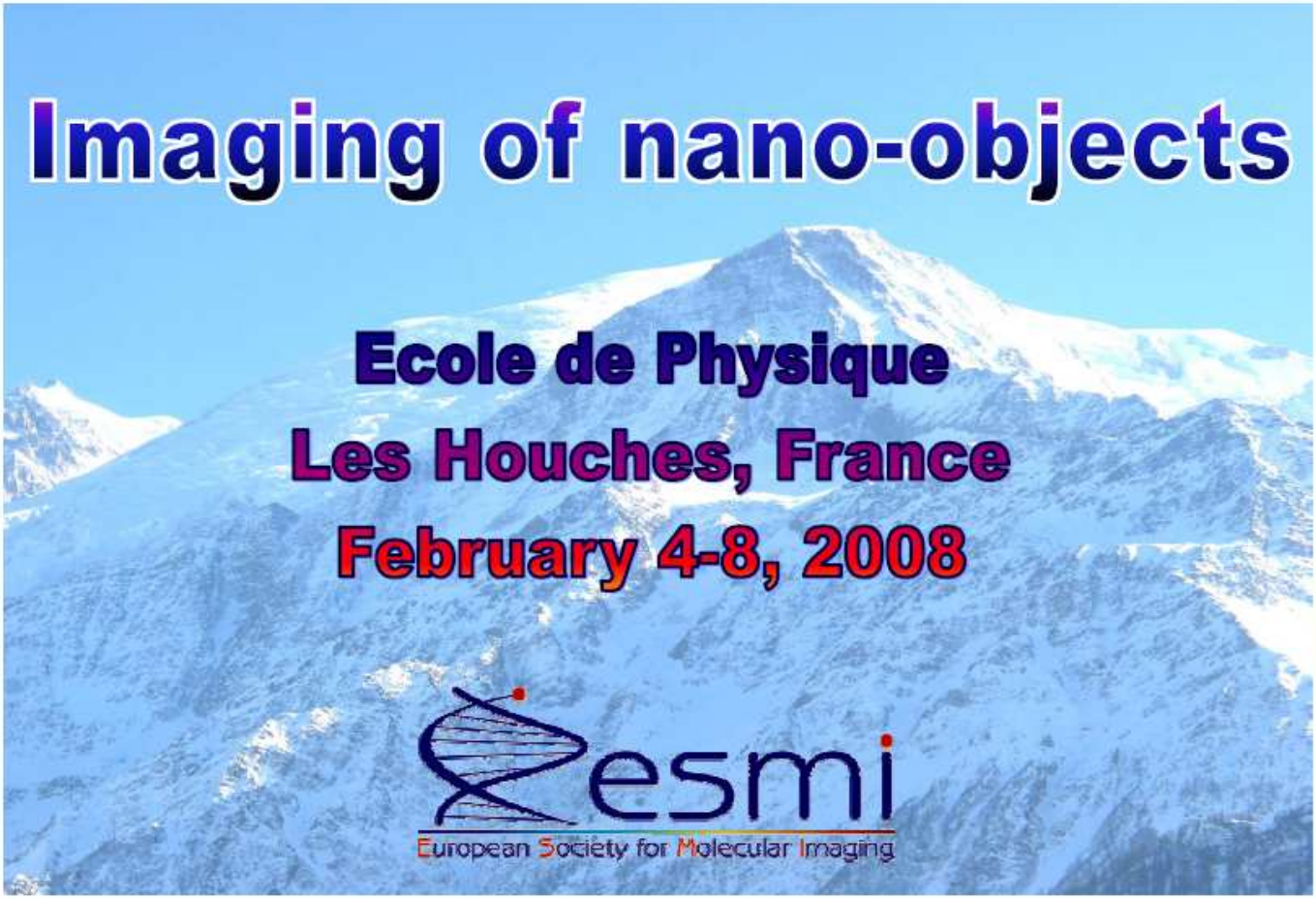
TOPIM'08

Hot Topics in Molecular Imaging 2008



Scientific committee

Bertrand Tavitian, Frédéric Ducongé, Andreas Jacobs, Patrick Curmi, Eva Pebay-Peyroula, Silvio Aime

esmi European Society for Molecular Imaging

European Molecular Imaging Laboratories

DiMI Diagnostic Molecular Imaging

Centre de Physique des Houches

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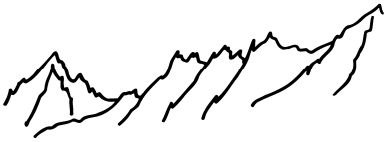
ECOLE DE PHYSIQUE DES HOUCHES	
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The **European Society for Molecular Imaging** (**ESMI**) is a non-profit and apolitical society, which promotes the development and practical application of Molecular Imaging within Europe. ESMI extends actions initiated in the scope of the 6th framework with the EMIL (European Molecular Imaging Laboratories) & DiMI (Diagnostic Molecular Imaging) Networks of Excellence and the Molecular Imaging Integrated Project, aspiring to be the premier body in its field within Europe and to promote research and practice of Molecular Imaging for benefits in healthcare, science and technology.
Membership in the organization is open to all persons who share the vision of the organization and have educational, research, or practical experience in some aspect of molecular imaging. For additional information on our Society, you can reach the ESMI website at: www.e-smi.eu.

All ESMI members receive the official journal of the Society, ***Molecular Imaging*** (www.journalsoft.com/molecularimaging/journal), the premier journal for imaging biochemical and cellular events in intact living organisms.

ESMI is proud to announce the venue of the **World Molecular Imaging Congress** (**WMIC**), the most important event in Molecular Imaging ever. The WMIC is co-organized by the ESMI, the Society for Molecular Imaging (SMI), the Academy of Molecular Imaging (AMI) and the Federation of Asian Societies for Molecular Imaging (FASMI) in **September 10-13**, **2008 in Nice** (France). In this context, we are pleased to announce that all ESMI members will benefit from a privileged access to the WMIC, including a substantial reduction on their registration fees. Please find additional information on the WMIC web-site: www.wmicmeeting.org.

2008 World Molecular Imaging Congress

FOREWORD

I like to think of molecular images as the scientific equivalents of paintings: a snapshot of organisms in a given situation (characters), depicted as spatial arrangements of chemicals (dabs of colours), produced by sophisticated techniques (artists). Molecular images bridge physical detectors with exquisite chemicals to explore life; just as paintings, they need not to be figurative to be meaningful.

Molecular imaging is a new science with already a strong influence on medicine and biology, leading the way in exploiting molecular, biological and genetic information to develop precise, precocious and predictive diagnostic methods. These methods are increasingly precious for the follow-up and the evaluation of new treatments of many pathological states.

These two features of molecular imaging: the bridge that it creates between biology, chemistry, physics and mathematics, and its growing importance in medicine, suffice to justify the creation of the European Society for Molecular Imaging (ESMI). But even more crucial is the fact that molecular imaging is asserting itself as an original means of discovery, and opens new avenues to address unexplored questions. This is what TOPIM, *Hot Topics in Molecular Imaging*, is all about: capture these assertions by providing an instant picture of the field, and foster new ones through the discussions between participants.

Naturally, it would be an incommensurable task to cover all aspects of the burgeoning molecular imaging field in just a few days, and there are already annual meetings which aim at doing that. As a consequence, rather than proposing a catalogue of recent releases in molecular imaging, the ESMI council decided to concentrate on one aspect at the forefront of the discipline, a *hot topic*. TOPIM is an annual rendezvous concentrating on one domain of application or technique of molecular imaging, chosen according to its pertinence and timeliness.

It is therefore no surprise that TOPIM'08 should deal with nano-imaging. Given the importance that nano-objects are likely to take in molecular medicine, it appears timely to combine the expertise in the analysis of distribution of large objects *in vivo* with the expertise in the fabrication and functionalization of these objects. Since the 1990s, progress made by physicists and chemists has provided a wealth of nano-scaled tools predicted to have a large impact on life sciences and particularly on cancer treatment. Nanoparticles are nowadays extensively exploited *in vitro* to achieve fundamental understanding of biological processes or disease diagnostic. In medicine, nanodevices offer important new possibilities, both for high contrast agents and therapeutic payloads. *In vivo* clinical applications of nano-objects are just beginning but their potential seems to be high and many efforts are provided to build and functionalized these nano-objects in order to achieve long circulation time, good biocompatibility and low immunogenicity, efficient penetration of physiological barriers, selective targeting, external activation or self regulating drug release... However, despite the appealing applications of nanotechnologies their use *in vivo* raises the question of the precise distribution and elimination pathways, as well as the toxicity associated with nano-scaled objects.

The organizers (Andreas Jacobs, Patrick Curmi, Eva Pebay-Peyroula, Silvio Aime, Frédéric Ducongé and myself) have been most fortunate to attract a panel of prestigious speakers, all at the fore point of research in their discipline. We would like to thank them heartily, especially those who have travelled a long way to the French Alps, for having accepted to share their knowledge with us. We are also indebted to the Ecole de Physique des Houches and to its committee for supporting the TOPIM project since the first *Hot Topics* event in February 2007. We would like as well to warmly thank our sponsors, many of them being with us from the very beginning of the ESMI, for their generous support. Last but not least, our warmest thanks go to Irina Carpusca for her precious help and dedication in organizing this event.

