non-invasive administration of these therapeutics will become more important. To develop successful technologies for mucosal delivery of nanomedicines, it is imperative that the mucosal barriers are fully understood. This work investigated the barrier properties of the BM, a component of the mucosal barrier, to mucosal delivery of nanomedicines for systemic effect.

Our data suggest that while only negatively charged, 100 nm model nanoparticles interact with the BM, the permeation of both positively-charged and negatively-charged systems is reduced by the BM barrier. These findings corroborate with limited studies in the area. For example, lining of a HeLa monolayer with BM followed by exposure to HPV-16 pseudoviruses reduced the percentage of infected HeLa cells by about 6-fold, highlighting the extent of the barrier that the BM presents to the movement of a 50 nm virus<sup>[16]</sup>. Extracellular matrix has also been shown to dramatically suppress the diffusion of both positively and negatively charged particles that were significantly smaller than the mesh size of the ECM<sup>[17]</sup>.

This work is important as it clearly highlights that the BM presents a barrier to mucosal delivery of nanomedicines for systemic effect. Therefore, drug delivery strategies for mucosal delivery of nanomedicines should also address this barrier, in addition to the widely recognised mucosal barrier components such as mucus. Ongoing work in our group is determining the effect of the BM barrier, incorporated into an intestinal epithelial model (Caco-2, which lacks the

ability to produce a BM) on nanoparticle permeation.

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# MULTISCALE OPTICAL IMAGING OF 10 NM POLYMER ACCUMULATION IN THE BRAIN UPON TEMPORARILY OPENING UP THE BLOOD-BRAIN BARRIER USING ULTRASOUND AND MICROBUBBLES

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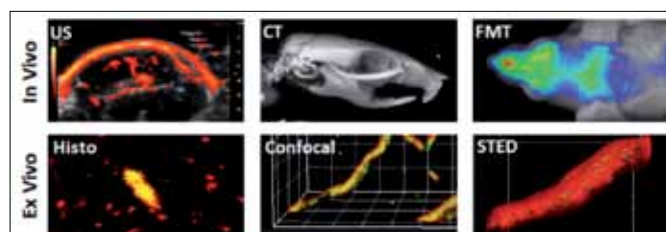
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The blood-brain barrier (BBB) is a major obstacle for drug delivery into the brain. The combination of ultrasound (US) and microbubbles (MB) can induce a spatially and temporarily controlled opening of the BBB, allowing for the extravasation of drug (delivery systems) out of the blood vessels into the brain. In this preliminary study, we used US and MB to permeabilize the BBB, and employed fluorophore-labeled polymers in combination with multimodal and multiscale optical imaging to assess the efficiency of sonoporation-mediated drug delivery to the brain.

## METHODS

Treated as well as control CD-1 nude mice received an i.v. injection of pHPMA polymers (10 nm) labeled with Alexa488 and Cy7. During US application, polymeric PBCA-based MBs were infused to open the BBB. Polymer accumulation was non-invasively and longitudinally monitored using computed tomography-fluorescence molecular tomography (CT-FMT). Prior to sacrifice, rhodamine-labeled lectine was injected to stain perfused blood vessels. Ex vivo analysis included fluorescence microscopy (FM), confocal microscopy (CM) and stimulated emission depletion nanoscopy (STED). Possible side effects and the overall extent of BBB opening were also investigated, using immunohistochemical (H&E) and immunofluorescence (directed against extravasated endogenous IgG) stainings.

Figure 1: Applied multimodal and multiscale imaging modalities. Fluorophore-labeled polymers were injected i.v. into healthy CD-1 nude mice and the BBB was opened via the combination of US and MB enabling the extravasation of nanocarriers. The used modalities include US for inducing the BBB opening, CT-FMT scans for the in vivo distribution and several microscope devices for a more accurate ex vivo evaluation

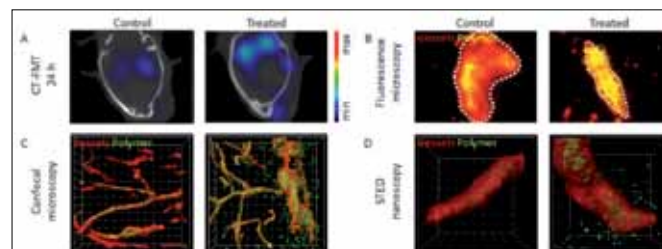


## RESULTS

The success of BBB opening upon applying US and MB was confirmed via the increased number of blood vessels showing IgG extravasation in sonoporated animals compared to control animals. Based on H&E stainings, the treatment did not induce any obvious brain damage. The extravasation and penetration of polymers upon sonoporation was clearly visible using all optical imaging modalities (Figure 2 A-D). Using software tools to quantify the extent of extravasation and penetration depth, we confirmed that 10 nm polymers penetrated efficiently and relatively deep into the brain.

Figure 2: Accumulation and extravasation of polymers upon BBB

opening. CT-FMT images of mice brains indicated an increased accumulation of polymers upon treatment compared to control animals (A). Ex vivo fluorescent microscopy of functional rhodamine lectine-perfused vessels and the fluorophore-labeled polymers verified their extravasation into the brain (B). Extravasated polymers were also detectable in z-stack-images acquired with a confocal microscope upon sonoporation (C). Specific regions of interest inside the confocal images were imaged using STED nanoscopy and confirmed the relatively deep extravasation of polymers upon sonoporation (D).



## CONCLUSIONS

US and MB can be employed to efficiently open up the BBB, enabling the delivery of drugs and drug delivery systems to the brain. Multimodal and multiscale optical imaging show that relatively small (10 nm polymers) carrier materials accumulate efficiently and penetrate deep into the brain. In an EuroNanoMed III - Project (NSC4DIPG), we will add several nanocarriers e.g. micelles and liposomes and compare their properties regarding accumulation and penetration into the brain. The best performing nanocarriers will be loaded with chemotherapeutic agents and a therapy study will be performed aiming for a proof-of-concept to translate this Nano-SonoChemotherapeutic approach into the clinic.

## ACKNOWLEDGEMENTS

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## PREPARATION AND CHARACTERIZATION OF MPEG-ZEIN MICELLES AS A DELIVERY VEHICLE FOR HYDROPHOBIC DRUGS

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Zein, a water-insoluble protein from corn, is generally recognized as safe (GRAS). It is biocompatible, biodegradable and confers properties that make it an attractive material as a delivery system for human use. However, its protein origin and hydrophobicity led to the rapid clearance of zein micro/nanoparticles by macrophages, which could limit its use for drug delivery. To overcome this limitation, we hypothesize that conjugating zein with polyethylene glycol (PEG) could provide steric shielding of the delivery system, thus preventing opsonization and providing sustained release for effective cellular uptake into cancer cells. The aim of this project was to prepare and characterize zein micelles conjugated with PEG and entrap a hydrophobic drug model, Nile red.

In this study, mPEG-succinimidyl carboxymethyl (SCM) was used to conjugate with yellow zein (a mixture of  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ -zein). Molecular weight of mPEG-SCM (5000 and 10000 Da) and PEG to zein ratio are summarized in Table 1.

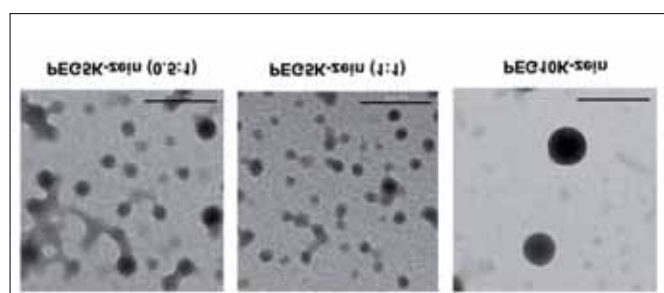
Table 1: Summary of PEGylation compositions used in the study

	Composition	Weight ratio	Molar ratio
mPEG5000-zein (0.5:1)	mPEG5000:zein	0.5:1	2.3:1
mPEG5000-zein (1:1)	mPEG5000:zein	1:1	4.6:1
mPEG10000-zein	mPEG10000:zein	1:1	2.3:1

Our work demonstrated that zein could be successfully conjugated with PEG and entrapping Nile red. PEGylated zein was prepared using a dialysis method through solvent exchange, resulting in micelles with critical micelle concentration values of about 80  $\mu\text{g/mL}$ . Attenuated Total Reflection - Fourier Transform Infrared spectroscopy (ATR-FTIR) spectra of zein, mPEG, and mPEG-zein conjugates confirmed that PEGylation was achieved. Since the bands corresponding to amide protein peaks of zein and methyl group of PEG appeared in the spectrum of mPEG-zein, while the N-hydroxysuccinimide (NHS) ester peak of PEG disappeared after the conjugation. Furthermore, core-shell structure of mPEG-zein micelles was confirmed by comparing Proton Nuclear Magnetic Resonance ( $^1\text{H}$  NMR) spectra in DMSO- $d_6$  and D $_2$ O. The changes in  $^1\text{H}$  NMR spectra suggest that mPEG-zein assemble into micelles with hydrophilic mPEG in the outer shell and hydrophobic zein in the core.

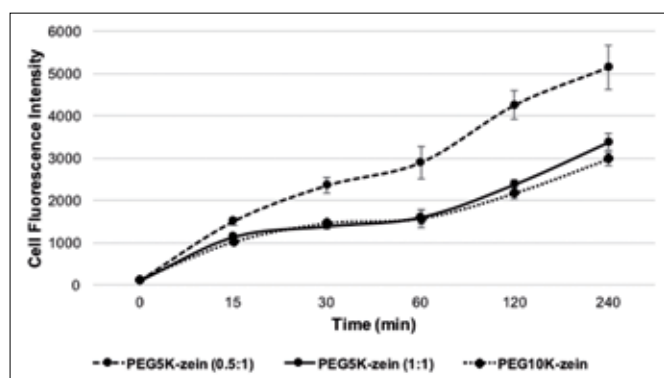
The micelles had spherical shape, as demonstrated by Transmission Electron Microscopy (TEM) images (Figure 1). Dynamic light scattering results showed the size of the micelles ranged from  $96.4 \pm 1.94$  to  $172.3 \pm 1.90$  nm, depending on the molecular weight of PEG used and PEG to zein ratio. The zeta potential of the micelles was positive in all formulations. Nile red-loaded mPEG-zein micelles exhibited an increase in particle size. Loading Nile red, however, did not change surface charge of the micelles, indicating that Nile red was encapsulated in the hydrophobic inner core of the micelles. The Nile red encapsulation efficiency of three mPEG-zein formulations was in the range of 70-85%.

Figure 1: TEM images of mPEG-zein micelles



In vitro studies revealed that mPEG-zein micelles could deliver Nile red into B16-F10-luc-G5 melanoma cells in a time-dependent manner (Figure 2). The Nile red uptake by the cells was the highest in PEG5K-zein (0.5:1), followed by PEG5K-zein (1:1) and PEG10K-zein, respectively. This implies that PEGylation with shorter PEG chain length and less PEG mass content could improve cellular uptake efficiency of the zein micelles. Cell viability study also indicated that the micelles were not toxic to the cells. Overall, the results suggest that PEGylated zein is highly promising as a delivery system for cancer therapy.

Figure 2: Cellular uptake of Nile red-loaded mPEG-zein micelles, as determined by flow cytometry



## INVOLVEMENT OF COMPLEMENT ACTIVATION IN THE PULMONARY VASOACTIVITY OF POLYSTYRENE NANOPARTICLES IN PIGS – PART II

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Complement (C) activation has been associated with hypersensitivity reactions (HSRs) to certain nano-pharmaceuticals, a phenomenon referred to as “C activation-related pseudoallergy” (CARPA). Many symptoms of these infusion reactions can be modeled in pigs, most sensitively the cardiopulmonary distress, with pulmonary hypertension taken as a quantitative measure of the drugs’ reactogenicity. A recent study reported that spherical polystyrene nanoparticles (PS-NPs) cause pulmonary hypertension in pigs without C activation, challenging the CARPA concept. This challenge was recently questioned on theoretical grounds, and here we sought experimental evidence for C activation playing a role in PS-NP-induced HSRs in pigs.

Physicochemical characterization of PS-NPs size, size distribution and zeta potential by Malvern Zetasizer, morphology by cryo-TEM, while hydrophobicity was assessed by Rose Bengal adsorption. In human serum C activation in vitro was measured using C3a, Bb, C4d and sC5b-9 markers determined by ELISA. CARPA in pigs was quantitated by real-time monitoring of pulmonary (PAP), systemic (SAP) blood pressures and heart rate (HR) changes.

PS-NPs are monodisperse, highly hydrophobic solid spheres with strong negative charge even at isotonic salt concentration. In human serum significant rises of C3a, Bb and sC5b-9 (but not that of C4d) indicated alternative pathway C activation, which was particle size dependent. PS-NPs caused major hemodynamic changes in pigs, primarily pulmonary hypertension, on the same minute scale as C activation and opsonization occurred. There was significant correlation between C activation by PS-NPs in human serum and pulmonary hypertension in pigs.

Our results clearly demonstrate that PS-NPs have extreme surface properties with no relevance to clinically used nanomedicines. The nanoparticles can activate C in human sera via alternative pathway and may play a causal or contributing role in the pulmonary reaction of pigs to PS-NPs. Our data are consistent with the double (or multiple) hit theory of HSRs, wherein C activation contributes to allergic mediator-secreting phagocyte activation via opsonization of reactogenic NPs.

## CONTACTPOINTNANO.CH: A STRATEGIC INITIATIVE TO TRANSFER THE NANOSAFETY KNOWLEDGE TO SWISS SMES

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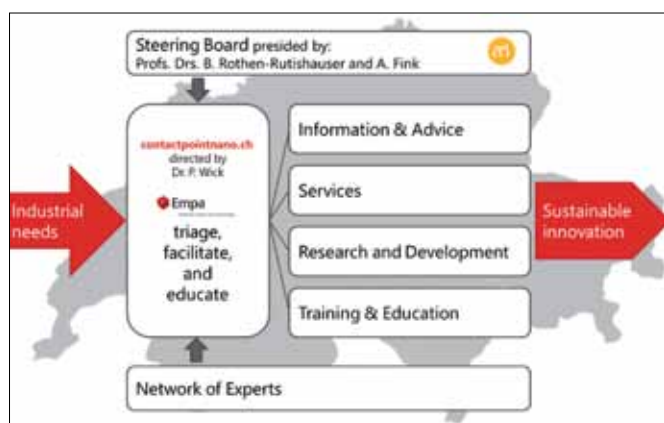
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As an independent national contact point, contactpointnano.ch pools and structures the scientific and regulatory expertise available in Switzerland on the safe handling of synthetic nanomaterials – from production and application to disposal. Its objective is to efficiently convey tailored high-quality information to companies (established companies, SMEs and start-ups), thus accelerating the transfer from invention to innovation and allowing Swiss companies to remain competitive in an international environment. con

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Figure 1. Organization of the contactpointnano.ch



## METAL ORGANIC FRAMEWORKS AS A POTENTIAL PAH DRUG CARRIERS

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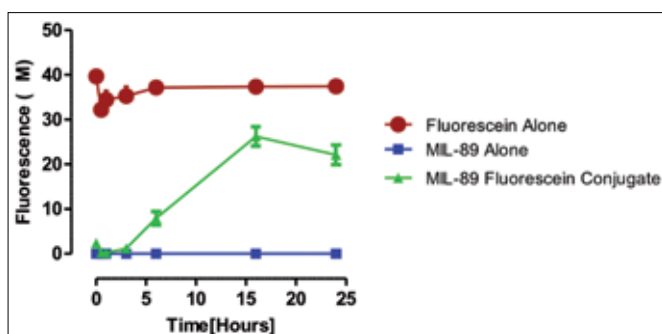


Figure 1: Fluorescein release rate overtime (24 hours) from MIL-89 compared to the fluorescein alone and MIL-89 alone.

**Introduction:** Nanomedicine is an attractive, promising and fast growing field that uses nanoparticles to serve as drug carriers. This field can aid in improving the treatment of debilitating and fatal complications of cardiovascular diseases including pulmonary arterial hypertension (PAH). PAH is an aggressive disease with poor

prognosis, no available cure and low survival rates of 2-5 years once diagnosed<sup>[1]</sup>. Whilst there is no cure there are several classes of drugs that are used to treat PAH such as: (i) endothelin-1 inhibitors, (ii) phosphodiesterase inhibitors, (iii) prostacyclin analogues and (iv) soluble guanylate cyclase activators. Despite the great potential, these therapies are hindered by their systemic side effects<sup>[1]</sup>. One way of overcoming this limitation is by encapsulating PAH drugs in a drug carrier using the nanomedicine technology. Currently, there are different types of clinically approved nanoparticles that could potentially be used to deliver PAH drugs. In particular, the iron (Fe) containing nanoparticles from the metal organic framework (MOF) class of modalities, namely MIL-89<sup>[2]</sup>, are considered as promising candidates for drug delivery. The MOF MIL-89 has two main advantages, (i) the cavity size is suitable to accommodate PAH drugs and (ii) it can be imaged using MRI<sup>[3]</sup>. Furthermore, we previously showed that MIL-89 is relatively non-toxic in a range of human cell types and well tolerated *in vivo*<sup>[4]</sup>. In this study, we assessed the loading capacity and the traceability of MIL-89 using fluorescein as a diagnostic dye selected for its similar size to PAH drugs. Methods: MIL-89 was loaded with fluorescein before 'drug' release was tested over 24 hours. The uptake of the loaded MOFs by endothelial cells was assessed using a fluorescent microscope. Results: MIL-89 was capable of taking up and releasing fluorescein over a period of 24 hours at room temperature and 37°C (Figure 1). Furthermore, endothelial cells readily took up fluorescein loaded MOFs (Figure 2). Conclusion: MIL-89 represents a non-toxic potential PAH drug-carrier with good pharmacodynamics properties. However, the pharmacokinetics of PAH-drug MIL-89 remains the subject of investigation.

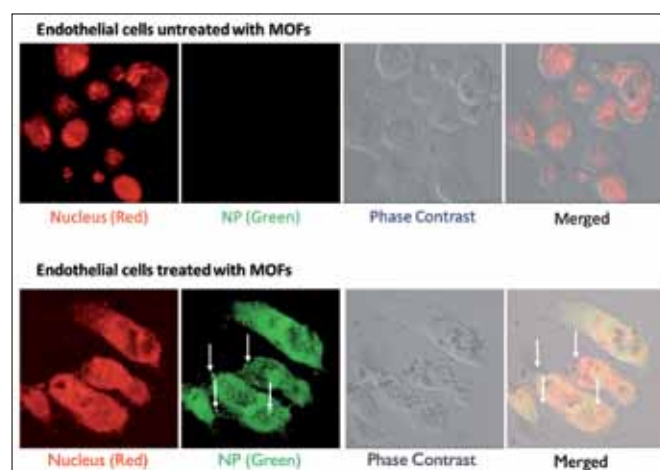


Figure2: MIL-89 Fluorescein conjugate uptake by endothelial cells.

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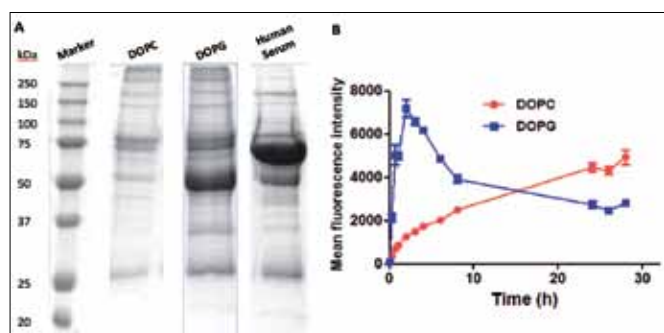


## ZWITTERIONIC MODIFICATIONS REDUCE CORONA FORMATION AND AFFECT UPTAKE KINETICS AND MECHANISM OF INTERNALIZATION OF LIPOSOMES

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Nanomedicine has great potential for targeted delivery of drugs to diseases. Nevertheless, it has been shown that in some cases targeting can be affected by the adsorption of biomolecules to the surface of nano-sized drug carriers once in contact with biological fluids such as for instance in blood<sup>1</sup>. Adsorption of biomolecules on nano-carriers can also lead to clearance by the mononuclear phagocytic system. An often used modification of nanoparticles to try to reduce the biomolecule adsorption and corona formation is PEGylation<sup>2</sup>. It is for example used in the first nanomedicine on the market, Doxil. Downsides of PEGylation are the non-biodegradability, tendency to induce aggregation of the nano-carrier in high salt solutions, and the potential to induce an immune response<sup>3,4</sup>. An alternative modification to decrease adsorption of proteins and induce the so called 'stealth' effect on nano-carrier surfaces are zwitterionic molecules. Zwitterionic molecules are overall neutral but contain a positive and negative charge to which water molecules are tightly bound and this, among others, has been suggested to reduce binding of proteins to zwitterionic surfaces<sup>5</sup>. Introducing zwitterionic modifications on nano-carriers surfaces will also affect corona composition and ultimately uptake kinetics as also the internalization mechanism of the nano-carrier. In order to study the effect of zwitterionic medication on all of these factors here we have used liposomes made of either 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) or 1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DOPG), both combined with cholesterol (Chol). The two liposomes are identical except for the head group of the lipid which is zwitterionic in the case of DOPC and negatively charged in the case of DOPG.



**Figure 1:** DOPG-Chol and DOPC-Chol liposomes were incubated in human serum (4mg/ml in PBS). After corona isolation similar protein amounts were run on a SDS-PAGE together with human serum and protein marker (A). HeLa cells were incubated with the DOPC-Chol and DOPG-Chol liposomes in medium containing human serum (4mg/ml) and uptake was measured over time using flow cytometry.

As expected the DOPC-Chol liposomes showed a reduced adsorption of proteins compared to DOPG-Chol liposomes. Also the composition of the corona was strongly affected (Figure A). Interestingly, exposure of HeLa cells to the liposomes revealed a very different uptake kinetics, especially in the first hours (Figure B). To further investigate the effect of the zwitterionic modification on internalization, inhibitors of various endocytic pathways were used and the results indicated that different mechanisms are involved in the internalization of the two liposomes.

Thus overall addition of zwitterionic molecules does not only affect the formation and composition of the protein corona but it also influences uptake kinetics and the mechanism of uptake.

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## FUNCTIONALISATION OF T LYMPHOCYTES FOR MAGNETICALLY CONTROLLED IMMUNE THERAPY

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Breast cancer is the most common cancer related cause of death in women. With many factors such as genetics, hormones or lifestyle having an effect on the development of breast cancer, there is a classification of various subgroups: triple negative, luminal A or luminal B, HER2 positive or HER2 negative, basal-like or normal-like. In addition, gene mutations e.g. in BRCA1 or BRCA2 can be found in every subgroup. The complexity of all those factors results in an individual treatment protocol consisting of chemotherapy, hormone therapy, antibody therapy, irradiation or surgery for every single patient.

On the contrary, the immune status of the tumour, especially the amount of tumour infiltrating lymphocytes (TIL) is important for all patients. The presence of CD8+ killer T cells in the tumour area directly correlates with the patient's clinical outcome, independent of molecular subgroup and treatment protocol. For this reason it seems desirable to increase the number of TIL.

One way to accumulate T cells in the tumour area is to make them magnetisable and attract them with an external magnetic field. Magnetisation can be achieved by superparamagnetic iron oxide nanoparticles (SPIONs) which can be bound to the cells' surfaces or can be internalised into the cells.

Superparamagnetic nanoparticles are characterised by the fact that the particles are magnetised by an external magnetic field, but

immediately demagnetise when the magnetic field is switched off or removed and thus do not form aggregates leading to the formation of thrombi.

With our promising preliminary data using modified lauric acid coated SPIONs, we already can demonstrate the possibility to attract SPIONs bearing T cells by an external magnet (fig. 1).



Figure 1: A small cylindrical magnet (8 mm x 5 mm, approx. 50 mT) was placed in a well with lauric acid coated SPIONs bearing T cells and filmed. Cells move towards the magnet's surface and accumulate there. (Colours and contrast have been adjusted for enhanced visualisation.)

Based on the proof of concept, various particle systems are now synthesised and tested to achieve the best possible combination of biocompatibility and attractability. Current SPIONs are coated with citrate or coated with poly(acrylic acid-co-maleic acid) (PAM). The difference to the previously used lauric acid particles is that these were absorbed into the cells, but the PAM or citrate coated particles are adsorbed on the cell surface. This makes it possible to load the cells with larger quantities of SPIONs within 15 minutes. Both particles show very low toxicity (fig. 2) and a concentration dependent increase of the side scatter which indicates absorption or adsorption of SPIONs (fig. 3).

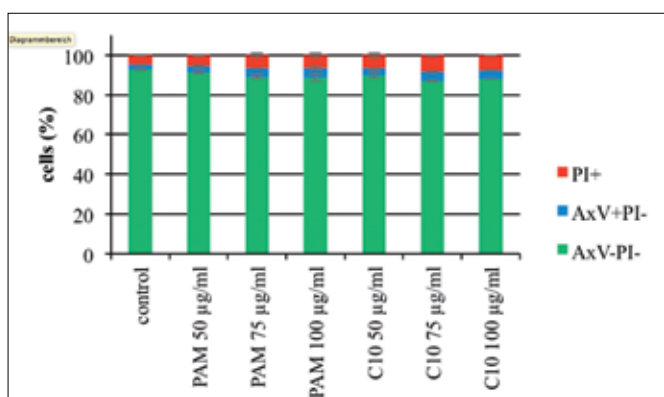


Figure 2: Determination of biocompatibility of SPIONs by flow cytometry.

Fluorescence staining after incubation with immortalised T cell line EL4 for 15 minutes in phosphate buffered saline with annexin A5 (AxV) and propidium iodide (PI) for detection of viable (AxV-PI-), apoptotic (AxV+PI-) and necrotic (AxV+PI+) cells displayed as a proportion of cells to detect effects on cell viability. Shown are the mean values with standard deviations.

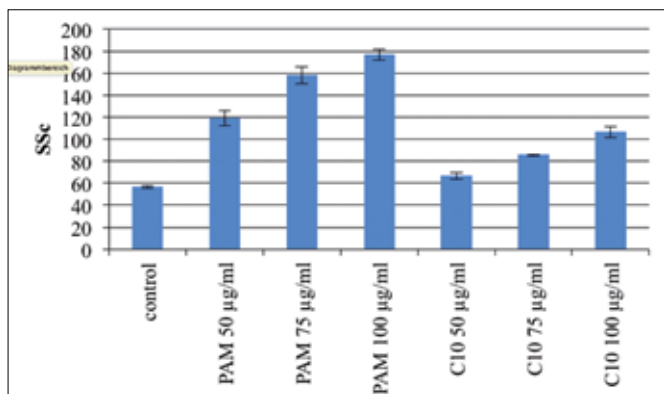


Figure 3: Side scatter increase indicates cellular SPION uptake.

Cellular granularity of viable EL4 cells after 15 minutes of incubation with SPIONs in phosphate buffered saline. SSc increase was determined in flow cytometry and provides information about cellular granularity, which can be used to estimate the SPION uptake into cells or attachment to cells. Shown are the mean values with standard deviations.

**Acknowledgement:** This work is supported by the EFI-project BIG-THERA (3\_Med\_05) and Manfred Roth Stiftung, Fuerth, Germany.

## COMPLEMENT ACTIVATORS AND AMPHOTERICIN-B LIPOSOMES CARDIOVASCULAR EFFECT IN MICE

ERIK ÖRFI

Undesirable C activation by some nanomedicines is known to cause complement activation related pseudoallergy (CARPA) in sensitive individuals. In CARPA, C split products result in a series of hemodynamic and other changes, which, when uncontrolled, may become fatal. Intravenous administration of liposomal amphotericin B (AmBisome and Abelcet) and high cholesterol multilamellar vesicles (HC-MLV) activate the C system in humans, pigs and rats and result in CARPA. No data, however, is available on the effect of these liposomes on the complement system in mice, which are widely used in preclinical research. The goal of this study was to understand the effects of these liposomal preparations in mice.

### METHODS

Blood pressure and heart rate were measured in anesthetized male NMRI mice (n=4-6/group). Blood was collected from the transected vena cava in isoflurane anesthetized mice at 3-30 min after treatment and blood count and plasma concentration changes were compared to a control group administered saline only (n=8-12/group).

### RESULTS

Liposomal amphotericin B formulations (AmBisome and Abelcet), and positive controls known to activate the complement (zymosan and cobra venom factor, CVF) but not HC-MLV led to transient hypertension, thrombocytopenia and elevation of plasma thromboxane concentration (TXB2) in mice. Hemodynamic changes progressed to hypotensive shock in animals treated with positive controls. Parallel measurement of plasma C3a concentration revealed massive C activation in zymosan and CVF treated animals but smaller activation was detectable in plasma of animals treated with liposomal amphotericin B formulations. Pretreatment of mice with the C3a inhibitor SB290157 attenuated the blood pressure raise caused by Abelcet and decreased blood pressure at later times.

### CONCLUSIONS

Treatment with liposomes caused a transient hypertension in mice. This change correlated with platelet activation, increased plasma TXB2 levels and complement activation. Hemodynamic changes induced by the positive controls were biphasic accompanied with considerable elevation of C split products. Activation of both the complement system and vasoactive mediator-secreting blood cells are responsible for the liposome-mediated hemodynamic changes typical of CARPA in mice.

M Schwarz, L Dézsi, R Urbanics, J Matuszak, E Örfi, T Fülöp, T Bäuerle, J Szebeni, C Journé, AR Boccaccini, C Alexiou, Stefan Lyer, I Cicha International Journal of Nanomedicine 12: pp. 5223-5238. 2017 doi: <https://doi.org/10.2147/IJN.S138108>, IF: 5,008

## IMMOBILIZATION OF CHEMICAL COMPOUNDS ONTO POLYMERIC NANOBACBONES AS A STRATEGY TO DECREASE THE INCIDENCE OF ALLERGIC CONTACT DERMATITIS

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Allergic contact dermatitis (ACD) is the manifestation of a sensitization and/or allergic response caused by the contact of the skin with a substance, and it is also among the most common forms of immunotoxicities in humans. Different chemicals, ranging from medicines, personal care products, specialty chemicals (e.g. food additives, adhesives etc.) and even agrichemicals (e.g. fertilizers) have been identified as the main triggers for ACD.

