# Nanoparticles for Early Diagnostics of Inflammatory Diseases

## New approaches in the field of soft and hard nanoparticles

## NANOFOL – NanoDiaRa Conference

7th Framework Programme

NANOFOL
diagnosis/therapy nanobiodevice

NanoDiaRA
Particles, Molecules & Cells · Diagnosis in-vitro & in-vivo · Rheumatoid Arthritis & Osteoarthritis

November 20th - 21st 2013, Lisbon

# NANOFOL – NanoDiaRa Conference

## Welcome letter from the NANOFOL and NanoDiaRa scientific coordinators

Two European Research Projects NanoDiaRA and NANOFOL, exploring novel techniques and scientific relationships in a very transdisciplinary way in the field of diagnosis and therapeutics of inflammatory diseases, have jointly organized a conference. The conference will present the results of more than 20 research groups and more than 70 scientist, engineers, technicians and trainees having had the opportunity to work together, to exchange, to learn and to profit from this interchange for their career. Starting more than 20 years ago with Cost Actions, Brite-Euram and the various Framework projects, the EU funding has allowed European researchers to work in close collaboration beyond national borders, and to exchange in science and culture. The EU Commission also encouraged academia and industry to work closer together already in research and development and this conference will highlight some of this collaborative work. We expect that presentations and discussions might help to create new concept ideas to exploit results of NanoDiaRA and NANOFOL and help to build new projects under the frame of Horizon 2020. In this sense we would like to thank all participants from both projects as well outside participants who are coming to listen, to learn, and to exchange, and the EU Commission for funding these projects and by this allowing more sustainable European partnerships.

*Artur Cavaco-Paulo* and *Margarethe Hofmann-Amtenbrink*

Key issues Nanoparticles, in vitro and in vivo nano-diagnostics, safety and risks aspects, molecular biology, pre-clinical research, biomarker development

## ORGANIZATION

NANOFOL

Scientific coordinator Artur Cavaco-Paulo

Secretariat Maritza Andrea Salas, Joana Cunha

NanoDiaRa

Scientific coordinator Margarethe Hofmann-Amtenbrink

Secretariat Alessandra Hool

Acknowledgements and support

## CONFERENCE VENUE

Associação Comercial de Lisboa - Câmara de Comércio Internacional

Salão Nobre D. Maria II

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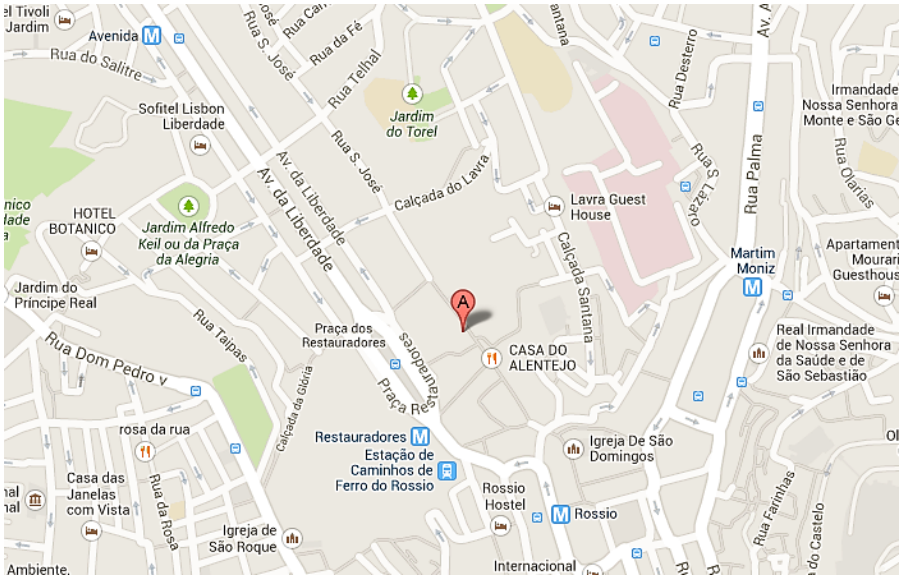
Rua das Portas de Santo Antão, nº 89

1169-022 Lisboa



# Program

# PROGRAM

## Wednesday, 20th November 2013

| | | | |
|---|---|---|---|
| 08:45 | **Registration** | | |
| 09:00 | **Welcome / Opening**<br>Artur Cavaco-Paulo, NANOFOL Scientific Coordinator<br>Margarethe Hofmann-Amtenbrink, NanoDiaRa Scientific Coordinator | | |
| CHAIRMAN Andreia Gomes | | | NANOFOL |
| 09:15 | **INVITED TALK: Liposomes for MR imaging and image-guided drug delivery**<br>Sander Langereis | | |
| 09:45 | O1 | **Protein-based nanoparticles**<br>Ana Loureiro | NANOFOL |
| 10:00 | O2 | **Liposomal-based nanoparticles**<br>Eugénia Nogueira | NANOFOL |
| 10:15 | O3 | **Magnetic nanoparticles**<br>Heinrich Hofmann | NanoDiaRa |
| 10:30 | **Coffee break and Posters** | | |
| 11:00 | O4 | **A new enzymatic approach for human serum albumin-antibody (HSA-mAb) conjugate production**<br>Alexandra Rollett | NANOFOL |
| 11:15 | O5 | **Influence of amino-PVA nanoparticles on survival and function of human immune cells**<br>Frank Buttgereit | NanoDiaRa |
| 11:30 | O6 | **What is needed to deliver safe nanodiagnostics: Part II – results from *in vivo* toxicity**<br>Thomas Broschard | NanoDiaRa |
| 11:45 | **INVITED TALK: The need for early diagnosis of arthritis: importance of developing new technology to make this possible**<br>Robin Poole | | |
| 12:15 | **EU INFORMATIVE LECTURE: Horizon 2020 – The EU Framework Programme for Research and Innovation – 2014-2020**<br>Maj-Inger Nilsson | | |
| 12:45 | **Lunch and Posters** | | |
| CHAIRMAN Robin Poole | | | |
| 14:00 | **INVITED TALK: Challenging the use of anticancer drugs with targeted n anotechnology-based strategies**<br>João Nuno Moreira | | |
| 14:30 | O7 | ***In vivo* molecular imaging with MRI using macrophages**<br>Lindsey A Crowe | NanoDiaRa |

