Tuesday, November 23th

Session Nano for Imaging, Diagnosis & Therapy

15:00 - 17:10

Abstracts

Thematic Session: Nano for Imaging, diagnosis, and therapy

Keywords: titanate nanotubes, polyethylene glycol PEG, docetaxel, cytotoxicity, cancer, radiotherapy

Influence of PEG Spacers on the Cytotoxicity of Titanate Nanotubes-Docetaxel Nanohybrids on a Prostate Cancer Cell Line

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Abstract

The association between chemotherapeutic drugs and metal oxide nanoparticles has sparked a rapidly growing interest in cancer nanomedicine. The elaboration of new engineered docetaxel (DTX)-nanocarriers based on titanate nanotubes (TiONts) for the drug delivery was reported. The idea was to maintain the drug inside cancer cells and avoid multidrug resistance mechanisms, which often limit drug efficacy by decreasing their intracellular concentrations in tumor cells. HS-PEG_n-COOH (PEG: polyethylene glycol, n = 3,000, 5,000, 10,000) was conjugated, in an organic medium by covalent linkages, on TiONts surface. The aim of this study was to investigate the influence of different chain lengths of PEG derivatives on the TiONts colloidal stability, on the PEG_n density and conformation, as well as on the DTX biological activity in a prostate cancer model (human PC-3 prostate adenocarcinoma cells). *In vitro* tests highlighted significant cytotoxicities of drug after loading DTX on PEG_n-modified TiONts (TiONts-PEG_n-DTX). Higher grafting densities of shorter lengths of PEGylated chains in a brush conformation were most favorable on cytotoxic activity of DTX by promoting both colloidal stability in biological media and the interaction of the therapeutic agent with microtubules. The internalization of TiONts-PEG_n-DTX have been studied by infrared-combined atomic force microscopy (AFM-IR). This promising strategy involves a better understanding of the nanohybrid engineering, in particular on the influence of the PEGylated chain length and can thus become a potent tool in nanomedicine to fight against cancer.

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Acknowledgments:

This work was supported by the Cancéropôle Est through the call “Emergence 2015” (Région Bourgogne Franche-Comté). This work is also part of the project “Nano2Bio”, funded by the “Université de Bourgogne” and the “Conseil Régional de Bourgogne” through the “Plan d’Actions Régional pour l’Innovation (PARI)”, the European Union through the PO FEDER-FSE Bourgogne 2014/2020 programs and this work has been supported by the EIPHI Graduate School (contract "ANR-17-EURE-0002").

The authors would like to thank Olivier Heintz (ICB) for XPS measurements, Rémi Chassagnon for TEM images and Myriam Heydel (Sayens) for ICP measurements. This work was performed within a regional center of excellence in pharmaco-imaging (Pharmimage GIE, Pharmacoimaging “groupement d’intérêt scientifique” (GIS).

Thematic Session: Nanos for Imaging, diagnosis and therapy
Keywords: cancer, ultra-small superparamagnetic nanoparticles, targeted nanotherapy, rotating magnetic field, mechanical forces

Remote magneto-mechanical destruction of cancer-associated fibroblasts using targeted ultra-small superparamagnetic iron oxide nanoparticles and low frequency magnetic fields

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In the tumor microenvironment, cancer-associated fibroblasts (CAFs) promote growth and resistance acquisition of tumors as well as limit the penetration and diffusion of chemotherapeutic agents into the tumor by secreting collagen and increasing extracellular matrix density[1]. In this context, the development of nanotherapy targeting tumor microenvironment represents a real opportunity to propose new therapeutic solutions. Alternatively to local heat release upon a high frequency alternating magnetic field exposure, magnetic nanoparticles generate torque or mechanical forces in response to a rotating low frequency magnetic field (RMF)[2]. Such approach constitutes a technically easier approach compared to magnetic hyperthermia and with potentially less undesirable effects for patients.
Here, we developed ultra-small superparamagnetic iron oxide nanoparticles (USPION, 6-nm crystalline core) decorated with gastrin, which successfully targeted pancreatic adenocarcinoma CAFs expressing the CCK2 receptor and accumulated within their lysosomes. A screening in the magnetic field amplitude, frequency and type (rotating, alternating, static) demonstrated that cell death is maximized when the field rotates, and displayed a maximum as a function of the field amplitude. These features were expected theoretically and are supported by kinetic Monte-Carlo simulations, permitting to estimate that the force generated by USPIONs assembly is of the order of 3pN under optimal conditions. Using a 40mT/1Hz RMF, a 34% cell death ratio is reached. Finally, we showed that cell death occurs through a lysosomal pathway. This study establishes the proof-of-concept that targeted USPIONs can disrupt the tumor microenvironment through mechanical forces generated by a low frequency RMF, that opens new opportunities for cancer therapy.

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The Nanoscience Meeting
TOULOUSE
Centre des congrès Pierre Baudis
December, 8, 9 and 10

Thematic Session: Nano for imaging, diagnosis and therapy
Keywords: iron oxide nanoparticles, synthesis parameters, dendronization, magnetic hyperthermia, photothermia, MRI contrast agent

MASTERING SIZE FOR THE DESIGN OF INNOVATIVE THERANOSTIC IRON OXIDE BASED NANOPARTICLES ENSURING MULTIMODAL THERAPY

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Abstract (**no longer than 250 words** (or 18 lines max. incl. figure), Calibri 11, single line spacing, black)

In nanomedicine, the goal is to develop multimodal nanoparticles (NPs) to speed up targeted diagnosis, to increase its sensitivity, reliability and specificity for a better management of the disease. Combination of therapies is a way to increase the efficiency of anticancer treatment. Therefore, besides precision diagnosis, other challenges for personalized nanomedicine are to develop tools to be able to test quickly different treatments and to follow-up the effect(s) of the treatments by imaging.

Besides being excellent T2 contrast agents for MRI[1], iron oxide NPs are promising as therapeutic agents by magnetic hyperthermia when correctly designed[2]. To be a good heating agent, iron oxide NPs have to display a high magneto-cristalline anisotropy and ways to increase it are to tune the NPs size and shape [2] [3] [4].Iron oxide nanoparticles have also an interest for photothermal treatment as they express a good photothermal response to laser irradiation[5].