Allergic contact dermatitis occurs in two phases. In the initial, clinically indiscernible sensitization phase, epidermal Langerhans cells or dermal dendritic cells are triggered to migrate to skin-draining lymph nodes and present the contact allergen covalently bound to skin proteins to naïve T lymphocytes. These recognize the chemically modified protein, leading to the production of skin-homing CD4<sup>+</sup> and CD8<sup>+</sup> effector T cells, which then enter the blood circulation. Subsequent exposure to the same contact allergen leads to the elicitation phase, characterized by the infiltration of T cells, neutrophils and macrophages and the appearance of skin lesions. Although even small amounts of allergen may be sufficient to start the sensitization cascade, there are two main determinants that have been proposed for the induction of contact sensitization: first, the penetration of a chemical through the outermost skin layer (i.e. the Stratum Corneum) to interact with the cutaneous immunological cells in the living epidermis; second, the reactivity of a compound towards skin proteins (mainly through an electrophilic-nucleophilic interaction).

In this study, we hypothesize that strategic functionalizations and increase in molecular size of a common allergen (para-Phenylenediamine (PPD)), and/or its immobilization onto a branched polymeric nanobackbone (Figure 1) are able to maintain the properties of the compound, possibly reduce the penetration into the skin, make it more resistant to metabolic and oxidative changes and therefore decrease the chance of allergic reactions tremendously. Polymers with high water solubility are used as backbone for incorporating photo- or chemical-crosslinking groups.

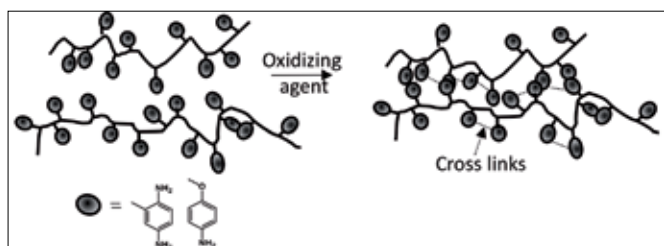


Figure 1: proposed PPD-incorporated water soluble polymers.

On the basis of preliminary experiments, we could identify the following significant advantages over conventional PPD-based products:

- 1) Since the skin does not allow the penetration of substances bigger than 500Da, bulkier PPD derivatives minimally penetrate the skin and thus decrease allergic reactions, simply because immune-competent cells in the skin don't get in contact with the allergen;
- 2) These compounds are able to maintain the properties of PPD as dye through a much more controlled chemical reaction;
- 3) Our oligomeric nanobackbone show less sensitization potential in the Direct Peptide Reactivity Assay (DPRA).

Taken together, our project provides an enormous commercial opportunity not only because it addresses an unmet need, but also

because it paves the way for applications to other products that cause skin allergies.

## EVALUATING THE EPR-EFFECT IN DIFFERENT TUMOR MODELS USING THE CLINICALLY APPROVED DIAGNOSTIC DRUG NANOTOP IN COMPARISON WITH LIPOSOMES

STEFANIE PEKTOR, Nicole Bausbacher, Stephan Maus, Andras Polyak, Tobias Ross, Mathias Schreckenberger, Matthias Miederer.

### AIM:

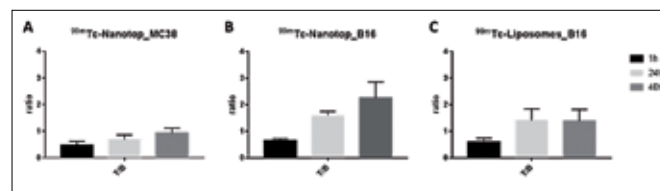
Pharmacokinetics and tissue distribution of nanoparticles largely define their therapeutic effect and toxicity. The physicochemical properties of the nanoparticles, including size, surface charge, and surface modifications are important factors that determine their pharmacokinetics and biodistribution and influence their EPR-effect. Furthermore, this effect is also strongly influenced by tumor-intrinsic factors like vessel diameter, number and permeability. Two subcutaneous mouse tumor models were used in this study to analyse how the EPR effect is influenced by the tumor composition itself using the clinically approved drug Nanotop as model system. Furthermore, in one tumor model the EPR-effect of Nanotop was analysed in comparison with that of liposomes.

### METHODS:

2x10<sup>5</sup> B16F10 melanoma or 1x10<sup>6</sup> MC38 coloncarcinoma were s.c. injected into the right flank of C57BL/6 mice (6 weeks old). After 14 days of tumor growth, mice were i.v. injected with the directly labeled 99mTc-Albumin-Nanokolloid Nanotop (NanoHSA-Rotop, average diameter: 10 nm; <sup>[1]</sup>) or the 99mTc-labeled Liposomes (average diameter: 80 nm). Radiochemical yield and stability over a time period of 48 hours in NaCl, serum and plasma was measured by ITLC. Organ distribution was determined after 1, 24 and 48 hours in tumor, liver, spleen and blood via gamma-counting. Tumors were fixed in PFA and analysed histologically by H&E staining.

### RESULTS:

The *in vitro* measured stabilities typically exceeded 95 %. In the B16 melanoma model the tumor enrichment of 99mTc-Nanotop was slightly increased compared to MC38 and the tumor to blood ratios increased more dominant over the time period of 48 hours (Fig. 1 A,B) while tumor enrichment of both models was independent of tumor size. The T/B ratios of 99mTc-Nanotop was furthermore comparable to the T/B ratios of the 99mTc-Liposomes (Fig. 1C) in the melanoma model, showing a comparable but relatively low EPR effect no matter which size and material of nanocarrier was used. In general, both particles showed higher accumulation into liver and spleen compared to tumor, concomitant with low blood retention. 99mTc-Nanotop enrichment in RES organs was slightly higher compared to the liposomes.



### CONCLUSION:

Despite different physicochemical properties like size and composition of the nanocarriers accumulation in tumors was not influenced in the B16 melanoma model. On the other hand the same nanocarrier showed different accumulation in dependency of the tumor model maybe due to other tumor morphology and number, size and permeability of tumor vessels <sup>[2]</sup>. For better translation into the human system we will use two different xenograft tumor models using human melanoma and colorectal coloncarcinoma cells for future studies. In order to improve the *in vivo* stability new labeling techniques will be evaluated using radionuclides with long half-lives to follow up tumor accumulation for several days.



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## WARM ISOSTATIC PRESSING TECHNOLOGY FOR ORTHOPEDIC IMPLANTS

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**Keywords:** warm isostatic pressing, hydroxyapatite, composites  
Orthopaedics employs implants made of materials that are characterised by high strength and are not resorbable in the organism, such as metal alloys, ceramic materials. Their application involves negative effects such as bone weakening, allergies, complications, another operation of implant removal. The currently applied bioresorbable implants include polymer materials, but their application is limited by poor mechanical properties. Numerous bioresorbable material solutions are known and employed but there is no solution of a bioresorbable implant that would be overgrown by bone tissue. The aim of the presented research is to produce an osteo-inductive composite of nano-hydroxyapatite and biodegradable polymer. This material is similar to natural bone as regards chemical, physical and mechanical properties.

Used warm isostatic pressing technology permits to obtain dense ceramic with mechanical properties close to the natural bone. The work shows mechanical properties of consolidated HAP-polymer composite. The consolidation process was carried out in extreme pressure up to 1GPa and temperature under 200°C. The mechanical properties of this nanoceramic were investigated and the compressive strength reach assign value above 130 MPa. The produced nano-hydroxyapatite / polymer composite is a promising biomaterial for application in orthopaedics in bioresorbable arthroscopic screws and wedges. The applied cryogenic milling method enabled obtaining a material containing 50% PLA and 50% GoHAP (by volume).

## ULTRASOUND MEDIATED SPATIAL DELIVERY OF CURCUMIN: IN VITRO AND IN VIVO STUDY

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Conventional chemotherapy leads to the systemic toxicity due to accumulation of drug at non-intended site. That is why the trigger based modality has gained much attention as it holds the spatio-temporal control over release of drug from nanoparticles. In this article we report 100 folds increase in cytotoxicity of curcumin in MDA MB 231 breast cancer cells in presence of microbubble and ultrasound. *In vivo* anti-tumor efficacy of the formulation was also studied in B16F10 melanoma subcutaneous tumor model generated on both the flanks of C57BL/6 mice. Ultrasound exposure to right tumor spatially enhanced the cytotoxicity and enabled the 5 folds higher regression of the tumor compared to unexposed left tumor which grew continuously in size. This study showed that the

ultrasound treatment can be used to selectively increase the drug uptake in tumor and minimize the systemic toxicity.

## INTRODUCTION

The low aqueous solubility of curcumin compromises its bioactivity<sup>[1]</sup>. In order to increase its solubility and bioactivity it has been encapsulated inside colloidal particles viz. liposome, micelles, solid lipid nanoparticles and nanoemulsions<sup>[2]</sup>. Ultrasound is typically used in field of diagnosis and its therapeutic potential as external trigger for drug delivery in cancer is being explored across the globe. Ultrasound in presence of microbubbles (MB) increases the permeability of cells through generation of small sonopores (1 nm to 5 μm) in the plasma membrane<sup>[3]</sup>. Generation of sonopores is an after effect of cavitation of MB under ultrasound wave. Hence, ultrasound would be very beneficial in enhancing the cellular uptake of drug encapsulated nanoparticles or free drug from extracellular to intracellular environment. We developed the curcumin encapsulated lecithin nanoemulsions (Cur\_NE) with diameter 70-90 nm and lipid shelled MB. These nano size Cur\_NEs can be internalized by cancer cells in higher amount as their diameter is quite lower than the diameter of reported sonopores. MBs were synthesized from lipid 1, 2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1, 2 dioleoyl-sn-glycero-3-phospho-L-serine (sodium salt) (DOPS-Na) whereas D-α-tocopherol polyethylene glycol 1000 succinate (TPGS) was used as stabilizer.

## MATERIALS AND METHODS

Cur\_NE was prepared by oil-in-water emulsion method followed by solvent evaporation. MBs were prepared by thin film hydration method using 20:1:1.5 molar ratio of DSPC:DOPS-Na:TPGS followed by purging with sulfur hexafluoride. To study cellular uptake, rhodamine 6 g containing nanoemulsions (NE\_Rh6g) were developed by same method as described for Cur\_NE but instead of curcumin, rhodamine 6g was dissolved in organic phase. NE\_Rh6g was incubated with MDA MB 231 breast cancer cells, ultrasound treatment was given and after 1.5 hours confocal laser scanning microscopic (CLSM) imaging and flow cytometry assay was performed for cellular internalization. To determine the cytotoxicity, Cur\_NE and MB were added to MDA MB 231 cells and the cells were exposed with ultrasound at intensity 2 W/cm<sup>2</sup>, 50 % duty cycle for 30 sec and thereafter incubated for 72 hours. After that cell viability was determined by MTT assay. To determine the *in vivo* antitumor efficacy, the 2x106 B16F10 melanoma cells were injected on both the flanks of mice C57BL/6 (n=6, 21-25 gm body weight). After 15 days, free Cur and Cur\_NE (dose: 40 mg/kg body weight) was administered p.o. and 2 hours after dosing 250 μg of MB was injected through tail vein and thereafter right tumor was exposed with ultrasound (2 W/cm<sup>2</sup>, 50% duty cycle for 60 sec) whereas left tumor was kept unexposed.

## PRINCIPAL RESULTS AND SIGNIFICANCE

The Cur\_NE had hydrodynamic diameter of 110.4 ± 5.3 nm and zeta potential of -34 ± 2.9 mV. The Cur\_NE were also characterized by cryo FEG SEM and the diameter was found to be 70-90 nm (fig. 1.A). The hydrodynamic diameter and zeta potential of MB was 1200 ± 200 nm and 15 ± 3 mV respectively. The size of MB obtained in FEG SEM analysis was in the range of size obtained in DLS (fig.1.B). The encapsulation efficiency of Cur\_NE for curcumin was found to be 98.8 ± 0.17%. Hydrophobic nature of both lecithin and curcumin would have complimented each other in achieving such high encapsulation efficiency. Ultrasound contrast property of MB was studied using agarose phantom model and MBs were found to be sonogenic in nature (fig.1.C). Internalization of NE\_Rh6g by MDA MB 231 cells was 2.15 folds higher to the one where ultrasound treatment was not given (fig.2). Such increase in cellular uptake is attributed to pores generated due to exposure of ultrasound in presence of MB. Moreover, the treatment of ultrasound also enhanced the fraction of cells which were internalizing the NE\_Rh6g. Hence, ultrasound treatment increased the cytotoxicity by 100 folds as compared to free curcumin (fig.3.A) and regressed the tumor effectively by ensuring internalization of adequate amount of Cur\_NE by maximum number of tumor's cell. *In vivo* anti-tumor ef-

ficacy study showed that the ultrasound treatment regressed the tumor to 5 folds lower volume as compared to the free curcumin (fig.3.B). Such decrease in size of tumor is attributed to cumulative uptake of Cur\_NE by both, conventional route as well as through pores generated after ultrasound exposure. Moreover, the ultrasound treatment ensured spatial delivery of drug to the tumor exposed with ultrasound. Consequently, the regression in size of right tumor was more than the left tumor which was not exposed with ultrasound (fig.3.C). Hence, the Cur\_NE has the potential to be used as carrier for spatial delivery of drug to the tumor and minimize the systemic toxicity in ultrasound mediated drug delivery system.

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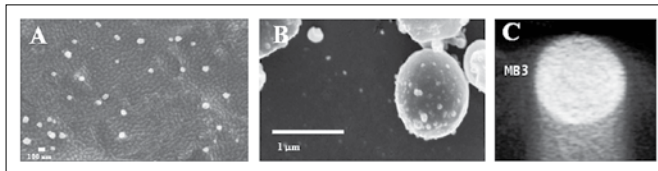


Fig.1. (A) SEM image of Cur\_NE, (B) SEM image of MB, (C) ultrasound contrast property of MB.

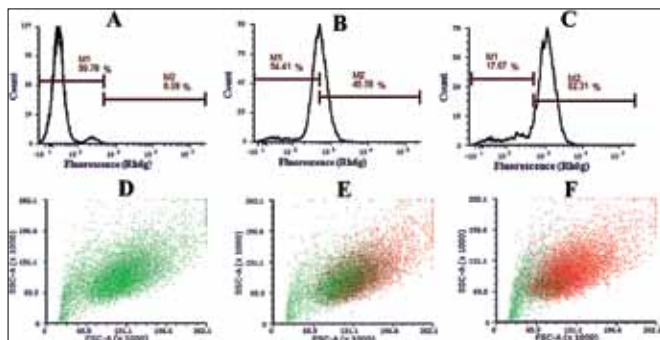


Fig.2. Quantification of fluorescent dye containing NE\_Rh6g internalized by MDA MB 231 cells through flow cytometry. (A) Only cells, (B) cells+ NE\_Rh6g, (C) cells+NE\_Rh6g+MB+US 0.9 W/cm<sup>2</sup>. Forward scatter (FSC-A) and side scatter (SSC-A) plot showed gradual increase in population of NE\_Rh6g positive cells (red dots), (D) only cells, (E) cells+ NE\_Rh6g, (F) cells+NE\_Rh6g+MB+US 0.9 W/cm<sup>2</sup>. M1 stands for NE\_Rh6g negative cells (green dots) and M2 for NE\_Rh6g positive cells (red dots).

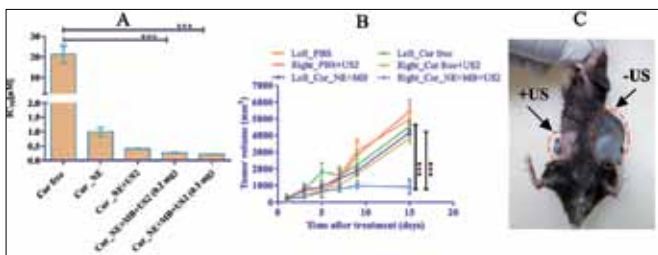


Fig.5. (A)  $IC_{50}$  concentration of Cur and Cur\_NE in presence of MB and ultrasound treatment in MDA MB 231 breast cancer cells. US2: 2 W/cm<sup>2</sup> ultrasound intensity at 50% duty cycle for 30 sec; 0.2 mg and 0.3 mg defines the amount of MB used for treatment. (B) B16F10 melanoma tropical tumors in C57BL/6 mice on both right and left flank and size reduction of tumor post treatment. Left and right specify the tumor developed on left and right flank respectively. US2: 2 W/cm<sup>2</sup> ultrasound intensity at 50% duty cycle for 60 sec.

(C) Reduction in size of right tumor post ultrasound treatment was observed whereas left tumor did not show size reduction.

## REAL-TIME SIMULTANEOUS MONITORING OF TUMOR SITE AND ALTERNATING MAGNETIC FIELD CONTROLLED DRUG RELEASE WITH DUAL MODAL MRI AND THERMOSENSITIVE MULTIFUNCTIONAL LIPOSOMES

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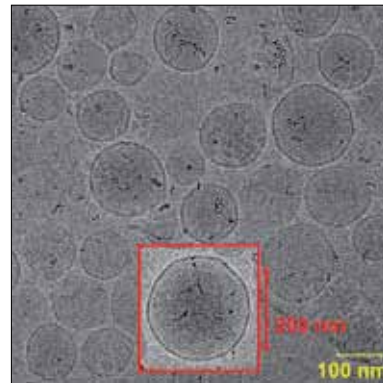


Fig. 1. A schematic diagram of drug (Fluorescein) and contrast agents (Magnevist and SPIO) release from the thermosensitive PEGlyted liposome under mild hyperthermia with alternating magnetic field (AMF).

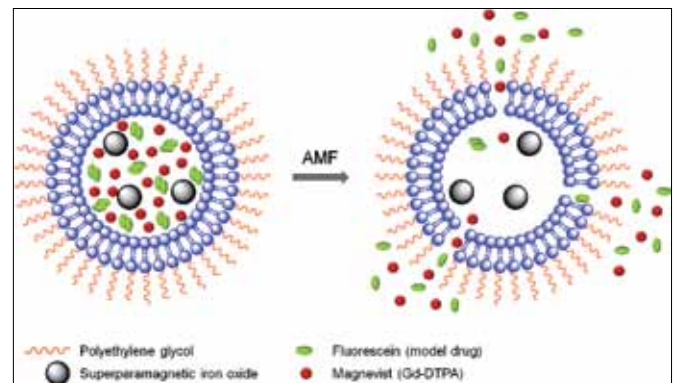


Fig. 2. CryoTEM images shows the homogeneous distribution of liposomes. The magnified one in red box clearly shows the morphology and encapsulation of SPIO inside liposome.

**Introduction:** Cancer is the second highest leading cause of death worldwide. One of the major problems to treat cancer by chemotherapy is to deliver the required high dose at tumor sites while minimizing the toxic effects on the benign tissues. Nanovehicles could provide means for such targeted drug delivery and controlled drug release. Liposome<sup>1</sup> is a promising nanovehicle because of its biocompatibility and ability to deliver drugs. It was found in clinical trials that the use of liposome reduced side effects, but did not show significant therapeutic advantage.<sup>2</sup> To alleviate this problem, liposome extravasation and bioavailability could be increased with the use of mild hyperthermia.<sup>2</sup> Besides, increasing the concentration of drug at tumor site through leaky vascular permeability, chemotherapy at a slightly higher temperature has also shown improved efficacy of drug. To complete such a drug delivery scheme, it is essential to have an efficient real-time monitoring system to observe and control the drug release at the target site. Magnetic resonance imaging (MRI) is the most powerful noninvasive tool for such monitoring purpose. In this study, we have developed a thermosensitive liposome co-encapsulating dual contrast agents<sup>3</sup> superparamagnetic iron oxide (SPIO) and Gd(III)-DTPA (Magnevist) co-loaded with fluorescein as a model drug. We have demonstrated real-time monitoring of drug release and tumor site simultaneously by observing dual MRI parameters R1 (longitudinal relaxation



rate) and R2 (transverse relaxation rate) under mild hyperthermia with alternating magnetic field (AMF) (Fig. 1). The use of AMF to create mild hyperthermia has various advantages like, higher penetration capability, particularly for blood brain barrier<sup>4</sup>, over other approaches like high intensity focused ultrasound. R1 is sensitive to the amount of drug release and R2 monitors the position and condition of the tumor site. So, this novel theranostic approach will simultaneously serve all the three important purposes 1) on demand drug release using AMF controlled mild hyperthermia 2) monitoring the position of tumor throughout the measurement 3) real-time monitoring of drug release.

**Experiments and Results:** Thermosensitive liposome was synthesized by thin layer evaporation method with transition temperature<sup>3</sup> (Tm) 41.4 °C and co-loaded with Magnevist, dextran coated SPIO and fluorescein (model drug). The surface was further engineered with polyethelene glycol (PEG) for prolonged circulation and passive targeting. The homogeneous distribution and SPIO encapsulation are shown in cryogenic transmission electron microscopy (cryoTEM) images (Fig. 2). Dynamic light scattering (DLS) measurements gave an effective diameter of 231 nm with polydispersity index 0.134.

The drug release profile was monitored by using the self-quenching property of fluorescein. The fluorescence yield is quenched when fluorescein is inside the liposome at a high concentration compared to when it is outside the liposome with a lower concentration. This property was used to quantify the percentage of fluorescein (which has similar size as cancer drug molecules) release due to the leakage through the liposome membrane under AMF heating. The liposome sample (2 ml) was exposed to an AMF with a five-turn copper coil (5 cm height and diameter) at a power of 5 kW and a frequency of 370 kHz and the fluorescence yield of the fluorescein was measured every 15 minutes. The characteristic drug release pattern is displayed in Fig. 3. It shows the drug release rate is fastest between 30 and 45 minutes and saturates after 1 hour, indicating complete release. The bulk temperature was around  $37 \pm 2$  °C during the procedure. All fluorescence measurements were done at room temperature and a control liposome sample was measured before and after the treatment procedure to ensure, there is no leakage otherwise. Finally, the sample was completely lysed with 10% Triton X-100. The percentage drug release was calculated according to the equation:

$$\frac{\text{Fluorescence of treated sample} - \text{Fluorescence before treatment}}{\text{Fluorescence after lysing with Triton} - \text{Fluorescence of Triton background}} \times 100\%$$

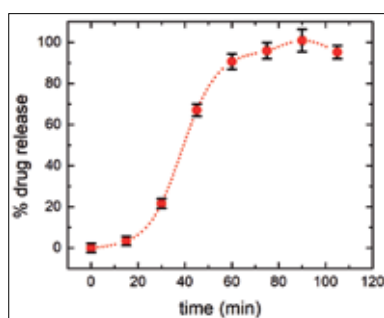


Fig. 3. Drug release profile as a function of heating time.

The samples with different drug release were collected and NMR study was done using a 600MHz (AV600, Bruker) NMR spectrometer with saturation recovery pulse sequence for R1 measurements and CPMG pulse sequence for R2 measurements.

The amounts of gadolinium (Gd) and iron (Fe) were quantified using inductively coupled plasma optical emission spectrometry (ICP-OES). Background relaxation rates were measured from the supernatant obtained after precipitating all the liposomes by centrifugation (20,000 g). Supernatant was checked with TEM and DLS to ensure it is free from liposomes. Background correction was done on the relaxation rate and the corrected relaxation rates were determined for each sample. All the measurements were done in phosphate buffer saline (PBS 100 mM), pH=7.4 and at room temperature.

In Fig. 4, we find that the percentage change of R1 increases linearly with the percentage change of drug release and the increase of R1 (red curve) saturates with complete drug release. After complete

drug release, R1 increases significantly by 69.8% due to the release of Magnevist, having higher relaxation rate outside the liposome. It implies that the local heating increases the permeability of liposome membrane, thus allowing leakage of both the Magnevist and the drug. Hence, R1 increases linearly with the drug release. On the other hand, R2 remains essentially constant, independent of drug release, because SPIO is not leaking through the liposome membrane<sup>3</sup> under this mild condition and hence R2 monitors the position and condition of the tumor site. In Fig. 5, we show plots of R1 versus Gd concentration and R2 versus Fe concentration both before (black curves) and after (red curves) AMF heating. It is clear from the plots that AMF heating significantly affects R1 versus Gd concentration curve and increases relaxivity (r1) of AMF treated sample by ~70%, whereas the heating has almost no effect on R2 versus Fe concentration curve, thus confirming our earlier conclusion.

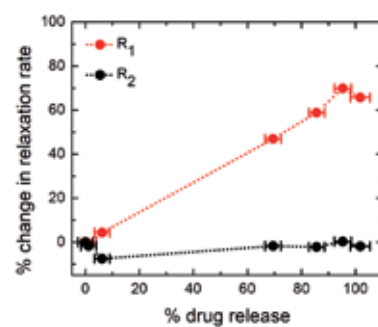
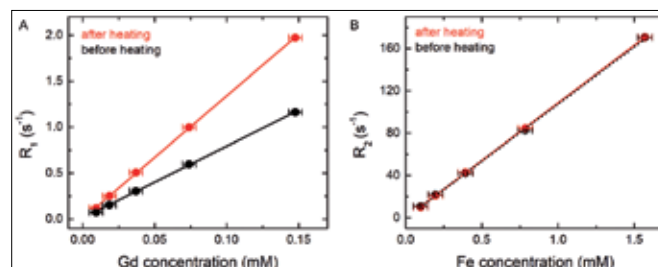


Fig. 4. Correlation between changes in relaxation rates with % drug release, where R1 (red) changes linearly with drug release, R2 (black) held constant.

Relaxation rates were measured at different dilutions before and after complete magnetic heating. R1 versus Gd concentration

curve both before and after complete AMF heating shown in left panel (panel A). It shows the slope of the fitted linear plot after heating (red)  $r1=13.38 \text{ s}^{-1} \text{ mM}^{-1} (\text{Gd})$  and before heating (black)  $r1= 7.84 \text{ s}^{-1} \text{ mM}^{-1}(\text{Gd})$ . R2 versus Fe concentration curve both before and after complete AMF heating shown in right panel (panel B). It shows the slope of the fitted linear plot after heating (red)  $r2=108.93 \text{ s}^{-1} \text{ mM}^{-1} (\text{Fe})$  and before heating (black)  $r2= 108.01 \text{ s}^{-1} \text{ mM}^{-1}(\text{Fe})$ . (The uncertainties on R1 and R2 are <1%, whereas the uncertainties on the concentration are ~5%.)



**Conclusion:** We have demonstrated a novel theranostic approach to deliver drug while simultaneously monitoring both the drug release and condition of the tumor site in real-time. A model drug fluorescein was used in the experiment and the drug release can be easily seen from the fluorescence yield. AMF heating was used for the drug release and these radio waves are highly penetrating and could be used deep inside the body. We have found very significant increase of MRI relaxation parameter R1 with the drug release, whereas, R2 parameter remained essentially constant monitoring the position and condition of the tumor site. Further works are needed to validate this hypothesis at *in vitro*, *in vivo* and clinical settings. We have used all clinically approved materials in this experiment. Thus, this method could be implemented for clinical trials and has the potential to get readily adapted with any existing targeting approach.

**Acknowledgements:** We gratefully acknowledge Mr. Ivo Atanasov, Dr. Peng Ge for technical support and Prof. William Gelbart, Prof. Charles M. Knobler, Prof. Neil K. Garg, Prof. Andre Nel for lab facility. This work was supported by NSF grant CHE-1112574 and CHE-1416598, University of California Cancer Research Award (CRR-13-201412), and the Hirshberg Foundation for Pancreatic Cancer Research.



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## NANOMEDICAL TECHNOLOGIES AND APPLICATIONS – SHOWCASES IN IMAGING/DIAGNOSTICS, THERAPEUTICS AND REGENERATIVE MEDICINE

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Complex illnesses like cancer, cardiovascular diseases, multiple sclerosis, Alzheimer's and Parkinson's disease as well as different kinds of serious inflammatory or infectious diseases (e.g., HIV) have a tremendous negative impact, not only on the patient himself but also on the whole society and linked social and insurance systems. These diseases have been key topics in the broad field of biomedical research in the past decades on a global level, and large amounts of investigations and studies have been carried out. However, appropriate diagnosis, treatments as well as preventive methods still pose one of the biggest challenges that society faces. Moreover, medical treatments targeted to the specific individual needs of a patient based on his/her own genetic, biomarker, phenotypic, or psychosocial characteristics becomes more and more important <sup>[1]</sup>.

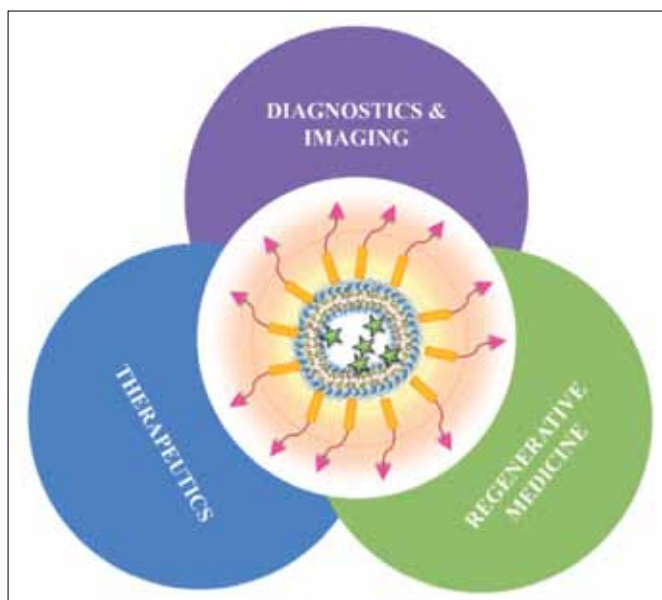


Figure 1. Nanomedical Technologies and Applications facilitate new methods and multidisciplinary approaches in Imaging/Diagnostics, Therapeutics and Regenerative Medicine.

Advanced nano- and biomaterials for medical applications that facilitate new methods and multidisciplinary approaches promise to tackle these challenges and to further enable precision medicine <sup>[2]</sup>.

As one of the most promising Key Enabling Technologies, nanotechnology facilitates major breakthroughs in different application sec-

tors. Nanomedicine, the application of nanotechnology to health, raises high expectations for millions of patients for better, more efficient and affordable healthcare, and promises new solutions to improve medical treatments. Moreover, fundamental research in nanomedicine allows a better understanding of biological processes in the human body at molecular and nanometric level. Several areas of medical care are already benefiting from the advantages that nanotechnology can offer <sup>[3]</sup>.

The BioNanoNet Association's Nanomedicine Expertise Compendium compiles expertise of different research groups in the field of (i) Diagnostics & Imaging, (ii) Therapeutics and (iii) Regenerative Medicine <sup>[4]</sup>.