I would like to address my warmest welcome to all of you, and to encourage you to participate without restriction in the scientific debates as well as to enjoy the beauties of the mountains surrounding us. As hot as the topics and discussions may be – and I hope they will – there is not one chance that they will melt the snow under your skis.

Bertrand Tavitian, *President of the European Society for Molecular Imaging*

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09:45 - 10:30	**Imaging-guided Drug Delivery** Silvio AIME, Torino	**Core-Shell Silica Nanoparticles as Fluorescent Labels for Nanobiotechnology and Nanomedicine: C Dots** Ulrich WIESNER, New York	**The influence of the chemical Coating on the Bio-distribution of Quantum Dots *in vivo*** Toufic DAOU, Grenoble	**Luminescent Oxide Nanoparticles : Synthesis, Properties and Applications** Thierry GACOIN, Palaiseau	**Chromatic Polymer Nano-patches for Imaging Membrane Processes in living Cells** Raz JELINEK, Beer Sheva
10:30 - 10:50					
10:50 - 11:35	**Quantum Dots for *in vivo* imaging** Benoît DUBERTRET, Paris	**Fluorescent Proteins and fundamental Aspects on Photophysics** Carlheinz ROECKER, Ulm	**Nano-objects, Properties, Assemblies and Applications** Luisa DE COLA, Münster	**Shape specific Nanoparticles as Contrast Agents for opto-acoustic Detection** Jean-François GREISCH, Liège	**Dendrimer Nanoplatforms for effective Tumor Targeting** Ella JONES, San Francisco
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12:05 - 12:20	Presentation submitted abstract **Use of Optical Imaging for Detection, medical Imaging and Treatment of Cancer** Sandrine DUFORT, Grenoble	Presentation submitted abstract **Micro-axial Tomography** Udo BIRK, Heraklion	Presentation submitted abstract **Novel Organometallic Complexes for multimodal Imaging** António PAULO, Sacavém	Presentation submitted abstract **The Size of Magnetic Nanoparticle finely tunes their heating Power under a high Frequency alternating Magnetic Field** Michael LEVY, Paris	
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PROGRAM

	Monday, 4th February
08:00 - 08:45	**BREAKFAST**
SESSION	**SCHOOL**
09:00 - 09:20	**INTRODUCTION** **Bertrand TAVITIAN, President ESMI** **Patrick BRESSLER, Representative ESF**
09:20 - 09:45	**Fluorine-18 Chemistry : from FDG to the Labelling of Macromolecules and Nano-objects** Frederic DOLLE , CEA, Orsay, France
09:45 - 10:30	**Imaging-guided Drug Delivery** Silvio AIME, Universita degli Studi di Torino, Torino, Italy
10:30 - 10:50	**BREAK**
10:50 - 11:35	**Quantum Dots for *in vivo* imaging** Benoît DUBERTRET, Ecole Supérieure de Physique et de Chimie Industrielles, Paris, France
11:35 -11:50	Presentation submitted abstract **Photoswitchable Fluorescent Proteins - Tools for Far-Field Microscopy at the Nanoscale** Hannes BOCK, Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany
11:50 - 12:05	Presentation submitted abstract **Iodinated Emulsion for multimodality Imaging using spectral CT and SPECT** Anke DE VRIES, Eindhoven University of Technology, Eindhoven, The Netherlands
12:05 - 12:20	Presentation submitted abstract **Use of Optical imaging for Detection, medical Imaging and Treatment of cancer** Sandrine DUFORT, Institut Albert Bonniot, La Tronche, France
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16:15 - 17:00	**Iron Oxide Nanoparticles in MRI Imaging** Marc PORT, Research Center Guerbet, Roissy, France
17:00 - 17:45	**Investigating cellular Processes at the single Molecule Level using Semiconductor Nanoscrystals** Maxime DAHAN, Département de Physique de l'Ecole Normale Supérieure, Paris, France
17:45 - 18:30	**Silica Nano-particles** Luca PRODI, Università di Bologna, Bologna, Italy
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18:45 - 19:30	**Optical Detection and Spectroscopy of single nonfluorescent Nanoparticles and Demonstration of a long-distance nanometric Ruler** Philipp Kukura, ETH Zürich, Switzerland
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09:45 - 10:30	**Core-Shell Silica Nanoparticles as Fluorescent Labels for Nanobiotechnology and Nanomedicine: C Dots** Ulrich WIESNER, Cornell University, New York, USA
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10:50 - 11:35	**Fluorescent Proteins and fundamental Aspects on Photophysics** Carlheinz ROECKER, Ulm University, Institute of Biophysics, Ulm, Germany
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11:50 -12:05	Presentation submitted abstract **Dendronised Magnetite Nanoparticles as Contrast Agent for MRI** Sylvie BEGIN-COLIN, Institut de Physique et Chimie des Matériaux, Strasbourg, France
12:05 -12:20	Presentation submitted abstract **Micro-axial Tomography** Udo BIRK, Foundation for Research and Technology-Hellas (FORTH), Heraklion, Crete, Greece
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17:00 - 17:45	**Integrated molecular Imaging : from Probes and Cameras to Human; the CEA-LETI Experience** Patrick BOISSEAU, CEA-Léti-MiNaTec , Grenoble, France
17:45 - 18:30	**Cell Labelling with magnetic Nanopartiocles : Mechanisms, Biocompatibility, MRI Cell Imaging and magnetic Manipulations** Florence GAZEAU , Université Paris-Diderot, Paris, France
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18:45 - 19:30	**STED Microscopy** Christian EGGELING, Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany
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10:30 - 10:50	**BREAK**
10:50 - 11:35	**Nano-objects, Properties, Assemblies and Applications** Luisa DE COLA, Westfälische Wilhelms-Universität, Münster, Germany
11:35 - 11:50	Presentation submitted abstract **Zeolite L Nanocrystals for Imaging Applications** Manuel TSOTSALAS, Westfälische Wilhelms-Universität, Münster, Germany
11:50 -12:05	Presentation submitted abstract **Determination of the depth Resolution Limit of Fluorescence diffuse optical Tomography** Matthieu BOFFETY, Laboratoire EM2C, CNRS, Ecole Centrale Paris, Châtenay-Malabry, France
12:05 -12:20	Presentation submitted abstract **Novel Organometallic Complexes for multimodal Imaging** António PAULO, Instituto Tecnológico e Nuclear, Sacavém, Portugal
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17:45 - 18:30	**Colour Centers in Nanodiamonds** Fedor JELEZKO, Stuttgart University, Stuttgart, Germany
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09:45 - 10:30	**Luminescent oxide Nanoparticles : Synthesis, Properties and Applications** Thierry GACOIN, Laboratoire de Physique de la Matière Condensée Ecole Polytechnique, Palaiseau, France
10:30 - 10:50	**BREAK**
10:50 - 11:35	**Shape specific Nanoparticles as contrast Agent for Opto-Acoustic Detection** Jean-François GREISCH, Liège University, Functionalized Nanoparticles, Liège, Belgium
11:35 - 11:50	Presentation submitted abstract **Controlling single Molecule Fluorescence through plasmonic Slabs** Cédric VANDENBEM, Laboratoire EM2C, CNRS, Ecole Centrale Paris, Paris, France
11:50 -12:05	Presentation submitted abstract ***In vivo* Imaging of vascular Permeability using Nano-objects in Mice Tumor** Masaaki KAWAI, Graduate School of Medicine, Tohoku University, Sendai, Japan
12:05 - 12:20	Presentation submitted abstract **Magnetic Nanoparticle Size finely tunes their heating Power under a high frequency alternating magnetic Field** Michael LEVY, Université Paris 7, MSC, Paris, France
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16:15 - 17:00	**Nanoscale mMRI Contrast Agents** Arne HENGERER, Siemens, Erlangen, Germany
17:00 - 17:45	**Iron oxide Nanoparticles as multifunctional Imaging and Delivery Agents** Zdravka MEDAROVA, MGH/MIT/HMS Athinoula A. Martinos Center for Biomedical Imaging,Massachusetts, USA
17:45 - 18:30	**Persistent Luminescence Nanoparticles (PLNs) : a new imaging tool for biologists ?** Quentin LE MASNE DE CHERMONT, Université Paris Descartes & Biospace, Paris, France
18:30 - 18:45	**BREAK**
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09:45 - 10:30	**Chromatic Polymer Nano-patches for Imaging Membrane Processes in living Cells** Raz JELINEK, Ben Gurion University, Beer Sheva, Israël
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11:35 - 11:50	Presentation submitted abstract **Paramagnetic Collagen binding Contrast Agents** H.M.H.F. SANDERS, Eindhoven University of Technology, Eindhoven, The Netherlands
11:50 -12:05	**CONCLUSION** Bertrand TAVITIAN, President ESMI
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ABSTRACTS OF PRESENTATION

IMAGING OF NANO-OBJECTS

FLUORINE-18 CHEMISTRY: FROM FDG TO THE LABELLING OF MACROMOLECULES AND NANOOBJECTS

Frédéric Dollé

CEA, Institut d'Imagerie Biomédicale, Service Hospitalier Frédéric Joliot, Orsay, France

Molecular *in vivo* imaging with the high-resolution and sensitive positron emission tomography (PET) technique requires the preparation of positron-emitting radiolabelled probes or radiotracers. For this purpose, fluorine-18 ($T_{1/2}$: 109.8 min) is becoming increasingly the radionuclide of choice due to its adequate physical and nuclear characteristics. The successful use in clinical oncology of 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG), the currently most widely used PET-radiopharmaceutical, is manifestly also the motor behind the growing availability and interest for this positron emitter in radiopharmaceutical chemistry. Fluorine-18 chemistry, however, presents some drawbacks, in particular the limited options in labelling strategies, which today are dominated by nucleophilic substitutions using no-carrier-added (high-specific-radioactivity) [^{18}F]fluoride as its K[^{18}F]F-K_{222} complex. The bases and some recent advances of fluorine-18 radiochemistry will be presented and the potential of this radioisotope in the design and preparation of fluorine-18-labelled probes for PET imaging will be highlighted. Particular attention will be given to an emerging research line with a bright future, which is the radiofluorination of macromolecules of biological interest such as peptides, proteins and oligonucleotides but also nanoobjects. Often performed by prosthetic conjugation, the labelling of these complex and high molecular weight structures with the short half-lived positron-emitter fluorine-18 remains a challenge.