The goal of this project is to develop iron oxides NPs with different sizes by the thermal decomposition method and by tuning synthesis parameters such as the reaction temperature, the heating rate and the nature of surfactant [2][3]. Main difficulties were the reproducible synthesis of NPs with mean size higher than 12 nm, a homogeneous spinel composition and their dendronization. NPs with different sizes in the range 5-20 nm were thus synthesized and coated with dendron molecules. Their magnetic properties as

well as their MRI properties were determined. Then, the effect of the NPs size on magnetic hyperthermia and photothermia has been investigated allowing to establish the optimal NPs design to combine both therapies.

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The Nanoscience Meeting
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December, 8, 9 and 10

Thematic Session: Nano for Imaging, Diagnosis 1 Therapy
Keywords: Nanothermometry, Silver sufite nanoparticles, Magnetic hyperthermia

Ag2S nanoparticle-based thermal sensing for hyperthermia therapy

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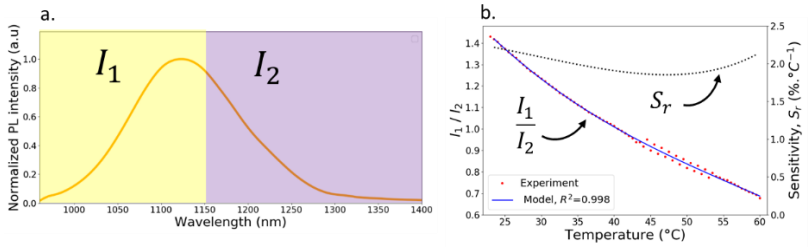
Biocompatible nanoheaters are of great interest as they can act on tumor microenvironment by changing local temperature distribution [1]. This therapeutic strategy is called Hyperthermia. Thermometry based on phosphor nanomaterials appears to be the solution of choice to perform *in-vitro* and *in-vivo* temperature readings at cellular scale and in real-time during hyperthermia treatment [2]. Nanothermometer photoluminescence (PL) spectrum is a fingerprint of material intrinsic temperature [3].

Our study uses silver sulfide phosphors (Ag_2S) as nanothermometer. Ag_2S nanoparticles are synthetized following a new green-synthesis route in aqueous solution. Low cost metal salts and surfactant to control particles growth and surface state are used. Prepared Ag_2S are 5-nm monodispersed spheres, directly stable in water. While excited at 730 nm, Ag_2S nanospheres emit in the second biological window (*figure a*. broad band centered @ 1120 nm at 23°C). Ag_2S temperature increase strongly quenches PL intensity. In addition, a 30-nm redshift is observed between spectral position at 23°C and 60°C. A temperature-dependent parameter, coming from hyperspectral information, is monitored to perform temperature readings. The selected parameter is based on ratiometric measurements (*figure a.*) to allow for concentration-independent temperature sensing. In addition, it maximizes both the relative thermal sensitivity (S_r) and the signal-to-noise ratio *(figure b.).*

In the present talk, maghemite nanoparticles (γ-Fe_2O_3) are used as nanoheater and silver sulphide as nanothermometer to monitor, in real time, temperature at different distance of γ-Fe_2O_3 nanoparticle surface, both in aqueous suspension and cells. This study brings key preliminary results to understand how nanoheaters act on tumor microenvironment. In the future this research work can be used to improve hyperthermia therapeutic strategy towards higher benefits and lower invasiveness for patients.



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Thematic Session: Nano for imaging, diagnosis and therapy

Keywords: Elastin-like polypeptides, RAFT polymerization, plasmid DNA, polyelectrolyte complexes, macromolecular click chemistry

Hybrid Cationic Elastin-like Polypeptides for Nucleic Acids Delivery

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Nanotechnology has been widely developed during the recent years for the transport and release of genes in cancer therapies. The principle of gene transfer is based on the use of DNA complexing agents, which can compact and protect the genetic material against degradation and allow delivering it into the target cell. In this work we explored the use of positively charged elastin-based polypeptides (ELPs) for the complexation of genetic material [1,2]. Recombinant ELPs were functionalized to graft 11 alkyne groups per chain and cationic oligomers were introduced by Huisgen's azide-alkyne cycloaddition reaction. For this, a series of cationic oligomers was prepared from a positively charged methacrylic monomer. These oligomers were synthesized by Reversible Addition-Fragmentation chain-Transfer (RAFT) polymerization using an azide containing chain transfer agent. Four oligomers with polymerization degrees of 6, 9, 15 and 23 were synthesized and coupled with the alkyne-functionalized ELP. Hybrid cationic ELPs were isolated and characterized by ^{1}H NMR, SEC and zeta-potential measurements to assess their purity and determine their degree of functionalization, molar mass and overall charge. Then, electrostatic complexation was achieved between these hybrid cationic ELPs and plasmid DNA, allowing the determination of the optimal conditions for obtaining stable nanoparticles having a controlled size and surface potentials at different N/P charge ratios. Preliminary biological tests showed the reliability of such hybrid cationic ELPs to internalize efficiently genetic material into living cells.

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Acknowledgments:

Authors wish to thank A. Vax (SEC), M. Rosselin (RMN) and C. Lebleu (TEM). Région Nouvelle Aquitaine, French National Research Agency (ANR-15-CE07-0002) and the Cancéropole Grand Sud-Ouest (Emergence 2018-E18) are gratefully acknowledged. L.M. Bravo-Anaya acknowledges the fellowship grant given by CONACYT (CVU 350759).

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December, 8, 9 and 10

Thematic Session: Nanos for Imaging, diagnosis and therapy
Keywords: controlled drug release, magnetic field, thermo-cleavable, magnetic nanoparticles

Thermo-stimulated drug release under alternating magnetic field

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Thanks to their unique properties, magnetic nanoparticles (MNPs) have emerged as an innovative platform in oncology, combining roles as contrast agents in medical imaging, radio-therapeutic agent and drug nanocarriers (NC) for targeted drug delivery[1,2]. To overcome issues arising from conventional chemotherapy and radiotherapy, drug releasing nanoplatforms activated under external application of an alternating magnetic field (AMF) have recently been reviewed by Mertz et al.[3] More specifically, magnetic NC to which the drug is attached *via* covalent thermo-responsive bonds such as diazo bonds[4] or Diels Alder cycloadducts[5] have reported encouraging results in terms of drug release and limited premature leakage of the drug. Despite their potential, the number of magnetic NC in clinical market remains quite low due to lack of efficacy as ascribed to premature clearance and low accumulation in desired areas. The present study involves the development of an "all-in-one" hybrid NC simultaneously capable to highly accumulate in tumour environment as well as to transport and release a therapeutic agent. The NC made of a magnetic core coated with a dense polyethylene glycol shell is covalently attached to a therapeutic drug via diazo thermocleavable bonds. An important part of this study comprises the control of "PEGylation" of MNPs as a crucial step in order to avoid opsonization and ensure higher accumulation in pathological zones. Also, the heat generated by the MNPs once exposed under AMF induces diazo bond breakage thereby releasing the therapeutic drug once the tumourous zone is reached. Lastly, our recent results show the successful PEGylation of MNPs presenting the diazo bond as well as a fluorescent probe mimicking the behavior of a therapeutic drug. Drug release experiments followed by fluorescence spectroscopy demonstrate enhanced release when the MNPs are placed under AMF or in a hot water bath, thus respectively by local (nanoscale) or global hyperthermia.