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## ULTRASONICALLY DEPOSITED NANOPARTICLE COATINGS ON POLYMERIC BIOMATERIALS FOR MEDICAL APPLICATION

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**Keywords:** nanohydroxyapatite, ultrasonic coating, surface modification

**Introduction.** The fundamental requirement of a biomaterial is that the material and the surrounding physiological environment should coexist without having any undesirable effect on one another. Surface is the interface where the biomaterials meet and interact with the biological environment (i.e., bone, soft tissue, blood), the surface properties are the major factors that ultimately determine the rejection or acceptance of a biomaterial in the body. Currently used polymeric resorbable and non-resorbable materials possess many structural, mechanical and bio-functional limitations <sup>[1]</sup>. Nevertheless, surface treatment of biomaterials is possible and offers the ability to improve material and biological responses through changes in a material's surface chemistry, topography, energy, and charge, while still maintaining the bulk properties of the biomaterials <sup>[2]</sup>. Ultrasonic coating method of various materials dedicated for implantology patented by authors of this study can induce osteoinductive properties of material by changing surface wettability and limit bacteria adhesion typical for most of hydrophobic surfaces. This type of surface modification is based on cavitation phenomenon occurring in liquid medium under ultrasonic wave of high frequency <sup>[3]</sup>. Cavitation is a process of formation and violent collapse

of steam voids. Particles present in vicinity of the collapsing bubble jet are naturally speed up to the coated surface direction, which result in uniform coverage of the surface with nanoparticles layer of nanometric thickness.

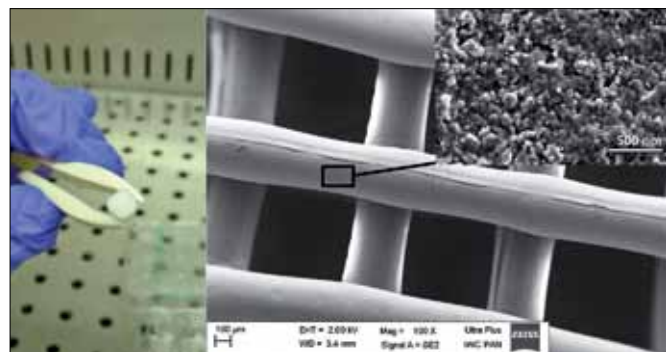


Figure 1. Optical and Scanning Electron Microscopy image of the nHA coated polymeric structure

**Materials and Methods.** Ultrasonic coating method of nanohydroxyapatite particles was performed in aqueous solutions according to the patent [3]. We used highly biocompatible hydroxyapatite ceramic nanoparticles of 10-50 nm size for implant coating in present study. It was proved by authors that application of ceramic nanoparticles of high specific area could significantly increase water contact angle and cellular activity on polymeric scaffolds as well as new tissue formation when implanted *in vivo* [3,4]. Results and Discussion. Imaging of the materials before and after coating procedure revealed significant differences. As a result of ultrasonic wave induced nearby the textile substrate homogenous nanohydroxyapatite coatings of 200-500 nm thicknesses were obtained on polymeric structures (Fig.1). Scanning Electron Microscopy imaging revealed that nHA particles cover materials but do not interfere with the structure or porosity of the base material. The porous structures are maintained unchanged and wettability of the surface was highly increased.

**Conclusions.** The novel method of nHA deposition on the polymeric structures can serve as an alternative for currently known tissue engineering implants modifications. It can also enhance the bone regeneration process in bone/implant interface region. The method of ultrasonic coating has been proved to be suitable for coating highly porous, thermally and mechanically sensitive polymeric fibres. Method can be used also for deposition of antibacterial nanoparticles. Thus, the obtained materials show a great potential as implantable scaffolds for tissue engineering applications.

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## DEVELOPMENT OF TRANSFERRIN-BEARING VESICLES ENTRAPPING PLUMBAGIN FOR CANCER THERAPY

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Plumbagin is a naphthoquinone mainly found in three families of plants, Plumbaginaceae, Droseraceae, and Ebenaceae. It has a wide spectrum of pharmacological properties, both *in vitro* and *in vivo*, including anti-tumour effect. Several studies reported that plumbagin played significant roles in inhibiting tumour growth and metastasis by reducing angiogenesis and mediating cellular apoptosis and autophagy. However, plumbagin has some limitations which significantly hampered its clinical translation, such as toxicity, low oral bioavailability, lack of stability and sensitivity to high temperatures. Moreover, this compound failed to specifically reach tumour at a therapeutic concentration due to its lack of tumour specificity and short biological half-life, with rapid elimination *in vivo*.

To overcome these limitations, we hypothesize that loading of plumbagin in a tumour-targeted delivery system would increase the specific delivery of the drug to cancer cells. The aim of this project was to prepare and characterize tumour-targeted delivery systems entrapping plumbagin.

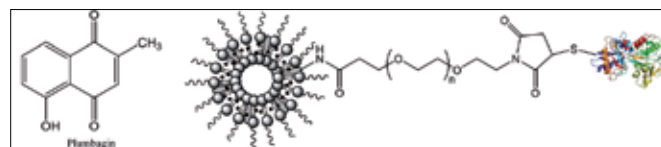


Figure 1. Chemical structure and graphical of plumbagin-entrapping vesicles conjugated to transferrin via thioether linkage

In this work, we developed transferrin-bearing vesicles entrapping plumbagin composed of HPC, DSPE-PEG2K, cholesterol, cholesterol-PEG5K-maleimide and plumbagin at molar ratios of 53: 5: 29: 0.4: 8 (Figure 1). Vesicles entrapping plumbagin were successfully prepared by probe sonication and were conjugated with transferrin using the thiol-maleimide 'click' reaction, as confirmed by TEM imaging. The transferrin-bearing and control vesicles exhibited high percentage of drug entrapment (about 80%) with transferrin conjugated to the vesicles at a level of 50% of the initial transferrin added. As expected, the conjugation of transferrin to the surface of vesicles increased their size and decrease their zeta potential compared to that observed for control vesicles ( $113.5 \pm 2.3$  nm and  $-18.4 \pm 0.4$  mV for transferrin-bearing vesicles, and  $106.0 \pm 1.5$  nm and  $-17.2 \pm 0.1$  mV for control vesicles). Both vesicles allowed a sustained release of plumbagin, with the drug release from transferrin-bearing vesicles being slightly slower than for the control vesicles ( $87.5 \pm 2.0\%$  and  $95.5 \pm 2.0\%$  at pH 7.4 over 12 hours, respectively). Both transferrin-bearing and control vesicles were stable under storage condition at 4°C for 4 weeks, with the transferrin-bearing vesicles being more stable than control vesicles. There was no significant change in mean diameter size and zeta potential of transferrin-bearing vesicles, unlike control vesicles, that displayed a significant increase of mean diameter size and decrease in zeta potential. The drug entrapment decreased for both vesicles (from  $79.1 \pm 0.4\%$  to  $75.0 \pm 0.9\%$  for transferrin-bearing vesicles and  $78.5 \pm 0.4\%$  to  $73.6 \pm 0.9\%$  for control vesicles).

*In vitro*, the cellular uptake of plumbagin was significantly increased compared to that of control vesicles in all tested cell lines, respectively by 1.6-fold in B16-F10 cells, 1.5-fold in A431 cells and 1.4-fold in T98G cells. This result was also qualitatively confirmed by confocal microscopy (Figure 2). The anti-proliferative efficacy of plumbagin was significantly improved when formulated in vesicles (Table 1). The conjugation of transferrin to the vesicles further improved plumbagin efficacy, by 1.6-fold for B16-F10 and A431 cells, and at least 1.2-fold for T98G cells, compared to that of plumbagin solu-

tion. This result correlates well with the increased cellular apoptosis resulting from the treatment. The transferrin-bearing vesicles entrapping plumbagin (at 1  $\mu\text{g}/\text{mL}$  or 5.3  $\mu\text{M}$ ) led to higher cellular apoptosis effect in all three cell lines compared to control vesicles and free drug solution (with total apoptotic cells of  $88.4 \pm 0.4\%$  for B16-F10,  $43.3 \pm 3.5\%$  for A431 and  $24.9 \pm 0.8\%$  for T98G). Plumbagin solution, however, did not show any apoptosis effect on A431 and T98G cells, and only exerted a limited effect in B16-F10 cells.

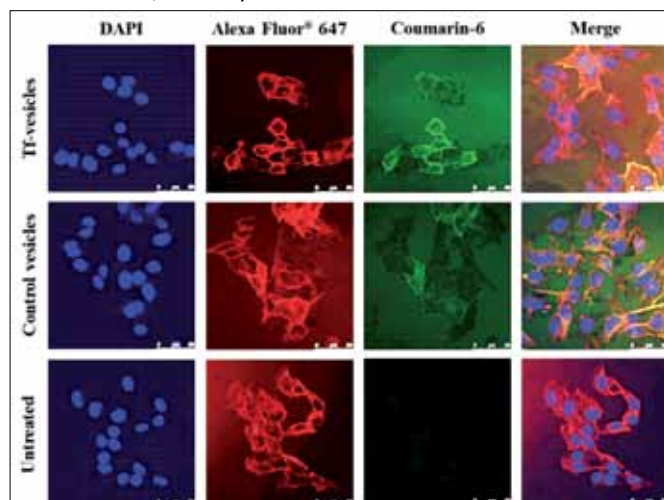


Figure 2. Confocal microscope images showing cellular uptake of transferrin-bearing and control vesicles in B16-F10 cells

Table 1. Anti-proliferative efficacy of plumbagin either entrapped in Tf-bearing vesicles, control vesicles or free in solution at 24h and 72h in B16-F10, A431 and T98G cells ( $n=15$ ).

Cell lines	$IC_{50}$ ( $\mu\text{g}/\text{mL}$ ) (mean $\pm$ S.E.M.)					
	Tf-bearing vesicles		Control vesicles		Plumbagin solution	
	24 h	72 h	24 h	72 h	24 h	72 h
B16-F10	$0.22 \pm 0.02$	$0.08 \pm 0.01$	$0.32 \pm 0.03$	$0.08 \pm 0.01$	$0.51 \pm 0.02$	$0.12 \pm 0.01$
A431	$0.41 \pm 0.02$	$0.25 \pm 0.00$	$0.64 \pm 0.03$	$0.27 \pm 0.02$	$1.78 \pm 0.20$	$0.39 \pm 0.02$
T98G	$1.48 \pm 0.27$	$0.87 \pm 0.04$	$2.05 \pm 0.35$	$0.90 \pm 0.10$	$6.19 \pm 0.20$	$1.09 \pm 0.13$

These results are promising and support the optimization of this delivery system to further improve plumbagin potential for cancer treatment.

## PH-SENSITIVE PU-BASED NANOCONTAINERS FOR ORAL DRUG DELIVERY

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Oral administration is the most common and convenient route of drug administration.<sup>1</sup> However, poor aqueous solubility of numerous drugs and their high sensitivity to the harsh conditions in the gastrointestinal (GI) tract limit their absorption and bioavailability.<sup>2</sup> In order to overcome these shortcomings plenty of (bio)polymer architectures have been developed for efficient increase of drug solubility and some of them are already marketed as drug delivery systems<sup>3</sup>

An important prerequisite of polymeric delivery systems, especially for oral drug administration is their stability at extreme pH conditions of the GI tract (gastric pH <2, neutral and basic pHs, 7.5-8 in the intestinal tract).<sup>1</sup> Owing to their long-term hydrolytic stability polyurethanes (PU) have been used for decades in the medical devices, i.e. implants, stents etc.<sup>4</sup> However, extensive studies have shown that the carbamate bonds are susceptible of (bio)degradation, especially in poly(ester urethanes), but also in poly(ether urethanes) that incorporate in their backbones water soluble building blocks, i.e. poly(ethylene oxide), PEO. Furthermore Ikeda et al.<sup>5</sup> demonstrated that the longer the PEO backbone content, the higher swelling and degradability of the poly(ether urethanes) might occur.

Here we report the synthesis and the characterization of hydrophobic PU-backbones incorporating ATRP-initiators sites and/or dimethylol propionic acid (DMPA) as chain extenders. Preliminary gel permeation chromatography (GPC) investigations showed increased hydrolytic stability of the PU backbones at extreme pH conditions (1, 5, 7, 8) and several days contact time, comparatively to the PU counterparts containing PEO in the main chain. The ATRP-initiating sites were used to further functionalize PU-backbone with several methacrylates as masked carboxylic groups that facilitate pH selective solubility, poor solubility at low pH and increased solubility at neutral to basic pH values. This pH-dependent water solubility is especially convenient for the drug protection under acidic conditions and pH-triggered drug release in the intestinal tract, where the pH values increase up to  $\sim 8.0$ . The PU-binding capacity to water insoluble molecules was investigated by UV-Vis measurements using sudan III and fenofibrat as model guest molecules that possess low intrinsic aqueous solubility and pH-constant solubility, i.e.  $7.4 \cdot 10^{-6}$  g/L and  $1.1 \cdot 10^{-3}$  g/L respectively).

The solubility of sudan III in phosphate buffer (pH 8) could be significantly increased in presence of PU polymers (see picture), whereas in control experiments at acidic pHs values or in absence of PU, sudan III remained undissolved in the aqueous solutions. Further scale up and pre-clinical experiments are underway.



Increased water solubility of sudan III in presence of PU, at pH 8.

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## DEVELOPMENT OF A LAB SCALE PROCESS TO SUPPORT CLINICAL MANUFACTURES OF POLYMERIC NANOPARTICLES

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## INTRODUCTION:

Drug delivery systems play a vital role in getting drug molecules to their targets in a controlled manner. Polymeric nanoparticles (nanoparticles) are one such system that enable the delivery of active molecules with improved safety and efficacy<sup>1</sup>.

In order to achieve the desired delivery attributes, the properties of the nanoparticle, such as particle size, shape, surface properties, drug loading and release rate need to be controlled. Using an emulsion process to manufacture the nanoparticles lends itself to a complex manufacturing process with a significant control challenge. Variability in raw materials, environment, and manufacturing conditions can have significant effects on the quality attributes of nanoparticle drug products. To this extent, it is important to have a lab scale manufacturing process that is representative of the clinical and commercial manufacturing process.



## METHODS:

Process understanding was obtained during early development, and through establishment of a manufacturing process at the clinical scale. This learning has been utilised to establish a lab scale manufacturing process that is capable of representing the clinical manufacturing process at one 600th scale. After each lab scale manufacture, characterization of the nanoparticles allowed for iterative improvements to be made on the manufacturing equipment and process.

## RESULTS:

The lab scale process is capable of producing nanoparticles that are representative of those manufactured at the clinical scale. The critical quality attributes, including size, and release rate, of the nanoparticles at the lab scale match those of the clinical scale. This enables small scale development manufactures to be performed to set process parameters ahead of full scale clinical manufactures, reducing the development cost, materials required, and time of plant.

## CONCLUSIONS:

Comparability between the lab scale and the clinical and commercial scale equipment has been demonstrated. As a result of this work, it is possible to define clinical and commercial scale manufacturing process parameters based on lab scale development for variable raw materials quality attributes. This reduces the time required on plant, usage of raw materials and API, and reduces the cost of manufactures.

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## LEARNING OBJECTIVES:

Explain the strategies for dealing with variability in starting materials.

Discuss the scale-up considerations for polymeric nanoparticle manufacturing processes.

Demonstrate the scalability of polymeric nanoparticle manufacturing processes.

## MONOCYTE DERIVED EXOSOME-LIKE VESICLES AS A PROMISING DRUG DELIVERY SYSTEMS

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**Introduction:** Extracellular vesicles are increasingly seen as possible alternatives to liposomes, as delivery carriers, since they could deliver their cargo across the plasma membrane, and delay premature drug transformation and elimination. Due to their nano- size, exosomes have shown a great potential, but their production and natural biodistribution can be a challenge in the development of drug delivery system<sup>1</sup>. Therefore, a new approach has been proposed to produce exosome-like vesicles (ELVs) by extruding monoblastic cells through different pore size membranes in order to increase the yield and generate exosome-like vesicles with retained cellular proteins<sup>2,3</sup>.

**Material and Methods:** In the present study, we have extruded U937 monoblastic cells to produce ELVs and purified them from free proteins using size exclusion chromatography. The engineered vesicles were characterized using dynamic light scattering (DLS), Nanoparticle Tracking Analysis (NTA), biconchonic acid (BCA) assay, Western blotting and Transmission electron microscopy (TEM). To assess their capability as drug delivery systems, ELVs were loaded with doxorubicin hydrochloride (DOX) using three different

methods: extrusion, mixing and remote loading. Antitumor activity of free DOX and DOX-loaded ELVs was assessed *in vitro* on the prostate cancer cell lines.

**Results:** ELVs extruded from the U937 cells showed an average diameter of 150–200nm as confirmed by DLS and NTA. Subsequently, ELVs showed the retention of the cellular membrane proteins and exosome markers after the extrusion, which was confirmed by Western blot. Encapsulation efficiency of DOX was the highest using the extrusion loading method, which was further used for production of DOX-loaded ELVs for *in vitro* cytotoxicity studies.

**Conclusion:** ELVs showed high similarity to naturally secreted exosomes, with good colloidal stability and drug loading capacity. Anticancer activity in prostate cancer cell lines showed promising results which indicate that ELVs could be used as a new nano-sized drug delivery system.

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## TUMOR PENETRATION PROMOTING POLYMERIC MICELLES STABILIZED BY $\pi$ - $\pi$ STACKING

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**Introduction:** Nanomedicines have been developed to promote drug accumulation in tumors. The actual clinical benefit of nanomedicines is however marginal due to the low targeting efficiency of many nanocarriers. Furthermore, the tumor penetration of nanomedicines remains modest because of inappropriate physicochemical properties (e.g., large size) of nanocarriers and dense tumor extracellular matrix<sup>1</sup>. We designed polymeric micelles (PM) with enhanced stability and drug retention promoted by  $\pi$ - $\pi$  stacking, which occurs between aromatic pendant groups of polymers and payloads. The PM showed long circulation and improved tumor targeting<sup>2</sup>. To further improve nanomedicine therapy, we engineered PM to enhance tumor penetration by optimizing the particle size and co-loading of penetration promoting agents.

**Methods:** Amphiphilic polymers were synthesized via free radical or reversible addition-fragmentation chain transfer polymerization. To prepare PM with optimal size for tumor penetration, polymers with different hydrophobic chain length were synthesized. PM were prepared by the nano-precipitation method, in which the hydrophobic chemotherapeutic drug paclitaxel and a tumor penetration enhancer losartan was loaded. The drug-loaded PM were intravenously injected in tumor-bearing mice, and the circulation kinetics and biodistribution of the carrier and the payload were characterized by HPLC and hybrid computer tomography-fluorescence molecular tomography (CT-FMT). The therapeutic efficacy of the drug loaded PM was studied in multiple tumor models.

**Results:** The PM prepared from polymers with aromatic pendant groups demonstrated excellent loading capacity for paclitaxel (~25 wt%) and sustained drug release, which was ascribed by  $\pi$ - $\pi$  stacking interaction. The PM showed significantly prolonged circulation time in mice and efficient tumor targeting. For paclitaxel loaded micelles, the half-life of the drug in the blood circulation was increased to around 8 hours, which resulted in >20 times higher tumor accumulation comparing to the free drug. This formulation induced complete tumor regression in multiple human tumor models (A431 and MDA-MB-468) established in mice and also showed sufficient tolerability after repeated injections. Furthermore, we observed that by varying the molecular weight of polymers, the size of PM was tuned between 30 and 80 nm, and losartan (27 wt%) was efficiently entrapped in the  $\pi$ - $\pi$  stacking stabilized PM.

**Conclusions:** We show that efficient tumor targeting was achieved by the PM. Furthermore, tumor penetration promoting PM with tunable size and loading of losartan were formulated, which will be tested in follow-up studies. Overall, these efforts together will pave the way towards translation of the  $\pi$ - $\pi$  stacking stabilized PM into clinical applications.

**Acknowledgements:** The author gratefully acknowledges financial support by the European Research Council (ERC), the German Research Foundation (DFG), and the China Scholarship Council (CSC).

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paclitaxel-loaded PM showed acceptable tolerability after repeated injection (h). PM with fluorescent labeling were used for assessments of tumor penetration and distribution (I and J, green: PM; grey: collagen; red: blood vessels), which efficiently extravasated into the tumor interstitium (k).

## THE ZEBRAFISH: A PRECLINICAL SCREENING MODEL FOR THE OPTIMIZATION OF NANOMEDICINE FORMULATIONS

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Nanomedicines have gained much attention for the delivery of small molecules or nucleic acids as treatment options for many diseases. However, the preclinical development of novel nanomedicines is a very cumbersome and often unsuccessful process. Among other things, this is due to the fact that formulation design and optimization is mainly based on *in vitro* studies which are not able to fully mimic complex biological conditions. Moreover, only a selected number of formulations can subsequently be assessed in rodent *in vivo* experiments, since such studies are expensive, time consuming and require regulatory approval at an experimental level. Obviously, there is a huge gap between *in vitro* cell culture and rodent *in vivo* studies. This does not allow a thorough formulation design and optimization under realistic biological conditions and hampers a detailed understanding of nanomedicine interactions with biological environments at a macromolecular level.

In order to overcome these limitations, zebrafish embryos were introduced as an early and easy accessible screening model during the development of nanomedicine formulations. Main nanomedicine clearance mechanisms (i.e. macrophages, hepatic scavenger receptors) were identified and validated in the zebrafish model which allowed the characterization of various nanoparticles regarding their pharmacokinetic behavior, biodistribution, and functionality under *in vivo* conditions. Selected findings of zebrafish studies have been verified in rodent *in vivo* studies in order to check the model's predictive power. Importantly, experimental data obtained in zebrafish and rodents were consistent.

Our findings highlight that the zebrafish model is a useful vertebrate screening tool to predict the *in vivo* performance of nanoparticulate drug delivery systems. First, this facilitates the selection of potentially successful formulations prior to subsequent rodent *in vivo* studies. Second, transparent zebrafish embryos allow the investigation of nano bio interactions at a macromolecular level. Altogether, this will broaden our understanding of basic nanomedicine formulation effects resulting in an increased translation of novel nanomedicines from bench to bedside.

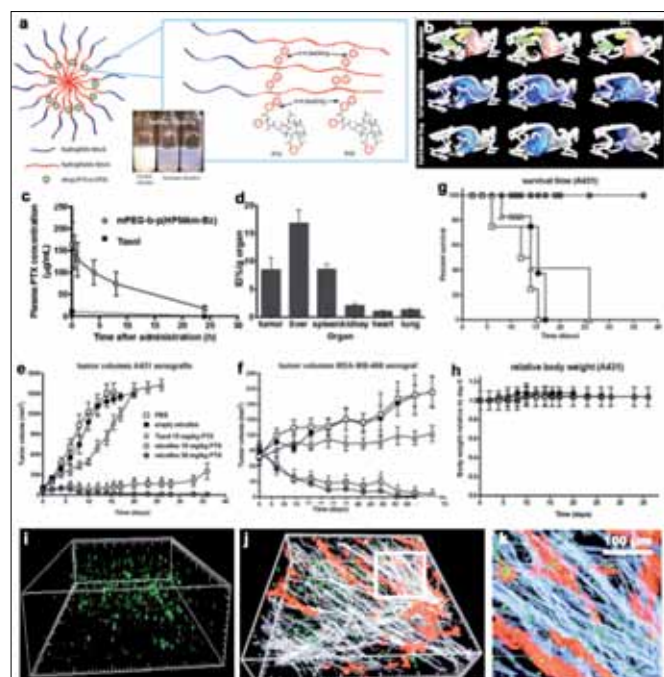


Figure 1: Polymeric micelles for improving tumor targeting and penetration. Schematic illustration of  $\pi$ - $\pi$  stacking stabilized PM (a), which showed efficient tumor targeting of the carrier (b) and significantly improved pharmacokinetics of paclitaxel in mice (c), resulting in efficient drug tumor targeting (d). The paclitaxel-loaded PM induced complete regression of both A431 (e) and MDA-Mb-468 (f) with substantially prolonged survival (g). The empty and

## THE ZEBRAFISH: A PRECLINICAL SCREENING MODEL FOR THE OPTIMIZATION OF NANO-MEDICINE FORMULATIONS

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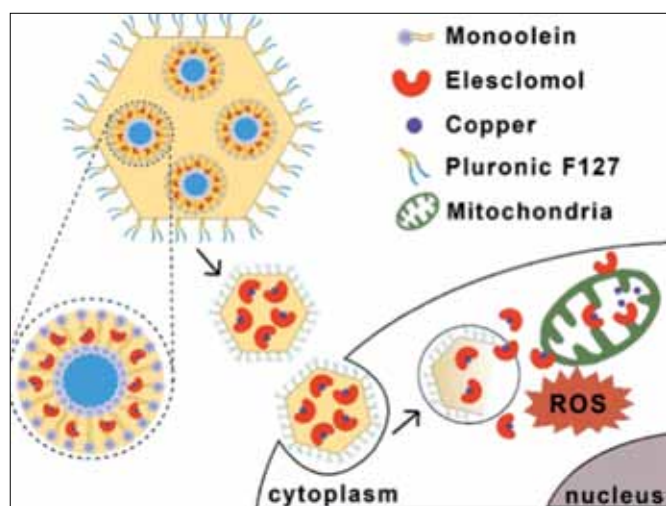
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Mitochondria have a major role in the energy metabolism and several other essential biological functions in the cell. Importantly, mitochondria have been proposed as a prime target for cancer therapy<sup>1</sup>. Tumor cells present aberrant mitochondria metabolism and exhibit altered redox status with increased reactive oxygen species (ROS) levels. Thus, more recent cancer therapeutic strategies include drugs that modulate ROS production<sup>2</sup>. Elesclomol (ELC), also known as STA-4783, is a promising anticancer drug that has been under clinical trials<sup>3</sup>. ELC chelates Cu(II) in the plasma, resulting in a Elesclomol-Copper complex (ELC-Cu). After entering the cell, ROS are generated via the reduction of Cu(II) to Cu(I) in the mitochondria. In cancer cells, ROS levels are already higher than in normal healthy cells, thus the action of ELC will raise ROS up to unsustainable levels, which induces cellular apoptosis<sup>4,5</sup>. An important issue to overcome is ELC's poor water solubility, which is a major obstacle for its pharmaceutical use<sup>6</sup>. The encapsulation into a lipid-based nanocarrier holds the promise of facilitating its distribution inside the body, improving drug bioavailability.

In our studies, for the first time, we encapsulate ELC and ELC-Cu in promising Monoolein (MO) cubosomes for drug delivery (Fig. 1). The resulting nanocarriers are monodisperse in size and with negative surface charge, dependent on the weight ratio between MO-lipid and Pluronic F127 stabilizer.



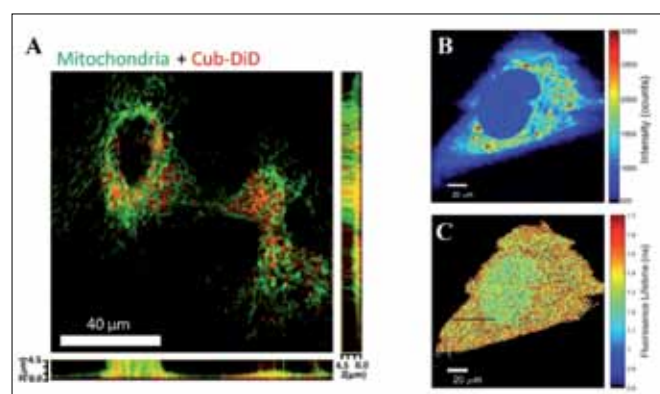
**Figure 1** Schematic illustration of Elesclomol-Copper (ELC-Cu) loaded cubosome nanocarriers and drug anticancer action. The nanocarriers are expected to be internalized by the cells with release of the drug and anticancer activity, by the generation of reactive oxygen species (ROS) in the mitochondria, resulting in cell death.

We report *in vitro* studies of such nanocarrier system using two cancer cell lines and confirm suited biocompatibility of cubosomes with lipid concentration up to 100 $\mu$ M. Cellular uptake is demonstrated via flow cytometry and accumulation close to the mitochondria via confocal microscopy with DiD-labeled cubosomes (Fig. 2A).

To monitor metabolism and ROS in cancer cells, fluorescence of endogenous NAD(P)H cofactors has been applied for label free imaging of the mitochondria<sup>7</sup>. Multi-photon Fluorescence Lifetime

Imaging Microscopy (MP-FLIM) is a valuable technique to discern NAD(P)H compounds, whose emission spectra are indistinguishable, while characteristic fluorescence lifetimes allow to distinguish free or protein bound NAD(P)H cofactors in the cell<sup>7</sup>. We use for the first time MP-FLIM to follow endogenous NAD(P)H cofactors with sub-cellular resolution on live cells exposed to anticancer drug-loaded nanocarriers (Fig. 2B and 2C). The relative presence of bound/unbound NAD(P)H lifetime contributions upon incubation with the ELC-Cu cubosomes indicates the therapeutic action. This technique is promising since it might contribute to optimize drug delivery systems and also elucidate about specific metabolic pathways and heterogeneity involved in both cancer resistance and treatment efficacy.

Cell viability *in vitro* analysis of cubosomes loaded with ELC-Cu complex show higher cytotoxicity compared to free ELC-Cu for equivalent copper concentration, demonstrating advantageous properties of the ELC-Cu nanocarrier system for drug delivery. Based on our results we propose a novel cubosome nanocarrier for the delivery of ELC-Cu complex showing anticancer activity and promising characteristics for enhanced drug bioavailability in systemic administration.



**Figure 2** Intracellular tracking and metabolic monitoring of cubosome nanocarriers. (A) Confocal fluorescence microscopy image showing close localization of DiD-labeled cubosomes (Cub-DiD) in relation to the mitochondria network inside the cells. Tracking of endogenous NAD(P)H was performed on a custom-built inverted Multi-photon Fluorescence Lifetime Imaging Microscopy (MP-FLIM) system. Representative (B) fluorescence intensity and (C) corresponding fluorescence lifetime image of a live cell.