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|---|---|---|---|
| 14:45 | O8 | *In vivo* tracking of SPION labeled mesenchymal stem cells in antigen induced arthritis<br>Azza Gramoun | NanoDiaRa |
| 15:00 | O9 | Strategies for genetic reprogramming of macrophage subsets in inflammatory diseases<br>Alexandre do Carmo | NANOFOL |
| 15:15 | O10 | Novel macrophage subsets with potential implication in inflammatory diseases<br>Anna Repic / Hannes Stockinger | NANOFOL |
| 15:30 | O11 | *In vivo* therapeutic assessment of targeted nanoparticles in mice models of rheumatoid arthritis: contribution of optical and ultrasound imaging<br>Gilles Renault | NANOFOL |
| 15:45 | O12 | Labelling of MSCs with PVA-SPIONs does not result in toxicity but triggers functional changes<br>Frank Schulze | NanoDiaRa |
| 16:00 | O13 | Ethical, social, and legal issues related to nanoparticles in medicine<br>Felix Thiele | NanoDiaRa |
| 16:15 | | Coffee break and Posters | |
| 16:30 | | CLUSTER PROJECT PRESENTATION: Contrast agent for early diagnostics and monitoring of progression of liver cancer (hepatocellular carcinoma)<br>Dmitry Grishenkov | |
| 16:45 | | CLUSTER PROJECT PRESENTATION: A modular nanosystems platform for advanced cancer management – The SaveMe Project<br>Louis Shenkman | |
| 17:00 | | Posters and apéro | |
| 20:00 | | Dinner at Casa do Alentejo | |

## Thursday, 21st November 2013

| CHAIRMAN Ana Preto | | | NANOFOL |
|---|---|---|---|
| | | THEMATIC SESSION: Technology Transfer and Exploitation | |
| 09:00 | O14 | Technology Transfer: From Bench to Market<br>Ion Arocena | NANOFOL |
| 09:20 | O15 | Some reflections on the commercialization of academic research in the interphase between University and Industry<br>Jan Hed | NanoDiaRa |
| 09:40 | | ROUND TABLE DISCUSSION<br>Ion Arocena and Jan Hed | |
| 10:00 | | INVITED LECTURE: Publishing in Nature<br>Natascha Bushati | |

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|---|---|---|---|
| 10:45 | **Coffee break** | | |
| | **THEMATIC SESSION: Nanotechnology in Medicine** | | |
| 11:00 | O16 | **Nanotechnology: definitions and concepts I**<br>Artur Cavaco-Paulo | NANOFOL |
| 11:20 | O17 | **Nanotechnology: definitions and concepts II**<br>Heinrich Hofmann | NanoDiaRa |
| 11:40 | O18 | **Characterization of folate-based nanodevices**<br>Florentina-Daniela Munteanu | NANOFOL |
| 12:00 | O19 | **Drug distribution and formulation**<br>Michael Burnet | NANOFOL |
| 12:20 | **Lunch** | | |
| CHAIRMAN Margarethe Hofmann | | | NanoDiaRa |
| 13:20 | O20 | **From nanoparticle to therapeutic agents I**<br>Thomas Broschard | NanoDiaRa |
| 13:40 | O21 | **From nanoparticle to therapeutic agents II**<br>Michel Dreano | NanoDiaRa |
| 14:00 | O22 | **Chemoselective transformations in benign aqueous systems for bioimaging and targeted therapeutics**<br>Gonçalo Bernardes | NANOFOL |
| 14:20 | O23 | **Nanoparticles: functionalization and characterization**<br>Lionel Maurizi | NanoDiaRa |
| 14:45 | O24 | **Biomarkers in RA and OA as tools for molecular imaging and immunoassays**<br>Patrik Önnerfjord | NanoDiaRa |
| 15:00 | O25 | **Biomarker assays in array format**<br>Hans Sigrist | NanoDiaRa |
| 15:15 | **ORAL PRESENTATION OF POSTERS (5 minutes each)**<br>Gaio Paradossi, Sofia Costa Lima, Daniela Barros, Andreia Valente, António Paulo, Johan Härmark, Maria de Deus Carvalho, João Neves Silva, Dmitri Grishenkov | | |
| 16:00 | **Farewell** | | |

# Résumés

## INVITED SPEAKERS RÉSUMÉS

Sander Langereis PhD

Dr. Sander Langereis is a Senior Scientist at the department of Minimally Invasive Healthcare at Philips Research Europe (The Netherlands). He obtained his PhD in Chemistry at the Eindhoven University of Technology under the supervision of Prof. E.W. Meijer. In 2006, he was employed by Philips Research Europe. His research focuses on molecular MRI contrast agents and MR-HIFU mediated drug delivery using temperature-sensitive liposomes. He has written more than 29 publications in internationally refereed journals (h-index = 17). Sander was responsible for nanoparticle related activities in two former EU projects (*i.e.* Meditrans (FP6) and Sonodrugs (FP7)) and will be the responsible project manager for another EU project on image-guided pancreatic cancer therapy (iPaCT, proposed starting date 1st of January 2014).

Anthony Robin Poole PhD, DSc

Robin Poole got his BSc and PhD degrees from Reading University, UK. He was a full professor at McGill University, Canada, since 1981 and during most of his career. His research has resulted in 246 peer reviewed papers and 96 invited reviews or book chapters. His work on molecular markers of cartilage matrix metabolism, joint damage and repair in arthritis has led to their commercialization and use in research and drug development in academia and industry. He wrote the white paper on biomarkers for the NIH Public/Private osteoarthritis Initiative in 2000 and recently he was involved in the preparation for the FDA of a guidance document on biomarker usage in clinical trials for osteoarthritis. He was a co-founder (1998), then Associate Director and later Scientific Director of the Canadian Arthritis Network, a National Centre of Excellence composed of almost 200 principal investigators. He maintains his numerous scientific and research interests being involved in the creation of new research programs, collaborative research programs, mentoring, reviewing, lecturing , editorial and scientific advisory boards in Canada and internationally and also as a consultant to biotech and pharmaceutical companies.

João Nuno Moreira PhD

João Nuno Moreira received his BSc in Pharmaceutical of Sciences, MSc in Cellular Biology and PhD in Pharmaceutical Technology from the University of Coimbra. During his PhD studies has spent three years at the Department of Pharmacology, Faculty of Medicine, University of Alberta, Edmonton, Canada. At present, he is an Assistant Professor at the Faculty of Pharmacy and Principal Investigator at the Department of Vectors and Gene Therapy, Center for Neuroscience and Cell Biology, University of Coimbra. Scientific activity is focused on the design of lipid-based nanosystems for drug and nucleic acid targeted delivery, namely towards cancer. He is co-founder of TREAT U, which is a spin-off from the University of Coimbra, since January 2010.

Maj-Inger Nilsson PhD

Directorate-General for Research &Innovation, European Commission, EU

Maj-Inger Nilsson is working as a Research Programme Officer in the area of Nano Sciences and Nano Technologies, DG Research & Innovation, European Commission, Brussels. By training she is a pharmacist, and after her PhD studies, she joined the pharmaceutical industries, working extensively with global drug development programmes. She also has experience from research policy and funding, first from the national level in the Sweden, later on the European level. She is an Associate Professor at the Faculty of Pharmacy of Uppsala University, Uppsala, Sweden.