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Thematic Session: Nanochemistry & Nanoparticles
Keywords: Magnetic nanoparticles, Anisotropic nanoparticles, Nanorods

Optimized Synthesis and Characterization of Magnetic Nanorods

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Magnetic nanoparticles (MNPs) are widely studied for bio-applications such as magnetic hyperthermia or magnetogenetics. MNPs with elongated shape such as nanorods are of great interest for such applications. For example, they exhibit higher heating efficiencies when subjected to alternative magnetic field than their spherical equivalents[1]. In addition, anisotropic nanoparticles are characterized by a higher blood circulation time[3] and a prolonged retention[4] in tumor sites compared to spherical nanoparticles. Moreover, membrane translocation phenomena have already been observed with other types of anisotropic nanoparticles, allowing the internalization of these objects in the cytoplasm without being trapped by endocytosis[5]. In this study, a simple and robust synthesis method was developed to produce magnetic nanorods of controlled composition in a large range of lengths and diameters. This three-step synthesis method was optimized in order to obtain iron oxide nanorods colloidally stable in water, with high aspect ratio and strong magnetic properties. The first step consisted in the formation of akaganeite nanorods in presence of polyethyleneimine (PEI). Then, a controlled reduction of the akaganeite into magnetite with oleylamine was performed and optimized to keep a good aspect ratio while improving the magnetic properties of the nanorods. Finally, the phase transfer of the magnetite nanorods into water by ligand exchange between oleylamine and 3,4-dihydroxyhydrocinnamic acid lead to water-stable magnetic iron oxide nanorods. At each step of the synthesis, the nanorods were fully characterized by transmission electron microscopy (TEM), powder X-ray diffraction (XRD) and magnetic measurements (SQUID). The final water-suspended objects were also characterized by magnetic birefringence measurements

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Acknowledgment:
The authors thank Mr. David Hrabovsky (MPBT) for his assistance in SQUID measurements.

Wednesday, November 24th

Session Nano for Imaging, Diagnosis & Therapy

16:00 - 18:30 Keynote

Carmen Isabel ALVAREZ LORENZO, USC – Pharma. Tech., Santiago de Compostela – Spain

Abstracts

Keynote Speakers

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Biography

Carmen Alvarez-Lorenzo (PhD Pharmacy, 1998) is Professor of Pharmaceutical Technology at the University of Santiago de Compostela. She was a postdoctoral fellow at Massachusetts Institute of Technology (MIT, USA) (1998-2001) and Ramón y Cajal researcher at the University of Santiago de Compostela (2001-2006). Her research interests include drug and gene nanocarriers, stimuli-responsive and imprinted networks, biomimetic materials, scaffolds, and drug-eluting medical devices. She has coauthored more than 300 papers, 30 book chapters, 17 patents, and +350 contributions to scientific meetings, and co-edited two books. She has supervised 21 PhD students and 8 more are on-going. h-index: 50 (Web of Science Core Collection); 52 (Scopus); 61 (Google Scholar). She is a member of a number of international committees and editorial advisory boards. Activities of her research group can be followed in the web site *https://www.idfarmausc.es/en.*

POLYMERIC MICELLES FOR TOPICAL TREATMENT OF OCULAR DISEASES

The eyes can suffer a variety of diseases, but their treatment is still a challenge due to the numerous anatomical barriers and eye defense mechanisms. The access of drugs through the blood stream is limited by the blood-ocular barriers. Periocular and intraocular injections may allow in situ management of diverse ocular pathologies, but the need of attenuating risks demands the development of more patient-friendly formulations. Topical formulations, mainly eye drops, are comfortable and safe, but only 1-10% of the dose can penetrate into the eye structures. Such poor ocular bioavailability is caused by low cornea permeability, short residence time, rapid tear fluid turnover, and efflux pumps. To overcome these hurdles, a variety of nanocarriers are being investigated. This talk focuses on the advantageous performances that polymeric micelles may offer for both anterior and posterior segments treatments [1]. A variety of amphiphilic polymers exhibit spontaneous self-assembly into nanomicelles that can encapsulate hydrophobic drugs and withstand the sterilization protocols and the subsequent dilution in the lachrymal fluid without premature disassembly. Moreover, polymeric micelles favor drug partition toward the corneal epithelium while the unimers may inhibit efflux pumps. Prolonged permanence on the ocular surface can be achieved through in situ gelling phenomena. The drug-loaded polymeric micelles can penetrate through different pathways into the ocular structures and may reach the posterior segment of the eye through the conjunctival–scleral route. Relevant examples of nanomicelles for lipoic acid, acyclovir [2], cyclosporine and progesterone [3] ocular delivery are discussed, paying attention to the preclinical tests suitable for predicting in vivo performance.

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Acknowledgments: This work was supported by MINECO [SAF2017-83118-R], Agencia Estatal de Investigación (AEI) Spain, Xunta de Galicia [AEMAT ED431E 2018/08] and FEDER.

Keywords: nanomicelles; ocular delivery; eye treatment; ex vivo permeability.

Thematic Session: Nano for Imaging, Diagnosis & Therapy

Keywords: Drug delivery, nanocomposite, electropermeabilisation, skin, non-invasive delivery.