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## SONOPORATION ENHANCES DELIVERY OF NANO-MEDICINE FOR IMPROVED CANCER TREATMENT

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Chemotherapy is limited by inadequate delivery to the tumor and severe side-effects due to accumulation in healthy tissues. Encapsulation of drugs in nanoparticles can enable a more targeted delivery, improved efficacy and reduced toxicity. However, delivery of nanoparticles is often insufficient due to various biological barriers in the tumor. Ultrasound in combination with microbubbles has emerged as a promising non-invasive and localized method to enhance delivery of nanomedicine to tumors and the brain. During ultrasound treatment, the biomechanical effects from the oscillating microbubbles enhance permeability of the vascular wall and improve extravasation and distribution of the nanoparticles in the tumor. We investigated a multifunctional drug delivery system consisting of microbubbles stabilized by polymeric nanoparticles<sup>[1,2,3]</sup> as illustrated in figure 1.

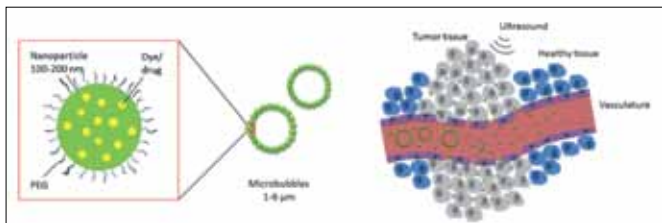


Figure 1: Schematic illustration of the nanoparticle-stabilized microbubble concept (left) and how it can be used for targeted drug delivery in combination with ultrasound (right). The poly (alkyl cyanoacrylate) (PACA) nanoparticles encapsulate drugs or fluorescent dyes and are surface coated with poly (ethylene glycol) (PEG).

The aim was to investigate if focused ultrasound combined with the novel nanoparticle-stabilized microbubbles could enhance uptake of nanoparticles in tumors, and to determine if increased tumor uptake of nanoparticles had a therapeutic benefit. The nanoparticles and microbubbles were characterized with respect to *in vitro* cellular uptake and toxicity, and *in vivo* circulation and biodistribution. Breast cancer xenografts were grown in mice, before nanoparticle-stabilized microbubbles were injected intravenously and the tumors were treated with ultrasound. Tumors were excised, and microdistribution of nanoparticles was imaged on frozen tumor sections and the total uptake was quantified. The therapeutic effect of ultrasound in combination with the nanoparticle-stabilized microbubbles encapsulating the anticancer drug cabazitaxel was investigated in a proof of concept study by measuring tumor growth. To understand more of the involved mechanisms, real-time intravital multiphoton microscopy was performed. Cancer xenografts from osteosarcoma cells were grown in dorsal window chambers in mice. Fluorescent dextrans were injected for visualization of the vasculature, before nanoparticle-stabilized microbubbles or SonoVue in combination with nanoparticles were injected. Four different ultrasound pressures were studied, and in-

travital imaging was done before, during and after sonication. The intra- and extravascular distribution and kinetics of extravasation of nanoparticles and dextrans were studied.

Ultrasound (frequency of 1 MHz, pressure of 0.5 MPa, bursts of 10 000 cycles (10 ms) every 100 ms (local pulse repetition frequency [PRF] = 10 Hz) for 0.5 sec, followed by a 1.5 sec break (global PRF = 0.5 Hz, total duty cycle = 2.5%) improved tumor uptake of nanoparticles 2.3 times, without tissue damage, resulting in enhanced therapeutic efficacy as shown in figure 2. Untreated animals showed a continuous tumor growth. Tumors treated with nanoparticle-stabilized microbubbles with cabazitaxel showed a reduced growth compared to control animals, but continued to grow 4 weeks after treatment. The tumors that received both nanoparticle-stabilized microbubbles with cabazitaxel and ultrasound, however, exhibited regression into complete remission.

The real-time observation using intravital multiphoton microscope reveals new knowledge on the temporal and spatial extravasation of nanoparticles and dextrans during ultrasound exposure, which is highly useful for optimizing such treatments. High pressures induced a violent and rapid extravasation of both nanoparticles and dextran as shown in figure 2. Using lower pressures, the extravasation was less violent and occurred in capillaries with larger diameters compared to the high pressures. The rate of extravasation and mean displacement of both nanoparticles and dextrans from vessels into the tumor tissue correlated with increasing pressure. The observed extravasation events seemed to appear randomly in time and space, and happened within seconds to minutes after starting the ultrasound exposure. Altogether, this demonstrates that ultrasound treatment improves the accumulation and distribution of nanoparticles and drugs, and that the nanoparticle-stabilized microbubble-platform is promising for targeted drug delivery applications.

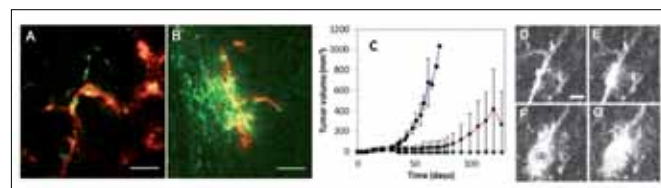


Figure 2: Confocal microscopy images of tumor vasculature (red) and nanoparticles (green) without (A) and with ultrasound (B), demonstrating increased uptake of nanoparticles after ultrasound. Scale bar is 50  $\mu$ m. Tumor growth with time after implantation of cells (C), showing growth of untreated tumors (blue), tumors treated with nanoparticle-stabilized microbubbles with drug (red), and tumors treated with ultrasound in addition to nanoparticle-stabilized microbubbles with drug (green). Treatments were done on day 21 and 29. Intravital microscopy images of extravasation of nanoparticles (white) from a vessel in a dorsal window chamber at 0 sec (D), 0.48 sec (E), 10 sec (F) and 3 min (G) after starting ultrasound treatment. Scale bar is 30  $\mu$ m.

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# PEGYLATION OF POLYPROPYLENIMINE DENDRIMERS: EFFECTS ON CYTOTOXICITY, DNA CONDENSATION, GENE DELIVERY AND EXPRESSION IN CANCER CELLS

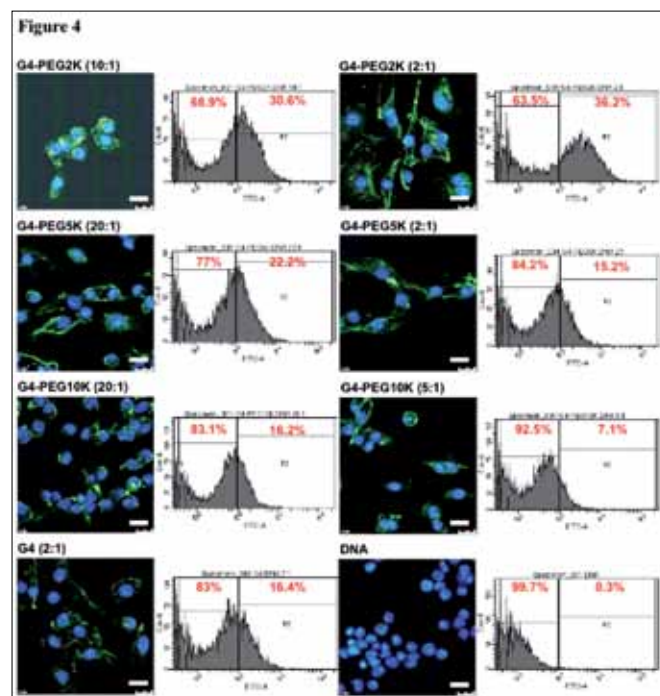
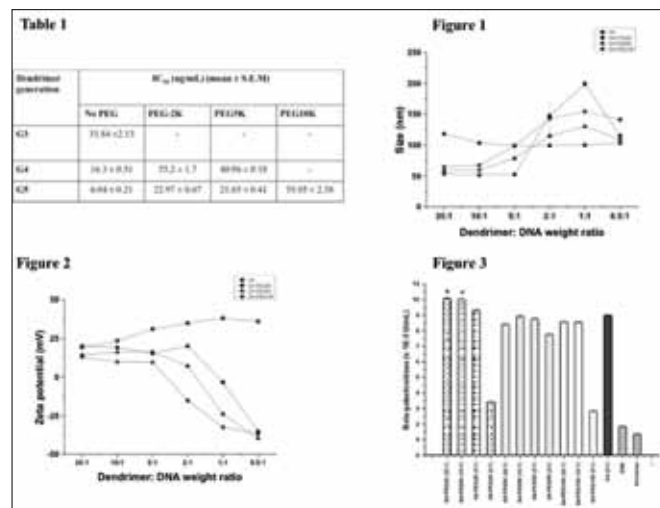
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Gene therapy has become one of the most intensively studied strategies for the treatment of various diseases, ranging from monogenic diseases such as cystic fibrosis to complex disorders such as cancer<sup>1</sup>. Despite numerous advances, the use of therapeutic genes in cancer treatment is still limited by the lack of safe and efficacious gene delivery vectors<sup>2</sup>. Diaminobutyric polypropylenimine (DAB) dendrimer has been demonstrated to be an efficient non-viral vector for targeted gene delivery to cancer<sup>3,4,5,6,7</sup> and to the brain<sup>8,9</sup>. However, DAB dendrimer shows concentration- and generation-dependent toxicity, caused by the presence of surface primary amines, which impedes their clinical development<sup>10</sup>. PEGylation, the conjugation of polyethylene glycol (PEG) to cationic polymers, is one of the most widely used strategies to shield positive charges and therefore reducing toxicity, macrophage uptake, aggregation and opsonisation, ultimately improving circulation times<sup>11</sup>. The aim of this study is therefore to investigate the influence of the conjugation of PEG with various molecular weights to DAB dendrimers with various generations, on the cytotoxicity, physicochemical properties, DNA condensation, cellular uptake and transfection efficacy of the dendriplexes.

PEGylated dendrimers were synthesized following the one-step reaction of generation 3, 4- or 5- DAB dendrimers with the amine-reactive methoxy PEG succinimidyl carboxymethyl esters (molecular weights: 2 kDa, 5 kDa and 10 kDa) (M-PEG). <sup>1</sup>H NMR confirmed the successful conjugation. The cytotoxicity of the PEGylated DAB dendrimers was assessed using a MTT assay. The conjugation of PEG with various molecular weights to DAB dendrimers of various generations led to a decrease in the cytotoxicity of the dendrimers by shielding their surface positive charges (Table 1). The ability of PEGylated DAB dendrimers to successfully complex plasmid DNA was assessed using a PicoGreen assay, following the protocol provided by the supplier. The charge-shielding effect of PEG had a substantial effect on the DNA condensation efficiency of the different generations of DAB dendrimers. Increase in the molecular weight of PEG conjugated to DAB dendrimers led to a decrease in the DNA condensation efficiency at lower dendrimer: DNA weight ratios. At higher ratios, stable dendriplex formation with DNA condensation with more than 70% DNA condensation efficiency was recorded. The size and zeta potential of the PEGylated DAB dendriplexes were measured for various dendrimer: DNA weight ratios (20:1, 10:1, 5:1, 2:1, 1:1, and 0.5:1) respectively by photon correlation spectroscopy and laser Doppler electrophoresis. PEGylated G4-DAB dendriplexes showed a lower size compared to the non-PEGylated dendriplexes only at high dendrimer: DNA weight ratios (20:1 and 10:1). The conjugation of PEG chains imparted flexibility to the dendrimers, leading to the formation of more compact dendriplexes at high dendrimer: DNA weight ratios (Figure 1). At low dendrimer: DNA weight ratios (from 2:1 and below), all PEGylated dendriplexes showed an increased size due to the weaker interactions between PEGylated DAB dendrimers and anionic plasmid DNA. PEGylation of DAB dendrimers resulted in a decrease in the zeta potential of all dendriplexes at all the tested dendrimer: DNA weight ratios. The decrease in the zeta potential was directly proportional to an increase in the molecular weight of PEG (Figure 2)

The transfection efficiency of the DNA complexed by PEGylated DAB dendrimers on various cancer cell lines was assessed using a plasmid DNA encoding  $\beta$ -galactosidase. The PEGylation of DAB dendrimers led to an increase in transfection for some specific generation-dependent dendrimers, PEG molecular weight, dendrimer: DNA weight ratio, and depending on the cell line (Figure 3). To de-

termine if there was a correlation between levels of transfection and cellular uptake of the dendriplexes, we treated B16F10-Luc cells with various dendriplexes that showed high levels and low levels of transfection and determined



the cellular uptake qualitatively using confocal microscopy and quantitatively using flow cytometry. No such correlation was observed, as some PEGylated dendriplexes that showed higher transfection efficiency had lower cellular uptake and dendriplexes that showed lower transfection efficiency had higher cellular uptake (Figure 4). Various rate-limiting steps such as cellular uptake, endosomal escape and nuclear entry hindered dendriplex-mediated transfection. These rate-limiting steps are controlled by a complex interplay between the DNA condensation efficiency, size, zeta potential and surface characteristics of the dendriplexes, which ultimately depend on the generation of dendrimer and the PEG molecular weight. In this study, we reported significantly higher transfection levels by PEGylated dendrimers at higher dendrimer: DNA weight ratios (20:1 and 10:1) compared to unmodified dendrimers. The conjugation of PEG to the DAB dendrimers imparts higher flexibility and steric stabilization of the complexes. In addition, PEGylation creates charge separation between the positively charged dendrimers and negatively charged DNA, which aids the cytoplasmic release of plasmid DNA from the dendriplex, thus facilitating nuclear entry. However, the correlation between cellular uptake and transfection is complicated, cell line-dependent and governed mainly by internal cellular machinery, more than the extracellular factors.

In this study, we have investigated *in vitro* a series of PEGylated DAB dendrimers, with various PEG molecular weight and dendrimer generations, for potential use as gene delivery systems. More details about this study could be found in the following publication: PEGylation of polypropylenimine dendrimers: effects on cytotoxicity, DNA condensation, gene delivery and expression in cancer cells. Somani S, Laskar P, Altwaijry N, Kewcharoenpong P, Irving C, Robb G, Pickard BS, Dufès C. *Sci Rep.* 2018 Jun 20;8(1):9410

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## EFFECT OF VASCULATURE AND EXTRACELLULAR MATRIX ON NANOPARTICLE ACCUMULATION IN TUMOR XENOGRAFT MODELS

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Preclinical research has shown that nanoparticles and macromolecules can accumulate in solid tumors due to the enhanced permeability and retention effect and give therapeutic efficacy superior to the standard formulation of the drug. However, not all tumor models respond equally well to nanomedicines, and clinical trials with nanomedicines typically fail to show improved efficacy across

an unstratified patient group. Further understanding of barriers to delivery of nanoparticles and tools that can be used to pre-select patients for nanomedicines would be valuable. In this study we aimed to see whether various imaging modalities of the tumors could be used to predict nanoparticle accumulation, and subsequently how solid stress and the extracellular matrix affected perfusion and nanoparticle delivery (figure 1).

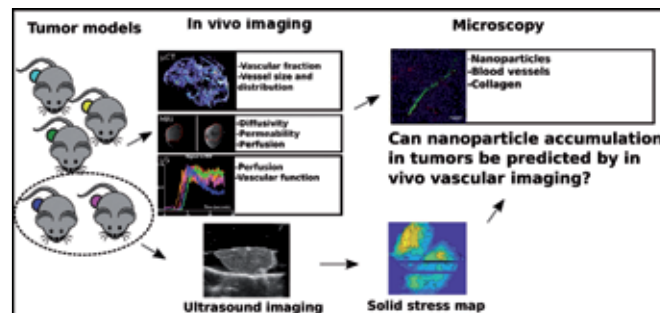


Figure 1: Study design.

## METHODS

Five different tumor models were grown subcutaneously in athymic mice and imaged with  $\mu$ CT, diffusion weighted and dynamic contrast enhanced MRI and contrast enhanced ultrasound. Parameters obtained from the *in vivo* imaging were then evaluated for correlation with the accumulation of 100 nm polystyrene nanoparticles. The tumors were also characterized for density of blood vessels and collagen by microscopy of tumor frozen sections. Subsequently, the prostate carcinoma model PC3 and osteosarcoma model OHS, tumor models with very different vascular density were characterized for solid stress by imaging cutting plane expansion with ultrasound. Solid stress and relative amount of functional blood vessels were compared to the accumulation and distribution of a more clinically relevant nanoparticle made from poly(alkyl cyanoacrylate).

## RESULTS

It was found that NP accumulation correlates with vascular properties. The blood vessel morphology, evaluated through the inflow time in the time-intensity curves from contrast enhanced ultrasound imaging correlated significantly ( $p = 0.041$ ) with the nanoparticle accumulation. This indicates that chaotic vasculature with shunts and dead ends that cause microbubble retention also is more susceptible to nanoparticle accumulation. The vascular density, when quantified by microscopy of lectin-perfused blood vessels also correlated significantly ( $p = 0.0056$ ) with the accumulation of nanoparticles, but this was not captured by the *in vivo* imaging, probably due to lack of sensitivity towards the smaller vessels. The effect of solid stress on NP accumulation and blood vessel perfusion is currently being analyzed (figure 2).

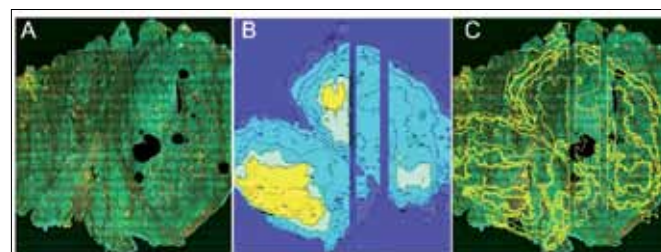


Figure 2: A, Confocal laser scanning microscopy tile scan of a section from the OHS tumor. B, solid stress map from the same area. C, overlay of the two. Nanoparticles are depicted in green, blood vessels in red and nuclei in blue.

## CONCLUSION

Our study shows that the blood vessel architecture and density, and not only vascular permeability can be important factors for nanoparticle accumulation. Thus contrast enhanced ultrasound imaging of tumors could be a useful approach to predict nanoparticle uptake and thereby stratifying patients for therapy with nanomedicine.



cines. Interesting we found that significant amount of solid stress was detected even in these relatively small, and subcutaneous tumors.

## ACKNOWLEDGEMENT

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## ANAPHYLATOXIN-DEPENDENT AND INDEPENDENT CIRCULATORY CHANGES IN MICE CAUSED BY COMPLEMENT ACTIVATORS AND AMPHOTERICIN-B-CONTAINING LIPOSOMES

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## BACKGROUND

Undesirable C activation by some nanomedicines is known to cause complement activation related pseudoallergy (CARPA) in sensitive individuals. In CARPA, C split products result in a series of hemodynamic and other changes, which, when uncontrolled, may become fatal. Intravenous administration of liposomal amphotericin B (AmBisome and Abelcet) and high cholesterol multilamellar vesicles (HC-MLV) activate the C system in humans, pigs and rats and result in CARPA. No data, however, is available on the effect of these liposomes on the complement system in mice, which are widely used in preclinical research. The goal of this study was to understand the effects of these liposomal preparations in mice.

Methods: Blood pressure and heart rate were measured in anesthetized male NMRI mice (n=4-6/group). Blood was collected from the transected vena cava in isoflurane anesthetized mice at 3-30 min after treatment and blood count and plasma concentration changes were compared to a control group administered saline only (n=8-12/group).

## RESULTS

Liposomal amphotericin B formulations (AmBisome and Abelcet), and positive controls known to activate the complement (zymosan and cobra venom factor, CVF) but not HC-MLV led to transient hypertension, thrombocytopenia and elevation of plasma thromboxane concentration (TXB2) in mice. Hemodynamic changes progressed to hypotensive shock in animals treated with positive controls. Parallel measurement of plasma C3a concentration revealed massive C activation in zymosan and CVF treated animals but smaller activation was detectable in plasma of animals treated with liposomal amphotericin B formulations. Pretreatment of mice with the C3a inhibitor SB290157 attenuated the blood pressure raise caused by Abelcet and decreased blood pressure at later times.

## CONCLUSIONS

Treatment with liposomes caused a transient hypertension in mice.

This change correlated with platelet activation, increased plasma TXB2 levels and complement activation. Hemodynamic changes induced by the positive controls were biphasic accompanied with considerable elevation of C split products. Activation of both the complement system and vasoactive mediator-secreting blood cells are responsible for the liposome-mediated hemodynamic changes typical of CARPA in mice.

## NORMALIZATION OF TUMOR BLOOD VESSELS BY DELIVERY OF DOPAMINE USING PH-SENSITIVE NANOPARTICLES

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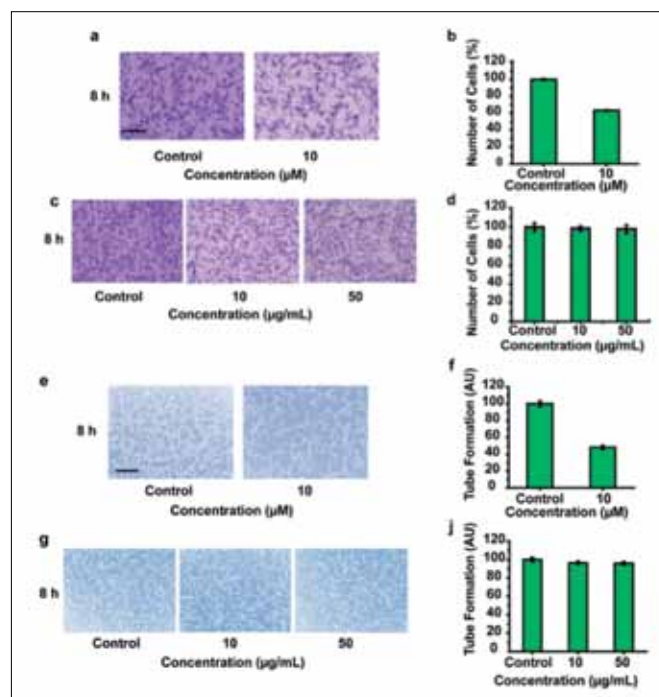


Figure 1. a-d. Trans well assay demonstrate that DA can inhibit the migration of HUVEC cells to another side of channel but free nanoparticles have no effect on them. e-j. tube formation analysis show that same as trans well assay DA has inhibition effect but NPs no. a, e related to free DA c, g allied to free NPs. Scale bar shows 200µm.

For supply nutrient and oxygen to cells, tissues need blood and definitely vessels to carry compounds and respectively remove waste compounds from them. In body by different mechanisms vessels are not only generated in normal tissues but also in tumors. Vessels existing by affecting from already ones by making special condition and releasing different signaling molecules such as Vascular Endothelial Growth Factor (VEGF). VEGF is one of the most important signal molecules that released from endothelial cells by the hypoxic condition. These signals causes generating new vessels in high speed. Due to these conditions, structure of vessels in tumors are not normal. Abnormal structure will hinder the perfusion of drugs and chemotherapeutic compounds, because of unevenly assorted and chaotic. Another hand in these structures, vessels are leakier; endothelial cells and pericyte cells do not have normal coverage as well as other tissues.<sup>1,2</sup> By controlling the tumor microenvironment specially normalization of tumor vessels, could be overcom-

ing tumor growth. Anti-angiogenesis described for the first time by Folkman. Dopamine (DA) is an effective molecule on inhibition of angiogenesis and could improve the tumor vessels normalization. DA effect on receptors on endothelial cells (ECs). This sympathetic nerve compound has a very short half life time and has some side effects on heart, kidney and other organs. Nanotechnology is facilitating the delivery of drugs, chemotherapeutic compounds, peptides, nucleic acids and proteins to increase the efficiency of them and decrease the side effects to body. Whereas DA has receptor on cell membrane we designed carrier with pH sensitive bond to deliver it inside tumors by affecting by acidic pH of tumor microenvironment.<sup>3</sup> DA can regulate the angiogenesis by inhibiting the VEGF.<sup>4</sup> Mesoporous Silicon Nanoparticles (MSN) is a biodegradable and multifunctional carrier that has high capacity, good dispersity in water, same pore size and easily modified by other molecules and chemical groups.<sup>5-7</sup> In our project MSN synthesized in 100 nm and modified by some molecules to release DA by pH sensitive bond. Our results not only *in vitro* but also *in vivo* shown that by releasing it, vessels normalized and endothelial cells migration was inhibited. Different migration assays have done and confirmed us hypothesize (Figure 1.).

**Keywords:** vessel normalization, dopamine, tumor targeting, tumor microenvironment

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## CELLULAR UPTAKE AND BIOCOMPATIBILITY OF STEALTH, PROTEIN-BASED NANOCARRIERS

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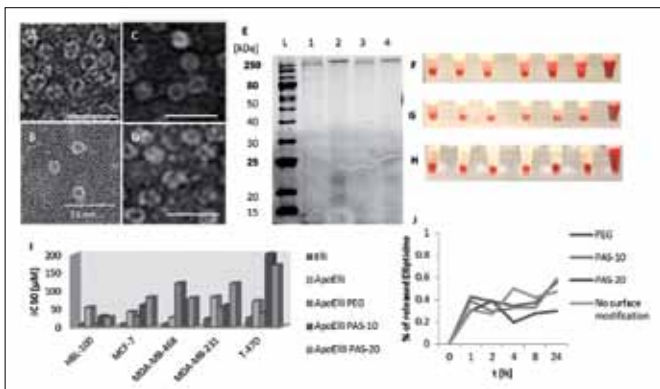
Nowadays, there is an increasing trend of studying the biological identity of nanoparticles in order to improve the ability to predict their biological outcomes. This could be used to potentially speed up of their translation to the clinic<sup>[1]</sup>. One of the typical signs of nanoparticles' biological identity is their protein corona, which occurs after exposition to high protein concentrations (about 60-80 g/l<sup>[2]</sup>) that are present in *in vivo* conditions. Protein corona can be defined as a natural interface between nanomaterials and living matter in biological milieu<sup>[3]</sup>. The formation and composition of protein corona is influenced by many ways, for example the way that the nanoparticle enters body, nanomaterial properties, temperature, etc. The importance of protein corona investigation is currently increasing as protein corona forms the actual biological identity of a nanoparticle and also defines its biological interactions.

Surface modifications of nanoparticles can be used in order to reduce or eliminate the binding of additional biomolecules leading to formation of protein corona. In this contribution, we focused on two very promising surface modifications: PEGylation and PASylation. PEGylation can be generally defined as modification of proteins, peptides or small organic molecules by covalent binding with one or more poly-ethylene glycol (PEG) chains<sup>[4]</sup>, a Food and Drug Administration (FDA) approved polymer for human oral, intravenous and dermal pharmaceutical use<sup>[5]</sup>. Even though PEG is the only one clinically approved surface modification of drug, PEGylation still has some drawbacks including PEG potential immunogenicity PEG<sup>[6]</sup>, decreased biological activity of a drug after PEGylation<sup>[7]</sup>, non-biodegradability of PEG and its possible cellular vacuolization<sup>[8]</sup>. Therefore, it is necessary to come up with new a surface modification that eliminates the main drawbacks of PEGylation. PASylation can be defined as a biological alternative to PEGylation with very similar biophysical properties. PAS sequences are hydrophilic, uncharged, comprising of small residues of amino acids Proline, Alanine and Serine (PAS)<sup>[9]</sup>.

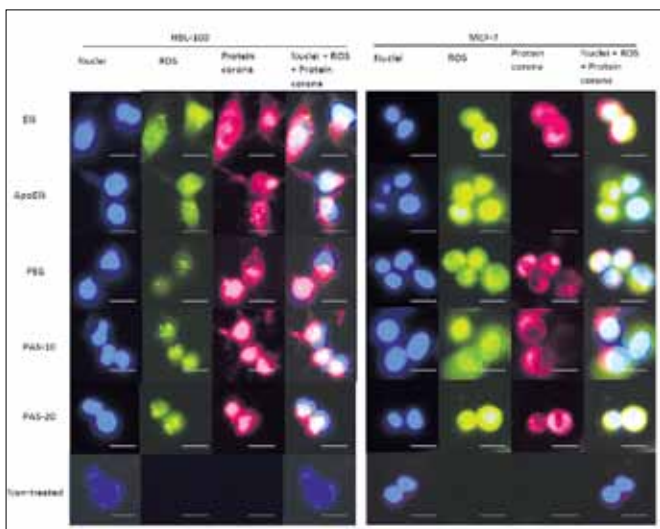
As a nanocarrier was chosen apoferritin (Apo), which is a biocompatible ubiquitous protein naturally presented in human body<sup>[10]</sup>. Ferritins without iron content usually self-assemble into a hollow rhombic dodecahedral cage called Apo. Apo weights between 450 and 475 kDa, the shell has an outer diameter of 12-13 nm and an inner diameter of 7-8 nm and has 24 subunits<sup>[11]</sup>. The structure of Apo is pH dependent, researchers from our research group of Molecular Biology and Nanomedicine published a study<sup>[12]</sup>, where they described easy-to-use encapsulation of doxorubicin (Dox) into Apo. For the purpose of this study was used experimental drug ellipticine (Elli). Elli has not been approved by FDA due to many side effects, such as its mutagenicity<sup>[13]</sup>. Elli encapsulation into Apo leading to formation of ApoElli was also optimized by our group.

According to Fig. 1A-D, obtained from transmission electron microscopy (TEM), it is obvious that the performed surface modifications did not cause any structural changes of Apo. Results from SDS-PAGE gel electrophoresis (Fig. 1E) showed that PASylation, same as PEGylation, eliminated the formation of protein corona. The need of Elli encapsulation into Apo is demonstrated on Fig. 1F, because after encapsulation (Fig. 1G, H) the degree of hemolysis decreased rapidly, which also proves that neither PEGylation, nor PASylation are hemotoxic. MTT assay (Fig. 1I) was performed in order to determine value of 24IC50 and via this value we compared the cytotoxicity of PEGylated and PASylated ApoElli. 24IC50 was determined for four malignant breast cell lines (MDA-MB-468, MDA-MB-231, MCF-7, T-47D) and for one non-malignant breast cell line (HBL-100). The results from MTT assay showed that surface modification with PEG was more cytotoxic than PASylation for all cell lines and also that PAS-10 is more cytotoxic than PAS-20, except for the cell line T-47D. Fig. 1J showed that neither PEGylation, nor

PASylation caused any changes in the release kinetics in off-target plasma environment of Elli after 24 h study.