IMAGING-GUIDED DRUG DELIVERY

Silvio Aime

Department of Chemistry & Center for Molecular Imaging, University of Torino

There is a large gap between the “magic bullet”, that is the dream of pharmacologists, and the currently available therapeutic treatments. Nanotechnology will help to fill this gap, as it provides the pharmacist with an armoury of tools that will allow the delivery of relatively high drug loads to their site of action. Through the use of specific reporters suitably co-added to the pay-load of nano-sized delivery structures, imaging offers the possibility of an *in vivo* visualization of drug targeting and drug releasing phases. Furthermore it may provide information on the microenvironment of the diseased region (pH, temperature, enzymatic activity, metabolites’ concentration, etc.). Thanks to its superb spatial resolution MRI appears to be the most appropriate technique although important insights for a detailed understanding of the drug delivery process and for monitoring the therapeutic output may be gained with other imaging modalities.
The synergy between the characteristics of the nano-carrier and the potential of the molecular imaging assessment prompts the development of innovative interventional procedures for operating drug release through the use of external devices that expose the region to ultrasound or heat. The external control of drug release, together with the monitoring of the biomarkers, will offer the clinician the possibility of providing the patient with a much safer and more efficient drug administration.

QUANTUM DOTS FOR *IN VIVO* IMAGING

T. Pons[1], B. Mahler[1], F. Ducongé[2], C. Pestourie[2], B. Tavitian[2], B. Dubertret[3]

[1]*Laboratoire Photons et Matière, CNRS UPRA0005, ESPCI, Paris*
[2]*CEA SHFJ, Inserm, ERM 103, Orsay*
[3]*Laboratoire Photons et Matière, CNRS UPRA0005, ESPCI, Paris*
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Semiconductor quantum dots are fluorescent crystalline nanoparticles with an emission wavelength that can be tuned between 400 nm and 1500 nm depending on their size and their composition. We will discuss recent results that we have obtained in QD core/shell synthesis and new understanding of the QD physico-chemical properties. We will detail several applications of these QDs for *in vivo* imaging. We will stress the importance of QD surface chemistry and present the synthesis of QD multimodal probes that could find useful applications for multifunctional imaging.

PHOTOSWITCHABLE FLUORESCENT PROTEINS – TOOLS FOR FAR-FIELD MICROSCOPY AT THE NANOSCALE

H. Bock, S. Jakobs, C. Eggeling, S.W. Hell

Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany

Fluorescence far-field microscopy is a very sensitive analysis tool. However, its resolution is limited by the diffraction of light: similar objects closer than about 200 nm cannot be discerned. **Methods:** The key of breaking the diffraction barrier in far-field microscopy is the exploitation of the fluorophore properties, in particular of their states. Specifically, by utilizing at least two distinguishable molecular states, such as a ‘bright’ and a ‘dark’ state, it is possible to ensure that the measured signal stems from a region of the sample which is much smaller than these 200 nm. **Results:** We implemented two different approaches to super-resolution imaging using photoswitchable proteins: 1) repeated sparse activation, excitation and localization of single isolated fluorophores and 2) scanning the sample with an effectively sub-diffraction sized structured excitation pattern created by specific saturated fluorescence depletion. **Conclusion:** Photoswitchable fluorescent proteins constitute a powerful tool for super-resolution imaging of biological specimen at comparatively low light intensities. We anticipate that they will play a vital role in addressing a multitude of questions at the interface of molecular and cellular biology.

IODINATED EMULSION FOR MULTIMODALITY IMAGING USING SPECTRAL CT AND SPECT

A. de Vries[1], J. P. Schlomka[2], E. Rössl[2], K. Nicolay[1], H. Grüll[1,2]

[1]Biomedical NMR, Department of Biomedical Engineering, Technical University Eindhoven
[2]Philips Research Europe

CT is one of the most frequently used clinical imaging modalities for diagnosis of bone fractures, lesions, and also cardiovascular diseases. CT contrast agents are usually based on iodinated small molecules that extravasate rapidly and show fast urinal excretion. For many CT and interventional x-ray applications, a longer circulating blood pool agent is desired. Furthermore, x-ray/CT can not differentiate between contrast generated by agents and radio-opaque structures such as lesions with for example a high degree of calcification in coronary plaques. We will present work on iodinated emulsion based CT contrast agents that show prolonged blood circulation. The emulsions are based on an iodinated hydrophobic oil using lipids as emulsifier. The lipid layer offers also the advantage to incorporate PEG modified lipids to extend blood circulation or to introduce labels for nuclear imaging. Secondly, we will show results obtained with a new imaging technique “spectral CT” that allows to only image the contrast agent by making use of the k-edge absorption of high z-elements. The technique allows quantification of the used contrast agent and can differentiate between for example contrast originating from calcium. Several different emulsion formulations were characterized *in-vitro* for size and stability using cryo-TEM, light scattering and CT. First *in-vivo* studies were performed using CT to test the blood circulation and organ uptake. A first scan of a mouse injected with an iodinated emulsion was performed using a spectral CT scanner at Philips Research. The k-edge imaging technology is able to visualize all well perfused tissues and structures containing the iodinated agent without interference from e.g. bone structures (Figure 1). Work is in progress on multimodal imaging with SPECT/CT using radiolabeled emulsions in order to correlate the contrast enhancement in (spectral) CT with the absolute amount of emulsion particles present in the organ or lesion of interest.

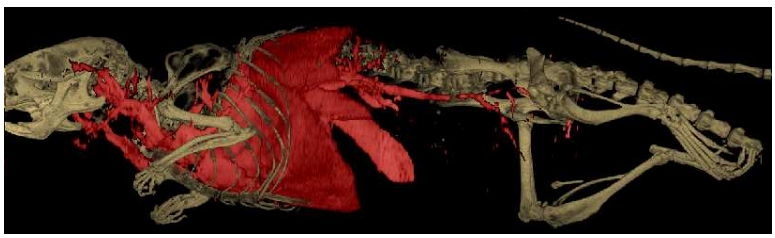

Figure 1: Spectral CT image visualizing the contrast agent and bone structure separately

USE OF OPTICAL IMAGING FOR DETECTION, MEDICAL IMAGING AND TREATMENT OF CANCER

Sandrine Dufort[1], Véronique Josserand[1], Lucie Sancey[1], Claire Rome[1], Zhaohui Jin[1], Stéphanie Foillard[2], Didier Boturyn[2], Pascal Dumy[2], I. Texier[3], Anne-Laure Faure, Stéphane Roux[4], Olivier Tillement[4], Philippe Rizo[3], Jean-Luc Coll[1]

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Our former involvement in non-viral gene therapy prompted us to specialize in the development of targeting molecules, which could function as a vector for drug delivery, medical imaging and when attached on the surface of a nanoparticle (NP). Indeed, synthetic gene therapy vectors form NP of 50 nm in width. Our previous work demonstrated that the “simple” covalent loading of an antitumor antibody or peptide may provide a certain specificity for the desired target but is not sufficient for in vivo activity. The main reason is that these ligands are not presented correctly in orientation, conformation and density on the surface of the NP and lose most of their activity after coupling. Since this time, we developed and patented RAFT - a more suitable scaffold. Coupling innovative targeting peptides on the RAFT and then on the NP provide targeting to tumour cells.
RAFTs in combination with oxime technology have provided fast chemical access to complex molecular systems with tailor-made recognition properties, useful in the field of drug targeting. In this context, we have devised a very selective and effective αvß3 integrin targeting system based on the multivalent presentation of the conformationally constrained RGD-containing cyclopeptide, c[-RGDfK-], by RAFT molecule.
In parallel we developed several 2D or 3D optical machines, enabling us to provide original solutions for pre-clinical imaging, therapy and surgery of tumours in association with the use of the RAFT-probe.