Natascha Bushati PhD

Associated Editor, Nature Communications, UK

After a period as a Scientific Editor at EMBO Molecular Medicine, Natascha joined the Nature Communications team in February 2013. Natascha first studied chemistry at the University of Vienna and subsequently investigated the roles of microRNAs in *Drosophila* development during her PhD at the European Molecular Biology Laboratory in Heidelberg and Temasek Life Sciences Laboratory in Singapore. Her postdoctoral work took her to the National Institute for Medical Research in London, where she studied the gene regulatory networks underlying neural development in chicks.

# Abstracts

## INVITED TALKS

Sander Langereis

*Minimally Invasive Healthcare (MIH), Philips Group Innovation, Eindhoven, The Netherlands*

### *Liposomes for MR imaging and image-guided drug delivery*

Liposomes are a versatile class of nanovesicles that have been explored extensively in the biomedical arena for early diagnosis and treatment of disease [1]. In recent years, the concept of hyperthermia-mediated drug delivery from temperature-sensitive liposomes (TSLs) has been explored under Magnetic Resonance Image (MRI) guidance (Figure 1) [2]. Mild hyperthermia of the tumor can be accomplished using MR-guided High Intensity Focused Ultrasound (MR-HIFU), while TSLs can serve as drug vehicles that release their payload upon heating. The co-encapsulation of a drug and an MRI contrast agent in the lumen of TSLs provides the ability to monitor the drug delivery process under MR image-guidance [3].

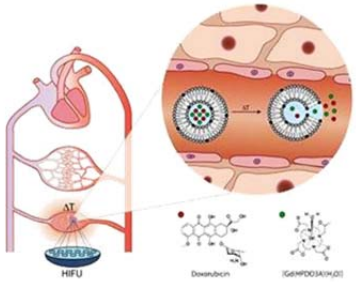



Figure 1. Heat-induced release of drugs and MRI contrast agents from a temperature-sensitive liposome (TSL).

References

[1] S. Langereis, T. Geelen, H. Grüll, G.J. Strijkers, K. Nicolay, *Paramagnetic liposomes for molecular MRI and MRI-guided drug delivery*, NMR Biomed, 26 (2013) 728-744.

[2] H. Grüll, S. Langereis, *Hyperthermia-triggered drug delivery from temperature-sensitive liposomes using MRI-guided high intensity focused ultrasound*, J Control Release, 161 (2012) 317-327.

[3] M. de Smet, E. Heijman, S. Langereis, N.M. Hijnen, H. Grüll, Magnetic resonance imaging of high intensity focused ultrasound mediated drug delivery from temperature-sensitive liposomes: an in vivo proof-of-concept study, J Control Release, 150 (2011) 102-110.

Robin Poole

*Department of Surgery, McGill University, Montreal,Quebec, Canada*

## *The need for early diagnosis of arthritis: importance of developing new technology to make this possible*

Arthritis involves the progressive destruction of joints such as those of the hands, spine, knees and hips. It can be both inflammatory, such as rheumatoid arthritis and ankylosing spondylitis, and degenerative such as osteoarthritis. As the different types of arthritis are very disabling, together affecting to 18 percent of the population, it is essential that the disease be detected as early as possible to enable effective treatment (where possible) and thereby minimize pain and disability. The presentation will discuss the ways in which arthritis is presently detected and the new opportunities offered by new technology being developed by scientists in NanoDiaRA and other research laboratories.

João Nuno Moreira

*Center for Neuroscience and Cell Biology and Faculty of Pharmacy, University of Coimbra, Portugal*

Co-authors:

Vera Moura

Lígia Gomes-da-Silva

Sérgio Simões

*Center for Neuroscience and Cell Biology and Faculty of Pharmacy, University of Coimbra, Portugal*

## *Challenging the use of anticancer drugs with targeted nanotechnologies-based strategies*

Choosing more than one target from the pool of tumor-stroma interactions, such as the blood vessel network, which ensures tumor survival, growth and metastases, can profoundly benefit therapeutic approaches. Blood vessels are excellent targets, since they are readily accessible to intravenously administered therapies and overexpress specific molecular markers at their surface, enabling treatment selectivity. The prevailing new rationale aims at the development of targeted therapies to the tumor microenvironment on the basis of characterized mechanisms, with the possibility of directing and concentrating a therapeutic agent only at the desired target site, while improving access to intracellular sites of action. If the same targeted system is capable of identifying a common target and perform its therapeutic action in different cell populations within a tumor, improved therapeutic outcomes are expected. Within this scope, in this communication the potential of ligand-mediated targeted delivery towards solid tumors, with nanotechnology-based approaches, will be discussed.

## INVITED LECTURES

Natascha Bushati

*Associate Editor, Nature Communications*

### *Publishing in Nature*

Selective scientific journals have high rejection rates, but few authors know what exactly happens to their manuscript after submission. In this presentation I will give an overview over the Nature family of journals and describe the individual stages a manuscript passes from submission to acceptance. In particular, I will highlight the role of the editors of in this process. I will explain the editorial criteria that determine whether a manuscript is sent out to peer-review, describe how editors go about choosing suitable reviewers, and provide insight into our decision-making process when a manuscript has completed peer-review. I will aim to provide tips that may help you maximize the potential for success when submitting your manuscript to the Nature family of journals.

Maj-Inger Nilsson

*DG Research & Innovation, European Commission, Brussels, Belgium*

## EU Informative lecture
## *Horizon 2020 – The EU Framework Programme for Research and Innovation – 2014-2020*

Horizon 2020 is the new Framework Programme for Research and Innovation in the European Union. The programme will start in 2014 and end by 2020. The presentation will give the objectives behind the programme and the achievements so far. The programme will be a single programme, bringing together three separate programmes / initiatives; coupling research to innovation – from research to retail, including all forms of innovation; focus on societal challenges facing EU society, e.g. health, clean energy and transport and simplified access for all companies, universities, institutes in all EU countries and beyond. The Work Programmes are still in progress and the latest developments will be discussed.

## CLUSTER PROJECT PRESENTATIONS

Dmitry Grishenkov

*Karolinska Institutet, Division of Medical Imaging and Technology – CLINTEC, Huddinge, Sweden*

Co-authors:

Gaio Paradossi

*Dipartimento di Scienze Tecnologie Chimiche, Università di Roma Tor Vergata, Italy*

Torkel Brismar

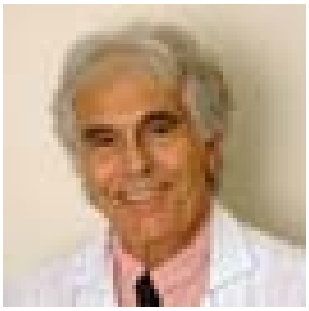
Birgitta Janerot-Sjoberg

*Karolinska Institutet, Division of Medical Imaging and Technology – CLINTEC, Huddinge, Sweden*

### *Contrast agent for early diagnostics and monitoring of progression of liver cancer (hepatocellular carcinoma)*

Evaluation of liver lesions is a challenge to every radiologist. Novel microbubble (MB) based targeted multimodal contrast agent, support several diagnostic approaches, including ultrasound, MRI and SPECT.