**Fig. 1:** Characterization of PEGylated and PASylated ApoElli: A) TEM image of ApoElli without any surface modification; B) TEM image of PEGylated ApoElli; C) TEM image of ApoElli modified with PAS-10; D) TEM image of ApoElli modified with PAS-20; E) 1D SDS-PAGE: 1 - NEB Protein Ladder 10-250 kDa, 2 - protein corona on PAS-10 modified ApoElli; 3 - PAS-10 modified ApoElli; 4 - protein corona on PAS-20 modified ApoElli, 5 - PAS-20 modified ApoElli; F) Hemolytic assay of ApoElli without using Apo (from left to right: negative control; 6.25; 12.5; 25; 50 and 100  $\mu\text{g}\cdot\text{mL}^{-1}$  Elli; positive control); G) Hemolytic assay of ApoElli modified with PEG (from left to right: negative control; 6.25; 12.5; 25; 50 and 100  $\mu\text{g}\cdot\text{mL}^{-1}$  Elli modified with PEG; positive control); H) Hemolytic assay of ApoElli modified with PAS-10 (from left to right: negative control; 6.25; 12.5; 25; 50 and 100  $\mu\text{g}\cdot\text{mL}^{-1}$  Elli modified with PAS-10; positive control); I) MTT Assay; J) Release kinetics study.



**Fig. 2:** Internalization of Elli into Apo, ROS/nuclei staining (the length of scale bar is 25 nm)

The internalization of Elli into Apo is shown in Fig. 2 with ROS/nuclei co-staining and fluorescence microscopy analysis of tested cell lines after 6 h of treatment. Cell nuclei were stained with blue fluorescent dye Hoechst 33258 according to manufacturer's instructions. Green colour represents fluorescence of Elli. Fig. 2 shows successful internalization of Elli into nuclei, especially to malignant cell line MCF-7. The internalization of Elli into non-malignant cells was 84% lower than into malignant cell line MCF-7. It must be noted that only surface modification with PAS-10 increased the internalization of Elli (166% compared to 100% of Elli), in case of PEGylation the internalization was 52% lower than for Elli and in case of PAS-20 5% lower than for Elli. ROS were detected via staining with CellROX<sup>®</sup> Deep Red Reagent (Thermo Fischer Scientific). Fig. 2 illustrates that all three performed surface modifications induced increased formation of ROS within both tested cell lines, but, overall, the

highest ROS formation was found in PAS-10 modified ApoElli for non-malignant cell line HBL-100 (218% compared to 100% of Elli) and in PAS-20 modified ApoElli for malignant cell line MCF-7 (269% compared to 100% of Elli).

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## LIPOBRID-BASED NANOCONSTRUCTS FOR TOPICAL DELIVERY OF ANTI-INFECTIVES: EVIDENCES OF IMPROVED ANTIMICROBIAL EFFICACY AND DERMATOKINETIC ATTRIBUTES IN BURN WOUND BACTERIAL INFECTIONS

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**Introduction:** Topical anti-infective like Fusidic acid (FA) is widely employed in the treatment of burn wounds infected by methicillin-resistant staphylococcus aureus (MRSA). However, the currently available topical formulations of FA fail to penetrate across the wound eschar leading to sub-optimal therapeutic efficacy. Moreover, topical formulation of FA has to be applied 2–3 times daily for several days leading to poor patient compliance. Taking the clinical situation into consideration, it becomes imperative to explore prospects of novel nanomedicine-based formulation strategies to improve the therapeutic activity of FA. The current research study intends to explore the combined potential of lipid nanoconstructs and chitosan as an optimum therapy for the management of wound infections. Fusidic acid (FA) was encapsulated within the nanoen-



gineered lipid-polymer hybrid nanoconstructs (FA-LPHNs) for the treatment of deep second degree burns leading to improved therapeutic efficacy and enhanced patient compliance.

**Methods:** The lipid-polymer nanoconstructs were prepared by hot microemulsion method and characterized with respect to their size, surface morphology, drug loading and entrapment efficiency. The selected system was incorporated into gel and subjected to skin compliance, ex vivo permeation and dermatokinetic studies. The developed carriers were further characterized for antibacterial activity, cytotoxicity studies in HaCat cell lines and evaluation of pharmacodynamic efficacy in murine burn wound model. The pharmacodynamic model comprised of determination of bacterial burden, wound contraction rate, histopathological examination, estimation of antioxidants and pro-inflammatory cytokines.

**Results and Discussion:** The developed systems were monophasic and whitish in color. The optimized formulation exhibited particle of  $284.67 \pm 5.67$  nm and PDI of  $0.354 \pm 0.56$  assuring narrow distribution of the particles. The % EE and drug loading were found to be  $80.76 \pm 4.0\%$  and  $25.45 \pm 5.78\%$ , respectively. The developed systems were found to possess spherical shape as depicted in Figure 1.

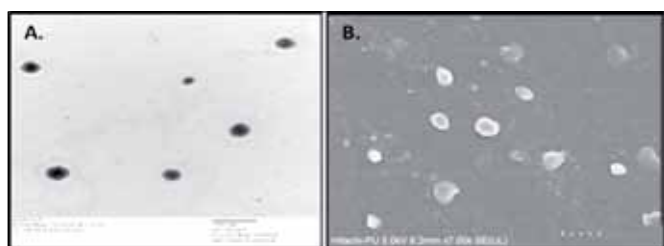


Figure 1: Morphological and topographical characteristics of the developed system as visualized through (A) TEM (B) FESEM

The changes in viability of HaCat cells were insignificant indicating the safety profile of developed formulation. The administration of FA encapsulated within the nanoconstructs resulted in 5-times and 4-times decrease in its inhibitory concentration against MRSA 33591 and MSSA 25921 respectively, along with antibacterial activity for a longer duration. Further, hydrogel incorporated nanoconstructs were found to be topically applicable and compatible with mice skin. The dermatokinetic studies confirmed enhanced skin bioavailability of FA to epidermis as well as dermis vis-à-vis the marketed product. The pharmacodynamic evaluation revealed improved wound healing and reduction in bacterial count vis-a-vis the marketed product.

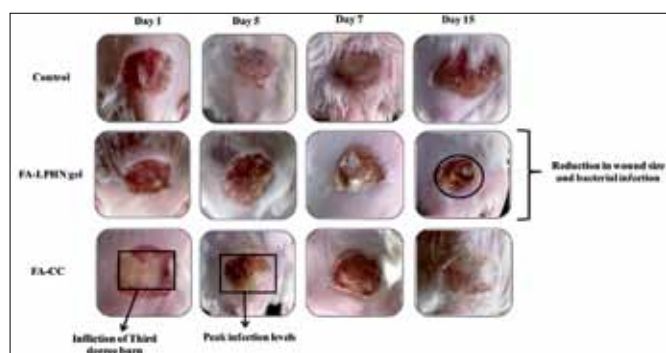


Figure 2: Comparison of partial thickness burn wounds in different groups during the course of treatment with FA-LPHN gel

**Conclusions:** The present study show the effectiveness of FA incorporated within lipid-polymer hybrid nanoconstructs in inhibiting bacterial wound infection. The studies indicated the superiority of FA-LPHNs for better management of wounds and associated infections over the conventional marketed product. The results encourage the exploration of the potential of lipid-polymer hybrid nanoconstructs and chitosan in treating resistant microorganisms such as MRSA involved in wound infections.

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## ACETALATED DEXTRAN NANOPARTICLES FOR CARDIOMYOCYTES PROLIFERATION

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Cardiovascular diseases represent one of the most burden diseases worldwide, due to the inability of cardiomyocytes to replace the loss of functionality of the cardiac tissue after an insult. Unfortunately, current therapies are still inefficient at treating these diseases and their prognosis is still quite poor. Nanomedicines gained an increasing attention during the last recent years and could represent a valid alternative to current therapies. In this work, we developed pH-responsive acetalated dextran (AcDEX) nanoparticles, with L-Alanine as a linker for further surface modification with polyethyleneglycole (PEG) and atrial natriuretic peptide (ANP). The particles are then loaded with two leading compounds for cardiac reprogramming and repair, SB431542 and CHIR99021, and cardiomyocytes proliferation is then assessed *in vitro* and *in vivo*.

### 1. INTRODUCTION

The inability of human cardiomyocytes to replicate and replace the loss of functional cells after an insult<sup>1</sup> and the following scar tissue formation, are the causes of the development of an irreversible decompensated state, named heart failure<sup>2</sup>. Nonetheless CVD represent a leading cause of death worldwide, counting over 17.7 million deaths globally in 20153. Unfortunately, current therapies are not able to recover that loss of functionality caused by an infarct or an ischemic insult<sup>4</sup>, so new approaches and strategies are urgently needed to solve this big issue from its roots. Recently, cardiac reprogramming has been regarded as a promising strategy, able to go beyond the barely symptomatic treatment offered by current therapies, as it allows for to replace death cardiac cells, by converting myofibroblasts into beating cardiomyocytes. For an efficient cell regeneration/repair, it has been reported that at least 6-9 model compounds, which could be small molecules, miRNAs and transcription factors (TFs), administered in simultaneously would be necessary for human fibroblast reprogramming into cardiomyocytes<sup>5</sup>. One problem related to the administration of these small molecules is that they are extremely hydrophobic. In addition to that, the simultaneous administration of all these compounds is challenging due to their chemical instability and it is difficult to properly formulate them for i.v. injections.

Nanomedicines can represent a solution to overcome all this drawbacks, since it has been shown that they can carry different payloads and improve their therapeutic efficacy meanwhile minimizing their side effects. Nanoparticles indeed, enabling the targeted delivery of drugs in specific sites, allow to obtain a specific concentration of a certain drug in the right point, at the right time<sup>6</sup>. One heart targeting molecule, described in literature, is ANP, which is a circulating cardiac hormone produced physiologically, responsible for the regulation of physiological homeostasis, with cardioprotective

properties, antiapoptotic effects and ability to inhibit hypertrophy of cardiomyocytes<sup>7</sup>.

Here, we present a new system based on ACDEX, modified with Alanine as linker and conjugated with PEG and ANP. The system is further loaded with two leading compounds for cardiac reprogramming and cardiac proliferation, SB431542 and CHIR99021. SB431542 is an activin receptor-like kinase (ALK) inhibitor, which inhibits the phosphorylation of Smads and then, their translocation to nucleus<sup>8</sup>, thus avoiding the transcription of target genes, which is the cause, in the canonical pathway, of fibrosis processes<sup>9</sup>. CHIR99021 is a potent inhibitor of the glycogen synthase kinase 3 (GSK3). This inhibition leads to the stabilization of  $\beta$ -catenin in the cytoplasm and then its translocation to the nucleus, where it regulates targeted gene expression<sup>10</sup>.

## 2. EXPERIMENTAL DESIGN, RESULTS AND CONCLUSIONS

The AcDEX polymer with L-Alanine as linker is synthesized as described in literature and the successful conjugation is then assessed by <sup>1</sup>H NMR spectroscopy in DCl/D<sub>2</sub>O according to the method. The AcDEX nanoparticles are prepared by an o/w single emulsion method, as described in literature<sup>11</sup>, and then are characterized by DLS, ATR-FTIR, TEM and EDX. Then the biocompatibility of the system is assessed. It has already been demonstrated, that Acetalated dextran spermine modified (AcDXSp) nanoparticles, loaded with these two compounds and decorated with PEG and ANP, are able to induce fibroblasts conversion into cardiomyocytes<sup>11</sup>. However, the same nanoparticles showed a significant toxicity towards cardiomyocytes (Fig. 1). Taking this into account, here we keep the advantageous pH-responsiveness of AcDEX, which enables the release of the payloads once taken-up by the cells, but we improved the biocompatibility of the nanoparticles by eliminating the presence of spermine groups, which was the cause of toxicity, since it endows nanoparticles with highly positive charge (Fig. 2).

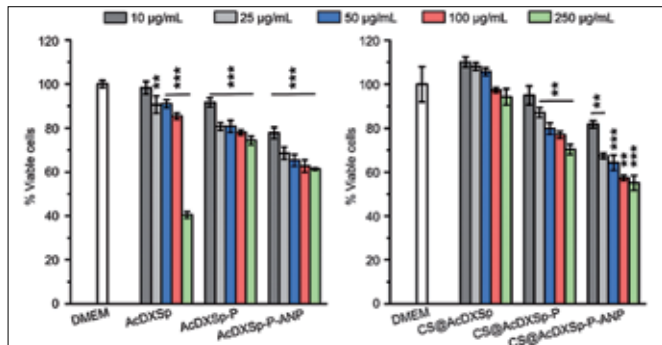


Fig. 1. Cell viability of primary cardiomyocytes in complete serum-free medium, determined by MTT assay, after incubation for 6 h with empty and drug-loaded nanoparticles at concentrations of 10–250  $\mu\text{g/mL}$ .

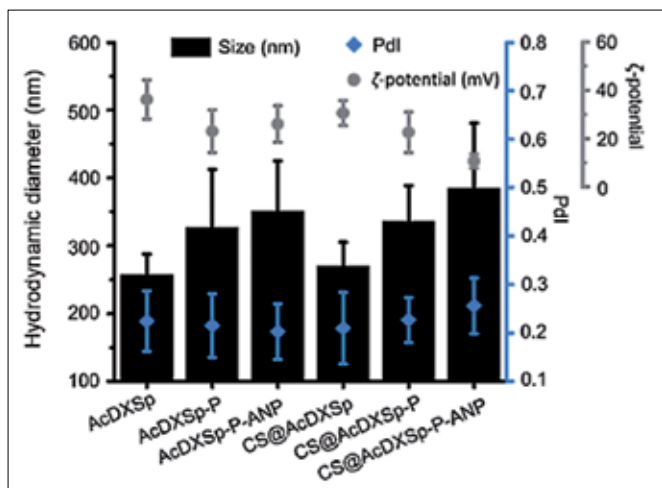


Fig. 2. Size, polydispersion index (PDI) and  $\zeta$ -potential of empty and

loaded AcDXSp NPs.

Preliminary results show that AcDEX nanoparticles are not toxic toward cardiomyocytes after both treatment for 24 and 48 h (Fig. 3). Moreover, the decoration with PEG and ANP, allows for to the accumulation of the nanoparticles in the infarcted area, in which they can release their cargoes, after degradation in the endoplasmic compartment.

The successful delivery of the two leading compounds is thus followed by the proliferation of cardiomyocytes, assessed and quantified by BrdU staining and imaging.

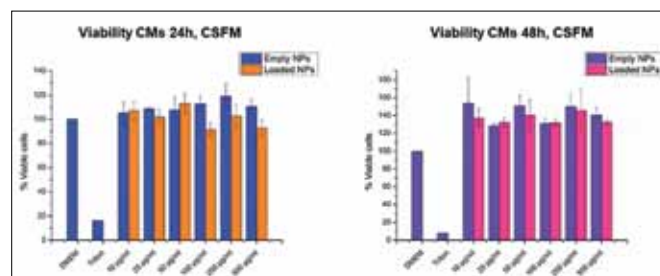


Fig. 3. Cell viability of primary cardiomyocytes in complete serum-free medium, determined by MTT-test, after incubation for 24 and 48 h with empty and drug-loaded nanoparticles at concentrations 10–250  $\mu\text{g/mL}$ .

## ACKNOWLEDGEMENTS

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# INFLUENCE OF STABILIZING COMPONENTS ON THE INTEGRITY OF ANTITUMOR LIPOSOMES LOADED WITH LIPOPHILIC PRODRUG IN THE BILAYER

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Liposomes like other nanoparticles become covered with proteins very rapidly upon contact with blood plasma [1]. Liposome-protein interactions could lead to destabilization of the membrane. In this work we studied how several amphiphilic molecules incorporated in the bilayer of liposomes loaded with lipophilic prodrug (Mlph-DG) — dioleoylglycerol ester of melphalan (chemotherapeutic alkylating agent) — influence the integrity of liposome membrane in human serum (Fig. 1). Phosphatidylinositol (PI) has been shown to decrease liposome uptake by cells of reticuloendothelial system [2], which may be due to the negative charge of the lipid with a relatively bulky head group, along with the steric hindrances caused by highly hydrated inositol moieties on surface of liposome [3]. Ganglioside GM1 has been shown to be even more effective for protection against premature withdrawal by mononuclear cells than PI due to the voluminous and rigid negatively charged pentasaccharide residue [2,4]. Grafting of polyethylene glycol (PEG) chains on the surface of liposomes in the form of PEG2000-conjugated phosphatidylethanolamine is a proven method of their stabilization [5]. Conjugate of an acidic oligopeptide CMG with dioleoylphosphatidylethanolamine (CMG-PE) is a new molecule that we chose to test for the ability to protect lipid bilayer surface.

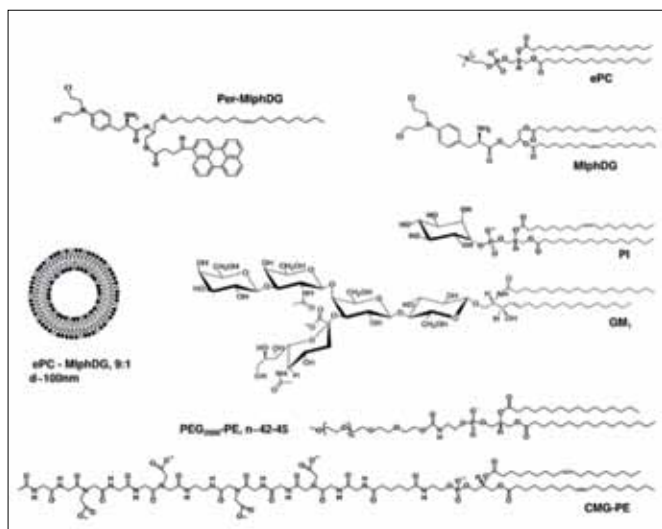


Fig. 1. Molecular structures of lipids used for liposome preparation.

Liposomes were prepared by standard extrusion procedure using 100-nm-pore polycarbonate membranes. Description of samples and their physicochemical characteristics are presented in Table 1. All liposomes had similar mean hydrodynamic diameter of 86–90 nm regardless of their composition. In order to control prodrug retention in the bilayer, we prepared liposome samples with 3-perylenoyl fluorescent analog of the lipophilic conjugate of melphalan (Per-MlphDG, Fig. 1).

Table 1. Physicochemical characteristics of liposomes.

Sample ID	Sample composition, mol	Mean size, nm (NTA)	Zeta potential, mV
L	ePC-MlphDG, 9:1	90 ± 2	+18
L-PI	ePC-MlphDG-PI, 8:1:1	90 ± 2	-25
L-GM1_2	ePC-MlphDG-GM1, 9:1:0.2	88 ± 2	+5
L-GM1_10	ePC-MlphDG-GM1, 8:1:1	89 ± 2	-30
L-CMG_2	ePC-MlphDG-CMG-PE, 9:1:0.2	86 ± 2	-31
L-CMG_10	ePC-MlphDG-CMG-PE, 8:1:1	87 ± 1	-66
L-PEG_2	ePC-MlphDG-PEG-PE, 9:1:0.2	88 ± 2	+9
L-PEG_10	ePC-MlphDG-PEG-PE, 8:1:1	88 ± 1	-10

Liposomes were incubated in PBS and serum at 37°C. Then they were separated from unbound proteins using gel chromatography on a calibrated sepharose column. For PBS samples, no decrease of fluorescence in the peak of liposomes after gel chromatography, nor appearance of any new fluorescence peaks were detected in the elution profiles (Fig. 2a, only data for L-PI are shown). For serum samples, decrease with time of fluorescence in the peak of liposomes after gel chromatography without occurrence of new ones is an evidence of bilayer interaction with proteins resulting in quenching of the perylenoyl group emission (Fig. 2b, only data for L-PI are shown). Oppositely, fluorescence growth in the course of elution of smaller complexes and micelles was observed for L-PEG10 liposomes (in serum, not in PBS), which apparently lose a part of the prodrug with time (Fig. 2c, d).

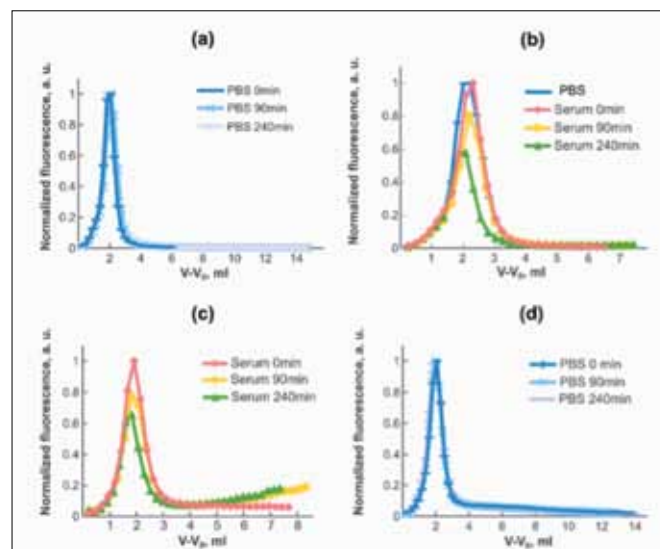


Fig. 2. Examples of elution profiles for liposomes L-PI with Per-MlphDG

Liposome integrity was evaluated by calcein-leakage assay. We prepared samples of liposomes listed in Table 1 with calcein encapsulated at self-quenching concentration. After extrusion, non-encapsulated calcein was removed by size exclusion chromatography on a Sephadex G-50 column equilibrated in PBS. Our results show that the best stabilization of liposomes with fluid lipid bilayer is achieved by incorporation of ganglioside GM1 or lipidic conjugate of oligopeptide, CMG-PE, in the membrane. Even small amounts (2 mol %) of these molecules can protect liposomes from the destructive action of blood plasma proteins at least for 24 h. The data for 4 h of incubation are presented in Fig. 3. When the content of these amphiphiles in the membrane increases (up to 10 mol%), water-soluble drugs encapsulated in the internal volume of liposomes can leak through widened water channels, or cracks, formed in the bilayer due to interactions of proteins with bulky negatively charged residues of amphiphiles protruding outwards. But for our prodrug in the form of a lipophilic conjugate anchored in the membrane of liposomes, there is no exit from the matrix of the lipid bilayer, as we can see in experiments with Per-MlphDG (Fig. 2b). The inclusion of phosphatidylinositol (10 mol%) ensures stabilization of the liposomal formulation in serum only for the first 4 h, which, however, may be significant in terms of therapeutic efficacy (Fig. 3).

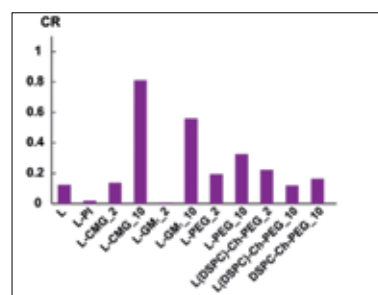


Fig. 3. Calcein release (CR) from different types of liposomes in serum after 4h incubation.

Calcein release was calculated as follows:

$$CR = \frac{(I(t) / I_{\max}(t) - I_0 / I_{\max,0}) \times [I_{\max}(t) / (I_{\max}(t) - I_0)]}{I_{\max}(t) - I_0}$$

where  $I_{\max}(t)$  is the intensity after Triton X-100 addition, performed immediately after dilution,  $I_{\max}(t)$



is the intensity after TritonX-100 addition at different time points and I(t) is intensity at chosen time point, I<sub>0</sub> -intensity immediately after liposome addition to serum or PBS.

The presence of PEG-lipid conjugate in the membrane of fluid-phase liposomes promotes exit of the bilayer components as we have seen by wavelike calcein release (data not shown) and PerMlphDG detection in later micellar fractions (Fig. 2c). This can result in both loss of a water-soluble drug and a lipophilic (pro)drug. In accordance with known data and argumentation, cholesterol-containing liquid ordered bilayers supplemented with sufficient amounts of PEG-lipid show good stability in serum (Fig. 3). However, the rationale to apply such a composition of lipids for inclusion of the drug in the membrane remains in question: a small leak of active pharmaceutical ingredient can occur, and tight packing of the lipid bilayer can prevent the inclusion of additives and reduce the payload of liposomes. This was not the case for MlphDG owing to the suitable molecule structure. (More information about the work: D. Tretiakova, N. Onishchenko, I. Boldyrev, I. Mikhalyov, A. Tuzikov, N. Bovin, E. Evtushenko, E. Vodovozova Influence of stabilizing components on the integrity of antitumor liposomes loaded with lipophilic prodrug in the bilayer // Colloids and Surfaces B: Biointerfaces 166 (2018) 45–53)

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## INTESTINAL UPTAKE AND TRANSPORT OF ALBUMIN NANOPARTICLES: POTENTIAL FOR ORAL DELIVERY

### DRITON VLLASALIU

Oral administration is the ultimate drug delivery route, but is currently not viable for advanced therapeutics, such as biologics or nanomedicines. Both biotherapeutics and nanomedicines suffer from poor penetration across the formidable barrier of the intestinal epithelium. Current approaches to improve oral delivery typically employ 'absorption enhancers' that non-selectively disrupt and increase intestinal permeability. However, safety concerns have hindered the clinical translation of this approach in general. Key to safe and effective oral delivery of biologics and nanomedicines is the engineering of systems capable of delivering the drug selectively across the intestinal mucosa without disrupting this physiologically important barrier. Such systems typically explore biological transport processes as potential routes to deliver therapeutics across the intestinal barrier. Albumin-based nanocarriers could present a new and simple way of achieving transcytosis-targeted nanocarriers for oral drug delivery as the biological transport mechanism of albumin is expressed in the intestinal epithelium. Albumin-based nanocarriers may serve both as a drug carrier and a 'transport-enabling' entity, bypassing the requirement for another component (e.g. transcytosis ligand), making the overall drug delivery system simpler and less expensive, in addition to improving drug loading and potentially drug transport properties.

This study fabricated sub-150 nm human serum albumin (HSA) nanoparticles of low polydispersity and investigated their uptake and transport across Caco-2 monolayers. We show that HSA nanoparticles displayed a different cell uptake and transintestinal transport behaviour to albumin in solution, suggesting a shift in the trafficking route with nanoformulation. Notably, a greater amount of HSA traversed Caco-2 monolayers following the application of HSA nanoparticles, as compared to equivalent concentration of solution, suggesting improvement of transintestinal delivery via nanoformulation.

## METHODS

**Fabrication and characterisation of HSA nanoparticles.** Albumin nanoparticles were prepared by the established desolvation technique via dropwise addition of ethanol and glutaraldehyde to induce particle cross-linking. Particle size and polydispersity were measured by dynamic light scattering (DLS).

**Cell culture.** Caco-2 cells were cultured using Dulbecco's Modified Eagle's Medium (DMEM) with supplements. Cells were seeded on Transwell permeable plates at 105 cells/cm<sup>2</sup> and cultured for 21–23 days, prior to their use as differentiated monolayers.

**Albumin uptake and permeability.** This experiment was conducted by applying fluorescently-labelled HSA solution to Caco-2 monolayers and determining its cell uptake and permeability.

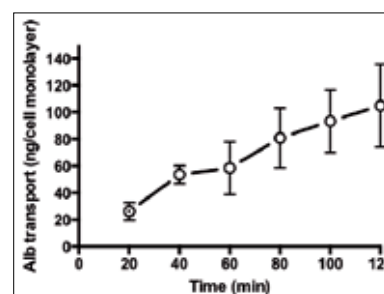
**Albumin nanoparticle uptake and epithelial transport.** Fluorescently-labelled HSA nanoparticles were applied to Caco-2 monolayers in Hank's Balanced Salt Solution (HBSS). Transepithelial transport and cell uptake were determined by quantifying HSA nanoparticles in the basal solution or following cell permeabilization, respectively. Cell uptake of FITC-albumin nanoparticles was also determined qualitatively using confocal microscopy.

## RESULTS

The mean HSA nanoparticle diameter in HBSS (buffer in which they were applied to cells) was 142.3 ± 1.3 nm.

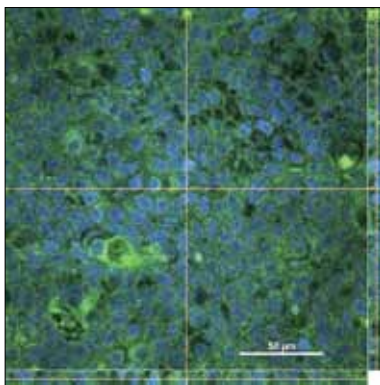
Figure 1 shows that HSA (applied in solution) permeated Caco-2 monolayers, with basal levels reaching approximately 68 ng after two hours.

Figure 1. Permeability of FITC-human serum albumin ('Alb') across differentiated Caco-2 monolayers. FITC-albumin was applied at 40 µg/ml and sampled from the basal side every 20 min. Data shown as the mean ± SD (n=3).

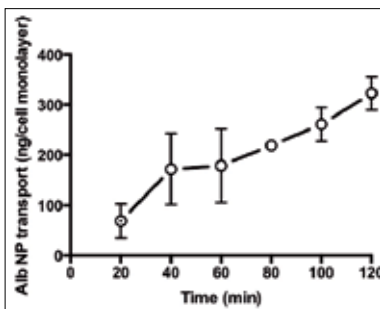


Confocal microscopy study of cell internalisation of HSA nanoparticles is shown in Figure 2. Strong fluorescence signal can clearly be seen in Caco-2 monolayers across the depth of the imaged area, suggesting fluorescence in cell interior.

Figure 2. Imaging of HSA nanoparticle uptake by Caco-2 cells. Confocal micrographs depicting Caco-2 monolayers treated with FITC-HSA nanoparticles. Orthogonal view showing the depth of the cell monolayer. Blue: cell nuclei stained with DAPI; green: FITC-HSA nanoparticles.



Fluorescently-labelled HSA nanoparticles were then applied to Caco-2 monolayers and their transepithelial transport established. Figure 3 indicates that HSA nanoparticles traversed Caco-2 monolayers, with HSA levels on the basal side reaching over 300 ng (per 1.1 cm<sup>2</sup> cell monolayer area).



**Figure 3.** HSA nanoparticle transport across differentiated Caco-2 monolayers. FITC-HSA nanoparticles were applied in HBSS.

Transepithelial transport was determined by regular sampling of the basal solution (every 20 min). Data shown as mean  $\pm$  SD ( $n=3$ ).

## DISCUSSION AND CONCLUSION

Fabrication of transcytosis-exploiting nanosystems for oral delivery requires complex systems, typically composed of the drug, the nanocarrier and the ligand directing the system to the transcytosis receptor of interest, presenting challenges in terms of formulation and stability of the system. HSA nanoparticles are potentially interesting as they may serve both as drug carriers and ‘transport-enabling’ entities – assuming they traverse the epithelium by transcytosis in the same manner as albumin in solution – hence removing the requirement for the ‘transport-enabling’ ligand. This would make the overall drug delivery system simpler and potentially more efficient. However, while albumin nanoparticles have demonstrated potential for delivery of hydrophobic chemotherapeutic agents, studies that fully explore the potential of albumin nanoparticles for oral delivery are limited to the use of bovine albumin, e.g. by coating as a ‘sacrificial’ component to protect drug degradation or provide nanosystem stability [20]. We explored whether albumin nanoparticles may be utilised as potential nanovehicles for oral therapeutic delivery based on the potential targeting of albumin transcytosis in the intestinal epithelium and the small size of albumin nanoparticles, achieved via a simple fabrication approach. The issue of stability of albumin nanoparticles in the gastrointestinal environment is appreciated, but the delivery of albumin nanoparticles to the intestinal mucosa is envisaged to take place via enteric delivery technologies, which are now well established.