Carbon nanotubes based composite for non-invasive transdermal drug delivery

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Unlike small molecules (*e.g.* nicotine), larger molecules such as insulin are too big to be able to cross the skin and reach the blood flow through passive diffusion. Needles are thus required to cross that natural barrier and properly deliver those molecules. In order to improve the life quality of patients using regular hypodermal injections, alternative delivery methods are needed. Carbon-based nanoparticles, free or associated with a polymer matrix, are increasingly used in innovative biomedical applications and recently emerged as a versatile tool to answer this need [1]. As such, in this work we explore a non-invasive transdermal delivery route, using a two-in-one nanocomposite device and the reversible permeabilisation of the skin, thanks to electroporation. The device we are developing, a nanocomposite with a biocompatible hydrogel matrix containing carbon nanotubes, combines both purposes of assisting the permeabilisation and storing the molecule to be delivered. The inclusion of the carbon nanotubes in the hydrogel matrix is also intended to prevent their release during operation of the device, with a safe(r) by design strategy in mind. Previous work from our group described an enhanced drug delivery of dextran FITC through permeabilised skin for devices containing carbon nanotubes [2]. Later, the electrical characterisation of those devices was performed [3]. However, although the proof of concept was validated, such devices are still far from practical applications and the optimisation of electroporation parameters, depending on the intended use, is still in progress. We will describe and discuss our most recent advances.

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Acknowledgment: This research is funded by Région Occitanie and Université Fédérale de Toulouse

Thematic Session: Nano for imaging, diagnosis & therapy
Keywords: thermoresponsive polymers, microstructure, colloids, mesoglobules, hydrogels

Effect of the microstructure of *n*-butyl acrylate/*N*-isopropylacrylamide copolymers on their thermo-responsiveness, self-organization and gel properties in water

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Abstract
Polymer composition and microstructure composition, molar mass, architecture… critically affect the properties of thermoresponsive polymers in solution. The behaviour of *N*-isopropylacrylamide-based copolymers of variable composition and structure (statistical, diblock or triblock) was studied in solution at different temperature and concentration with turbidimetry measurements, differential scanning calorimetry, electronic microscopy and scattering experiments. This study illustrates how it is possible through chemical engineering on the microstructure of amphiphilic thermoresponsive polymers to modulate significantly the self-assembly, morphological and mechanical properties of these materials in aqueous media. Statistical structures induced a strong decrease of cloud point temperature compared to block structures with similar composition. Moreover, block structures lead below the transition temperature to the formation of colloidal structures. Above the transition temperature, the formation of colloidal aggregates is observed at low concentrations, and at higher concentrations the formation of gel. Significant differences on colloidal structures and mechanical properties of gel issued from polymer microstructure were evidenced.[1] Lastly these families of polymer were successfully used as stabilizing agent for the stabilization of gold nanoparticles.[2]

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Acknowledgment:
The authors acknowledge CSC Chinese government scholarship and EU for financial support (FEDER-35477: Nano-objets pour la biotechnologie). This work benefited from the use of the SasView application, originally developed under NSF Award DMR-0520547. SasView also contains code developed with funding from the EU Horizon 2020 programme under the SINE2020 project Grant No 654000.

C'NANO 2020
The Nanoscience Meeting
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Centre des congrès Pierre Baudis
December, 8, 9 and 10

Thematic Session: Nano for Imaging, diagnosis & therapy
Keywords: Self-assembly, block copolymers, amphiphilic, thermoresponsive, pH-sensitive

Synthesis and self-assembly of amphiphilic multi-responsive block copolymers for drug delivery applications

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Over the past decade, block copolymers have raised a strong interest in polymer science and biomedical engineering as they can be efficient drug delivery nanocarriers.(1) Among the different synthesis pathways available, RAFT/MADIX technology is often acknowledged as the most robust and versatile technology in the design of well-controlled block copolymers.(2-3) Amphiphilic block copolymers are especially interesting because of their self-assembly properties in aqueous media and their ability to carry bioactive agents.(4) Such amphiphilic copolymers may also display multi-responsiveness, e.g thermo- or pH-sensitivity. Multi-responsiveness results in appropriate conditions to a change of macromolecular conformation of one or each block and thus to a triggered and controlled release of loaded drugs.
This work consists in the synthesis and physico-chemical properties characterization of a multi-responsive block copolymer, displaying both pH-sensitivity of a polybase block and thermoresponsiveness of a LCST block. According to a reported method,(5) RAFT/MADIX polymerization of N-vinylphtalimide in presence of xanthate yielded a first block from which chain extension was carried out with a LCST monomer.
Phtalimido groups were deprotected to recover poly(vinyl amine) –b- poly(LCST) copolymer. ^{1}H-NMR and SEC confirmed structural nature of the diblock copolymer and its behavior in aqueous media was studied with UV-vis spectroscopy, DLS, temperature-dependent NMR and DSC. Results include the determination of a cloud point temperature of 37 °C and a good reversibility of the LCST transition.

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Thematic Session: Nano for Imaging, diagnosis and therapeutics
Keywords: Hybrid nanoparticles, Bio-inspired apatite, Organic corona, Nanomedicine

Bio-inspired apatite-based nanoparticles: a smart platform for nanomedicine

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Apatitic calcium phosphates are naturally present in many biomineralizations in living organisms [1]. Synthetic bio-inspired apatite nanoparticles (NPs) close to the composition of bone mineral can be prepared and stabilized in colloidal form via the decoration of the particles by an organic corona. It is possible to tailor the physicochemical characteristics and related biological properties of such hybrid NPs by modifying adequately the chemical composition of the apatitic core of the particles on the one hand, and of the grafted organic molecules on the other hand. The intrinsically biocompatible nature of the NPs allows imagining a wealth of biomedical applications, far beyond bone applications for which calcium phosphates were initially studied.
In this contribution, after reminding the background of nanocrystalline apatite physicochemistry, we will present several features of such NPs relevant to different domains of use, and we will illustrate how apatite-based NPs may prove helpful in the fields of hematology, oncology and dermatology. By designing appropriately their properties, the NPs were for example shown to either allow increased drug permeation into live cells, or instead limit a drug transcutaneous permeation for a local, topical delivery to the skin. Conferring antimicrobial activity can also be explored, with the possibility to allow a dual kinetic release of active agents, and smart drug delivery using stimuli-responsive approaches are being developed. By combining their bio-inspiration, high related biocompatibility, tailorable nano-carrier features and cell-membrane modifying capabilities, hybrid apatite-based colloidal NPs appear as appealing systems for tomorrow's nanomedicine.