Hepatocellular carcinoma (HCC) is the most frequently diagnosed type of liver cancer and is the second most common liver lesion after cirrhosis. Size, staging, and surgical radicality severely affect the prognosis. Today it is impossible to mix different contrast agents, requiring diagnostic tests such as ultrasound, SPECT, MRI, biopsy to be made in different days. In clinical practice this means that the time from suspected HCC to diagnosis can be several weeks. Novel procedure allows to perform all tests in a single day with immediate response. Moreover, MBs functionalized with HCC specific ligands, allow diagnosis even on sub-cm lesions. These would have a significant impact on healthcare. Unsuccessful treatment can be given up earlier, in favor of more aggressive treatment or surgical approaches.



Louis Shenkman

*Tel Aviv University, Israel*

Co-authors:

Hans Hebert

Philip J.B. Koeck

*School of Technology and Health, KTH Royal Institute of Technology and Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden*

## *A modular nanosystems platform for advanced cancer management – The SaveMe Project*

The SaveMe project funded under the EU's FP7 Program is addressing major urgent needs for early cancer diagnosis and therapy by designing and developing a novel modular nanosystems platform that integrates advanced functionalized nano-core particles and active agents. We are developing active nanosystems for diagnostic or therapeutic applications as defined by their active agent compositions. For the early diagnosis of pancreatic cancer, somatostatin and galectin-1 receptors (SSTR and Gal-1/tPA) are utilized for targeting the tumor and visualization by PET, MRI or combined scanning. Similarly labeled nanoparticles containing specific antibodies or silencing RNA directed against tumor genes will be used for advanced therapeutics. To date we have developed a panel of potentially useful nanoparticles for imaging and therapeutic applications. Targeting agents have been loaded on the NPs and preliminary data in tumor cell lines are promising. Preliminary animal studies have demonstrated the feasibility of this system in imaging tumors. One of our NPs efficiently binds siRNAs and is capable of silencing mRNA, microRNA and long non-coding RNAs without toxicity. We have demonstrated the feasibility of NPs as carriers for siRNA and have successfully silenced selected pancreatic cancer genes using our system in cell lines and in animal models.

## ORAL COMMUNICATIONS

Ana Loureiro
*University of Minho, Portugal*

Co-authors:
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*University of Minho, Portugal*

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### *O1*
### *Protein Based Nanoparticles*

Protein nanoparticles have a huge potential as possible vehicles for drug delivery due to their proven biocompatibility and biodegradability, sustained release, increased drug stability and targeting of specific tissues. In this study, we have produced several formulations of bovine serum albumin (BSA) nanoparticles using a high-pressure homogenizer. Different parameters were optimized in order to obtain monodisperse formulations of small nanoparticles, which are compatible with a potential application as drug delivery systems. The different formulations were extensively characterized and in vitro tests were performed. The incorporation of targeting and imaging agents and the encapsulation of drugs in nanoparticles were also performed. Specific internalization and effect of drugs encapsulated in small BSA nanoparticles obtained indicated that these protein nanoparticles exhibit suitable characteristics for application as drug delivery systems.

Eugénia Nogueira
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*University of Minho, Portugal*

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## *O2*
## *Liposomal formulations for specific drug delivery*

Liposomes have received considerable scrutiny as possible vehicles for drug delivery due to properties such as sustained release, increased drug stability, ability to overcome drug resistance and targeting of specific tissues.

In this study, we have produced several liposomal formulations prepared by thin film hydration method. The different formulations were extensively characterized and preliminary tests were performed to evaluate their potential as specific drug delivery systems. The liposomes present very small values of size and polydispersity index, as well as lower cytotoxicity, which are compatible with intended in vivo applications. The incorporation of imaging and targeting agents in the liposomes was performed, what improved selectivity to the system, being the liposomes specifically internalized in target cells. The encapsulation of drugs was also performed and their effect analyzed in the target cells. In summary, the liposomal formulations obtained exhibit suitable characteristics to be used in vivo as specific drug delivery systems.

Heinrich Hofmann

*Ecole Polytechnique Fédérale de Lausanne, Switzerland*

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## *O3*
## *Magnetic nanoparticles*

Nanosized inorganic nanoparticle shows interesting and unique magnetic properties, like superparamagnetism, which give access to novel application. Especially superparamagnetic iron oxide nanoparticles, SPION, are mostly used for application like contrast agent, vector for drug delivery or separation and “medicament” for cancer treatment. Essential for any biomedical application is, beside the physical properties of the inorganic particle, the coating. Several coatings are used to reach high colloidal stability, biocompatibility and finally the possibility to add biomolecules for the control of the biodistribution, targeting and up take by cells. In this presentation, important aspects regarding synthesis, coating and application of SPION will be presented whereas especially the use of the particles as contrast agent and as heating elements for hyperthermia will be treated.

Alexandra Rollett

*Institute for Environmental Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria*

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Anna Repic

Christian Machachek

Hannes Stockinger

*Institute for Hygiene and Applied Immunology, Medical University of Vienna, Austria*

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## *O4*
## *A new enzymatic approach for the production of human serum albumin-antibody (HSA-mAb) conjugate*

Targeted drug delivery to chronic activated macrophages can be obtained with HSA-mAb conjugates. However, their conventional production involves several reaction steps including toxic cross-linking chemicals.

Here we established a green alternative for the production of HSA-mAb using the enzyme tyrosinase from *Agaricus bisporus* (AbT).

The reaction was optimized and progress was followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis and mass spectrometry. Enzymatic cross-linking in the presence of natural low molecular weight phenolic compounds (e.g. caffeic acid) resulted in reaction products in the molecular weight range of ~216 kDa, corresponding to HSA-mAb conjugates. Successful binding of HSA-mAb conjugates (in contrast to free HSA) to MHC II molecules, located on antigen-presenting cells, was demonstrated by both enzyme-linked immunosorbent assay (ELISA) and flow cytometry analysis.

Frank Buttgereit

*Charité University Medicine Berlin, Germany*

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Timo Gaber

Cindy Strehl

Martin Hahne

Roman Rauch

Manuela Jakstadt

Kerstin Schönbeck

Cam Loan Tran

Peggy Kunath

*Charité University Medicine Berlin*

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## *O5*
## *Influence of amino-PVA nanoparticles on survival and function of human immune cells*

Nanotechnology in medical applications provides new opportunities for diagnostic and therapeutic interventions in a variety of human diseases such as in rheumatology (see www.nanodiara.eu <http://www.nanodiara.eu>).

However, safety aspects represent crucial questions for the further development of nanotechnology based products. Therefore, the focus of our work here was to identify putative effects of amino-PVA-coated super paramagnetic iron oxide nanoparticles (SPIONs) on human immune cell functions.