HSA nanoparticles fabricated in this study displayed a size below 150 nm, low polydispersity and stability in a commonly used biological buffer, HBSS. Cell uptake of FITC-albumin nanoparticles was confirmed and shown convincingly by confocal microscopy, where fluorescence intensity of nanoparticles can clearly be seen in the cell interior. Comparing transport amounts between HSA solution and HSA nanoparticles, it is apparent that a notably higher amount of albumin permeates the cell monolayers following the application as nanoparticles, as compared to HSA solution. This is important regarding the system’s transport capacity, although it must be confirmed *in vivo*.

Overall, our study demonstrates the potential of HSA nanoparticles, fabricated via a simple process, as drug delivery systems capable of traversing the intestinal epithelium. Although the mechanism of intestinal transit of these systems is not clear, work showed remarkable level of internalisation of HSA nanoparticles by intestinal Caco-2 cells and more efficient apical-to-basal mass transport of albumin when formulated as nanoparticles compared to the so-

lution form. To fully evaluate the potential of this approach for oral delivery, the stability of HSA nanoparticles in different regions of the intestinal mucosa, diffusion across the intestinal mucus, potential immunogenicity and the mechanism of trafficking by intestinal epithelial cells should be studied.

## NATURAL ANTIBODIES DETERMINE BETWEEN-SUBJECT VARIABILITY AND EFFICIENCY OF COMPLEMENT C3 DEPOSITION ON PRECLINICAL AND CLINICAL NANOMEDICINES

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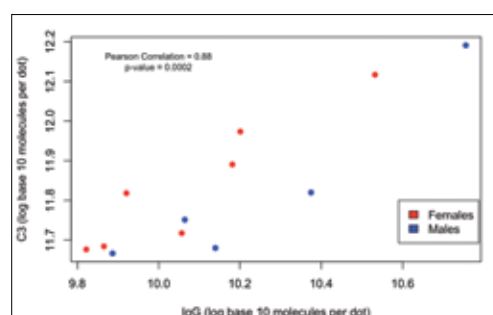
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Systemically injected nanomedicines trigger immune and proinflammatory responses through activation of the complement cascade. Levels of complement activation vary between human subjects; however, the mechanisms behind these differences are not well understood. We previously demonstrated that serum protein corona plays a role in enhancing the binding of third complement component (C3) to superparamagnetic iron oxide nanoworms (SPIO NWs). We show that immunoglobulin (IgG) deposition promotes significant complement activation and C3 deposition on SPIO NWs in a cohort of healthy human subjects ( $n=12$ ). IgG depletion from normal sera reduced C3 deposition 2-5 fold, while reconstituting the depleted sera with polyclonal human IgG restored C3 opsonization to a normal level. The dependency of C3 on IgG binding was shown through different sera and plasma (healthy and breast cancer patients) with SPIO NWs as well as clinically approved nanomedicines such as iron oxide Feraheme liposomal irinotecan Onivyde and PEGylated liposomal doxorubicin LipoDox, regardless of the activation pathway. This evidence indicates that few surface-bound IgG molecules trigger complement deposition. Although the level of complement activation is not determined by the total amount of absorbed protein, we demonstrated that the corona is necessary for effective IgG binding to nanoparticles. These results suggest that natural antibodies serve as a link between protein corona and C3 deposition, and may be used to predict interindividual immune responses to nanomedicines.

**Fig. 1.** Correlation between complement activation and IgG in protein corona of different healthy donors. IgG levels on SPIO NWs significantly correlated with C3 levels.



## SUGAR-FUNCTIONALISED AND FAR-RED FLUORESCENCE EMITTING GRAPHENE OXIDE FOR ANTIGEN DELIVERY AND MONITORING

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### INTRODUCTION

This project aims to design and synthesize fluorescent multi-glycosylated graphene oxide (GO)-based nanocarriers with special focus on the delivery of antigens for vaccination purposes (e.g. cancer or infectious diseases vaccines). The main hypothesis of the work is that graphene-based nanocarriers can, due to their small size and 2D structure, absorb a substantial amount of antigens *in situ* and offer efficient drainage into sentinel lymph nodes following intratumoural injection. After migration to lymph nodes, vaccine constructs can be internalised by antigen presenting cells, e.g. dendritic cells (DCs) which will result in generation of cellular immunity. The incorporation of far-red fluorescent probe can allow *in vitro* and *in vivo* tracking of the conjugate in a non-invasive manner. Surface functionalisation of the oligomer with multiple sugar moieties can improve the overall water solubility of the graphene nanocarrier and active-targeting of DCs via the mannose receptor, resulting in an enhanced immunoprotection *in vivo*. The aim of the work is to establish the feasibility of using GO as a protein (antigen) carrier and optimise the conjugation/adsorption of these oligomers on GO platforms.

### METHODS

GO was prepared at high yield using the previously optimised method. The propargylated GO was prepared by esterification reaction between GO and propargyl alcohol using EDC and DMAP [1]. Far-red emitting fluorophore oligomer with 4 pendant azide groups was synthesised and sugar motifs were added via Cu(I)-catalyzed azide alkyne click (CuAAC) chemistry with propargylated mannose and galactose to prepare tri-sugar functionalised oligomers (oligomers, fig. 2). Multi glycosylation sites were included to increase the water solubility and also the effectiveness of the nanocarriers due to multi ligand effect (better binding with the sugar receptors present on APC). At least one azide group was left unreacted and used for conjugation to propargylated GO via CuAAC reaction. Click reaction between GO and oligomer at differing ratios was performed both with and without click reagents (CuSO<sub>4</sub> and L-ascorbic acid) as negative control. Loading efficiency was determined by quantification of unreacted oligomer in supernatant. Whole cell lysate (a potential source of protein antigen) loading on GO was tested to confirm GO's propensity to non-covalently bind the hydrophobic pockets of proteins. Loading studies were carried out at room temperature overnight at 1-5 fold protein to GO mass ratios. Un-adsorbed protein was removed by multiple cycles of centrifugation/washings. Protein loading on GO was quantified by TGA analysis and compared to unloaded GO.

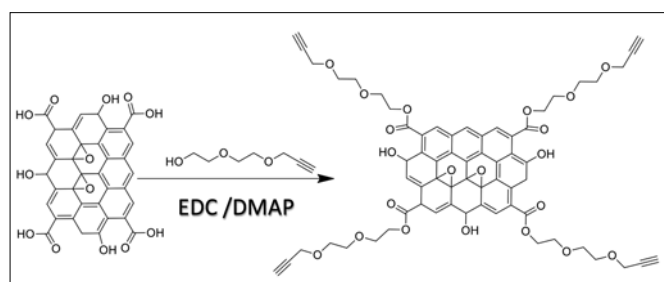


Figure 1: Esterification of GO. GO produced using Mei's method modified from the Kovtyukhova-Hummer method is esterified to produce propargyl functionalised GO.

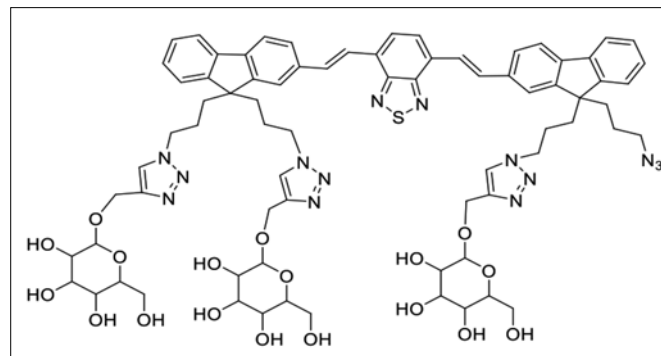


Figure 2: Structure of far red emitting oligomer functionalised with sugar moieties while maintaining a free azide residue for further click reactions.

### RESULTS

Using this method a high yield of GO was obtained with approximately 60% of starting graphite being converted to GO. GO was analysed by thermogravimetric analysis and shown to be highly oxidised exhibiting c. 70% weight loss between 100-1000°C. Especially notable was the degradation from 600-800°C representing introduced oxygen residues. GO was further functionalised with alkyne residues using the methods described and underwent physical changes associated with this process such as increased viscosity and aggregation. The Oligomer was synthesised as described and was shown to be soluble in polar solvents and was highly soluble in water (>30mg/ml). Oligomer exhibited strong fluorescence in the far infrared spectrum as predicted by its structure with an excitation and emission of 351/483nm and 614nm, respectively. This suggests it is suitable for tissue penetration and therefore *in vivo* imaging. GO was shown to bind to Oligomer both with and without click reagents though binding could be greatly enhanced through the inclusion of click reagents. The exact means of binding could not be identified however there is good evidence to suggest the two molecules are undergoing the click reaction rather than non-specific absorption. Protein could be loaded on to the constructs as demonstrated by a strong thermal degradation event at 400°C, using this method it was shown that protein represented 26-45% of the final construct and loading was dependant on the starting concentration of protein. Together these data suggest that GO can be functionalised with both candidate vaccine antigens in addition to the novel oligomer. The next steps will be to evaluate constructs *in vitro* and *in vivo* for their imaging and delivery properties.

### ACKNOWLEDGEMENT

Funding is provided from the Newton Institutional Links Grant (ImmunoGoRed) to support collaborations between King's College London and Bilkent University, Ankara.

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## ABROGATION OF SiO<sub>2</sub> NANOPARTICLE-INDUCED INFLAMMATORY LIPID CHANGES IN THE LUNG BY PHOSPHONATE COATING: A MALDI-MS BIOIMAGING STUDY

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The (phospho)lipid composition of the broncho-alveolar-lavage fluid (BALF) indicates the disease state of the human lung. While typical changes in the ratio of phosphatidylinositol (PI) and phosphatidylglycerol (PG) have been observed upon quartz, no information is available for effects of silica nanoparticles (NP) or detoxifying surface modifications thereof. Here we used matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) to analyse changes of the local (phosphor)lipid composition.

Animals (n=5 per group) were intratracheally instilled with 0.36 mg/lung of either fluorescein (FITC)-labeled silica ( $\text{SiO}_2$ -FITC), unmodified silica ( $\text{SiO}_2$ ), or trihydroxy silylpropyl methylphosphonate (TPMP)-coated silica ( $\text{SiO}_2$ -p). Particle sizes amounted to 10-50 nm. Control animals received instillation fluid only (0.5 mL NaCl). BALF was analyzed for protein and differential cell counts 3 days post application. Particle toxicity was also assessed *in vitro* using alveolar macrophages (Wiemann et al. J. Nanobiotechnology 14:16). Cryo-sections of the lungs were prepared 0.5 and 72 h post instillation to study NP distribution and lipid composition by MALDI-MSI.

Compared to unmodified and FITC-labeled silica NP, TPMP-silica elicited far less inflammation in the lung and less cytotoxicity and macrophage activation *in vitro*. Cryo-sections revealed a typical patchy distribution pattern of administered  $\text{SiO}_2$ -FITC after 30 min, whereas after 72 h fluorescent particles were confined to alveolar macrophages. At this point in time MALDI-MSI revealed a local increase in relevant phosphatidylinositol (PI) species (PI 34:1; PI 36:2; PI 36:4) and relative decreases in phosphatidylglycerol (PG) and triacylglycerol (TAG) upon  $\text{SiO}_2$  and  $\text{SiO}_2$ -FITC. These alterations were highly co-localized with the initial particle distribution pattern and were not seen in control animals. Changes in PI were further confirmed by Fourier transform infrared spectroscopy imaging followed by principal component analysis. Importantly,  $\text{SiO}_2$ -p elicited no such changes.

Results show for the first time that amorphous silica NP locally increase the PI/PG ratio, which is a long-known, though neglected diagnostic tool in human BALF analysis. As the TPMP-coating of  $\text{SiO}_2$  abrogated both, its inflammatory and lipid changing effects, we suggest that phospholipid changes are secondary to inflammation. The study highlights MALDI-MSI as a valuable method to study effects of clinical nanomaterials in human tissue.

Parts of the study were financed by the BMBF (project "NanoBio-Detect", FKZ 03X0146)

## LIPID COATED CALCIUM CARBONATE/PHOSPHATE NANOPARTICLES AS A NOVEL GENE/DRUG CO-DELIVERY PLATFORM FOR CANCER TREATMENT

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Calcium carbonate/phosphate based nanoparticles (NPs) are widely considered as promising non-virus gene delivery vehicles. The coating of lipids outside endows these NPs with the colloidal stability, targeting ability, and long blood circulation. Inspired by the pH sensitivity of calcium phosphate and calcium carbonate, we herein developed lipid-coated calcium carbonate/phosphate (LCCP) hybrid NPs for gene/drug co-delivery to cancer cells (Figure 1A). The TEM image shows that the LCCP NPs were around 40 nm in size, with the core diameter of 15 nm (Figure 1B). The coating lipids can be observed with negative staining (insert in Figure B). The loaded genes can be released from LCCP in a pH-dependent manner, and the released dsDNA band intensity increased from pH 7.4, 6.0, to

5.5 at the same time point (Figure 1C). With further modification with folic acid (FA) via PEG in the outer layer lipid, the LCCP-PEG-FA NPs can be effectively taken up by B16F0 melanoma cells. The mean fluorescence of cells treated with LCCP-PEG-FA was 3 times stronger than that of unmodified NP (LCCP-PEG), suggesting the FA-mediated specific internalization of LCCP-PEG-FA by B16F0 cells (Figure 1D). Moreover, we demonstrated the LCCP NPs can be used for cancer treatment via gene/drug co-delivery. Here, cell death siRNA (CD siRNA) and  $\alpha$ -tocopheryl succinate ( $\alpha$ -TOS) were used as model gene and hydrophobic drug in our study. The viability of B16F0 cells was decreased to 85% upon single treatment with gene or drug loaded NPs (CD/FA or TOS/FA) at 6 nM of CD siRNA or 5  $\mu\text{M}$  of  $\alpha$ -TOS, respectively. Much stronger inhibition was observed in cells treated with CD/TOS/FA NPs (55%) than treated with CD/FA or TOS/FA (85% each) at 6 nM of CD siRNA or 5  $\mu\text{M}$  of  $\alpha$ -TOS (Figure E), demonstrating the combinational synergy for cancer therapy. In consistence, the LCCP based NPs present a great potential as a platform for gene/drug co-delivery and cancer therapy.

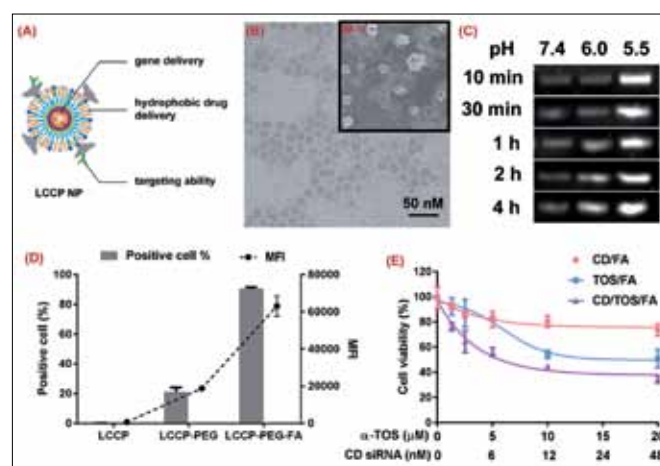


Figure 1. The design and properties of LCCP NP. (A) Schematic illustration of LCCP structure; (B) TEM image of LCCP NP, with (B-1) negative stained image showing the outer layer lipids; (C) the dsDNA release profile; (D) the cellular uptake of LCCP NPs with 50 nM dsDNA-cy5 for 4 h; and (E) the inhibition of B16F0 cancer cell growth.

## MTHPC-IN-CYCLODEXTRIN-IN-LIPOSOME NANODELIVERY SYSTEM

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Application of meta-tetra(hydroxyphenyl)chorin (mTHPC), one of the most potent photosensitizer (PS), in the photodynamic therapy (PDT) of solid tumors encounters several complications resulting from its insolubility in aqueous medium. It requires low light doses and concentrations to be photoactive (Senge & Brandt, 2011), however mTHPC aggregation results in reduced photodynamic activity, moderate selectivity and skin photosensitivity (Kachatkou et al., 2009). To improve the transport of mTHPC to target tissue and to strengthen its intra-tissue accumulation, several nanoconstructions have been investigated such as liposomes, polymeric nanoparticles, inclusion complexes etc.

In the present study, we suggested the coupling of two independent delivery systems by encapsulating cyclodextrin/mTHPC inclu-

sion complexes into liposomes to achieve drug-in-cyclodextrin-in-liposome (DCL) nanoparticles. DCL could circumvent the drawbacks of each separate system. Liposomes offer an excellent opportunity to achieve selective drug targeting which is expected to optimize the pharmacokinetic parameters, prevent local irritation, and reduce drug toxicity. In its turn, cyclodextrins (CDs) are cyclic oligosaccharides, which have been utilized as independent carriers for improvement of pharmaceutical properties such as solubility, stability and bioavailability of various drug molecules, including mTHPC (Yankovsky et al., 2016, Yakavets et al., 2017). Therefore, encapsulation of CD-complexed drug into liposomes may increase the drug loading capacity, entrapment efficiency, restrain the dissociation of drug-CD complexes and prolong its systemic circulation. The aim of this study was to evaluate the effect of DCLs on mTHPC behavior at various stages of its distribution in tumor models *in vitro*.

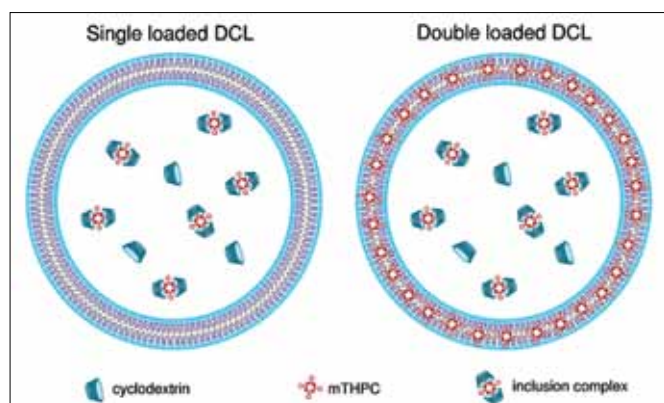


Figure 1. Scheme of drug-in-cyclodextrin-in-liposome nanoparticles with single and double loading of mTHPC

We have prepared DCL with various compositions to optimize DCL structures in terms of mTHPC tumor cells targeting. We have studied the influence of DCLs on mTHPC accumulation, distribution and photodynamic efficiency in human adenocarcinoma HT29 cellular monolayer and spheroid models. We have demonstrated that mTHPC-DCLs are stable and almost all PS is bound to  $\beta$ -CDs in the inner aqueous liposome core. We are also prepared double loaded DCL (DCL-DL), which includes mTHPC in lipid bilayer as well as in the inner aqueous core in inclusion complexes with cyclodextrin. We studied mTHPC accumulation and localization in both HT29 monolayer and spheroid cells. It was demonstrated that cellular of DCL-DL was like liposomal formulation in monolayer cultures. However, using 3D multicellular HT29 tumor spheroids we showed that the application of DCLs significantly improved PS compared to other formulations resulting in homogeneous distribution of mTHPC across spheroids (Figure 2).

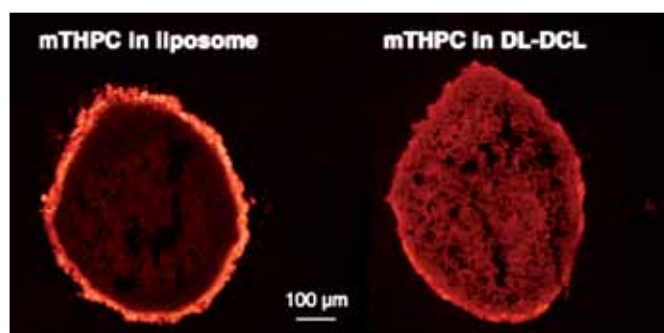


Figure 2. Fluorescence patterns of different mTHPC formulations in HT29 multicellular tumor spheroids

The data obtained confirm the interest of hybrid nanostructures in mTHPC-PDT.

#### ACKNOWLEDGEMENTS:

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## DEVELOPMENT OF BROAD SPECTRUM ANTIVIRAL GOLD NANOPARTICLES

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Infection diseases commonly lead to significant disability, morbidity, mortality and increasing burden for the health systems worldwide. Viruses are responsible for one third of these infections<sup>1</sup>. The most effective approach to prevent such infections is vaccination<sup>2</sup> because so far, most viral infections have no efficient treatment<sup>3</sup>. However, for many viruses no vaccines exist, or a spontaneous outbreak requires additional treatment with an antiviral. The effective antiviral therapies are often hampered by several factors, such as the high specificity of antivirals, high toxicity or the development of drug resistance following viral mutation. The development of novel treatment strategies is therefore required to overcome these limitations<sup>4</sup>.

Advances in nanotechnology have led to a new generation of broad-spectrum antivirals employing gold nanoparticles (AuNP) functionalized with small molecules<sup>5</sup>. These nanoparticles aim at preventing the first step of virus-cell interaction by mimicking viral attachment ligands, such as "heparan sulfate proteoglycans" (HSPGs), the highly conserved target of viral attachment ligands (VALs). However, these NPs have shown only limited efficacy in Dengue virus (DENV), another heparan sulfate binding virus or viruses which are not targeting HSPG as VAL such as enteroviruses.

DENV is the cause of dengue fever, an infectious tropical disease caused by the transmission to humans through the bites of infective female *Aedes* mosquitoes. The disease is becoming an increasing health problem also in the developed world due to the climate change and increased travel activities. There is no specific anti-DENV therapeutic or approved vaccine currently available, but early detection and access to proper medical care lowers fatality rates below 1%.

Enteroviruses are suspected to be responsible for some chronic diseases such as type 1 diabetes (CV-B3 and others) and for most of them no vaccines exist so far.

Our research activity focused on the preparation of AuNP coated with different small sulfonated molecules and biological molecules that act as potential inhibitors toward Dengue virus (DENV) and/or enteroviruses.

AuNPs with long ligands have shown promising activity not only as broad spectrum antiviral but also as virucidal drug (destruction of the virus) rather as virustatic agents (reversible binding to the viral capsid). Most of our AuNP have been produced by using a green chemistry approach, a simple, fast, and low-cost method to pro-

duce highly biocompatible AuNPs. Moreover, we produced antiviral nanoparticles with different types of sulfonated ligands varying in length and complexity in order to promote strong multivalent binding with the Dengue virus.

We found highly efficient virustatic nanoparticles for Dengue and virucidal nanoparticles for enterovirus CVB3. The low toxicity and the high efficacy makes the novel nanoparticle approach for viral diseases a versatile platform for broad spectrum attachment targeting antivirals.

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## DEVELOPMENT OF SELF-DEGRADABLE LIPID-LIKE MATERIAL EQUIPPED WITH ENVIRONMENT SENSING UNITS

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An in-vitro transcribed messenger RNA (mRNA) is now becoming one of the most powerful tools for introducing a desired protein to cells and organisms. We previously developed a lipid-like material (COATSOME<sup>®</sup> SS-series) for the successful cytoplasmic delivery of the mRNA. COATSOME<sup>®</sup> SS-series contain two environment sensing units; tertiary amines for endosomal escape and a disulfide bonding for cytoplasmic collapse<sup>[1,2]</sup>. In this study, we further improved the cytoplasmic release of the mRNA from the lipid nanoparticles by modifying the structure of COATSOME<sup>®</sup> SS-series.

We have recently discovered that an enrichment of hydrophobic thiols in the particle under reducing environments induces an intraparticle hydrolysis reaction. In this study, we introduced a hydrolysable phenyl ester group to COATSOME<sup>®</sup> SS-OP (Fig. 1) as a self-degradation unit.

The self-degradation reaction was confirmed using high performance liquid chromatography, liquid chromatography–mass spectrometry, and nuclear magnetic resonance spectroscopy. Transmission electron cryomicroscopy observation revealed that the mRNA-encapsulating particle has a spherical and homogenous morphology. The COATSOME<sup>®</sup> SS-OP showed potent gene expressions in both in-vitro and in-vivo assays.

## IN VIVO ERYTHROPOIETIN-ENCODING MRNA DELIVERY TO LIVER

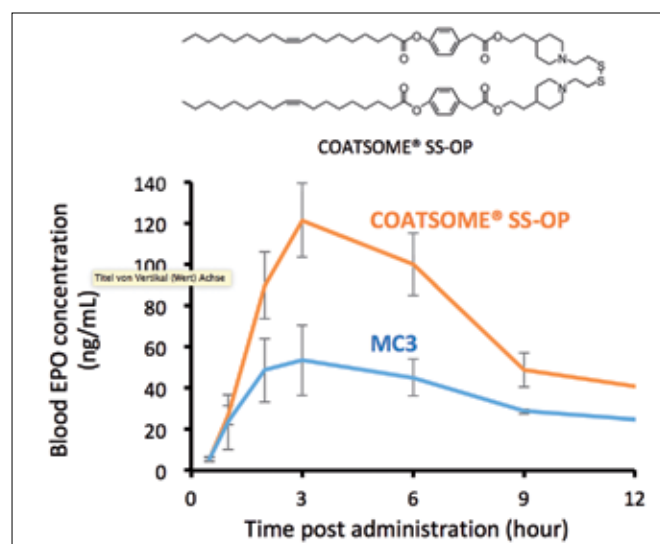


Figure 1. Erythropoietin (EPO) concentrations after delivery of 0.05 mg/kg EPO mRNA using DLin-MC3-DMA<sup>[3]</sup> or COATSOME<sup>®</sup> SS-OP based LNPs, i.v. injection into a mouse tail. Data points are presented as the mean  $\pm$  SD of experiments ( $n = 3-4$ ), normalized to those obtained with nontreated group.

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## EMULSOME-BASED TARGETED DELIVERY OF ANTI-LEISHMANIAL COMPOUNDS TO MACROPHAGES

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**Introduction:** Leishmaniasis is a neglected tropical diseases, caused by the protozoan parasites of the genus *Leishmania* that affect numerous species including humans, and transmitted by the bite of phlebotomine sandflies. Leishmaniasis is endemic in over 98 countries year, including Turkey and its subcontinental region, i.e. Southern Europe, the Middle East and North Africa, with more than 350 million people at risk as estimated that 1.3. million new cases of leishmaniasis occur every year. The spread of the Leishmaniasis is mostly caused by movement of populations that expose non-immune people to transmission and also are assumed to be important cases in travellers and migrants.

Similar to other parasitic diseases, chemotherapy is the most efficient strategy for leishmaniasis. However, the high toxicity of many antiparasitic compounds restricts their utility, and the emergence of drug resistant strains often impairs the lifespan of a given drug.



Among alternative drug candidates, bisnaphthalimidopropyl (BNIP) derivatives have been recently shown to have anti-leishmanial activities, which even surpass the standard and most common amphotericin B therapy. However BNIP derivatives have some drawbacks including low aqueous solubility and toxicity [1].

In addition to alternative drug candidates, curcumin is a natural hydrophobic polyphenol, obtained from the rhizome *Curcuma longa* (turmeric) herb, is known to have anti-parasitic property. However, in spite of the promising antiparasitic activity of curcumin, the clinical use of curcumin remain largely limited mainly due to its extremely poor water solubility and low bioavailability following oral administration and low stability in human metabolism, as well as its rapid metabolism and systemic elimination [2].

Addressing these limitations, this study applies the nanocarrier-based drug delivery technology approach together with targeted drug delivery approach. The former approach will focus on both BNIP1 and Curcumin that have higher efficacy and bioavailability, whereas the latter will be used to deliver the drug specifically to the parasite, thereby decreasing the side effects of the chemotherapy, in particular on macrophages.

The delivery of BNIP derivatives and Curcumin into the macrophages will be achieved by encapsulating the active molecule in a lipid-based nanocarrier system, so-called tripalmitin-based emulsomes with average diameter around 300 nm [3]. Emulsome is preferred mainly because of its four major features. Firstly, owing a solid lipid core like the solid lipid nanoparticles, emulsome may offer high loading capacities for hydrophobic substances such as BNIP [3,4]. Secondly, composed of only lipids and in the absence of any surfactants, emulsome is highly biocompatible [4]. Thirdly, the solid character of the nanocarrier provides a prolonged drug release profile, which can be controlled, or tuned, by the selection of the lipid composition as well as by surface modifications [5]. Lastly, but most importantly, the natural feature of lipids allows emulsome to accumulate in the organs of the reticuloendothelial system (RES) instead of the kidney, which will not only largely reduce toxicity, but will also improve the anti-leishmaniasis efficacy of the loaded drug, as parasites are also located in the organs of RES.

The development of the emulsome-BNIP1 and emulsome-Curcumin nanoformulations facilitating the targeted delivery to the macrophages is expected to substantially contribute to the improvements in treating parasitic disease Leishmaniasis in European region as well as worldwide.

**Methods:** CurcuEmulsome and BNIP1-Emulsome formulations have been synthesized by the same procedure as described before with slight modifications [3]. Briefly, rotary evaporation technique was used where lipids, i.e. tripalmitin, dipalmitoylphosphatidylcholine and cholesterol, together with curcumin/BNIP1 were first dissolved in organic solvent, i.e. chloroform. Solvent was completely removed and dry lipid film was rehydrated with an aqueous solution. Ultrasonication bath at 70°C replaced the final extrusion step [3] to homogenize the particle size. The formulations were analyzed in Zetasizer (Zetasizer Nano ZS, Malvern Instruments Ltd, UK) for their particle size distribution (dynamic light scattering; DLS) and zeta potential characteristics (Phase Analysis Light Scattering; M3-PALS). The shape and the integrity were analyzed under Scanning Electron Microscopy (Supra VP35 Carl Zeiss, Jena, Germany). Curcumin and BNIP1 encapsulation were quantified through absorbance analysis at 430 nm and 330 nm, respectively [4,5,6]. Cytotoxicity of CurcuEmulsomes and BNIP1-Emulsomes on *Leishmania* parasites were examined by Alamar Blue cell viability assay (Resazurin sodium salt (R7017), Sigma-Aldrich, Turkey). Cell viability of *Leishmania*-infected macrophages were investigated using flow cytometer (Becton Dickinson (BD), US).