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December, 8, 9 and 10

Thematic Session: Nano for imaging, diagnosis & therapy

Keywords: Mesoporous silica nanoparticles, zwitterion, catalysis

Encapsulation of bio-inspired Mn complexes in mesoporous silica nanoparticles with improved biocompatibility for the regulation of oxidative stress

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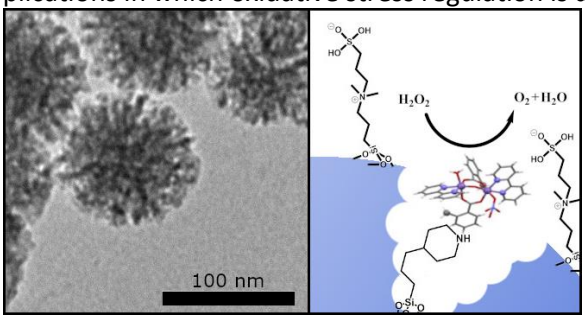
Abstract:

Nature remains a major source of inspiration for designing of catalysts with great interests. Unfortunately, most of these bio-inspired catalysts are inactive in water because of instability or insolubility. This is the case for all the bio-mimics of the Mn-catalase (Mn-CAT), an enzyme involved in the regulation of oxidative stress.[1] One may solve this problem by recreating the confinement brought by the 3D-structure of the proteins through the incorporation of these catalysts inside the pores of mesoporous silica nanoparticles (MSN).[2, 3]

Accordingly, our hybrid MSN's contain two organic functions: 1,4-pyridinium selectively anchored on the internal surface to fix the bio-inspired Mn complex, and sulfobetain zwitterion on the external surface to ensure the biocompatibility and avoid the flocculation.[4] The material synthesis was adapted from a previous work using cetyltrimethylammonium tosylate (CTATos) as surfactant and triethanolamine ($TEAH_3$) as catalyst for the sol-gel reaction. [5] A series of nanoparticles with various amounts of organic functions were synthesized. They present an unprecedented colloidal stability in the biologic PBS buffer, mimicking the ionic media of blood while the anchored Mn complexes exhibit an excellent catalyze activity probed by the dismutation of hydrogen peroxide (H_2O_2) in aqueous media. These results are very promising for biomedical applications in which oxidative stress regulation is at stake.



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Thursday, November 25th

Session Nano for Imaging, Diagnosis & Therapy

10:20 - 10:50 Keynote

Laurent COGNET, CNRS – LP2N, France

Abstracts

Keynote Speakers

Laurent COGNET

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Biography

Laurent Cognet is CNRS Research Director at Institute of Optics in Bordeaux where he leads a group in nano&biophotonics. After a PhD in atom optics with A. Aspect (Orsay) and a postdoc in biophysics with Th. Schmidt's (Leiden University, NL), he was tenured as CNRS junior researcher in Bordeaux in 2000 to develop the emerging field of single-molecule detection and super-resolution microscopy in the context of biological applications. In 2006-7, he was a Fulbright scholar at Rice University (Houston, TX) and initiated original studies on carbon nanotube optics. He was promoted Research Director in 2009 and actively participated in 2011 to the creation of LP2N at Institute of Optics in Bordeaux. His current research interests include the nanoscale investigation of the biological matter based on innovative nanostructures and high-resolution optical microscopy. He has published over 90 papers totalizing more than 7000 citations and received several prizes of his achievements.

NANOSCALE EXPLORATION OF LIVE BRAIN TISSUE BASED ON SUPER-RESOLUTION MICROSCOPY AND NEAR-INFRARED EMITTING CARBON NANOTUBES

Single-molecule localization microscopy (SMLM) is a key approach used nowadays to study structural and dynamic arrangements of the matter at the nanoscale in a wide range of applications. As a member of the "super-resolution microscopy" family SMLM indeed provides optical images with resolutions well beyond the diffraction limit. Yet, it remains challenging to study more complex systems than isolated nanostructures or isolated living cells in biology with such approaches. For instance, SMLM in thick and intact brain tissues is penalized by the limited brightness of fluorescent emitters, the optical aberrations induced by the samples and/or the poor penetration of the light into biological tissue at visible wavelengths. To circumvent these limitations and investigate live brain tissues at the nanoscale, we developed a framework based on SMLM [1] and single-wall carbon nanotubes imaging [2] which luminesce in the near-infrared. Nanotube detection and tracking at the single nanotube level allow the extracellular space of intact live brain tissues to be revealed at the nanoscale and its modifications to be studied in the context of neurodegenerative diseases [3]. Building on this strategy, I will present how a toolbox of SMLM nanoprobes can be engineered in the near-infrared to study complex biological tissues through (i) the creation of photoswitchable carbon nanotubes [4] and (ii) ultrashort carbon nanotube displaying localized emission centers that could be revealed by super-resolution microscopy of the nanotube themselves [5]. Other applications in life and medical science beyond neurosciences will be presented.

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See also: *www.cognet-research.com*

Keywords: single molecule detection; carbon nanotube; nanoparticles; superresolution microscopy; neurosciences; near-infrared; tissue imaging; extracellular space.

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December, 8, 9 and 10

Thematic Session: Nano for Imaging, diagnosis and therapy
Keywords: Photodynamic Therapy, nanovectors, self-assembly, amphiphilic copolymer, model membranes.

Influence of polymer micelles in the delivery of a photosensitizer to model membranes and cells

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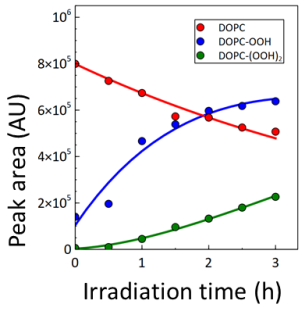
Abstract (**no longer than 250 words** (or 18 lines max. incl. figure), Calibri 11, single line spacing, black)

In the context of Photodynamic therapy, where light is indirectly used to generate localised tissue damage, the use of polymer-based nanocarriers allows for much greater efficiency when highly hydrophobic drugs are used as photosensitizers. [1] This compartmentalization of the drug adds extra complexity and interactions between the carriers and the target cells should be taken into account, but are still poorly understood. In this study, we focus on two amphiphilic block copolymers (poly(ethylene oxide)-*block*-poly(ε-caprolactone) PEO-PCL and poly(ethylene oxide)-*block*-poly styrene PEO-PS) that are known to form well characterized micelles and are able to incorporate Pheophorbide a, a highly efficient singlet oxygen generator. First, we have used artificial lipid self-assemblies as model targets, and physical chemistry techniques to evaluate several key characteristics such as lipid oxidation rate (figure) of the ménage-à-trois between nanocarrier, drug and target. In order to confront these model studies to *in vivo* situations, the Pheo-loaded carriers were used in contact experiments monitored by flow cytometry, and PDT experiments on two different cancerous cell lines. Even though PEO-PCL carriers led to higher concentrations of PheO in cells, this did not translate into greater PDT efficiency.[2] We will describe our methodology and discuss hypotheses to rationalise the subtle differences observed.