To this end, we analyzed the effects of various concentrations of amino-PVA-coated SPIONs in a whole blood assay on survival and cell activation, and in isolated immune cell fractions on survival, cell activation and functionality also under several different conditions (such as normoxic *versus* hypoxic incubation conditions). We investigated blood from healthy donors and from patients with rheumatoid arthritis and, to some extent, also osteoarthritis. As a result, investigations of cellular toxicity of amino-PVA-coated SPIONs did not show any measurable effects on the measured parameters describing survival and functionality in a whole blood assay and also on isolated monocytes and T cells at concentrations less than 1000µg/ml. However, cytokine secretion assays revealed that pro-inflammatory cytokines, such as TNFα, IL1β, IL6, IL8, and IFNγ were induced at varying SPION concentrations in whole blood assay cultures yet measured only after 20h. Further results indicated that PVA alone is a similar inducer of such cytokines as compared to the amino-PVA-coated SPIONs. Therefore, alternative modifications of these SPIONs are indicated. It should also be noted that the SPION amounts used in these *in vitro* tests were much higher than the quantities usually used in humans e.g. when applying contrast agents.

Thomas Broschard

*Merck Serono, Darmstadt, Germany*

Co-authors:

Michel Dreano

*M3DC Sàrl, Geneva, Switzerland*

Sven Lindemann

*Merck Serono, Darmstadt, Germany*

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## *O6*
## *What is needed to deliver safe nanodiagnostics:*
## *Part II – results from* in vivo *toxicity*

The increasing number of medical applications based on nanoparticulate polymers, metals or metal oxides as drug carriers, for diagnosis purposes or for the treatment of cancer, raises the question whether these novel compounds are safe for patients and have favorable benefit-risk balances. Therefore, the investigation of the toxicological profile of nanoparticles in medical applications using *in vitro* and *in vivo* assays is one of the main pillars in the safety evaluation of these new product classes. In this presentation, the regulatory framework on which the safety assessment of nano-based medical imaging drugs is based on will be described. Moreover, toxicological data from superparamagnetic iron oxide nanoparticles (SPION) derived from the NanoDiaRa project and from literature will be presented with a focus on single dose toxicity and genotoxicity and the safety of these medical imaging drugs will be discussed.

Lindsey A Crowe

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## *O7*
## In vivo *molecular imaging with MRI using macrophages*

The main objective of this study is the validation of *in vivo* imaging of SPIONs serving as a tool for monocyte/macrophage tracking and molecular or cellular imaging during inflammation in rheumatoid arthritis (RA) models in small animals. SPION imaging using MRI facilitates monitoring of disease detection and progression as well as possibilities for verification of targeted drug delivery and biosensing.

In studies of *in vivo* SPION-loaded macrophage tracking, we observed changes in quantifiable SPION signal with time in serial MRI. Complementary effects were observed to those generally seen with conventional gadolinium chelate contrast agent.

These results illustrate the potential of SPIONs as a tool to detect specific cellular changes in RA and other disease models using MRI, monitoring their delivery to the site of inflammation in a non-invasive and longitudinal fashion.

Azza Gramoun

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Lindsey Crowe

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## *O8*

## In vivo *tracking of SPION labeled mesenchymal stem cells in antigen induced arthritis*

Objective: To track SPION labeled human mesenchymal stem cells (hMSCs) *in vivo* during antigen induced arthritis (AIA). Methods: hMSCs were labeled *in vitro* using SPIONs (51±11 nm). After verifying cell viability, SPION labeled hMSCs or SPIONs were injected intra-articularly (i.a.) post-AIA induction. The rats were MR scanned on days 3, 6 and 10. At the end of the experiment, the knees were isolated and processed for histology. The resulting sections were Prussian blue stained and immunostained for CD44. Results: A strong SPION signal was detected on MR images 24 hours after the i.a. injection of SPION labeled hMSCs and up to day 10. This signal significantly changed between the different timepoints. Furthermore, SPIONs alone had a specific distribution in the synovium, which was different from that of the SPION labeled hMSCs. Co-localization of Prussian blue particles and CD44 positive cells on histological sections confirmed a persisting uptake of the SPIONs by hMSCs *in vivo*. Conclusion: Our findings demonstrate the feasibility of MRI tracking of SPION labeled hMSCs in AIA.

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## *O9*
## *Strategies for genetic reprogramming of macrophage subsets in inflammatory diseases*

Macrophages have crucial roles both in the building up of inflammation as well as in the resolution phase. Macrophages are known for their plasticity, which is also evident at the transcription level where many genes contribute to the phenotype acquired. Macrophage function may be altered with a well-targeted genetic reprogramming, which is of crucial importance in the therapy of chronic inflammatory diseases. Genes involved in the pro-inflammatory profile of macrophages or in the survival of activated macrophages are potential targets to be silenced in macrophages involved in the maintenance of inflammatory diseases. Using locked nucleic acids, we are targeting anti-apoptotic genes to induce macrophage apoptosis. A list of genes with a potential in the reprogramming of macrophages will be available by a deep-sequencing transcriptional analysis of different macrophages sub-populations. Genes that discriminate the various functionally distinct macrophage subsets could be considered as optimal targets for reprogramming cellular behavior.

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## *O10*
## *Novel macrophage subsets with potential implication in inflammatory diseases*

Macrophages are strategically located within the tissues where they play a crucial role in immune surveillance. In response to tissue damage or invading pathogens, macrophages effectively ingest and eliminate the source of danger and by concomitant release of proinflammatory cytokines they attract and activate other immune cells including macrophage precursors and T cells. However, exacerbated macrophage responses underlie the pathology of several inflammatory diseases including rheumatoid arthritis. A subset of macrophages found within the affected joints was characterized by expression of folate receptor β (FRβ). In order to study the function of FRβ-positive macrophages within the diseased tissue in more detail, we prepared *in vitro*-differentiated macrophages from peripheral blood monocytes. Our analyses, comprising a comprehensive gene expression profiling, revealed that FRβ is strongly upregulated in several subsets of macrophages that can be distinguished by other markers as well as by functional properties. While FRβ-positive subsets share high phagocytic potential, they display marked differences in their capacity to stimulate T cells. Our results revealed an important aspect of macrophage plasticity and potentiating a transition to an immunoregulatory phenotype *in vivo* could lead to a novel way of treatment for inflammatory diseases.

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## O11

## In vivo *therapeutic assessment of targeted nanoparticles in mice models of rheumatoid arthritis: contribution of optical and ultrasound imaging*

Evaluation of therapeutic efficacy of new drugs against Rheumatoid Arthritis (RA) relies mainly on small animal models of disease. These animal models are classified as severe in terms of pain evaluation. In such studies, great attention is given by ethics committees to reduction of the number of animal and maximisation of information gathered on each animal (refinement). Imaging is a way to enrich the data collected in such studies, and possibly reduce the number of animals.

Among all imaging modalities, ultrasound and optical imaging remain the less expensive and the most widespread, with reasonable throughput.