**Results:** An increased in size of emulsomes were observed upon encapsulation of Curcumin and BNIP1. A delay in response time was attributed to the gradual release of curcumin from the solid

tripalmitin core of the nanocarrier inside the cell. Curcuemulsome and BNIP1-emulsomes were indicated to be effective against *Leishmania* parasites and demonstrated the prolonged therapeutic effect which is comparable to free curcumin and BNIP1, as assessed by cell viability studies (Figure 1). As the cell culture studies investigated, CurcuEmulsomes and BNIP1-emulsomes have lower the half maximal inhibitory concentration (IC<sub>50</sub>) values against *Leishmania* parasites than that of free form of the compounds (Table 1). The formulations displays no significant toxicity, as testified on healthy macrophages. The efficacy of emulsome formulations on *Leishmania*-infected macrophages will be investigated in on-going studies.

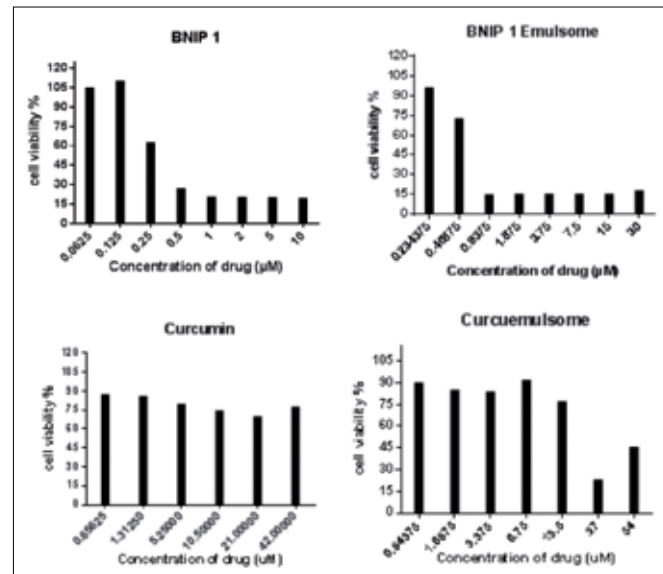


Figure 1. Viability assay of *L. infantum* parasites treated with BNIP1, BNIP1-emulsome, Curcumin and Curcuemulsome for 72 h.

<sup>a</sup>. Viability determined by Alamar Blue assay. The results were obtained after treatment of the promastigote form of the parasite with BNIP1, BNIP1-Emulsome, Curcumin and CurcuEmulsome at various concentrations (0.5 - 10 µM) after 72 h of incubation.

Table 1. The half maximal inhibitory concentration (IC<sub>50</sub>) of BNIP1 and Curcumin as compared to the value of BNIP1-emulsome and CurcuEmulsome. (IC<sub>50</sub> values for 72-hour treatment of *Leishmania infantum* promastigotes)

Compound *	IC <sub>50</sub> (µM) 72 h
<b>BNIP1</b>	0.84 ± 0.03
<b>BNIP1-Emulsome</b>	0.59 ± 0.03
<b>Curcumin</b>	29 ± 0.11
<b>CurcuEmulsome</b>	25.64 ± 0.19

**Conclusion:** In conclusion, the promising efficacy of CurcuEmulsomes and BNIP1 emulsome on *in vitro* model highlights the potential of the system as a new approach for the delivery of lipophilic drugs. Curcuemulsome and BNIP1 emulsomes were found to be effective against *Leishmania infantum* promastigotes. When Curcumin and BNIP1 were used as CurcuEmulsomes and BNIP1 emulsome, it was more efficient against *Leishmania* parasites resulted from the effects of enhanced bioavailability of the Curcuemulsome and BNIP1-Emulsome in certain ratios. As the results demonstrated, CurcuEmulsome and BNIP1-Emulsome have lower IC<sub>50</sub> values on *Leishmania* parasites. High degree of compatibility, prolonged release profile and tailoring properties feature CurcuEmulsomes and BNIP1-Emulsome for further therapeutic applications of the leishmaniasis *in vivo* and will be the focus of our future studies.

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## MAGNETIC TISSUE ENGINEERING (MTE) USING SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES IN HUMAN VOCAL FOLD CELLS – BASIC STUDIES FOR TRANSLATIONAL PERSPECTIVES

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### INTRODUCTION

Until now, there is no successful approach for functionally regenerating the vocal fold (VF). As the voice is the most important instrument of communication, losing one's ability to speak, due to tissue loss at the VF, leads to serious impairment in the quality of life in a significant number of patients.

Briefly, the voice is produced by vibrations of the VFs via an air-stream from the lungs. In order to function properly, the VF tissue requires a complex interplay between the epithelial cells and fibroblasts, which are responsible for the unique VF lamina propria production. Conventional tissue engineering methods for the VF are so far ineffective. Thus, techniques to manipulate and remotely control cells and their behaviour can deliver a powerful tool for regenerative medicine. Such a method is Magnetic Tissue Engineering (MTE), with the basic concept of magnetic nanoparticle uptake by cells, which can be subsequently controlled with a magnetic field.

### METHODS

The innovative approach of MTE provides a fast and precise method for generating functional multi-layered cell-constructs. Superparamagnetic iron oxide nanoparticles (SPIONs) are used to magnetize cells, which can then be manipulated and remotely controlled with an external magnetic field. After we established MTE as proof of concept in the rabbit model, we were able to translate this method in a human model. Therefore, we used human cadaver larynxes provided from the Institute of Legal Medicine in Munich, with a post mortem time no longer than 48 h, and were able to isolate liv-

ing cells from the human vocal fold (hVF). Both, fibroblasts and epithelial cells could be characterized and were used for MTE. Same as in the rabbit model, human cells took up 20 µg/cm<sup>2</sup> SPIONs, which are hence coated with human serum albumin (HAS), for 48 h and were used for MTE. After the cell-constructs are generated by MTE for 24 h, 3D-epithelial cell- and fibroblast constructs were merged to form a functional multi-layered VF cell-construct.

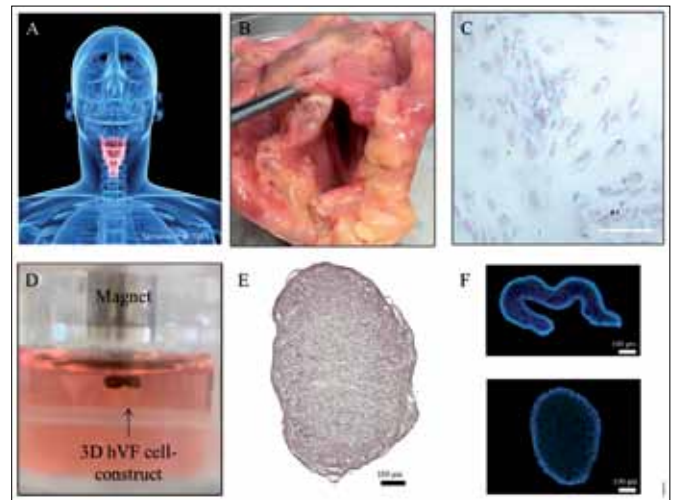
### RESULTS AND CONCLUSION

The biocompatibility of SPIONs coated with HSA was proven to be significantly better, compared to BSA coated SPIONs in hVF cells for higher concentrations (up to 320 µg/cm<sup>2</sup>). After we established the best ratio between SPION uptake, good cell-magnetisation in both, epithelial cells and fibroblasts, we were able to generate multi-layered 3D VF cell-constructs (figure 1). Immunohistology using antibodies to visualize epithelial structures and antibodies against extracellular matrix components as well as cell-cell interaction proteins identified the maturity of the 3D hVF cell-construct. Therefore, using human VF fibroblasts and epithelial cells is a successful onset for the translation of this established method into clinics.

This is particularly important for patients, who suffer from dysphonia as a consequence of a VF tissue defects, and will help to improve their quality of life.

Transplantation of the 3D multi-layered VF cell-constructs into rabbit and human larynxes, imaging as well as functional and structural analysis will give further insights about the successful realisation and efficiency of this technique.

**Acknowledgments:** Deutsche Krebshilfe Nr.111332, Manfred-Roth Stiftung (Fürth, Germany)



**Figure 1: Magnetic Tissue Engineering (MTE):** After isolation and characterization of vocal fold (VF)-cells from a human larynx (A and B), cells are analyzed for best SPION<sup>HSA</sup> uptake conditions (C). 20 µg/cm<sup>2</sup> SPIONs are sufficient to generate a 3D human CF cell-construct (D). For characterization these constructs are fixed and cryosections for immunohistological staining (E and F).

A) Location of the human larynx (red). B) Human cadaver larynx (PMI 46 h) view from cranial. C) Light microscopic picture of hVF fibroblasts; red: nuclei; blue: SPIONs stained with Prussian Blue for iron. D) MTE of human vocal fold cells (hVF). A magnet (5 mm in diameter) with the magnetic remanescence of 1.28 T was used to generate a 3D hVF cell-construct in a 24 well plate. E) Elastic van Gilsen staining exhibited elastic fibers on the edges of the construct (nuclei: black; collagen: red; elastic fibers: violet). F) Cryosections of 3D hVF cell-construct stained with Hoechst 33142 for nuclei displays different viewpoints depending on intersecting plane of constructs (terete, when cut laterally, or oval shaped, when cut apically). Scale bar: 100 µm.

## A SUPRAMOLECULAR PLATFORM FOR NANO-SCALED MULTIFUNCTIONAL ANTITUMOR VACCINES

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Nanomedicine has attracted enormous interest in the recent years. These nano-sized entities can incorporate multiple functionalities enabling the controlled interaction with biological systems which renders them a promising class of materials. Peptides are ubiquitous macromolecules in biological systems where they are involved in the control and modulation of various processes. Artificial cognates can exhibit unique pharmacological properties which render them highly interesting for biomedical applications. The function of polypeptides is closely related to their structure which originates from the chemical properties of the primary sequence. Knowledge of favorable morphologies such as  $\alpha$ -helices or  $\beta$ -sheets can be harnessed to design monomers capable of self-assembling in a defined way into large, nano-scaled supramolecular structures in aqueous media.<sup>[1,2]</sup> Modulating the surface by decoration with immunogenic molecular patterns results in pathogen-mimicking entities and potential vaccine candidates.

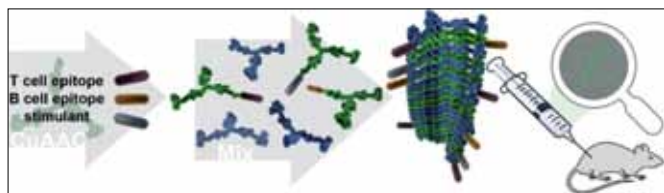


Figure 1: Pathogen-mimicking, self-assembled peptide supramolecules in aqueous solution. The modular platform gives full control with respect to antigen presentation. Structural Monomer (SM) in blue, functional monomer (FM) in green.

In the scenario of antitumor vaccines, the challenge is to overcome self-tolerance mechanisms to enforce an immune response against endogenous, tumor-associated structures. Different approaches can be pursued. Antibody dependent cell-mediated cytotoxicity focusses on the induction of a humoral immune response which involves an activation and maturation of B lymphocytes to antibody secreting plasma cells. To achieve this, a co-stimulation of the B cells by Th cells is mandatory. From this relationship the requirement of a co-presentation of different epitopes and immunostimulating agents in a vaccine candidate arises. Mimicking a pathogen-like structure which features different epitopes on the surface requires an elaborate molecular design. We aimed for an anisotropic rod-like morphology using a structural monomer (SM) with a three-armed star shape was employed. This scaffold consists of a 1,3,5-triazine core which bears three amphiphilic ethylene glycol (EG) modified tri-L-phenylalanine (F3) on the 2,4,6 positions as the central motif. This monomer can be mixed with structurally related functional monomer (FM) carrying the immunogenic structures. The latter are conjugated via CuAAC to FM through an alkyne terminated F3 sequence, instead of EG in SM.

A fully synthetic MUC1 derived glycopeptide, consisting of 22 amino acids bearing the Tn and 2,3-Sialyl T tumor associated antigens was used as B cell epitope.<sup>[3,4]</sup> As T cell epitope p30, a small fragment (AA947-967) from highly immunogenic tetanus toxin was chosen. Additionally, an imidazoquinoline, as potent TLR7/8 agonist,<sup>[5]</sup> was coupled to FM. The aforementioned three functional and one structural monomer can be readily mixed in water to yield an injectable formulation. The resulting vaccines were intraperitoneally administered into C57BL/6 mice and the antisera collected after 3 boosts. High antibody titers of the IgG type were observed

in all mice, determined by ELISA. Furthermore, FACS analysis confirmed the high binding affinity of the generated antibodies to T47D tumor cells. These results highly encourage further investigations and highlight the potential of this modular supramolecular platform approach.

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The **BioNanoNet Forschungsgesellschaft mbH** is European key player in the field of nanosafety, specialised in developing nano-safety-by-design strategies together with researchers and industry, and coordinates the national technology platforms **NanoMedicine-Austria** and **SusChem-AT**.

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## NANO MEDICINE AUSTRIA

Bio- and nanomedicine opens up fascinating new chances for medical applications by offering novel methods for drug delivery systems, imaging techniques, diagnostic tools, etc. The diversity of these topics encourages interdisciplinary expertise and connects a variety of scientific fields. The use of smallest particles in the nanometer range enables new methods for improved treatment of numerous diseases. Through Austria's excellent scientific expertise in these fields, it has also high economic potential.

In 2015, BioNanoNet founded the Austrian platform **NanoMedicine-Austria** to ensure Austria a leading position in this promising and economically highly interesting field. The aim of this platform is to bring together Austrian bio- and nanomedical experts into one platform in order to create appropriate structures to promote science and research and to strengthen the scientific and business location Austria sustainably.

We kindly invite you to become part of NanoMedicine-Austria contributing your relevant expertise in the platform; if you are interested, please contact [office@nanomedicine-austria.at](mailto:office@nanomedicine-austria.at).



## Biomedical Research Networking Center (CIBER) on Bioengineering, Biomaterials and Nanomedicine

The **Biomedical Research Networking Center (CIBER)** is a public research consortium created in 2006 under the leadership of the Carlos III Health Institute (ISCIII, the main public research entity responsible of funding, managing and carrying out biomedical research in Spain), to promote research excellence and build a critical mass of researchers in the field of Biomedicine and Health Sciences. CIBER-BBN is one of eleven thematic areas of the consortium, specialised in bioengineering, biomaterials and nanomedicine being **CIBER the largest biomedical research network in Spain**.

The **CIBER-BBN** ([www.ciber-bbn.es](http://www.ciber-bbn.es)) is the research area devoted to the study of Bioengineering, Biomaterials and Nanomedicine. Organized in three research fields: i) **Bioengineering & Medical Imaging** includes research groups devoted to the studies of Multimodal Diagnosis and Intelligent Devices & Biomedical Systems, ii) **the area of Biomaterials & Advanced Therapies** develop research on Cell and Gene Therapy, Tissue Engineering and Prostheses and Implants, while iii) **the area of Nanomedicine** involves groups devoted to the study of Nanodiagnostics, Therapeutic Nanosystems and NanoSafety. CIBER-BBN scientific strategy uses the Research programme as an instrument to set out the main research lines to be developed by the groups, the medical specialities to be focused on and major scientific challenges to be undertaken in the different research areas. The BBN Research programme is mainly focused on:



CIBER-BBN employs nearly 100 researchers directly hired by CIBER and more than 350 associated researchers who conduct their research at any of the **46 participating groups**. Its purpose is to conduct translational research and to transfer the results to industry. CIBER-BBN also manages a Research Infrastructure (RI) in Biomedicine which is constituted by clusters of high-tech equipment, which has been recognized as a Singular Scientific-Technological Infrastructure (ICTS) by the Spanish Government ([www.nanbiosis.es](http://www.nanbiosis.es)).



European Foundation for Clinical Nanomedicine

**CLINAM was founded and registered 2007 in Switzerland by Beat Löffler and Patrick Hunziker.**

**Its goal is to contribute to patients and society by:**

- uniting the global community of nanomedicine and targeted medicine
- performing nanomedical and clinical research and promoting its clinical applications
- setting nanomedicine into the broad context of related medical procedures, technologies and therapeutic trends
- promoting and supporting the global transfer of knowledge
- networking in nanomedicine and targeted medicine by international summits, national conferences, summer-schools and seminars
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# Deutsche Plattform NanoBioMedizin

Trotz immenser Fortschritte in der modernen Medizin besteht immer noch großer Bedarf an neuen diagnostischen und therapeutischen Innovationen, um Krankheiten früher und gezielter diagnostizieren sowie besser und kostengünstiger therapieren zu können.

Eine Schlüsseltechnologie auf diesem Weg ist die Nanotechnologie, weil sie einerseits die Instrumente für eine noch detailliertere Analyse und ein daraus resultierendes besseres Verständnis der Krankheitsursachen und -abläufe liefert, und andererseits die Materialien zur Verfügung stellt, Krankheiten gezielter behandeln zu können.

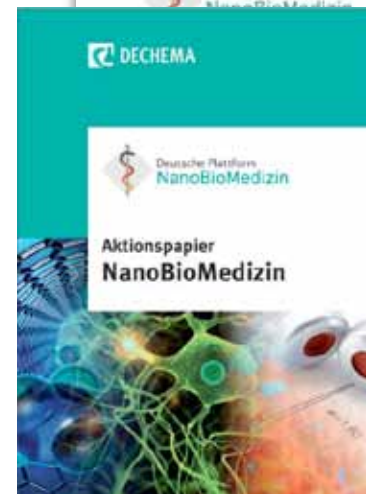
**Megatrends wie Personalisierte oder Regenerative Medizin sind ohne nanotechnologische Lösungen nicht zu realisieren.**

Die Integration der Nanotechnologie in biomedizinische Anwendungen, kurz NanoBioMedizin genannt, erfordert allerdings neue **Forschungsdisziplin-übergreifende Konzepte** und **Wertschöpfungsketten**. Dazu müssen Technologieentwickler aus den Ingenieurwissenschaften, Chemie, Biologie und Physik mit medizinischen Anwendern wie Pharmazeuten, Klinikern und Industrieunternehmen zusammenarbeiten, um am Ende bedarfsgerechte Innovationen für Patienten auf den Markt bringen zu können.

Als Rahmen für den erforderlichen Dialog all dieser Akteure ist **die Deutsche Plattform NanoBioMedizin gegründet worden**. Das Ziel ist **eine integrierte Forschungsförderung von der Nanomaterialentwicklung bis zur Klinik**, mit der das zweifellos vorhandene wissenschaftliche und wirtschaftliche Potenzial in der deutschen NanoBioMedizin durch nachhaltige Forschungsthemen und Strukturen weiterentwickelt und **eine Spitzenposition Deutschlands in dieser Zukunftstechnologie gefestigt** werden kann.

## Aktivitäten

- **Netzwerk- und Informationsveranstaltungen**
- **Plattform-Webseite** mit aktuellen Bekanntmachungen und Veranstaltungshinweisen (<http://www.dp-nbm.de>)
- **Diskussion mit Fördermittelgebern** und der **Fachcommunity**, **Ihre Mitwirkung ist sehr willkommen!**



**Sprecher** der Deutschen Plattform NanoBioMedizin: **PD Dr. Klaus Michael Weltring**, Gesellschaft für Bioanalytik Münster e. V., Münster

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# Engaging Publics on Nanomedicine with a Democs Card Game



Donald Bruce, Edinethics Ltd.

## The NanoAthero Project

**NanoAthero** is an EC FP7 medical research project which uses nanotechnology to develop ways to test and treat people for unstable plaques and blood clots, caused by the heart disease atherosclerosis. This is the largest cause of death in Europe. Specially created nanoscale particles, small enough to enter body cells, locate affected cells in the heart, which can be seen on MRI or CT scans. Other nanoparticles are made to encapsulate medicines to deliver them directly to the damaged cells. It is an example of how nanotechnologies are becoming used in practical medicine, which is changing how people experience health care: moving towards a more predictive medicine, rapid point-of-care diagnostics, targeted and more 'personalised' medicines, remote monitoring of vulnerable patients.

## Nanomedicine & Publics

Nanotechnology is unfamiliar to many people. How safe are these tiny nano devices in my body? How much advanced warning do I want about my future health? Is it empowering me or just giving extra stress for my family? And who gets to see my data? The NanoAthero project assesses ethical aspects as an integral part of its work. We are seeking to enter into dialogue with members of the public and patients. We want to know how they respond to the potential of nanomedicine, and we have devised a Democs card game to help us do this.

## Democs : Engaging with people with cards

A Democs card game is a discussion method for groups of 6-8 people, using sets of cards specially written to provide basic facts and explore the ethical and social issues of a new technology. It needs no previous expertise: the 'expert' is the cards. The aim is to promote informed, open and balanced discussion to enable people come to their own opinions.



**We are looking for people who would like to host this Democs game with friends, neighbours, colleagues, patients, etc.**

**The game is available in English, French, Dutch and German and is available free, as a boxed set or downloadable.**

**Come to this stand at coffee breaks or lunch time during Clinam and see for yourself how a game works.**

**Dr Donald Bruce Edinethics Ltd**  
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Email [info@edinethics.co.uk](mailto:info@edinethics.co.uk)  
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## What is Democs?

for small groups  
anywhere ... any time ... any people



All you need is the box of cards and instructions, a table, some friends, and about 1½ hour to play. A person sends off for the kit (free), or downloads and prints it, reads the instructions, to be able to act as 'dealer', invites people round, and you begin.

Three types of cards are dealt to the players.

**Story Cards** - imaginary stories about people who are involved in nanomedicine or affected by it, based on real situations or that might happen in future, often presenting an ethical dilemma. Each person gets a different card and reads it aloud to the group.

b). **Information Cards** - essential information to explain the basic technology. Everyone gets a hand of cards. When their turn comes, each player selects 2 cards, reads them out loud and says why the cards interest them. Then do the same thing with the Issue Cards:

c). **Issue Cards** - explore ethical and social implications, to cover a fair range of opinions and attitudes known or likely to exist.

**Clustering and Voting** - As discussion progresses, the group is asked to crystallise its ideas into 3-4 statements which people would like to make as a group, clustering the cards, and working for consensus as far as possible. Then each person votes as an individual on the acceptability (or not) of applications stating their reasons if they wish to. The results are then sent for analysis.

*The Democs concept was devised by Perry Walker in 2001 and many games have been created on subjects from cloning to climate change, played with a wide range of publics and languages. It is also a useful pedagogical tool for students 16+ years, and for training science researchers about ethical issues.*

# ESNAM

**The European Society for Nanomedicine** aims to promote the research and the application of nanomedicine and its implication for humanity and for the environment, keeping focus on the welfare of individuals and society.

**ESNAM concentrates** its efforts in facilitating the exchange between its members. It also represents the clinical nanomedical community to governmental and international boards as well as to associations and industry oriented networks.

**Society members** represent different sectors of the scientific community: clinicians, biologists, chemists, medical doctors, pharmacists and generally all stakeholders in Nanomedicine.

The society has currently more than 800 members. ESNAM contributes to the dissemination of nanomedicine and related fields and strengthens the improvement of clinical research.

**The technical office** of the ESNAM is located in Barcelona in the Vall d'Hebron Hospital, under the presidency of Dr. Simó Schwartz who aims at increasing the number of members and to promote cooperation and interaction between them so that new European international projects arise. He also strives to increase the list of sponsors that allow the promotion of activities of the nonprofit society, establishing courses specialized in nanomedicine and nanotechnology or workshops.

**The office address of ESNAM** is CIBBIM-Nanomedicine Passeig Vall d'Hebron 119, Barcelona 08035, phone +34-489 4055





FOUNDED 1996

**THE CHALLENGE:**

- LOTS OF SCIENCE, LITTLE BUSINESS KNOWHOW
- CAPITAL INTENSIVE AND LONG DURATION OF DEVELOPMENT TO MARKET
- DIFFICULT FINANCING IN EARLY PHASE
- TOO SMALL FOR VENTURE CAPITAL
- TOO EARLY FOR BIG PHARMA OR MEDTECH CORPORATE FUNDS
- NO LEAD INVESTOR FOR PRIVATE STOCKHOLDERS / BUSINESS ANGELS

**THE SOLUTION:**

WHEN EVA SELECTS COMPANIES, WE PARTNER WITH THEM RIGHT FROM THE START. WE HELP TO COMMERCIALIZE LIFE SCIENCES PROJECTS, TECHNOLOGY AND INNOVATIVE IDEAS WITH INDIVIDUALIZED COACHING, SEED MONEY INVESTMENTS AND PROVIDE A VAST INDUSTRIAL AND FINANCIAL NETWORK.

THIS MIX OF OFFERINGS ALSO DIFFERENTIATES US FROM OTHER PLAYERS, BE THEY CORPORATE OR PUBLIC.

OUR BOARD OF DIRECTORS, CEO AND NETWORK BRING TOGETHER PROFOUND KNOWLEDGE IN LIFE SCIENCES AND FINANCE.

WE ACTIVELY SUPPORT THE CREATION OF NEW COMPANIES BY ADVISING ENTREPRENEURS-TO-BE ON ALL GENERAL MATTERS, BE IT LEGAL, FINANCE, PRODUCT DEVELOPMENT, HR, SUPPLY CHAIN ETC. AND CONNECTING THEM ALSO TO SPECIALISTS IN THE AREA, IF NEEDED.

IN THE COMPANIES WE SELECT, WE INVEST INTO EQUITY OR GIVE CONVERTIBLE LOANS OCCASIONALLY. WE ALSO HELP IN THE SEARCH FOR ADDITIONAL INVESTORS IN CURRENT AND FUTURE ROUNDS. EVA TAKES A BOARD SEAT INITIALLY TO ENSURE ONGOING COACHING OF THE FOUNDING TEAM.

**THE TRANSLATION AND TRANSFORMATION OF GOOD SCIENCE INTO GOOD BUSINESS IS AT THE HEART OF ERFINDUNGSVERWERTUNG AG (EVA). IT SUPPORTS AND INVESTS INTO LIFE SCIENCE STARTUPS IN NORTHWESTERN SWITZERLAND PRIMARILY.**

STARTED AS A VISIONARY PROJECT BY THE CANTONAL BANKS OF BASEL-STADT (BKB) AND BASELLANDSCHAFT (BLKB), AND THE THREE ENTREPRENEURS MAAG, GROGG AND ENDRESS.

34 COMPANIES SUPPORTED TO DATE  
22 COMPANIES STILL IN BUSINESS  
14 COMPANIES IN PORTFOLIO  
9 BOARD OR OBSERVER SEATS  
CREATED OVER 200 JOBS IN HIGH-TECH  
MOST KNOWN EXIT: MEDGATE

ERFINDUNGSVERWERTUNG AG  
HOCHBERGERSTRASSE 60C  
CH-4057 BASEL  
SWITZERLAND

**CEO: DR. WERNER M. ENZ**  
  
E-MAIL: WERNER.ENZ@EVA-BASEL.CH  
INTERNET: WWW.EVA-BASEL.CH

**SELECTION OF COMPANIES**

SCIENCE BASED AND IP PROTECTED INNOVATIVE BUSINESS IDEAS IN LIFE SCIENCES SUCH AS E.G. NEW DRUGS, MEDICAL DEVICES, DIGITAL HEALTH, IMAGING, NANOTECHNOLOGY.

THE STARTUP NEEDS A STRONG & POLYVALENT TEAM WITH DEDICATION, PERSEVERANCE, THE WILL TO WIN AND OVERCOME RESISTANCE.





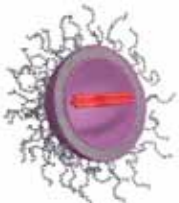
# Innovative Nanomedicine for Targeted Therapy

## First cancer therapy shortly before admission to the clinic, Parkinson's drug new in the pipeline

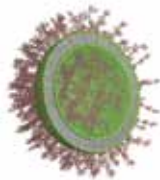
Despite major advances in medicine, there is still high unmet medical need in cardiovascular diseases, central nervous system disorders, and cancer. Although many potent molecularly targeted drugs are available, they often do not reach the disease-specific tissue in the body in sufficient quantities and therefore cannot exert their full medical potential. For instance in chemotherapy often less than one percent of the drug load reaches the actual tumor tissue. The problem with developing successful therapeutic approaches is therefore not a lack of potent drugs, but rather their biological distribution within the body.

InnoMedica has successfully developed a means of transport for drugs that mimics the natural transport system of the body. Similarly as cells package molecules into phospholipid bilayer microvesicles, InnoMedica packages pharmaceutical agents into a biocompatible phospholipid nanoparticle - in a so-called liposome. The ultrasmall liposomes of 30 nanometers in diameter are manufactured in a proprietary process that enables beneficial drug biodistributions in a multitude of medical applications. This facilitates treatments, increases therapeutic effect, and reduces unwanted side effects.

InnoMedica is pioneering the field with its own clinical translation of TLD-1/Talidox and targets a chemotherapeutic agent to tumors. In neurology, InnoMedica has successfully developed TLGM-1/Talineuren, a novel nanodrug for the treatment of Parkinson's disease.



Talidox liposome: Doxorubicin is packaged into a liposome, thereby shielding the drug from premature reactivity in the blood stream and enabling transport and uptake to tumor tissue.



Talineuren liposome: GM1 is spiked into the membrane of a liposome, thereby GM1 is protected from excessive early degradation and is transported to the brain, even after oral intake. GM1 plays an important role in the regulation and function of nerve cells.

### InnoMedica

Patent-protected nanotechnology platform: Therapeutic agent reaches the target site in the body thanks to liposomal packaging.

Initial development: Talidox addresses a large medical need for chemotherapy with a better efficacy/side effect profile.

- Preclinical phase: Talidox delivers up to four times more active substance to the tumor. Thus, Talidox works better than conventional chemotherapy and reduces stressful side effects.

- Clinical study in Swiss hospitals: The application dossier for the Phase I clinical trial with Talidox was submitted to the SAKK at the end of March 2018.

- Registration: Simplified admission for Talidox was assured by Swissmedic thanks to well-known active substance.

Second development: Talineuren is to stop the progression of Parkinson's disease with a regenerative active substance.

Production: InnoMedica has its own GMP production facility certified by Swissmedic.