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Acknowledgment: TRI-GENOTOUL is acknowledged for the flow cytometry experiments

Thematic Session: nanomaterials
Keywords: Inorganic nanoparticles, Hafnium oxide nanoparticles, X-ray imaging, fucoidan, Polyéthylène Glycol, atherothrombosis.

Hafnium Oxyde nanoparticles synthesis for the detection of atherothrombosis through X-ray imaging

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Cardiovascular diseases such as atherothrombosis are chronic pathologies that progress silently in the body for decades. They are responsible for 30% of death in the world due to a lack of an early detection of the location of the vascular diseases by the conventional imaging system. Recent studies showed that molecular imaging for cardiovascular diseases using a probe to target key biological markers such as P-selectin are required for an early diagnosis. The diagnostic imaging system used in this pathology is mainly Computed Tomography (CT) based from differences in X-ray attenuation of tissues [1]. The primary limitations of CT are relatively low soft tissue contrast and sensitivity. Using nanoparticles (NPs) as contrast agents can enable delivery of a greater mass concentration of the X-ray attenuating element per particle compared with molecular agents (i.e., iodine) [2].

Herein, inorganic NPs made of hafnium oxyde had been synthesized as nanocarriers and potential CT contrast agent to target the arterial thrombi overexpressing P-selectin and detect the activated platelets through X-ray imaging. The synthesis of hafnium oxyde NPs was optimized by using a sol-gel method, associated to microwave. The physico-chemical characterization of the obtained NPs showed a well-controlled size. These stable nanoparticles were then successfully either functionalized with polyethylene Glycol (PEG), or fucoidan, which showed in previous studies a good affinity and a strong binding for the P-selectin [3]. Due to this ability it has been considered as a good candidate to image vascular diseases.

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Thematic Session: Nano for Imaging, diagnosis and therapy
Keywords: Luminescent nanoparticles, intracellular imaging, single-particle tracking, delivery of nanoparticles

Luminescent Polymer Nanoparticles for Intracellular Imaging

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Imaging individual biomolecules in living cells can give a wealth of information on how biology functions at the molecular level, but requires bright probes to attain high spatial and temporal resolution.[1] Fluorescent and luminescent nanoparticles (NPs) are excellent candidates to achieve very high brightness, but making them also small and avoiding non-specific interactions remains challenging.

Here, we will give an overview on some recent developments by us of NP probes combining high brightness, very small size and stealth properties and their use for intracellular imaging. These probes are based on polymeric NPs loaded with high amounts either of salts of cationic dyes with bulky hydrophobic counterions[2] or of luminescent lanthanide complexes.[3] Through variation of nature and amount of charged groups on the polymers, NPs with sizes down to that of single proteins were obtained.[4] Introduction of zwitterionic groups strongly reduced interactions with proteins while maintaining a brightness 10 times higher than quantum dots.[5] Different ways of delivering these NPs directly to the cytosol of living cells will be presented, and the effect of particle size on delivery by electroporation will be discussed. Tracking these NPs at the single NP level within the cytosol showed the importance of combining small size and stealth properties to achieve free particle diffusion and to access all regions of the cytosol.

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Acknowledgment:
Supported by the Agence National de Recherche JC/JC grant "Supertrack" ANR-16-CE09-0007, the ERC Consolidator grant BrightSens 648528, and by the "Prix Espoirs" de l'Université de Strasbourg.

Thematic Session: Nano for Imaging, diagnosis & therapy

Keywords: molecular nanocrystals, core shell nanoparticles, nanohybrids, *In vivo* fluorescence imaging.

Molecular Nanocrystals for Bioimaging

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Fluorescence imaging may constitute a valuable tool for bio-imaging, provided highly fluorescent and safe biotracers are developed. In particular, three-dimensional, deep-tissue imaging may be improved following the development of two-photon emitting probes excited and emitting in the biological transparency window. Silica-based nanoparticles are widely used for bioimaging, as the silica matrix is biocompatible and can be easily surface-modified to target cancer tissues or induce stealth in biological fluids. However, the dye loading in such cases is usually very low, which limits the overall brightness. Following the groups' experience in organic nanocrystals, we were interested in developing nanoparticles of organic fluorophores displaying high two-photon absorption cross sections and bright emission in the red-NIR region in the crystal state following a dye engineering at ENS Lyon. This strategy allows a very high loading in organic fluorophores while keeping a strong brightness.[1] In this talk, we will show that nanoparticles featuring a crystalline organic core and an amorphous silicate shell can be produced in one step process thanks to the spray-drying technique. [2] The crystallinity of the organic cores will be discussed on the basis of electron diffraction studies. The functionalization of these hybrid core-shell nanoparticles in water with PEG chains using click chemistry reactions allows to provide colloidal stability in biologically-relevant media. *In vivo* fluorescence imaging of mice vasculature revealed the very strong brightness of such nanoparticles.[3] Furthermore, colloids of crystal-state emitters can be produced using an original sono-crystallization technique. The application of such colloids for imaging of cancer cells under two-photon excitation will be also introduced.

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Thematic Session: Nano for Imaging, diagnosis and therapy
Keywords: gadolinium complexes, silica, magnetic resonance imaging, cytotoxicity

Surface engineering of silica nanoparticles with a Gd-PCTA complex for efficient T_1-weighted MRI contrast agents.