We will present what benefits may be obtained from optical imaging and ultrasound to better depict the pathology and possibly provide early signs of therapeutic efficacy, with cost effective imaging.

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## *O12*
## *Labelling of MSCs with PVA-SPIONs does not result in toxicity but triggers functional changes*

Polyvinyl alcohol coated superparamagnetic iron oxide nanoparticles (PVA-SPIONs) were designed for biomedical applications and show high potential in the context of rheumatoid diseases. Every application in a clinical context will lead to the exposition of tissues with PVA-SPIONs. Mesenchymal stromal cells (MSCs) are key players of tissue regeneration, it is thus crucial to ensure that MSCs viability and their regenerative functions are not affected by exposure to PVA-SPIONs. After initial proof for PVA-SPION internalization by transmission electron and X-ray microscopy, a reproducible labeling protocol was established, including dosimetric information about administered, delivered and cellular dose. The PVA-SPION-labeled MSCs were subjected to assays concerning their viability and function, showing no effect on these parameters by PVA-SPIONs except an alteration in cell motility. Although PVA-SPIONs do not exhibit toxicity towards MSCs, an alteration in the cells biology should be carefully considered as a possible hazard sign even if its effect is favorable.

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## *O13*
## *Ethical, social, and legal issues related to nanoparticles in medicine*

Nanotechnology is frequently judged to be a key technology of the 21st century. Especially in the field of medicine nanotechnology, specifically nanoparticles may help to develop new and effective applications. However, as with many modern technologies, there are considerable moral, legal and social concerns about the consequences nanotechnology may have for humans and their environment. More than in many other areas of nanotechnology, nanomedicine stimulates stakeholders to raise concerns. Not only patients are concerned, but also in politics and administration, in industry and in the scientific community there are substantial debates about ethical, legal, and social implications (ELSI) of nanomedicine. The talk will discuss some of the major ELSI problems of nanomedicine, and reflect on suitable procedures for solving them.

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## *O14*
## *Technology Transfer: From Bench to Market*

Innovation means being able to transform scientific knowledge into products and services which can be marketed. This transformation is not an easy and straightforward process and usually, a great effort is needed in terms of both time and resources. That means investing big amounts of money.

In current western economies, private enterprises are meant to be the agents responsible for providing products and services to the marketplace. New technologies can be marketed by already existing companies or might the basis for the foundation of a new venture. Companies must decide in which technologies to invest and which not, based on the fit with their strategy, the investment needed to take the technology to the market and the expected returns. In fact, private companies must allocate their limited resources in those projects offering the best risk-reward equation.

For innovations arising from the academia, starting a new company might sometimes be the best way to take a new technology to the market, if not the only way. In fact, not all technologies are demanded by established enterprises, especially when these technologies are disruptive and in initial stages of their development, having a high risk profile. At the same time, not all the technologies are sound enough to launch a new venture. What does a technology need to establish new venture on it? What will the company need to do to take the technology to the market? How can this be funded?

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## *O15*
## *Some reflections on the commercialization of academic research in the interphase between University and Industry*

Exploitation and commercialization is an important output of an EU-project. This presentation is based on lessons learned from the NanoDiaRA-project in relation to earlier experience of this interphase between academic research and industry.

The focus will be on the following issues:

- Publish or Perish *vs* Protect the Know-How
- Discovery *vs* Invention
- Novelty and Nonobviousness
- Verify/define the utility/market *vs* product design and development
- Freedom to Operate
- File a patent or not
- Substance patent *vs* application patent
- Identify critical issues (“kill your baby”)

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## *O16 - O17*
## *Nanotechnology: definitions and concepts*

Massive amount of funds have been allocated for the research in Nanotechnology. It is not always clear the boundaries between “Micro” and “Nano” areas. Definitions are very broad depending on the disciplinary area. Bold names from several disciplines exist like bionanotechnology, nanomedicine, nanomaterials, nanoformulation, nanoelectronics, nanofluidics, nanoparticles, nanosensors and others. In spite of view of the huge impact of definitions on research funding, regulations, toxicity, investigation and accreditation of new products, it is important to know and to understand the origin and reasons for these different approaches to those definitions. Definitions and examples from the literature as well as from the own research are given. This tutorial aims to clarify those misconceptions.

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## *O18*
## *Characterization of folate-based nanodevices*

The formulation of nanodevices should have in attention that the final product has the characteristics that makes it prone for use in theranostics. Some of the main characteristics of the nanodevices are referring to the nanoparticle size, surface charge and permeability, biodegradability, biocompatibility, toxicity, drug solubility and stability, design of the drug release and antigenicity of the final product.

In our study, we present several methods used for the characterization of the obtained folate-based nanodevices. One of the methodologies was based on high resolution mass spectrometry (MS) with electrospray ionization for highly accurate determination of the product molecular weight and tandem MS using collision-induced dissociation at low energies for fragmentation analysis and complex structure confirmation.

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## *O19*
## *Drug distribution and formulation*

Drugs may be formulated for directed distribution, but once released, they are liable to the same elimination processes as unformulated material. To determine whether an advantage has been obtained, the only efficacy and dose response are useful. Formulants also have effects on compound elimination that explain part of the influence of particles: p-glycoproteins are inhibited by surfactants which can, thereby, extend the half-life of the substances. For compounds with low affinity to their targets, equilibration to plasma effectively removes them from the target area. Macro-molecular linkage helps but runs foul of regulatory strategies focused on administering "unchanged" substances to the patient.

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## *O20 – O21*
## *From nanoparticle to therapeutic agents*

The unique properties of nanoparticles make them attractive for development as novel therapeutic or diagnostic agents. Using the stepwise development of a "classic" therapeutic agent as a framework, this presentation illustrates the time consuming and exacting nature of the development of any novel agent. Highlighting that while the development of SPION is not a direct overlay, there is considerable and critical overlap and just as for a "classic" agent, the SPION research data has to support both internal "company" NanoDiaRA and external "regulatory agency" decisions. Although these two strands are intertwined, a discrete viewpoint for each must be maintained and this is particularly important when assembling the draft regulatory submission and considering whether or not to advance the novel agent into first-in-man clinical trials.

SPION specific toxicology data (NanoDiaRA WP7) will also be presented along with overall project conclusions and recommendations.

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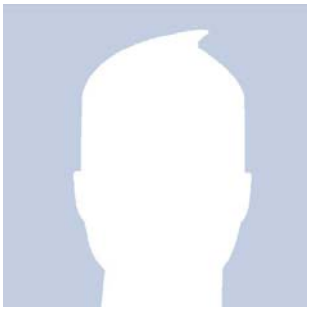
## *O22*
## *Chemoselective transformations in benign aqueous systems for bioimaging and targeted therapeutics*

Covalent protein modification is a key instrument in Chemical Biology: attachment of biophysical probes enables protein tracking and imaging; attachment of lipids to small therapeutic peptides extends their half-life in circulation; and protein conjugates are important biologic targets for the treatment of human diseases. In addition, proteins are modified after translation, increasingly significantly their structural complexity and functional capability. The use of efficient, complete, chemo- & regioselective methods in benign aqueous systems offers ways to redesign the structure and function of proteins of biological and therapeutic interest. This abstract features examples of (a) precise and controlled methods for site-specific protein labeling in vitro and in vivo; (b) development of chemically-defined, traceless therapeutic proteins for cancer therapy and (c) controlled delivery of therapeutic CO using chemically-defined carbonyl metalloproteins.