CLINICAL APPLICATION OF IRON OXIDE NANOPARTICLES IN MAGNETIC RESONANCE IMAGING AND RESEARCH PERSPECTIVES

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Superparamagnetic nanoparticles of iron oxides (SPIO and USPIO) have become a major tool for medical imaging with a wealth of applications. For nearly 20 years, research in the field of magnetic resonance imaging (MRI) contrast agents has been oriented towards the study and development of these iron oxide nanoparticles, because they are highly effective in MRI as strong enhancers of proton relaxation (1/T1 , 1/T2 and 1/T2*).
The multiple components which govern the efficacy of these agents require them to be characterised as accurately as possible by information such as the size of the iron oxide crystals, the charge, the nature of the coating, the hydrodynamic size of the coated particle, etc. These physico-chemical characteristics not only affect the efficacy of the superparamagnetic particles in MRI but also their stability, biodistribution and metabolism as well as their clearance from the vascular system.
The aim of this presentation is to illustrate:

1. the MRI efficacy of iron oxide nanoparticles;
2. the physico-chemical characterisation of SPIO and USPIO;
3. the clinical applications of iron oxide in MRI : macrophage imaging and blood pool imaging;
4. the research perspectives in the field of stem cell labelling and molecular imaging.

INVESTIGATING CELLULAR PROCESSES AT THE SINGLE MOLECULE LEVEL USING SEMICONDUCTOR NANOCRYSTALS

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Colloidal semiconductor quantum dots (QDs) are remarkable fluorescent nanoparticles with great interest for optics and biology. Their brightness and photostability have in particular enabled new experiments at the single nanoparticle level. Using a combination of physical or biochemical methods, single QDs can be inserted in various optical or biological environments and used as local probes. This has in particular found a wide range of applications for the tracking of individual biomolecules in live cells. We will first discuss the optical, chemical and biological techniques that are required to use QDs as markers for single molecule research. Next we will illustrate the principles and interest of single molecule experiments in cell biophysics with two examples: the diffusion of membrane proteins necessary for the transmission of neuronal signals and the dynamics of molecular motors involved in intracellular transport. Finally, we will present the perspectives offered by ultrasensitive microscopy techniques and the challenges that still need to be met to make single QD imaging a standard tool in cell biology.

LUMINESCENT SILICA NANOPARTICLES: ORGANIZATION AND VERSATILITY TOWARDS BRIGHTNESS

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Great efforts have been recently made towards the design of new labelling materials, endowed with high brightness, for medical diagnostics and imaging. The most studied ones are quantum dots and dye-doped nanoparticles (DDNs) and these last systems appear to be the most promising ones being more stable and less toxic. Silica, in fact, is a very good candidate as a protective matrix due to its proven biocompatibility and stability in most of the biosystems. In addition, the high versatility of the synthesis allows the organization of different shells in order to increase the photophysical performances of the system. The possibility to encapsulate thousands of dye molecules into one DDN and to control photoinduced energy- and electron-transfer processes inside each shell and/or between them can lead to systems having highly valuable features, such as high absorption, high quantum yield, large Stokes shifts, better photostability, and better protection from quenching processes by external species. In addition, high signal amplification processes can be obtained, and this could be particularly valuable in systems acting as chemosensors. In fact, allowing strong signal changes also at very low analyte concentration this can lead to unprecedented sensitivities. In this contribution, we will discuss our last results on the synthesis and characterization of dye doped silica nanoparticles; some applications in the field of *in vivo* imaging will be also presented.

OPTICAL DETECTION OF SINGLE *NON*FLUORESCENT NANOPARTICLES AND DEMONSTRATION OF A LONG-DISTANCE NANOMETRIC RULER

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The advent of various single molecule detection techniques in the 1990s has pushed fluorescence microscopy to its limit, where a single dye molecule is used to visualize the location, translation or rotation of nanoscopic biological entities such as proteins. At the single emitter level, however, all fluorescent systems confront the problem of limited photostability. We show that an interferometric microscopy method can be used to detect nonfluorescent nanoparticles such as gold particles down to a diameter of 5 nm [1], microtubules [2] or single viruses on membranes [3]. Furthermore, we shall show that the modification of the fluorescence lifetime of an emitter close to a gold nanoparticle can be used as a measure for changes in the separation between the two [4, 5]. This method extends the range of fluorescence resonant energy transfer (FRET) as a nanoscopic ruler to 10-40 nm. If time permits, we will also discuss the design of plasmonic nanostructures (nano-antennae) for achieving very large modifications of the spontaneous emission rate and thus improvement of the quantum efficiency of poor emitters [6].

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PHOTOLUMINESCENT CARBON DOTS AND BIOIMAGING APPLICATIONS

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Fluorescent semiconductor quantum dots have generated much excitement for a wide variety of promising applications, especially those in biology and medicine. For both *in vitro* and *in vivo* uses, however, the known toxicity and potential environmental hazard associated with many of these materials may represent serious limitations. Therefore, the search for benign nanomaterials of similar optical properties continues. For quantum-sized silicon, the discovery of Brus and co-workers on the strong luminescence in surface-oxidized nanocrystals has attracted extensive investigations of silicon nanoparticles and nanowires. We have discovered that small carbon nanoparticles (preferably less than 10 nm) can be made highly photoactive upon simple surface passivation, exhibiting strong photoluminescence in both solution and solid-state and with either one- or two-photon excitation.[1,2] These luminescent carbon nanoparticles ("carbon dots") may find applications alternative to or beyond those of traditional semiconductor quantum dots. For example, they may be derivatized to recognize and bind to biologically active species. Some representative experimental results will be presented, along with discussion on mechanistic issues.

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CORE-SHELL SILICA NANOPARTICLES AS FLUORESCENT LABELS FOR NANOBIOTECHNOLOGY AND NANOMEDICINE: C DOTS

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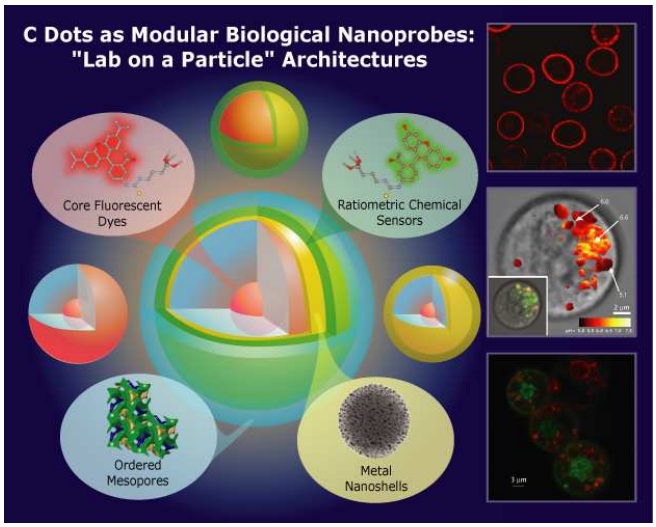
Fluorescent nanoparticles offer enormous scientific and technological promise as labels and photon sources for a range of biotechnological and information-technology applications such as biological imaging, sensor technology, microarrays, optical computing, and display technology. Many applications require size-controlled, monodisperse, bright nanoparticles that can be specifically conjugated to biological macromolecules or arranged and positioned in higher-order structures and devices. As an alternative to single molecule fluorophores and quantum dots, fluorescent silica-based particles derived through the Stöber process hold particular promise since they are more biocompatible, are water soluble, silica chemistry is well established and extremely versatile, and silica is compatible with semiconductor processing. The presentation will report on programs at Cornell's Center for Materials Research (CCMR) and Nanobiotechnology Center (NBTC) to develop a novel class of multifunctional silica-based fluorescent core-shell nanoparticles referred to as C-dots. Results on C-dot synthesis and characterization are discussed and various life sciences applications are demonstrated with developments towards labels for nanomedicine.



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FLUORESCENT PROTEINS AND FUNDAMENTAL ASPECTS ON PHOTOPHYSICS

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Since the discovery of the first green fluorescent protein (FP) in a hydromedusa almost 50 years ago, a wide variety of proteins from this family was identified and engineered into genetically encoded fluorescent markers. The DNAs coding for the FP and the target protein can be fused to obtain a functional fluorescently tagged protein for probing gene expression, protein trafficking, localization and protein-protein interactions within living cells. In recent years, photophysics and photochemistry of FPs was studied in great detail, and the advent of photoactivatable FPs opened further exciting application fields such as regional optical marking within living cells and ultra-high resolution imaging. Here, we discuss photophysical properties and cellular applications of FPs, with a focus on EosFP, a protein the fluorescence emission of which can be switched from green (516 nm) to red (581 nm) by irradiation with 400-nm light. Besides classical optical spectroscopies, fluctuation spectroscopy and single molecule methods are being employed to explore the photophysics of FPs. We demonstrate applications in embryogenesis and cell division using one-photon photoconversion and regional optical marking by two-photon conversion of EosFP within the mitochondrial networks of HeLa cells. The optically highlighted regions could be imaged with an axial resolution in the 100-nm range using dual-color 4Pi microscopy.