### Cooperations

Many active substances that are used in medicine today could be improved in their effect and side effect profile by liposomal packaging. In addition to InnoMedica's own clinical explorations, further new formulations could be developed and produced in the GMP-certified production facilities thanks to the broad applicability and excellent biocompatibility of the liposomal technology platform. We offer our expertise in liposomal formulations and in-vivo drug targeting through strategic partnerships.

## Overview of InnoMedica's pipeline projects

Product / Application	Preclinical development				Clinical studies	
	Discovery	Development	Animal studies	Toxicity and safety	Phase I	Phase II
Talidox (TLD-1) - Oncology	[Progress bar showing completion through Discovery, Development, Animal studies, and Toxicity and safety]					
Talineuren (TLGM-1) - Parkinson's disease	[Progress bar showing completion through Discovery, Development, and Animal studies]					
TLTX-1 - Oncology	[Progress bar showing completion through Discovery and Development]					
TLNIR-1 - Oncology / diagnostics	[Progress bar showing completion through Discovery and Development]					
TLFR-1 - Arteriosclerosis	[Progress bar showing completion through Discovery and Development]					
TLTS-1 - Bacterial infection	[Progress bar showing completion through Discovery and Development]					

## **Uniting the Worldwide Societies in Nanomedicine**

The International Society for Nanomedicine was founded in 2009 to bring together all at that time developing societies and platforms from all continents related to Nanomedicine. First members were the European Foundation for Nanomedicine, the European Society for Nanomedicine, the European Technology Platform on Nanomedicine and the American Society for Nanomedicine. In the years after Societies from Japan, Korea, Australia, Canada, South America, India, China and Australia joined this unique network.

## **Main Aim of the Society**

Let's shape together the transparent network of all globally large Nanomedicine Societies, to cooperate, share and bring forward research, characterization, training in summer schools and seminars, development and achieve great results to the benefit of patients and mankind.

## **Presently Active Members to Shape the Structure**

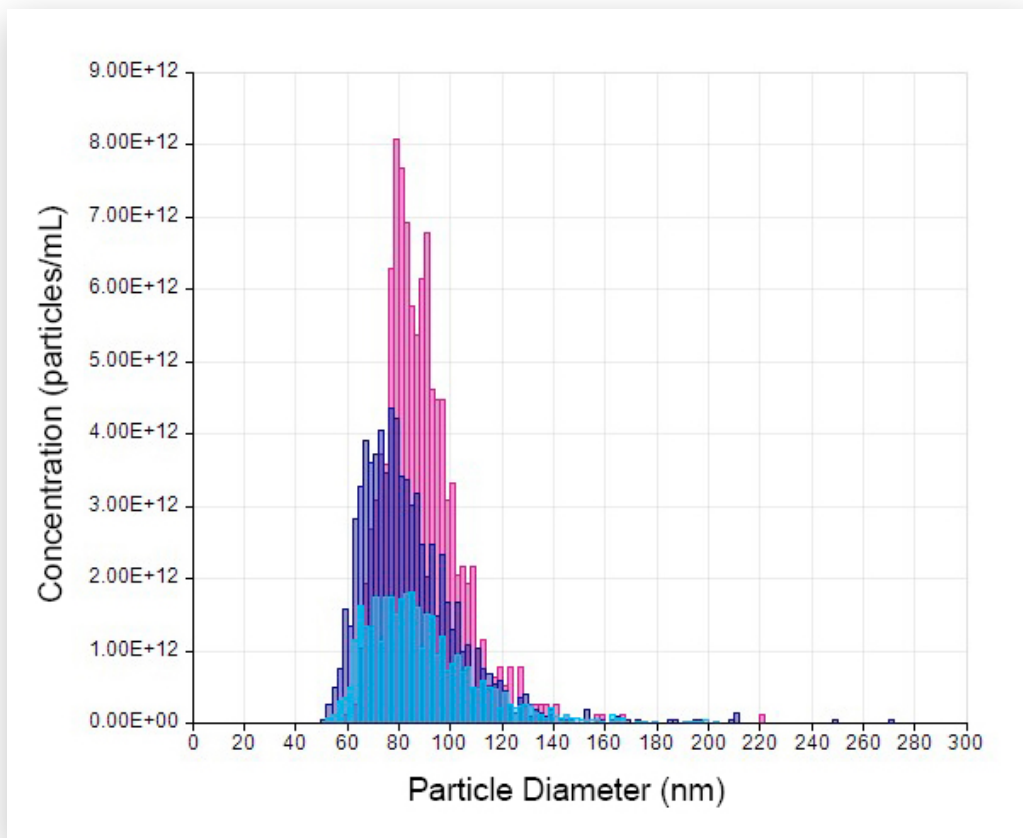
*President:* Prof. Dr. med. Patrick Hunziker (CH), *Vice President:* Dong Soo Lee (ROK). *Board Members:* Prof. Dr. André Nel (USA), Prof. Dr. Eder Lilia Romero (ARG), Prof. Dr. Simo Schwartz (E), Dr. Beat Löffler (CH), Dr. Panagiotis Trohopoulos (GR), Dr. Frank Weichold (USA), Prof. Dr. Moein Moghimi (DK), Prof. Dr. Chezy Barenholz (IL), Prof. Dr. Christoph Alexiou (D), Prof. Dr. Lajos Balogh (USA), Prof. Dr. Keon Kang (ROK), Dr. Georgette Beugelaar (NL), Prof. Dr. Amit Kumar Dinda (IND), Prof. Dr. Marianna Foldvari (CND), Prof. Dr. Hulda Swai (ZA), Prof. Dr. Guangjun Nie (CHN) ...and further members



# New Nanomedicine Regulation Is Coming.

Does your data include concentration measurement and will it meet new size resolution requirements?

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**IZON**  
science

## GMP Phospholipids

- Egg and Soybean Phospholipids
- Hydrogenated Phospholipids
- Monoacyl Phospholipids
- Synthetic Phospholipids
- PEGylated Phospholipids
- Delivery Systems
- Ready for Use Lipid Mixes
- Purified Oils and Fatty Acids
- Phospholipid Reference Standards

## Your Partner from Research up to the Approved Drug

Founded in 1977, Lipoid has gained an outstanding reputation in the development and industrial production of high quality lecithin and phospholipids for the pharmaceutical industry. Today, we are the only company worldwide offering the whole range of natural and synthetic phospholipids in industrial scale. Environmental protection and sustainability determines our strategy within all phases of the value chain. This long-standing expertise, in combination with the exceptional quality of our products, enables our customers to develop and market innovative products for the benefit of the general public.

### Contact Germany

#### Headquarters and Manufacturing

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Frigenstrasse 4  
D-67065 Ludwigshafen  
Phone + 49 (0) 621 538 190  
info@lipoid.com

#### Contact Worldwide

Lipoid AG  
Sennweidstrasse 44  
CH-6312 Steinhausen  
Phone + 41 (0) 41 741 740 8  
info@lipoid.com

**[www.lipoid.com](http://www.lipoid.com)**

## Nacamed – Delivering the green nano carrier system for the future

Nacamed is a Norwegian spin-off company of Dynatec AS. Dynatec has through the 10 last years investigated in the production of Silicon particles and has acquired unique competencies in this area. Nacamed aims to combine these physical competencies with the clinical competencies in the pharmaceutical industry to create a new generation of treatment methods. Our vision is to contribute to human health through a green targeted drug delivery system of Porous Silicon (PSi) nano particles. We have a unique production technology of silicon nano particles using chemical vapor deposition – CVD. This solves previous production issues and enables tailoring of particles with the desired physical attributes. The production costs are substantially decreased compared to previous production methods of Porous Silicon (PSi) nanoparticles, and production capacity can be scaled up to meet future needs.

For more information about Nacamed, see [www.nacamed.com](http://www.nacamed.com)

### The Silicon particles have desired inherent characteristics

- **Biodegradability:** The particle is shown to degrade completely. In in vitro studies, nearly all particle structures were gone after 13 days (E. Tolstik et al, 2016).
- **Excretion from the body:** The particles are transformed into silicic acid, which is excreted through the kidneys.
- **Toxicity:** Both Silicon and silicic acid is naturally present in the body and is believed to be non toxic.

### The physical characteristics of the Silicon particles can be closely controlled

- **Size and shape:** Size and shape of the particle is controlled by process conditions. This results in a narrow particle size distribution, and the possibility to make either spherical or disc shaped particles. Particles have been made in the range 50 nm-1000 nm
- **Zeta potential:** The zeta potential is controlled by surface modification within the reactor or post process, and can be tailored to control the hydrophobic/hydrophilic traits of the particle, or the affinity to certain tissues
- **Coating:** Because of the oxygen free production environment, the particle can be coated with a chosen element to achieve the right surface. For example can the particle be carbonized through the production process. The carbon layer can then be used for covalent bounding to ligands or other molecules.
- **Incorporating of other elements within the particle:** In the production process, other elements can be incorporate in the particle, like metals (e.g. gold (Au)), semiconductors, or other elements with desired characteristics

### The loading and unloading abilities of the particle can be controlled

- **Pore size:** The pore size can be controlled through the etching process
  - A variety of etching procedures to make different pore sizes and geometries
    - Depth
    - Width
    - Frequency
    - Shape (V or O groves)
  - Surface texturing in CVD process may control the secondary etching process
- **Coating to reduce pore openings:** Coating to reduce pore openings can be done in or post reactorprocess

### Contact information:

Christina Westerveld Haug, Chief Executive Officer: [cwh@nacamed.com](mailto:cwh@nacamed.com)

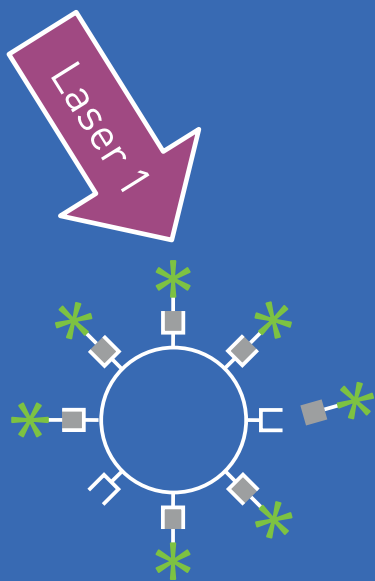
Lars Gunnar Fledsberg, Chief Commercial Officer: [lgf@nacamed.com](mailto:lgf@nacamed.com)



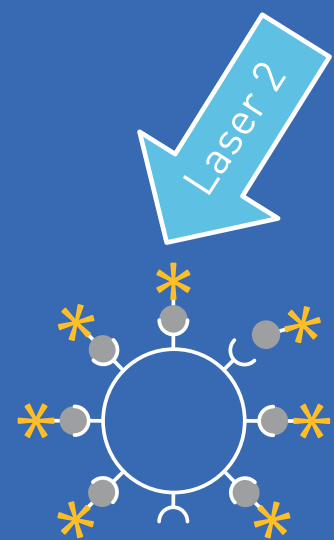
**NEW!**



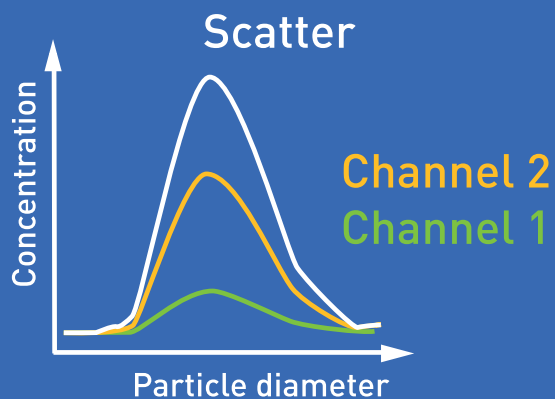
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e.g. CD63



e.g. CD81



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# Liposomal Formulation of Drugs

Polymun has unique know-how and technology for the development and manufacturing of liposomal formulations.

Polymun offers the development of liposomal Formulations for all kinds of pharmaceutically active ingredients such as oligonucleotides, small molecules and proteins as well as vaccine antigens. A broad spectrum of formulation techniques as well as analytical methods have been established for this purpose. Polymun produces GMP material including all necessary documentation for IMPD/IND.

We assist in planning and implementation of clinical trials. Finally, license agreements are offered for the respective substance on an exclusive basis. Contracts can be arranged step by step - proof of concept, in-depth analysis, GMP production, product license - or all in one.

## MAIN CHARACTERISTICS OF OUR TECHNOLOGY

**FULL SCALABILITY**  
The injection module is the heart of the liposome production. The process parameters determine the size of the liposomes regardless of the scale. Production of up to 1,000 liters of liposome preparation takes only 3 hours. This large scale can be achieved by using several injection modules in parallel.

**ASEPTIC PROCESS**  
A closed system is used for production. All components can be added via sterile filtration. Subsequent concentration by crossflow filtration is possible as well.

**HOMOGENEOUS, UNIFORM VESICLES**  
All process parameters are controlled precisely. This results in a very narrow size distribution, necessary for reliable targeting and transport characteristics.

**SINGLE STEP PROCESS**  
Liposome size is adjusted by modulating the process parameters during vesicle formation. No additional downsizing is required.

**EXCELLENT BATCH TO BATCH CONSISTENCY**  
High quality of raw materials and precisely controlled process parameters guarantee excellent reproducibility - essential for pharmaceutical products.

**MILD PROCEDURE - STABILITY**  
The crossflow injection technique is a very mild procedure that allows the processing of sensitive drugs. Together with the high quality of raw materials and narrow size distribution, we achieve long term stability of liposomes even at room temperature.

- SERVICES**
- Formulation Development
  - Analytical Method Development
  - Process Development
  - GMP Production
  - Filling
  - Clinical & Regulatory Support

- CLINICAL & REGULATORY SUPPORT**
- IMPD/IND, IRB Submission
  - Pre-/Clinical Development Concepts
  - Organisation of Clinical Studies
  - Legal Representative
  - Requests for Scientific Advice





## PRECISION NANOMEDICINE

The official Journal of CLINAM



There is an increasing need for scientist-led publishing to share reliable and reproducible knowledge which is translatable into practice.

While many societies hire for-profit publishers to run their media, we, a group of professionals and lead scientists, have decided to create our own non-profit publishing company and launch our own open access journal to ***promote all progressive and rational aspects of nanomedicine while exercising good publishing practices from basic science through commercialization.*** The journal is supported by CLINAM and the International Society for Nanomedicine. The first two issues of *Precision Nanomedicine* appeared in 2018 April and July.

### COMPETITIVE ADVANTAGES:

- The publishing **company is supervised by scientists** and experts
- PRNANO Editors have been **working together in publishing for years;**
- The Editorial Board is formed of **renown international scientists;**
- Authors may submit **original articles, replication studies, and negative results**
- Submissions only incur charges reflecting the actual costs of operating the system (typically \$350–500 for society journals). **Submissions are currently free** due to society support.
- Peer reviews focus on **how to make the manuscripts better**
- **Articles are published as soon as possible** (in 2-4 weeks), depending on how much time peer-review and authors' response requires. (Journal processing time is one week.)
- **Innovative approaches** such as:
  - HTML pages are optimized for computers scanning the web, while freely downloadable PDFs are formatted for human reading;
  - Using Standard Scientific Style (DS3) for accepted manuscripts allows us to translate copyedited and proofread manuscripts instantly to SEO-optimized files for immediate posting;
  - Articles are published immediately with DOI, and assigned volume, issue, and page numbers.
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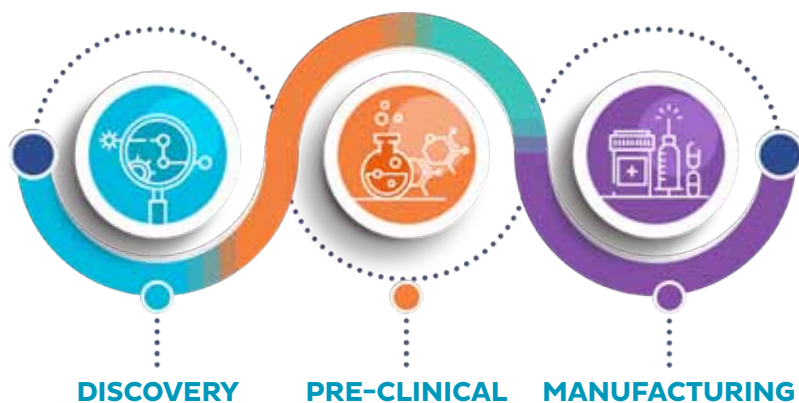




# Introducing Our Clinical Solutions Team

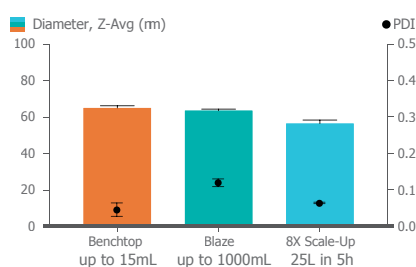
An integrated suite of proprietary technology, custom services and nanomedicine expertise to empower our clients to create transformative medicines that significantly improve human well being.

Our proprietary NanoAssemblr™ technology is trusted by over 70% of the worlds top pharmaceutical companies to accelerate their nanomedicine development programs. It uses advanced microfluidic technology to overcome key challenges in making nanomedicines at all scales from discovery to the clinic.

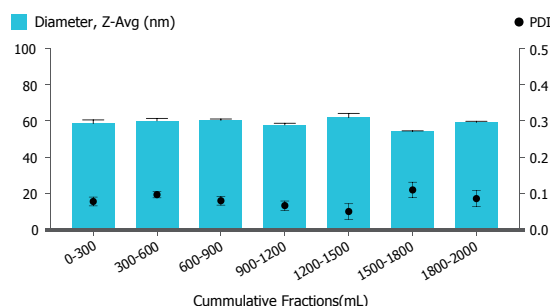


## Example: seamless scale-up of mRNA-LNPs

Size and PDI maintained across NanoAssmblr systems



Size and PDI were consistent between fractions collected from 8x Scale-Up



**Lloyd Jeffs, PhD**  
Director of Clinical  
Manufacturing Solutions

[ljeffs@precision-nano.com](mailto:ljeffs@precision-nano.com)

**“The Clinical Solutions Team is ready for your transformative drug candidates. Contact me today.”**

Dr. Jeffs has 20 years of industry experience in developing Lipid-based therapeutics and played a key role in manufacturing GMP RNA-LNP drugs for more than 10 Clinical Stage programs.

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# Nano-motion based antibiotic susceptibility test: *E.coli* and ceftriaxone

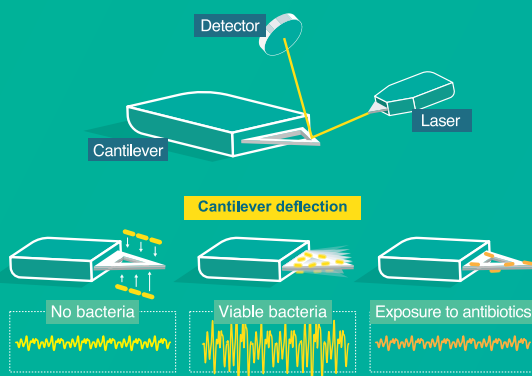
Petar Stupar<sup>1</sup>, Danuta Cichocka<sup>2</sup>, Sandor Kasas<sup>2</sup>, Onya Opota<sup>3</sup>, Gilbert Greub<sup>3</sup>, Giovanni Dietler<sup>2</sup>

1) Laboratory of Physics of Living Matter, EPFL, Lausanne

2) Resistell AG, Basel

3) Institute of Microbiology, Lausanne University Hospital – CHUV

## Nano-motion based rapid antibiotic susceptibility test

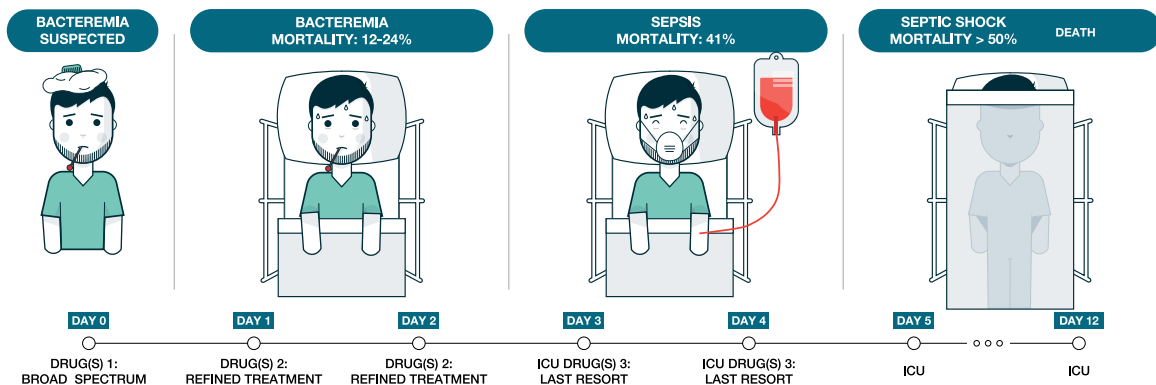


Blood stream infections (bacteremias) are life threatening if not treated immediately. Hence, fast diagnostics of antibiotic susceptibility is urgently needed to avoid the progression of the disease to sepsis and septic shock and to reduce mortality rate of the patients. In response to this health challenge we have developed a rapid, nano-motion based, phenotypic AST method.

We use AFM cantilevers as sensors. The bacteria are attached to the cantilevers, which are subsequently immersed in the fluid chamber. The oscillations of the sensor indicate whether the bacteria in the applied sample are metabolically active. When the antibiotic susceptible strains are exposed to the drug, they become non-viable within minutes and the oscillations of the sensor return to the level of an abiotic sample. The variance of the signal acquired in the growth medium and after exposure to antibiotic serves as a marker of susceptibility or resistance. The pre-clinical data for a wide range of fast and slow growing bacteria are available.

Here we show the results of nano-motion based test for *E.coli* and ceftriaxone. The detection of resistance can be completed within 1.5 hour. The full antibiogram shown here and prepared using one-channel device took around 3 hours.

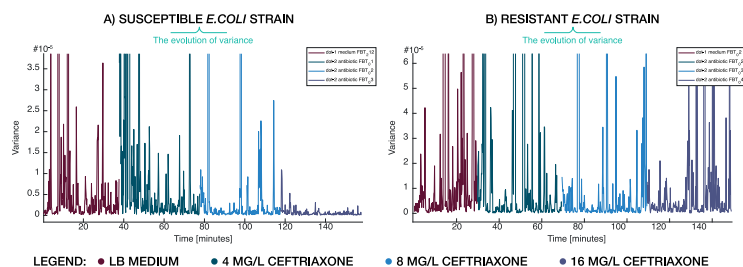
resistell.com | danuta.cichocka@resistell.com | +41 79 899 91 88



## RESISTELL PROTOTYPE



## NANO-MOTION BASED AST



References  
 G. Longo et al. *Nature Nanotechnology* 8, 522–526 (2013). doi:10.1038/nnano.2013.120  
 P. Stupar et al. *Clinical Microbiology and Infection* 23 (6), 400–405 (2017). doi: 10.1016/j.cmi.2016.12.028  
 M. I. Villalba et al. *Small* 14, 1702671 (2018) | https://doi.org/10.1002/smll.201702671



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## SeroScience Ltd (SRS)

is a small/micro biotech enterprise (SME), spin-off company of Semmelweis University Medical School in Budapest, Hungary, founded in 2006 by medical scientists. It provides research services and regulatory testing for pharmaceutical companies in regards to the safety of drugs in R&D. Specifically, SRS utilizes state-of-art in vitro technologies and large and small animal models for assessing the potential of drug candidates (most importantly intravenous drugs, biologicals, nanomedicines and contrast media) to cause hypersensitivity (allergic, pseudoallergic or infusion) reactions and other adverse immune effects, including antibody production (immunogenicity).

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[jszebeni@seroscience.com](mailto:jszebeni@seroscience.com)

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1089 Budapest  
Nagyvárad tér 4, Hungary  
Tel:  
36-30-415-0007  
36-20-825-9690  
Fax: 36-1-210-0100

## Expertise

SeroScience Ltd. has special expertise in the prediction of complement activation-related pseudoallergy (CARPA) that represents a major barrier to the clinical use of many nanomedicines and biologicals.

## Services

### IN VITRO STUDIES

Complement activation assays in vitro in human/animal sera/plasma/whole blood  
ELISA for human C3a, C5a, C4d, Bb, SC5b-9  
ELISA for all animal C3 (PAN-C3)  
SRBC (CH50) hemolytic assay for all animals  
ELISAs for serum/plasma TXB2, PAF, histamine, leukotrienes, triptase in man and animals  
FACS analysis of basophil activation  
ELISAs for anti-drug antibody (ADA) measurements

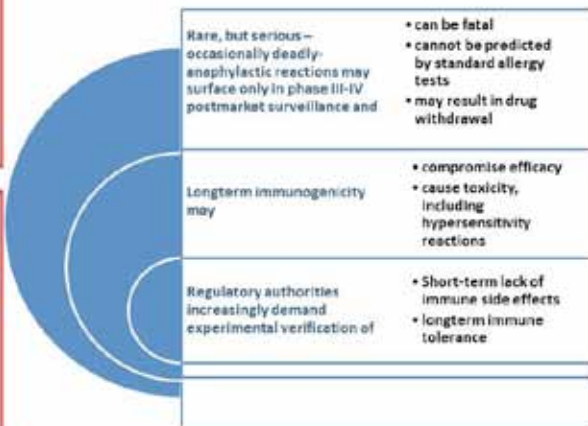
### IN VIVO STUDIES

Analysis of test drug-induced hemodynamic, hematological, laboratory and skin changes in pigs/minipigs/dogs/rats/ mice (CARPA assays)

- REGULATORY REPORTS
- R&D WITH LIPOSOMES AND OTHER NANOCARRIER FORMULATIONS
- SAFETY COUNCELING



## Why CARPA is important?



**See** SPECIAL SECTION ON CARPA IN THE CLINAM ISSUE OF THE EUROPEAN J. NANOMEDICINE



We deliver.



## SiBreaX develops and manufactures stimulus responsive silicate-based nanocarriers for drug/compound delivery

SiBreaX facilitates compound/drug delivery based on its platform technology of organosilicate forming nano-/microparticles that break upon distinct triggers at the desired site of action

### What we do:

#### Formulation/encapsulation

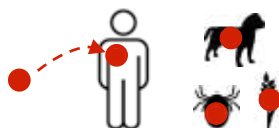
Encapsulation of active compounds in dispersible nano-/microparticles



- Tailored silicate-based particles
- Dispersible, highly stable particles
- Biocompatible, non-toxic particles
- High load factor incl. complex cargo
  - Hydrophilic small molecules
  - Hydrophobic small molecules
  - Peptides
  - Proteins
  - RNA, DNA, and PNA

#### Targeted delivery

Controlled targeting of tissues and compound delivery into target cells



- Controlled targeting of tissues and cells via the particle size/shape and functionalization of particle surfaces
  - Intracellular targeting
  - Blood cell targeting
  - Liver cell targeting
  - Lung cell targeting
  - Skin targeting


#### Controlled release


Controlled release of compounds, fast shell degradation and excretion




- Controlled release of compounds/ cargo at the targeted site of action via molecular release triggers
  - Intracellular triggers
  - Enzymatic activation
  - Release within blood stream
  - Intra-gastric release
- Controlled shell degradation for fast excretion without accumulation

### What we offer you:

**Co-development**  We partner to facilitate the formulation of your compounds and the targeted delivery of your compounds to the desired site of action

**Joint venture**  We form joint ventures with you to develop drugs and novel therapeutic approaches where targeted delivery adds major value

**In-licensing**  We seek promising compounds that require improved drug delivery for in-licensing and inhouse drug development

### What you should do:

Interested? Contact us! [info@sibreax.com](mailto:info@sibreax.com) [www.sibreax.com](http://www.sibreax.com)

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 Address Gewerbestrasse 10  
 Postal Code/City 4450 / Sissach / Switzerland  
 Telephone +41 61 985 81 00  
 Email info@tecomedical.com  
 Website www.tecomedical.com



Founded in 1984, the Swiss-based TECOmedical Group and the subsidiaries in Germany, France, Austria and Benelux provide assays and services for (pre)clinical studies, biosafety and toxicology studies, medical research and in vitro diagnostics. We offer an extensive portfolio of specialty assays, assay systems and services to Pharma and Biotech companies, CROs, medical and research centers.

#### Specialty assays for (pre-)clinical and medical studies

Drug development, research, diagnostics and therapy control

- bone/calcium/cartilage metabolism
- diabetes/obesity/metabolic syndrome
- liver disease & apoptosis
- drug-induced liver & kidney injury
- complement system
- cardiovascular disease
- oxidative stress

#### Specialty assays and test systems for biosafety of medical devices, transplants, implants, pharmaceuticals and blood products

- Haemocompatibility related to activation of the complement (C) system – Anaphylatoxins
- Complement C activation related to pseudoallergy (CARPA)
- Complement activation in animals (in vitro & in vivo)
- Cytotoxicity

#### Specialty assays for toxicology

- Detection of drug induced liver injury (DILI)
- Detection of drug induced kidney injury (DIKI)
- Vitellogenin assay for endocrine disruption potential of chemical substances according to OECD for laboratory and environmental use. This is the first Vitellogenin fish assay allowing non-destructive, non-invasive sampling from epidermal mucosa.

#### Custom Assay Development & Services

Custom ELISA Assay development and Services are offered to organizations like Biotech companies, CROs, Pharma and Research institutions, requiring specialty assays and studies based on customer specifications.

- Host Cell Protein testing for recombinant protein pharmaceuticals
- Immunogenicity assays to test for Anti-Drug Antibodies (ADA)
- High sensitive ELISAs
- Food safety assays
- Veterinary assays
- Environmental assays

Assay Services include measurement of study samples, validation of new and existing assays, test adaption, pilot to medium size manufacturing of ELISA kits and assay components.

**Impressum**

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# CLINAM was founded and registered 2007 in Switzerland by Beat Löffler and Patrick Hunziker. Its goal is to contribute to patients and society by:

- uniting the global community of nanomedicine and targeted medicine
- performing nanomedical and clinical research and promoting its clinical applications
- setting nanomedicine into the broad context of related medical procedures, technologies and therapeutic trends
- promoting and supporting the global transfer of knowledge
- networking in nanomedicine and targeted medicine by international summits, national conferences, summer-schools and seminars
- acting as non-for-profit neutral platform for all stakeholders and authorities in nanomedicine and related fields

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