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Magnetic Resonance Imaging (MRI) is a versatile imaging technique with impressive spatial resolution but poor sensitivity.[1] A large research effort thus focuses on developing more efficient and safer contrast agents,[2,3] designed to detect tumors at the earliest possible stage, or vascular deficiencies in the body and the brain. To bring some answers, we have synthesized new PCTA (Pyridine Containing TriAza; 3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3,6,9-triacetic acid) ligands presenting pendant carboxylic acid or alcohol functions and used them to form diaqua Gd(III) complexes, which have been immobilized onto dense silica nanoparticles. Cytotoxicity studies of these complexes (free or immobilized on the nanoparticles) on normal 1BR3G cell line and cancerous HCT 116 cells indicated a dependence on the pendant chemical group for the free complexes. However, this effect disappeared once the complexes were immobilized on the surface of the nanoparticles, leading to non-toxic nanomaterials. The interest of these complexes (free or immobilized on dense silica

nanoparticles) as contrast agents in Magnetic Resonance Imaging has been evaluated in comparison with DOTAREM® (currently, the most commonly used Gd-complex for contrast enhancement in MRI) by recording their nuclear magnetic resonance dispersion profiles, measuring their transversal and longitudinal relaxivities as well as recording images on phantoms at 37°C. This study has evidenced the high potential of these complexes: first it suggested the possibility to reduce the dose to be injected by a factor of 10, second it evidenced their high efficiency in high field T_1-weighted MRI (9.4T), the key towards images of higher resolution and shorter acquisition times.

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Acknowledgments: This research was funded through the C-NIM project (ESR_R&S_DF-000003/2017-006704) and by the Spanish Ministry of Sciences, Innovation and Universities through the project RTI2018-098027-B-C22. The CMMI is supported by the European Regional Development Fund and the Walloon Region (Wallonia-Biomed portfolio, #411132-957270). P.M. thanks University of Toulouse for his PhD grant. We thank Vincent Collière for his help in collecting the TEM data and Corinne Routaboul for her help in recording the IR spectra in DRIFT mode.

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December, 8, 9 and 10

Thematic Session: Nano for Imaging, diagnosis and therapy
Keywords: surface functionalization, gold nanoparticles, aryl diazonium salts, Raman imaging

Raman Reporters Derived from Aryl Diazonium Salts for SERS Encoded-Nanoparticles

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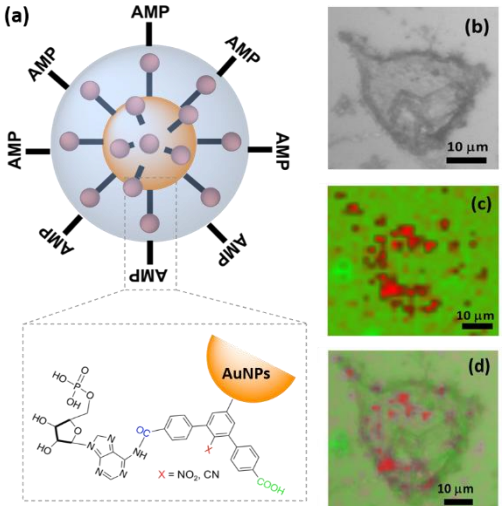
Abstract

The popularity of surface-enhanced Raman scattering (SERS) tags has increased in recent decades, as an alternative to fluorescent probes, due to their attractive properties.[1] Indeed, unlike the broad spectral feature of fluorescence probes, SERS tags provide multiple sets of narrow peaks, resulting in low spectral overlap and high multiplexing ability. In this talk, we describe the potential of aryl diazonium salt-encoded gold nanoparticles (AuNPs) as contrast agents for Raman imaging. Compared to thiol self-assembled monolayers which are commonly employed for the preparation of SERS tags, aryl diazonium salts present several advantages, such as: (i) the formation of strong interfacial bonds with the supporting NPs, (ii) the presence of intense SERS fingerprints, (iii) the possibility to create multilayers with various functions. The formation of multilayers has been exploited here to introduce straightforwardly several SERS labels along the grafted polyaryl chains and post-functionalization moieties at their end. This new generation of SERS encoded NPs, based on AuNPs functionalized by aryl diazonium salts, were shown to be efficient labels for Raman bioimaging inside cells. Therefore, this aryl diazonium salt-based approach will not only pave a new way for the functionalization of AuNPs by multilayers but also provide a general strategy to design SERS-encoded NPs[2].



Scheme 1. (a) Illustration of AuNPs functionalized using aryl diazonium salts. (b) bright-field optical and (c) Raman image of EGI-1 cell after 24h incubation with Au@CN@COOH@AMP. (d) Overlaid image of (b) and (c). The distribution of SERS-encoded nanocomposite is marked as red spot, obtained under 633 nm laser excitation.

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Thursday, November 25th

Session Nano for Imaging, Diagnosis & Therapy

14:00 - 15:10

Abstracts

Thematic Session: **Nano for Imaging, diagnosis & therapy**

Keywords: GB, nanocomposite hydrogel, nanoprecipitation, polymeric nanoparticles, *in vitro* studies,

Development of innovative nanocomposite hydrogels for the treatment of Glioblastoma

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Abstract

Glioblastoma (GB) is the most aggressive and lethal subtype of brain cancer. According to the WHO, it belongs to the Grade IV brain cancers. It is highly invasive and can infiltrate cerebral tissue, and therefore the complete resection of the tumor is difficult. GB tumor is removed by surgery, but it relapses in 90% of cases, 6 to 7 months after treatment due to radiotherapy resistance. Gliadel® associated to radiotherapy and Temozolomide increases the overall survival from 1 to 2 years for GB patients [1].

The current project is based on a new injectable nanocomposite hydrogel where a protein is encapsulated in polymeric nanoparticles (NPs). PLGA NPs are prepared via non-toxic and biocompatible solvents and are incorporated into nanocomposite hydrogels to achieve a controlled release of BMP-4, a cytokine involved in neurogenesis. Herein, a differentiating strategy is applied to lead cancer cells and more precisely cancer stem cells to acquire a less aggressive phenotype that may increase their radiotherapy sensitivity [2-3]. Since brain microenvironment is composed of a Hyaluronic acid (HA) enriched extracellular matrix (ECM), we expect to develop an injectable, biocompatible, bioadhesive and biodegradable HA scaffold. HA is thus a good candidate since it possesses all those functionalities [4].

Finally, the most challenging part of this innovative strategy is the encapsulation of the BMP protein and tuning its delivery in situ. The encapsulation yield and release from hydrogel will be studied in vitro to obtain a proof of concept regarding cytotoxicity and efficiency on U87MG cell

line and on primary patient cells. During this work, optimization of the formulation process and physico-chemical characterizations such as rheology, mechanical properties tests and other types will be performed. Bioperformance evaluation of our device on preclinical models is also expected.