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## *O23*
## *Nanoparticles: functionalization and characterization*

Superparamagnetic iron oxide nanoparticles (SPION) have become important for various *in vivo* and *in vitro* biomedical applications such as imaging, magnetic separation, biosensor devices and therapy.

To be used in biomedical applications, SPION are usually stabilized in physiological media with biocompatible surface coating. They can also be surface modified for specific targeting or detection [1].

The main goal of NanoDiaRA European project was to try to synthesize, in laboratories, functionalized SPION with methods always adapted for a scaling-up process for industrial partners.

Standardized operating procedures and repeatable processes were developed to obtain, in a reproducible way, polymers and/or silica coated nanoparticles. The chemical characterizations of these nanohybrids such as sizes, charges or molecules covering and pre-biological studies (cytotoxicity, biodistribution,…) were also standardized to fit with up-scaling development.

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## *O24*
## *Biomarkers in RA and OA as tools for molecular imaging and immunoassays*

To identify patients at risk for progressive joint damage there is a need for early diagnostic tools to detect molecular events leading to cartilage destruction. Cartilage proteins are cleaved and released from the tissue into synovial fluid and serum in diseases such as osteoarthritis (OA) and rheumatoid arthritis (RA).

We have identified several fragmentations of cartilage proteins in synovial fluid and cartilage tissue samples that are particularly abundant in pathological conditions. To achieve this, we have used quantitative methods utilizing tandem mass spectrometry. These fragmentations could be potential biomarkers and we have generated a number of antibodies that only recognize the cleaved protein. Through development of immunoassays for these neo-epitopes we generate tools for early detection of disease and increased understanding of the molecular events in disease progression.

The antibodies developed will be coupled to nanoparticles to be used *in vivo* as well as *in vitro* applications.

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## *O25*
## *Biomarker assays in array format*

The simultaneous detection of three arthritis biomarkers will be presented and novel analytical IncaArray procedures will be introduced. Applying competitive immunoassay formats on a single analytical chip, a selected set of indicators of arthritic disorders: Cartilage Oligomeric Matrix Proteins (COMP), Cartilage Intermediate Layer Protein (CILP) and a type II collagen neoepitope (C2C), can be detected and quantitated. IncaArray analytical multiplex immunoassays closely meet the performance of current monoplex analytical procedures (e.g. ELISA) and are applicable with relevant biological fluids. Multiplex IncaArray procedures advantageously use small sample volumes and biomarker profiles are obtained in short time.

## ORAL PRESENTATIONS OF POSTERS

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### *P1*
### *Biodistribution of polymer shelled microbubbles supporting multimodal imaging*

The search of high performance theranostic devices to be used in different fields of customized medicine has addressed the researchers toward the formulation of multifunctional systems to target a specific pathology, to support multimodality imaging and finally to sustain a controlled drug release. In this context, we report on the fate and the biodistribution of injectable poly(vinyl alcohol) shelled microbubbles, a microdevice with air filled core, which acts as an ultrasound enhancing contrast agent.

The potentialities of this device have been studied during the activities of two European projects, SIGHT and 3MiCRON. The main results of this research, *i.e.* the development of multimodal contrast agent for ultrasound, magnetic resonance and nuclear medicine, and the findings of the on-going research on the cytotoxicity, biocompatibility and biodistribution will be highlighted.

Different administration ways were tested: preliminary results show that microbubbles can be visualized in the liver, spleen and, for nasal administration, lungs. After a week they are cleared from mice through the urine.

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## *P2*
## *Preparation and characterization of polymeric nanospheres for rheumatoid arthritis treatment*

Recent developments in the field of immune-regulatory neuropeptides led to the description of the effective anti-inflammatory activity of endomorphin 1 (EM-1) as potential candidate for the treatment of inflammatory disorders [1]. The therapeutic use of peptides has been popularized in the last decade, however the problem is delivering peptides to the specific site, in an intact and biologically active form and without the peptide being integrated into other tissues. To improve peptide drug efficiency a selective drug delivery system is usually required.

In this study, polymeric nanospheres encapsulating EM-1 as novel prototypes for rheumatoid arthritis (RA) treatment were designed, characterized and evaluated using macrophages. Nanosystems composed of a biocompatible blend of poly(epsilon-caprolactone) and poly(D,L-lactic-co-glycolic acid), were prepared and characterized in terms of morphology, particle size and zeta-potential, encapsulation efficiency and *in vitro* release studies. Cellular uptake of nanospheres was also evaluated in macrophages using confocal fluorescence microscopy and flow cytometry.

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## *P3*
## *Surface functionalization of polymeric nanospheres modulates macrophage activation*

Functionalization of nanospheres with ligands targeting specific receptors on antigen presenting cells, such as the mannose receptor, can potentially improve their immunotherapeutic effect [1,2]. This work aims to evaluate the effect of polymeric nanospheres surface functionalization with three carbohydrates (mannose, mannan, and mannosamine), on the activation status of macrophages. The cellular uptake, internalization mechanism and intracellular trafficking of carbohydrate-functionalized nanospheres were also evaluated.

Our results show that, macrophage co-culture with carbohydrate-functionalized nanospheres lead to macrophage activation (↑MHCII, CD86, CD80 and CD40), triggering production of pro-inflammatory cytokines (IL-6, IL12p40 and TNF-α), after internalization using clathrin-mediated endocytosis, reaching the lysosome of bone marrow derived macrophages in less than one hour.

The use of ligands immune-modulators could be a promising way to forward the immune system to a favorable environment for the host.

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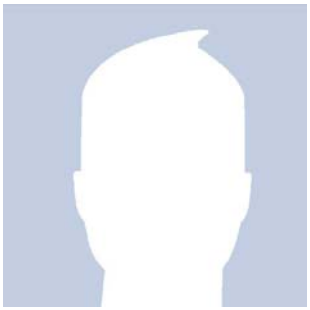
## *P4*
## *New polymer-ruthenium conjugates for targeted drug delivery in cancer chemotherapy*

One of the main problems of chemotherapy is the high noxious side effects caused by the lack of selectivity. The ideal situation is to have a drug that might only be delivered to the tumor without affecting the healthy tissues. In this frame, Polymer-Metal Complexes (PMCs) put forward an important contribution to the drug-delivery research in cancer therapy, using polymers as drug carriers. The great advantage is the easier accumulation of polymers and nanosized particles in the cancer cells relatively to the usual drugs. We will present new PMCs of the general formula [RuArLP]+(Ar=arene, L=heteroaromatic bidentate macroligand bearing a target vector and P=coligand), charged with our new emerging Ru compounds already recognized as an efficient cancer cell killers. The $IC_{50}$ values in several human cancer cell lines, together with a pH dependent hydrolysis suggests high potentialities for the application of RuPMCs as new drug delivery systems for RuIICp compounds.