OPTICAL PROPERTIES OF FLUORESCENT PROTEINS IN CONTROLLED ENVIRONMENTS

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In recent years, fluorescent proteins (FPs) have become indispensable tools in life science research. They are frequently used in studies of gene expression, protein-protein interaction and protein localization in cells. To further advance this technology, it is desirable to improve photophysical and photochemical properties of FPs, including brightness and photostability, by molecular engineering. To this end, the relation between molecular structure/dynamics and optical properties need to be known. Here we have analyzed properties of the photoactivatable protein EosFP[1,2] on different surfaces and under different environmental conditions.
The processes that affect light emission from fluorescent proteins are often hidden in measurements on large ensembles. Single molecule spectroscopy is a promising technique to gain insight into the details of the photophysical and photochemical processes that affect light emission from fluorescent proteins. With a high-pressure cell that enables us to study molecules at pressures up to ~4000 bar, we observed a pronounced influence of pressure on the quantum yield and the emission spectrum of EosFP. A similar profound effect on the optical properties originates from interactions of the protein with its environment. We have investigated the influence of protein-surface interaction on bleaching times and the blinking behavior of fluorescent proteins.

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DENDRONISED MAGNETITE NANOPARTICLES AS CONTRAST AGENT FOR MRI

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Magnetic resonance imaging (MRI) is one of the most powerful techniques in diagnostic clinical medicine and biomedical research. To enhance the contrast between normal and diseased tissues or to indicate organ functions or blood flow, contrast agents based on paramagnetic gadolinium or manganese ions, or superparamagnetic particles, consisting of colloidal magnetic iron-based nanoparticles, are intravenously administered to patients. The latter field of iron nanoparticles has attracted an increasing interest in the last 10 years due. However the main difficulty is to obtain homogeneous and stable aqueous suspensions of iron oxide nanoparticles without aggregates. Indeed sizes smaller than 200nm are required to avoid toxicity and smaller than 20nm to improve tissular diffusion. Iron oxide nanoparticles, mainly maghemite, are in general coated with dextran (sugar-based polymer) and prepared by a method leading to a wide particle size distribution. In this work, very stable aqueous suspensions of magnetite nanoparticles with an average size of 10 nm have been prepared by co-precipitation of iron chlorides by a base. These nanoparticles have then been covalently coated with a hydrophilic polyethyleneglycol-based dendron having a phosphonic acid as a focal point. The suspension stability has been studied as a function of the grafting rate and optimisation of grafting conditions has conducted to very stable suspensions of magnetite nanoparticles. The functionalized nanoparticles have been carefully characterized and the magnetic and relaxation properties of the colloidal suspensions have been studied in order to evaluate the possible use of these materials as MRI contrast agents.

MICRO-AXIAL TOMOGRAPHY

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Conventional far field light microscopes like confocal laser scanning microscopes have a lower resolving power in the axial direction. We are using a micro-axial tomographic (MAT) setup to overcome this impasse by rotating the sample, thus providing isotropic resolution in all three dimension of space, after application of suitable image reconstruction algorithms. Different approaches to combine the multiple views from a four-dimensional MAT data stack (three dimensions of space an the rotation angle) have been implemented. We present a newly developed algorithm using weighted average Fourier-Transforms to combine the different views, and compare this to results obtained from multi-view deconvolution reconstruction. When applied to fluorescently labeled specimens, this method can also be combined with structured illumination to further increase the optical resolution. Using photo-switchable or photo-activatable fluorophores, this technique allows the non-destructive investigation of biological specimens with a resolution only limited by the signal-to-noise ration, i.e. the photon count. Even though MAT data with photo-switchable fluorophores is not available so far, first measurements on fixed cells using this non-linear excitation technique have revealed the potential for an increased optical resolution with our structured illumination microscope setup.

INTEGRATED MOLECULAR IMAGING: FROM PROBES AND CAMERAS TO HUMAN. THE CEA-LETI EXPERIENCE

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Our technology roadmap in designing detection devices for optical imaging covers the following stages: a) optical imaging on small animals b) optical imaging on humans c) Fluo + X on small animals and d) Fluo + other modalities on humans. The main achievements are

- 3D tomograph on small animals
- Coupled X and fluorescence tomography
- Portable intra operative instrumentation for surface and deep fluorescence imaging with targeted applications in detection and surgery of prostate cancer and breast cancer

The corresponding IPR consists in 4 patents.
Simultaneously, adequate and high performance probes are developed. The corresponding technology roadmap identifies a) optical probes on small animals b) optical probes for imaging drug delivery c) optical probes on humans and d) probes for double modalities on humans. Different classes of nanoparticles have been developed so far:

- 3 generations of NP: RAFT (chemical scaffold), QD for optimisation of surface chemistry, and then nano-emulsions. The activation of imaging probes during internalization in cells has been developed.
- Highly specific, activable and non toxic nanoparticles including novel QD and nano-emulsions have been designed for deep fluorescence (3.5 cm), and will be tested in vivo very soon.

The next step consists in the development of probes for multimodal imaging like US+Fluo or MRi + Fluo or PET + Fluo. The corresponding IP consist in 3+ patents.
The next challenge consist in transferring this instrumentation and the associated markers to clinical application beginning in revisiting the operation theatre for specific use of optical molecular imaging in routine human surgery.

CELL LABELLING WITH MAGNETIC NANOPARTICLES: MECHANISMS, BIOCOMPATIBILITY, MRI CELL IMAGING AND MAGNETIC MANIPULATIONS

C. Wilhelm, F. Gazeau

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The lecture will provide an overview of the use of magnetic nanoparticles for cell labelling and biomedical applications. Magnetic labelling of cells is attracting growing interest, mainly in the field of MRI imaging. Indeed, cell magnetization has been shown to permit highly sensitive and non invasive MR detection of specific cell populations in living organisms. This approach is a method of choice for monitoring cell migration in cell therapy trials. Recent improvements in MRI resolution have even enabled single labelled cells to be detected *in vivo*. “Magnetic cells” can also be remotely manipulated, to control the movement of flowing cells in cell-sorting applications for example, and also to influence the migration and organization of cells in engineered substrates or tissues. Tissue engineering and cellular therapy could both benefit from magnetic targeting and control.
We will present the current methods for biocompatible and efficient magnetic labelling of cells, the basis of MRI cell detection and different MRI studies of cell therapy *in vivo*. We will also discuss the use of magnetically responsive vectors (synthetic and cell-derived vectors) for drug targeting and hyperthermia.

STED MICROSCOPY

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The resolution of far-field fluorescence microscopy is limited by the diffraction of light, which prevents the visualization of many (sub-cellular) details. **Methods:** A powerful approach for sub-diffraction far-field microscopy is based on specific fluorescence depletion by *St*imulated *E*mission *D*epletion (STED). In STED microscopy it is possible to ensure that the measured signal stems from a region of the sample that is much smaller than given by the diffraction limit. **Results:** We present recent applications of far-field fluorescence STED microscopy with image resolution down to the molecular scale, applying it to the determination of protein cluster sizes or the visualization of sub-cellular structures. Special attention is drawn to heterogeneous lipid diffusion on the plasma membrane of living cells. By detecting the fluorescence signal from single diffusing lipids with nanoscale resolution we reveal details about the heavily debated existence of lipid rafts. **Conclusion:** Nanoscale STED microscopy is helpful in solving fundamental biological problems that cannot be addressed by conventional diffraction-limited microscopy.

CARBON NANOTUBES: FUNCTIONALIZATION AND SELECTED EXAMPLES OF APPLICATIONS

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Since their discovery by Iijima in 1991, carbon nanotubes have attracted considerable interest due to their unique mechanical, electronic, and optical properties. Their potential in nanotechnology however, has been hampered by difficulties associated with processing and manipulation. Considerable efforts have thus been devoted to nanotube functionalization to improve solubility and to enhance compatibility in composite materials. Some functionalization methods recently developed in our laboratory will be presented. These include the self assembly of surfactants on the nanotube surface, leading to the formation of nanorings. The synthesis, the isolation and the potential use of these nano-objects will also be introduced.

THE INFLUENCE OF THE CHEMICAL COATING ON THE BIO-DISTRIBUTION OF QUANTUM DOTS *IN VIVO*

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The meeting of nano-materials with biology has produced a new generation of technologies that can profoundly impact biomedical research. The NIR emitting window (650-900 nm) is appealing for *in vivo* optical imaging because of the low tissue absorption and scattering in this wavelength range. The design of high-quality NIR-emitting quantum dots, with outstanding optical properties in comparison to organic dyes, should therefore lead to novel contrast agents with improved performance (higher fluorescence quantum yields and photo-stability). Quantum dots growth is controlled by the coordination of hydrophobic ligands. Hence, they have to be transferred in water and conveniently coated before their use *in vivo*. **Methods:** Several coating strategies are developed in order to: A) prevent quantum dots from flocculating during long-term storage, B) efficiently convert the organic-soluble quantum dots to water-soluble nanoparticles, C) maintain the quantum dot fluorescence quantum yield in biological buffers, and D) maintain the sub-10 nm particle size, necessary for their renal clearance. **Results**: The speed of first pass extraction of quantum dots towards the reticulo-endothelial system (liver, spleen, bone marrow) depends strongly on the particle size and surface coating (cationic, anionic, neutral, zwitterionic). **Conclusion:** The surface coating of quantum dots and their hydrodynamic diameter are shown to be the critical parameters in the development of new diagnostic agents.