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Acknowledgment:
The authors would like to thank for “La Ligue Contre Le Cancer” for providing the financial support of the project of the PhD grant. The authors are thankful for the European financial support (EACEA) in the frame of the NanoFar program, an Erasmus Mundus Joint Doctorate (EMJD) program in nanomedicine and pharmaceutical innovation. This work was also supported by “La Région Pays-de-la- Loire”(Nanofar+ projet strategy international), by the “Institut National de la Santé et de la Recherche Médicale” (INSERM), by the “University of Angers” and the “University of Liège” and by the “Cancéropôle Grand-Ouest” though the ”glioblastoma“ and ”vectorization and radiotherapies“ networks. E. Garcion is also a member of the LabEx IRON “Innovative Radiopharmaceuticals in Oncology and Neurology” as part of the French government “Investissements d’Avenir” program and head the PL-BIO 2014-2020 INCA (Institut National du Cancer) project MARENGO - “MicroRNA agonist and antagonist Nanomedicines for GliOblastoma treatment: from molecular programmation to preclinical validation”.

Thematic Session: Nano for Imaging, diagnosis and therapy
Keywords: Photoacoustic imaging, nanoparticle, Bodipy, photophysics, personnalized nanomedicine, polylactide

Biocompatible photoacoustic nanoparticular contrast agents based on BODIPY-scaffold and polylactide polymers

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Photoacoustic imaging is an emerging biomedical imaging modality combining optical and ultrasound waves to map optical-absorption contrast at centimetric depth with sub-millimeter resolution. The key is the photoacoustic (PA) effect: optically absorbing structures emit ultrasound waves when excited with a ns-laser pulse. To reach cm-depth, PA imaging operates in the near-infrared (NIR) window in biological tissue (650-1000nm). NIR optical absorbers can thus be mapped throughout the range of depths and resolution explorable with medical ultrasound. We have designed novel PA molecules based on the BODIPY scaffold. These PA-BODIPYs were used as initiators for the ring opening polymerization of lactide to yield BODIPY-polylactide, that were further formulated into nanoparticles (NP). We present here the full spectroscopic and photoacoustic characterizations of the PA-BODIPYs, the corresponding polymers and NPs. Results show BODIPY NPs are promising contrast agents for PA imaging.

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Thematic Session: Nano for Imaging, diagnosis and therapy
Keywords: Organic nanoparticles, Fluorescence, Stealth, Single Particle Tracking, Bioimaging

All-organic, intrinsically stealth nanoparticles for single particle tracking and bioimaging

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To date, quantum-dots are the most widely used nanoparticles for bioimaging thanks to their unprecedented brightness and photostability. However, their inorganic core is inherently water insoluble, requiring them to be coated by solubilizing agents such as polyethylene-glycol (PEG). Moreover, most contain heavy metals, raising environmental concerns and limiting their clinical potential.

To circumvent these limitations, our lab develops Fluorescent Organic Nanoparticles (FONs) as a promising, all organic, spontaneously water soluble alternative. Prepared by self-aggregation of rationally designed hydrophobic dyes in water, it is possible to tune the properties of FONs by molecular engineering of their constitutive dyes. We have previously reported on green[1] and near-infrared[2] emitting FONs that could enter and be tracked in living cells, making them good candidates for drug delivery systems. However, stealth emitters can be of interest for tracking of cell-surface receptors or exploring the extracellular space.

In this work, we describe spontaneously stealth, size-tunable, ultrabright, red emitting FONs made from a novel quadrupolar dye. We report on the characteristics and properties of these FONs and show that they have no unspecific interactions with living cells. Thanks to their brightness and stability, we also have achieved single particle tracking in solution. From these combined properties, we conclude that these novel FONs are promising candidates for the next generation of tools for bioimaging.

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Acknowledgments:

This work has received funding from the European Union's Horizon 2020 research and innovation program (Marie Skłodowska-Curie grant agreement No. 841379 to M.R. and 793296 to C.P.), from the US-France-Belgium iREU Site in Translational Chemistry (grant No. 1560390 to J. Fl), from the Agence Nationale de la Recheche (ANR-15-CE16-0004) and from the LAPHIA Cluster of Excellence. M.B-D. acknowledges an excellence Chaire d'Accueil grant from the Conseil Régional d'Aquitaine. We acknowledge the Bordeaux Imaging Center (CNRS-INSERM-UBx), a member of the France Bioimaging infrastructure, for transmission electron microscopy.

Thematic Session: Nano for imaging, diagnosis and therapy

Keywords: Elastin-like polypeptides, RAFT polymerization, plasmid DNA, polyelectrolyte complexes, macromolecular click chemistry

Hybrid Cationic Elastin-like Polypeptides for Nucleic Acids Delivery

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Nanotechnology has been widely developed during the recent years for the transport and release of genes in cancer therapies. The principle of gene transfer is based on the use of DNA complexing agents, which can compact and protect the genetic material against degradation and allow delivering it into the target cell. In this work we explored the use of positively charged elastin-based polypeptides (ELPs) for the complexation of genetic material [1,2]. Recombinant ELPs were functionalized to graft 11 alkyne groups per chain and cationic oligomers were introduced by Huisgen's azide-alkyne cycloaddition reaction. For this, a series of cationic oligomers was prepared from a positively charged methacrylic monomer. These oligomers were synthesized by Reversible Addition-Fragmentation chain-Transfer (RAFT) polymerization using an azide containing chain transfer agent. Four oligomers with polymerization degrees of 6, 9, 15 and 23 were synthesized and coupled with the alkyne-functionalized ELP. Hybrid cationic ELPs were isolated and characterized by ^{1}H NMR, SEC and zeta-potential measurements to assess their purity and determine their degree of functionalization, molar mass and overall charge. Then, electrostatic complexation was achieved between these hybrid cationic ELPs and plasmid DNA, allowing the determination of the optimal conditions for obtaining stable nanoparticles having a controlled size and surface potentials at different N/P charge ratios. Preliminary biological tests showed the reliability of such hybrid cationic ELPs to internalize efficiently genetic material into living cells.

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Acknowledgments:

Authors wish to thank A. Vax (SEC), M. Rosselin (RMN) and C. Lebleu (TEM). Région Nouvelle Aquitaine, French National Research Agency (ANR-15-CE07-0002) and the Cancéropole Grand Sud-Ouest (Emergence 2018-E18) are gratefully acknowledged. L.M. Bravo-Anaya acknowledges the fellowship grant given by CONACYT (CVU 350759).