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## *P5*
## *Small Core Gold Nanoparticles Stabilized by Thiolated DOTA Derivatives*

Gold nanoparticles (AuNPs) show very promising potential as diagnosis and therapeutic agents for cancer. AuNPs can be decorated with a wide range of distinct functionalizing molecules to enhance their target specificity and can be loaded with different diagnostic/therapeutic payloads. In this way, AuNPs can be considered attractive platforms for the development of theranostic agents.

In this work, we will describe the synthesis, characterization and biological evaluation of small core (3-5 nm) AuNPs (BBN-AuNP-TDOTA) stabilized with a DOTA based ligand (TDOTA) through thiol-to-gold covalent bonds, and also loaded with bombesin (BBN) as a target specific vector. AuNP-TDOTA and BBN-AuNP-TDOTA were successfully radiolabelled with 67Ga. Herein, it will be also reported the *in vitro* and *in vivo* evaluation of these 67Ga-labelled AuNPs in order to assess their interest for the design of nano-radiopharmaceuticals.

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## *P6*
## *Cryo transmission electron microscopy of liposome and protein based nanoparticles*

Transmission electron microscopy (TEM) can be utilized for resolving 3D structures of protein molecules or macromolecular complexes. In biological applications cryo-electron microscopy (cryo-EM) in combination with an image processing technique called single-particle reconstruction is widely used to study macromolecular complexes. The main advantage with this technique is that the complexes are visualized in a close-to-native state. Single-particle cryo-EM has resolved macromolecular complexes at near-atomic resolution.

In addition to 3D reconstruction approaches, TEM can also be utilized for extracting information like size and morphologies of nano-sizes particles. Nanometer-sized particles have recently been imaged on a system consisting of polyvinyl alcohol spheres, microbubbles. Moreover, as demonstrated herein, TEM imaging clearly visualizes the size and morphology of vitrified liposome and protein based nanoparticles. These particles have been modified to improve stealth and thus decrease their clearance by macrophages. Hence, these surface modified nanoparticles could be used for targeted administration of drugs.

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## *P7*
## *Core@shell nanostructures for medical applications*

Magnetic nanoparticles have gained increasing importance for applications in different areas of medicine, both in diagnostics and therapy.

Within the different magnetic materials, iron oxide nanoparticles (Fe3-xO4) are considered one of the best candidates for biomedical applications due to the good biocompatibility and strong magnetic properties, which are dependent on size and chemical composition [1]. The efficiency of the technical procedures depends on the control of the nanoparticles magnetic behaviour implying the good characterization of their structural and physical properties.

In order to manipulate and improve the magnetic properties of iron oxide nanoparticles, core@shell nanostructures have been synthesized combining different magnetic oxides.

This work presents the results of the structural, morphological and magnetic characterization of magnetite@hematite nanoparticles using XRD, TEM, SQUID magnetometry and Mössbauer spectroscopy. The purpose of this study is connected with applications in magnetic resonance imaging, drug delivery and cancer treatment by magnetic hyperthermia.

Acknowledgements

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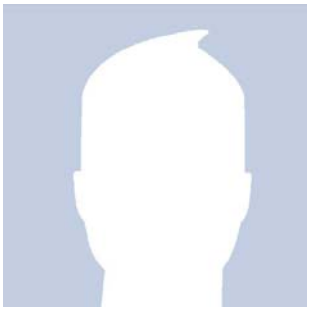

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NANOFOL
diagnosis/therapy nanobiodevice

## *P8*
## *Development of DODAB:MO Liposomes for Intracellular Gene Delivery*

The technology of DNA replacement therapy and siRNA silencing therapy is currently limited by the absence of efficient and safe viral/non-viral carriers that can condense, transport and release the genetic material into the cells. Liposomes such as the Dimethylammonium Bromide (DODAB) / Monoolein (MO) formulation present interesting properties of application in the gene therapy field. DODAB, a synthetic cationic lipid with an high melting temperature (Tm) of 45 °C, tends to form gel-lamellar phases at physiological temperatures, which are fluidized by the presence of the non-lamellar forming lipid MO, associated with the appearance of inverted cubic mesophases at high MO contents ($X_{MO} \geq 0.5$). This rich phase diagram gives rise to a versatile lipid structure in the presence of nucleic acids, characterized by cryo-TEM imaging, fluorescence spectroscopy and light scattering techniques. This system reveals a similar transfection efficiency to the commercial transfection reagent Lipofectamine, while presenting a very low cytotoxic profile.

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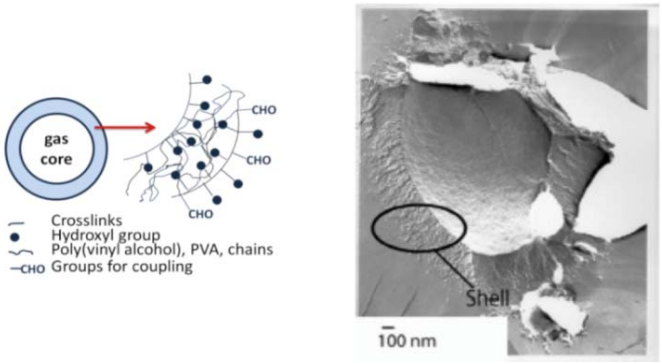
## *P9*
## *Ultrasound contrast agent loaded with nitric oxide as a theranostic microdevise for myocardial ischemia*

Ischemic heart disease is the most widespread cardiovascular disease, equally diagnosed in both genders with mortality exponentially increasing with age. Efforts of healthcare system should be primary focused on prevention, timely detection, efficient differentiation and instant treatment of the disease.

Current project introduces a new class of microdevice providing integrated diagnostic and therapeutic applications, i.e. theranostics, of ischemic heart disease using novel multifunctional polymer-shelled microbubbles (MBs) loaded with nitric oxide (NO). Proposed MBs can be manufactured according to clinical requirements of controlled size below 5 µm and narrow size distribution for improved ultrasound imaging. They are chemically and mechanically stable. Therapeutic core gas (NO) has anti-clotting effects on blood, dilates vessels and reduces myocardial injuries. Systemic treatment with NO has however a very narrow therapeutic window and may provoke critically low blood pressure. As a result, local, specific and controlled delivery of NO is of great clinical importance.




Left: Schematic representation of PVA MB. Right: Electron micrograph of freeze-fractured MB showing a shell thickness of about 200 nm with a microstructure of PVA microfibrils.

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