NANO-OBJECTS, PROPERTIES, ASSEMBLIES AND APPLICATIONS

Luisa De Cola

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In this talk I will describe the synthesis and properties of silica-based nano-containers such as zeolites L. In particular I will show how luminescent molecules can be encapsulated inside the nanostructures or covalently linked on their surfaces. Zeolites L, which are transparent, stiff, nanocontainers, made of hundreds of parallel aligned unidimensional channel, can be synthesized in different shape and size (30 nm to several micron). Their selective and spatial resolved functionalization can lead to multifunctional systems. In fact we are able to covalently link molecules on the coat of the zeolites as well as different systems at the channel entrances. *In vitro* experiments have shown that some of their properties are extremely interesting for MRI and nuclear imaging. Finally the selective functionalization of the channel entrances, lead to the self-assembling of the zeolites, and the assembly process can be extended to living organism such as bacteria.

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Adv. Mat. in press.

ZEOLITE L NANOCRYSTALS FOR IMAGING APPLICATIONS

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Novel hybrid materials comprising of nano and microscale inorganic scaffolds coupled to functional molecules can have direct applications in biomedical imaging. The modularity and self assembly approach used in their fabrication allows for a multitude of functionalities to be introduced and tuned. Magnetic and optical contrast agents are two properties of great interest. MRI contrast agent have been devised from dendrimer, viral capsids, carbon nanotubes and gold nano shells, important factors determining the efficacy of such a material, involve the incorporation of a high concentration of paramagnetic ions in hydrodynamic exchange with water. Optical imaging requires emission in the red to near-infrared, as not to interfere with cellular autofluorescence and great stability towards photobleaching. General factors to consider when developing such systems are the small size i.e ability to pass through the human membranes, toxicity of the introduced substances, chemical stability, and sensitivity of the imaging signal.
This work presents the synthesis, and spectroscopic characterization of novel nano-zeolite hybrid materials which can act as a dual optical and magnetic probe. The components responsible for imaging can be entrapped inside the zeolite channels or placed on the surface. Possible groups, which incorporate either the red emitting Eu-DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) or MRI contrast agent Gd-DOTA moiety, are presented.

DETERMINATION OF THE DEPTH RESOLUTION LIMIT OF FLUORESCENCE DIFFUSE OPTICAL TOMOGRAPHY

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We provide a methodology to determine the depth resolution limit of fluorescence diffuse optical tomography. **Method:** For a given fluorophore depth L, the Radiative Transfer Equation is solved numerically using a Monte Carlo algorithm. The measured signal and its sensitivity to L are calculated, accounting for the actual geometry of the detection optics. Then we use the Cramér-Rao analysis that provides an absolute lower bound for the variance of the reconstructed depth L, and therefore the resolution limit. **Results:** We considered a multilayer structure representing a 1D model of a brain skull model and an effective homogeneous slab. We show that the depth dependence is not correctly reproduced in the effective homogeneous model. For a quantitative calculation of the depth sensitivity, the multilayer structure has to be properly taken into account. **Conclusion:** we have developed a methodology to determine the resolution limits of fluorescence tomography imaging setups. The approach is based on an improved photon transport model and a rigorous statistical analysis using parametric sensitivities and a noise model.

NOVEL ORGANOMETALLIC COMPLEXES FOR MULTIMODAL IMAGING

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As part of our ongoing efforts to introduce fluorescence and radioactive probes suitable for multimodal imaging applications, we report in this communication the synthesis, characterization and biological evaluation of novel Re(I) and ^{99m}Tc(I) tricarbonyl complexes anchored by pyrazole-diamine chelators bearing anthracene or acridine fluorophores. The Re(I) tricarbonyl complexes were characterized by common analytical techniques, such as multinuclear NMR, ESI-MS and HPLC. The ^{99m}Tc complexes were identified by HLPC comparison with the fully characterized Re congeners. Fluorescence microscopy studies were used to follow *in vitro* the uptake of the Re complexes in tumour cell lines, while the biological evaluation of the ^{99m}Tc complexes comprised pharmacokinetics and biodistribution studies in mice. Our attempts to obtain related multifuncional complexes, bearing a pendant butyric arm for further coupling to a bioactive vector and/or to different nanoparticles, will be also discussed.

SINGLE MOLECULE FLUORESCENCE IN DISORDERED NANOSCOPIC ENVIRONMENTS

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Spontaneous emission of an emitter (e.g. fluorescent molecule, quantum dot) is strongly dependent on the environment. In complex disordered media, such as soft-matter or biological systems, the environment of a single emitter can change randomly, either because of the internal dynamics of the systems or the proper motion of the emitter. In this case, the lifetime can be studied statistically. Although fluorescence lifetime imaging techniques using averaged measurement have become important tools in biological imaging, little attention has been paid so far to the lifetime *fluctuations*. In this talk, we first discuss the basic mechanisms that induce lifetime modifications in structured environments, based on the simple example of an emitter coupled to a single nano-antenna. In the presence of absorption, we discuss the trade-off between radiative and non-radiative processes. Then, we describe the lifetime statistics of a single emitter in a nanoscopic disordered system (e.g. a cluster of nanoparticles), based on exact numerical simulations and simple models. We show that the relative fluctuations of lifetime (or decay rate) exhibit two well defined regimes dominated either by near-field scattering or by absorption processes. In both regimes, the averaged apparent quantum yield remains high enough to permit practical measurements. This suggests the use of lifetime fluctuations to probe the local environment in complex systems at the sub-micron scale.

MAGNETIC RESONANCE IMAGING OF SINGLE SPINS IN DIAMOND NANOCRYSTALS UNDER AMBIENT CONDITIONS

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Magnetic resonance imaging and optical microscopy belong nowadays to key technologies in life science. The best resolution attainable in conventional optical imaging is limited by diffraction to the order of the wavelength (Abbe criterion). For spectroscopic imaging methods (like MRI) the ultimate resolution is limited by the size of the detectable magnetic marker. Hence for nm-scale resolution the use ultrasensitive detection technique is crucial. This report shows that nanometer resolution at long standoff distances can be achieved when using single paramagnetic atoms as probe. We use spatially varying magnetic field shifting the frequency of a spin resonance of the single spin associated with defect in diamond. For a known field gradient we are able to localize magnetic marker with nanometer precision under ambient conditions.

TARGETED LIPID-BASED NANO-PARTICLES FOR MULTIMODAL BIOMARKER IMAGING

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In recent years, our group has developed the technology for the MRI-based *in vivo* detection of disease biomarkers, using paramagnetic nano-particles (NP) [1,2]. MRI is a versatile technique in biomedical research and clinical diagnostics, and provides high resolution images in a non-invasive manner. Due to the relatively low sensitivity of MRI, the detection of sparse biomarkers requires powerful contrast agents. We explore lipid-based NP's, as these can be equipped with a high payload of Gd^{3+}-containing lipid for MRI detection and can be prepared in a range of sizes. Apart from the Gd^{3+}-label the NP's also contain fluorescent entities, which are particularly useful for the fluorescence microscopic detection of the NP's on histological slices. Most often, we use fluorescent lipids but have also prepared lipidic NP's that contain a quantum dot in their core [3] and thus combine paramagnetic and fluorescent properties for parallel MRI and optical detection. The presentation will detail the preparation and characterization of lipid-based NP's, procedures for ligand conjugation and will demonstrate their application for *in vivo* detection of disease processes, including tumor angiogenesis [4,5].

References:

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NOVEL IMAGING TECHNIQUES WITH FUNCTIONAL NANO-OBJECTS FOR CANCER DIAGNOSIS

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Advances in nano-biotechnology have a great potential to improve prevention, diagnosis and treatment of human disease. Nano-objects for medical applications are expected to grasp pharmacokinetics and the toxicity for application to medical treatment on the aspect of safety of the nano-materials and nano-devices. **Methods:** We present generation of CdSe nano-particles conjugated with monoclonal anti-HER2 antibody (Trastuzumab), for single molecular *in vivo* imaging of breast cancer cells. We established a high resolution *in vivo* 3D microscopic system for a novel imaging method at molecular level. **Results:** With the use of the QT complex (Q-dot labeled with Trastuzumab; T) in mice, we successfully identified tracking of single nanoparticles and continuous processes of delivery; initially in circulation, then extravasation, extracellular region, cell membrane, intracellular and perinuclear region. The translational speed of QT-complexes in each process was highly variable, even in the vessel circulation (*Cancer Res, 2007*). The image analysis of single particles *in vivo* may provide valuable information for molecular-targeting in cancer therapy, with new insights into interactions and processes involved in transport of drug carriers. The cancer cells expressing HER2 protein were visualized by the nano-objects at subcellular resolution, suggesting future utilization in medical applications to improve drug delivery system to target the primary and metastatic cancers for made to order treatment. With the aim of clinical utilization in cancer surgery, we also report fluorescent nano-particles for sentinel node navigation in experimental model, with appropriate particle size and wavelength. **Conclusion:** Future innovation in cancer imaging, not only at cellular level but also at molecular level, by synthesizing diagnostic agents with nano-objects, is now expected.

LUMINESCENT OXIDE NANOPARTICLES : SYNTHESIS, PROPERTIES AND APPLICATIONS

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Aims: Our two groups have been involved since a few years on the development of rare earth doped luminescent nanoparticles for optical applications. Our research is devoted on the synthesis of nanoparticles through colloidal routes, the investigation of their properties with respect to size and surface effects, and their applications either for the elaboration of transparent coatings and for the use of single nanoparticles as original probes for biology. **Methods:** we focused mainly on europium doped yttrium vanadate particles that are made through simple colloidal synthesis from precursor salts. In a second step, particles are functionalized through surface modification using an organosilane. This allows either to disperse the particles in a sol-gel silica matrix that can further be deposited on glass to obtain transparent nanocomposite coatings, or to graft biological species such as toxins for *in vitro* tracking experiments. **Results:** YVO_4:Eu nanoparticles can be prepared with optimized emission properties that allow their detection at the single particle level. Composite films may be deposited for applications in transparent lightning devices. Concerning the use of these particles as biological probes, two applications were investigated. The first one concerns the original spectroscopic properties of these particles that allow their use for the local detection of H_2O_2 concentrations. The second one concerns the coupling of particles to toxins in order to study their individual actions and dynamics. **Conclusion:** results obtained on rare earth doped nanoparticles show the broad potentialities of these systems for different applications, opening a large field of investigation taking advantage of their original spectroscopic properties.

SHAPE-SPECIFIC NANOPARTICLES AS CONTRAST AGENTS FOR OPTO-ACOUSTIC DETECTION

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Aims: The development of an imaging technique improving the sensitivity and selectivity of the diagnostic to meet a major challenge in prostate cancer. **Methods:** The ADONIS project (FP6) intends to prove the concept of using opto-acoustic imaging in combination with biologically functionalized nanoparticles as an integrated biosensor-based system for the production of highly selective and sensitive data for accurate diagnosis of prostate cancer. This concept involves the use of contrast agents which transform light into local heating inducing a detectable pressure wave (comparable to echography). The nanoparticles used as a contrast agent must present a maximum of absorption in the optical transparency window of the human tissues in order to allow their excitation and subsequently cancer-selective detection while avoiding unwanted destructive energy transfer. **Results:** Based on the tissue absorption characteristics, rod-like gold nanorods with an absorption maximum of approximately 760 nm are produced. These nanoparticles are then coupled with an antibody directed against an antigen over-expressed by the cancer cells to provide selectivity and to ensure the accumulation of the particles at the tumour site. The selectivity of the functionalized particles is assessed using *in vitro* tests on cancer cell monolayers and spheroids using both scanning electron microscopy and two-photon luminescence as imaging techniques. Pressure wave generation upon optical excitation of the functionalized nanoparticles has been tested on spheroids incubated with the functionalized nanoparticles and embedded into a polymer matrix (phantoms). **Conclusions:** Based on the preliminary results obtained *in vitro*, it has been shown that opto-acoustics combined with functionalized nano-particles improves the diagnostic sensitivity. Pilot tests *in vivo* using human tumour xenografts will be used to confirm the increased selectivity.

CONTROLLING SINGLE MOLECULE FLUORESCENCE THROUGH PLASMONIC SLABS

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We have shown that the apparent quantum yield of a single molecule can be modified through a film made of either a metallic or a negative-index material. This is achieved by placing the emitter on one-side of the slab, and a metallic nanoparticle on the other side. **Methods:** The Green Tensor (or electric-field susceptibility) of the full system can be computed numerically, accounting for multiple scattering between slab interfaces and the nanoparticle. We use an angular spectral decomposition and appropriate Fresnel coefficients for the transmitted and reflected fields at the slab interfaces, and a couple dipole method to account for the coupling with the particle. **Results:** A transition from a regime of strong quenching to a regime of fluorescent emission is observed when approaching a metallic nanoparticle at nanometric distance from the slab surface, coupling the particle with a single emitter on the other side. The enhancement means a reduction of the overall absorption in the coupled slab-particle system. We have demonstrated the effect using both negative-index and purely metallic materials. **Conclusion:** Using numerical simulations, we have shown that the apparent quantum yield can be modified using plasmonic systems, providing an efficient control of the dynamics of a single emitter, with nanometer scale resolution. Based on this result, we discuss the feasibility of a dark near-field fluorescence microscopy.

IN VIVO IMAGING OF VASCULAR PERMEABILITY USING NANO-OBJECTS IN MICE TUMOR

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AIMS: We successfully identified the processes of nano-particle delivery in the interstitial space of the human tumor xenograft in mice and quantitatively analyzed them to understand the rate limiting constraints on single nano-particle delivery *in vivo*. **Methods:** We used nanocrystals, sizes of 20, 40, 100nm. A suspension of KPL-4 was inoculated subcutaneously into the dorsal skin of female Balb/c nu/nu mice. Several weeks after inoculation, mice with a tumor volume of 100 - 200 mm^3 were selected for observation. We used the optic system, consisted of an epi-fluorescent microscope equipped with Nipkow lens type confocal unit and an electron multiplier type CCD camera, which can captures images of single nanoparticles. Quantitative and qualitative information such as velocity, directionality and diffusion coefficients was obtained using time-resolved trajectories of particles analyzed by mean square displacement (MSD) method. **Results:** After the injection, many particles had extravasated and moved into the perivascular area close to the tumor vessels. Particle diffuses within a highly restricted area and then moves suddenly to the next point. We acquired trajectories of the three sizes of particles in the three parts of the tumor (perivascular, interstitial and intercellular). The plots of the MSD are in parabolic shape, indicating that the movements are consisted of convection with superimposed random diffusion. Velocity was weakly dependent on the size but dependent on the position from perivascular region. Diffusion was dependent on the size and position. **Conclusion:** Our results show that rate limiting constraints of single nano-particle delivery was its size and position from tumor vasculature.

THE SIZE OF MAGNETIC NANOPARTICLES FINELY TUNES THEIR HEATING POWER UNDER A HIGH FREQUENCY ALTERNATING MAGNETIC FIELD

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Since magnetic nanoparticles have unique features, the development of a variety of medical applications has been possible. Combining magnetic properties with nanosized biocompatible materials, superparamagnetic nanoparticles may serve as colloidal heating mediators for cancer therapy. This unique potentiality attracts attention for designing new magnetic nanoparticles with high efficiency heating properties. Iron oxide colloidal nanomagnets generate heat when subjected to a high frequency magnetic field. Their heating power is governed by the mechanisms of magnetic energy dissipation for single-domain particles due both to internal fluctuations of particle magnetic moment (Néel relaxation) and to the external Brownian fluctuations of the crystal itself with respect to the carrier fluid. Those mechanisms are highly sensitive to the crystal size, the particle material, and the solvent properties.
Here we explore the heating properties of iron oxide particles with particle sizes, in the range 5 nm-100 nm. Then, we try to experimentally separate the Brownian and Néel mechanisms in order to define their relative contributions to energy dissipation. Experiments with nanoparticles in cells were also carried to observe the effects of a biological environment on each mechanism in heat generation.

NANOSCALE MMRI CONTRAST AGENTS

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The concept of molecular MRI promises a significant improvement in health care due to better prevention, early detection and more efficient treatment of disease. However this poses a lot of new challenges to technology as MRI lacks sensitivity compared to nuclear or optical imaging. **Methods:** In principle there are three ways to improve the sensitivity of MRI. It is possible to increase the lower detection limit of the MRI scanner, to use contrast agents (CA), which yield a high signal deposition within the target tissue or to address targets (biomarkers) with high copy numbers. **Results:** In general the target concentration is given by physiology. MRI scanners continuously improve sensitivity, the trend to higher fields and parallel acquisition techniques support this. Still the main boost will come from CAs with higher relaxivities and higher enrichment within the target tissue. There are various experimental CA concepts, which results in pretty good sensitivity for MRI. The most advanced compounds are high payload nanoparticles, which can be classified into iron oxides or Gadolinium assemblies. Most of these CAs are used in animal models only or are even earlier in development process. Some iron oxides however are clinically approved or in clinical development. In 1996, Ferridex was introduced as the world's first organ-specific MR imaging agent. Resovist is approved for the detection of focal liver and Sinerem and VSOP are in clinical trials. **Conclusions:** The major challenges in nanotech CA design are tailoring of the specificity to the respective applications, develop strategies to circumvent physiologic barriers, assurance & control of *in vivo* degradation and manufacturing in the required quality and quantity at competitive costs.

IRON OXIDE NANOPARTICLES AS MULTIFUNCTIONAL IMAGING AND DELIVERY AGENTS

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Aims: Superparamagnetic iron oxide nanoparticles have traditionally been utilized as contrast agents for magnetic resonance imaging. Here, we explore their potential as delivery modules for small interfering RNA to target organs. Furthermore, we investigate the feasibility of combining the imaging and delivery capabilities of these nanoparticles for the tracking of siRNA bioavailability. **Methods and Results:** The iron oxide probe (MN-NIRF-siRNA) consists of magnetic nanoparticles (for magnetic resonance imaging), labeled with Cy5.5 dye (for near-infrared in vivo optical imaging), and conjugated to a synthetic siRNA duplex targeting model (*gfp/egfp*) or therapeutic (*birc5*) genes. In addition, it can be modified with myristoylated polyarginine peptides (MPAP) for enhanced membrane translocation. MN-NIRF-siRNA can be used for the in vivo delivery of functional siRNA to tumors. The delivery can be tracked by noninvasive magnetic resonance and near-infrared optical imaging. The tumoral accumulation of MN-NIRF-siRNA results in a remarkable level of target-gene down-regulation. Repeated treatment with MN-NIRF-siSurvivin, targeting the tumor-specific anti-apoptotic gene, *birc5*, results in the induction of apoptosis in the tumors but is not associated with systemic toxicity or inflammatory cytokine induction, reflecting the suitability of this probe for safe in vivo application. Another application of MN-NIRF-siRNA extends from the fact that these nanoparticles are also taken up by pancreatic islets following in vitro incubation. This uptake can be visualized by magnetic resonance and near-infrared fluorescence optical imaging and results in down-regulation of a target gene (*egfp*). This approach has relevance in the context of pancreatic islet transplantation, which is the most promising treatment against type 1 diabetes. A potential application of this method would involve the selective knock-down of genes implicated in islet graft dysfunction, such as pro-apoptotic genes and genes involved in immuno-recognition. **Conclusions:** RNA interference is emerging as one of the most promising new tools for tackling diseases amenable to manipulation at the gene expression level. The major challenge in implementing small interfering RNA (siRNA)-based therapies, however, is adequate delivery to the tissue of interest. To address this issue, we have developed a method for the concurrent delivery of siRNAs to target tissues and imaging of the delivery. This new methodology could enhance the potential of RNA interference as a therapeutic modality by providing a non-invasive means of assessing the bioavailability of the siRNA agent.

PERSISTENT LUMINESCENCE NANOPARTICLES (PLNS): A NEW IMAGING TOOL FOR BIOLOGISTS?

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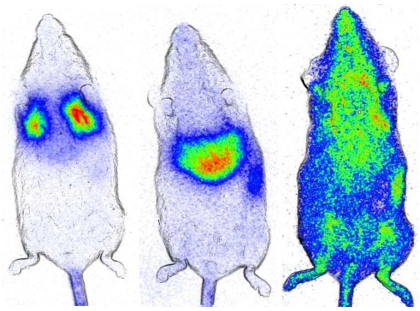
The goal of this work was to develop and to use in small animal imaging a new kind of optical probes that have the properties of persistent luminescence. Commonly referred as phosphorescence, persistent luminescence is the phenomenon encountered in materials which make them glow in the dark after the end of an excitation with UV or visible light. Due to growing demands of imaging tools for biomedical research, existing imaging systems have been rapidly improved and new imaging techniques have been developed during the past decades. Nowadays, imaging techniques such as MRI, scanner, ultrasound, scintigraphy and other major imaging systems are available to scientists. Each technique has advantages and disadvantages, making them complementary. Optical imaging is a rapid expanding field with direct applications in pharmacology and in the development of tools for diagnostic and research in molecular and cellular biology. Despite the increasing use of fluorescence for *in vivo* imaging, this technique presents several limitations, especially due to tissues autofluorescence under external illumination and weak tissue penetration of low wavelength excitation light. These drawbacks can limit the ability to detect fluorescent probes from background signal in deep tissues imaging.
We present here an alternative optical imaging system using near-infrared persistent luminescence nanoparticles (PLNs) suitable for small animal imaging. The main advantage of this technique resides in the absence of autofluorescence, as the nanoparticles continue to emit light in the animal without the need for any kind of excitation. Using these probes in small animal imaging, we demonstrate that nanoparticles can be excited prior to injection to follow their *in vivo* distribution for more than one hour without any external illumination source. Chemical modification of nanoparticles surface led to distinct biodistribution pattern. Tumor mass was also identified on a mouse model.



Differential biodistribution patterns are obtained for differently charged nanoparticles (from left to right: positive PLNs, negative PLNs and neutral PLNs.

VISUALIZING DYNAMICS OF CELL SIGNALING

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Cellular signaling relies on the control of a finely balanced, complex biochemical network. Understanding these central cellular processes requires quantification of the spatial and temporal changes of distribution of interacting signaling partners on the plasma membrane and inside of a cell. We present our approaches including (A) specific and covalent labeling of proteins in live cells with small organic fluorophores, (B) visualization of molecular chemical states with FRET, (C) measurement of molecular dynamics at equilibrium conditions using fluorescence correlation spectroscopy and single molecule tracking, (D) downscaling the investigation of cellular reactions on planar cellular membrane fragments and submicrometer-sized native vesicles, (E) the effect of protein expression and crowding in membranes on cellular signaling reactions.

Some recent publications of the group:

H. Pick et al*: J Am Chem Soc 127*, 2908 (2005).
R. Schmauder et al: *Chemphyschem 11,* 1381 (2005)
B.H. Meyer et al: *PNAS 103,* 2138 (2006).
V. Jacquier et al: *PNAS 103*, 14325 (2006).
C. Danelon et al: *Langmuir 22*, 22 (2006).

FULLERENES AND CARBON NANOTUBES LOADED WITH GD AS HIGH RELAXIVITY MRI AGENTS

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Carbon nanomaterials like fullerenes and single walled nanotubes can be loaded with paramagnetic gadolinium ions to build contrast agents for MRI imaging. **Methods:** Endohedral, water soluble gadolinium fullerenes have been investigated by ^{1}H relaxometry in vitro. Ultra-short single walled carbon nanotubes have been loaded with Gd^{3+} and relaxivities have been measured as a function of temperature and pH. **Results:** Gado-fullerenes give high relaxivities due to aggregation in aqueous solution. De-aggregated compounds show NMRD profiles similar to mid-weight poly-aminocarboxylate based contrast agents. Gd-loaded carbon nanotubes give rise to high relaxivities up to high magnetic fields. NMRD profiles look different from “normal” Gd-based compounds and cannot be described theoretically up to now. **Conclusions:** In-vitro relaxivity measurements show, that carbon based nanomaterials can be promising high-relaxivity MRI contrast agents. Especially gadolinium loaded fullerenes show perspective as high-field agents.

CHROMATOC POLYMER NANO-PATCHES FOR IMAGING MEMBRANE PROCESSES IN LIVING CELLS

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This seminar will describe the development of a new platform for imaging and analysis of membrane processes in living cells through cell-surface incorporation of chromatic “nano-patches” comprising lipids and polydiacetylene (PDA). Specifically, we show that the membrane-incorporated PDA patches undergo both visible colour transformations, as well as fluorescence induction, following interactions of external species with the cell membrane. PDA-labeled “sensor cells” have been employed for analysis of drug permeation, virus-cell interactions, vesicle fusion with the plasma membrane, and others. The new platform is highly generic, and different from conventional fluorescent dyes – PDA responds only to structural perturbation to the membrane bilayer, thus constitutes a useful “functional assay”.

DENDRIMER NANOPLATFORMS FOR EFFECTIVE TUMOR TARGETING

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High molecular weight nanoplatforms preferentially accumulate at tumor tissues through the enhanced permeation-and-retention (EPR) mechanism. In the past several years, with Professor J. Frechet and his group members at UC Berkeley, we have investigated the drug carrier potential of various polymeric scaffolds synthesized from a central core. These polymers are known as dendrimers or dendronized polymers. Our findings show that dendritic constructs exhibit desirable *in vivo* characteristics as carriers. In this lecture, we review the *in vivo* properties of dendritic polymers as drug carriers and comment on their structural attributes that make them ideal for delivery of molecular imaging reporters. **Methods:** Various scaffolds based on the 2,2 bis(hydroxymethyl)propanoic acid unit, polylysine or polyamidoamine have been synthesized. Pharmacokinetics of the polymer backbones were followed using ^{125}I radiolabeled dendrimers and the therapeutic effects of the drug-loaded versions were determined in tumor bearing mice. **Results:** The dendrimer backbones studied were water soluble and non-toxic. When conjugated with multiple copies of an anti-cancer drug, the dendrimer-drug constructs exhibit long serum half-lives, efficient tumor uptake, rapid drug release rate at acidic pH and little uptake by vital organs when compared to the free drug. **Conclusions:** Dendrimer nanoplatforms possess desirable *in vivo* characteristics for development of a tumor targeting delivery system for therapeutic and imaging agents.

PARAMAGNETIC COLLAGEN BINDING CONTRAST AGENTS

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Collagen, a major extracellular matrix component, plays a very important role in both normal and abnormal tissue development. Recently, we have developed several CNA-35- (a bacterial collagen adhesion protein) based contrast agents (CAs) capable of binding collagen specifically[1]. In vitro, binding of the CAs was characterized using cryo-TEM, fluorescence and magnetic resonance imaging (MRI). Also a new high-resolution MR depth profiling technique, combined with mathematical modelling was used to characterize and quantify binding. This technique, capable of quantifying the surface water longitudinal relaxation rate induced by bound CAs will be helpful in studying the behaviour of targeted CAs in general and yield valuable insights in the optimization of target-associated CAs. Binding on the molecular level using cryo-TEM was investigated, indicating that CNA-35 preferably binds collagen by folding around a single collagen triple helix, in excellent agreement with the 'collagen hug' model published by Zong et al.[2] All techniques showed collagen specific binding and therefore CNA-35 based CAs can be a valuable tool to monitor tissue maturation and remodelling.

[1] Krahn et al. Anal. Biochem. 2006: 350: 177-185
[2] Zong et al. EMBO J. 2005: 24: 4224-4236

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