

# Abstracts

## Poster Session

### P4 - NANO FOR IMAGING, DIAGNOSIS AND THERANOSTICS

**Thematic Session:** Nano for imaging, diagnosis and theranostics

**Keywords:** Chitosan, Hyaluronic acid, Nanohydrogel, Functionalization, MRI, optical imaging

**Fluorescent biocompatible nanohydrogels for Gd chelates encapsulation: polymer design, nanogel syntheses and *in vitro* evaluation of the resulting magneto-optical probes.**

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Hydrophilic bio-sourcing polymers, like chitosan (CS) or hyaluronic acid (HA), are good candidates to elaborate biocompatible nanogels (CS/HA NGs). We have previously shown [1, 2] that CS/HA NGs allow to boost the relaxivity of gadolinium contrast agents (GdCAs) between 20 and 60 MHz, when these GdCAs are encapsulated within these gels (Gd $\subset$ CS/HA NGs). In order to have an insight of Gd $\subset$ CS/HA NGs biodistribution, it is now mandatory to add an optical functionality to these objects.

CS and HA present the advantage to be easily functionalizable [3] which allows to graft optical probes on amino or carboxylic functions respectively. In this poster, we will present the functionalization of CS and HA polymers with a fluorescent organic dye as well as their characterisations by a combination of NMR and optical techniques.

The syntheses of the relevant nanogels will be described, the MRI efficiency and the first *in vitro* evaluation of the magneto-optical nanogels will also be presented.

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**Thematic Session:** Nano for imaging, diagnosis & theranostics

**Keywords:** theranostic, polymer, magnetic resonance imaging

## Evaluation of polyrotaxanes as novel contrast agents designed against arthritis by engineering of dynamic MRI.

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### Abstract

**Introduction:** In the context of the valorization and development of innovative theranostic imaging probes, we propose the development of new quantitative in vivo molecular imaging methods based on dynamic imaging to record the kinetic biodistribution of novel MR imaging probes. The method will be developed and applied to multifunctional polyrotaxanes with theranostic purpose against arthritis and cancer.

The theranostic probe is a supramolecular approach for the design of a bimodal agent based first on a polyrotaxane

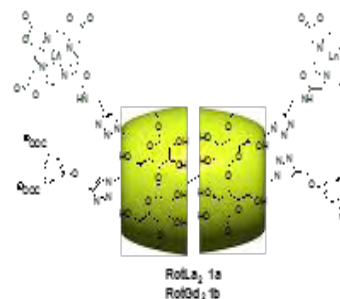


Fig 1. Polyrotaxane

**Methods:** The polyrotaxane will be composed of a polymeric chain threaded through cyclodextrins (CDs) functionalized by MRI Gd contrast agents or fluorescent probes, and targeting ligands with different sizes and functionalisations (Jere, Yui, Li). This project involved the synthesis of functionalized CDs following a route already developed to supply different polyrotaxanes with different size, polymer chain and functionalisation. The new compounds are tested on our bioimaging facility in vitro and in vivo. After structural and physico-chemical characterisations, cytotoxicity was performed in vitro by Alamar Blue test method on liver cell lines (TIB-75). In vivo biodistribution studies were performed on Balb-C mice after an IV injection of 100  $\mu$ L of molecule at 10 mM of Gd for 1 h, 3 h and several days by MRI (Bruker, 7T) with dynamic contrast-enhanced contrast DCE acquisition developed in our laboratory. Dynamic profiles were computed to provide pharmacokinetics quantitative values of the biodistribution.

**Results:** The relaxivity measurements reveal compatible values for further in vivo use as T1 and T<sub>2</sub> contrast agents in MRI. A first generation of rotaxanes has evidenced the strong potential of a bi and four rotaxane as moderately toxic, stable, bimodal optical and magnetic with longer circulating blood feature compared with commercial DOTAREM with preclinical in vivo MRI. A second generation of rotaxane was designed and synthesized, assessed with our in vitro protocol showing an increased biocompatibility for in vivo MRI to arthritis targeting.

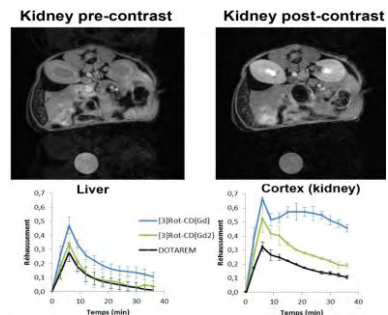
**Conclusion/ Discussion:** We have evidenced the efficiency of polyrotaxanes moieties as efficient MRI contrast agent in vitro and in vivo for further applications on arthritis murine models.

We have in particular developed an MRI method to study the kinetic biodistribution of Gd MRI contrast agent enabling in vivo studies. Our biodistribution protocol can be applied to all mid size Gd based contrast agent.

This bioimaging assesement will constitute a go/nogo step for further diagnosis and or therapeutical applications.

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**Fig. 1: T1 weighted image of polyrotaxane biodistribution during dynamic MRI**



**Thematic Session:** Nano for imaging, diagnosis and theranostics

**Keywords:** Nanovectorization, Nanodroplets, Hydrophobic drug, Cellular uptake

## Cellular uptake of perfluorocarbon nanodroplets: a potential nanocarrier for ultrasound-mediated drug delivery

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During the last decade, the field of nanotechnology has focused on the development of theranostic tools for early diagnosis and targeted therapy in oncology. In this context, perfluorocarbon (PFC) nanoemulsions are increasingly investigated as ultrasound contrast agents and sonosensitive drug delivery systems. Our theranostic agents consist of perfluorooctyl bromide (PFOB) droplets stabilized and dispersed in water thanks to a shell resulting from the self-assembling of tailor-made fluorinated surfactants. To encapsulate a hydrophobic agent/drug within the droplet core, a biocompatible oil is used. In addition, our PFOB droplets exhibit an interesting diameter for medicinal applications (less than 100 nm) and their size growth can be limited in time by a freeze-drying step following the emulsification process.

In this work, we performed *in vitro* safety tests on different murine and human normal or cancer cell lines to assess biocompatibility/toxicity of both droplet components and droplets themselves. Simultaneously, a dual-labelling approach was applied to confirm their capacity to carry a hydrophobic agent: the nanodroplet surface was endowed with fluorescein moieties grafted to the polar head of surfactants while a hydrophobic dye (DiD), solubilized in an oily phase, was encapsulated in the core. Then, the cellular uptake and kinetic of internalization of nanodroplets was studied in various normal and cancer cell lines using Confocal Laser Scanning Microscopy (CLSM) and flow cytometry. All together, these results confirm the potential of these nanodroplets as promising nanocarriers for hydrophobic agents/drugs.

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**Thematic Session:** Nano for imaging, diagnosis & theranostics

**Keywords:** Gold nanoparticles, PEGylation, theranostic and biodistribution

## Influence of PEGylation on the in vivo behavior of radiosensitizing gold nanoparticles

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Gadolinium coated gold nanoparticles have a promising potential for magnetic resonance imaging (MRI) guided radiotherapy. However this potential is not exploited plentifully because of a too fast renal elimination. In order to postpone the renal clearance, which is essential for non-biodegradable nanoparticles, gold nanoparticles coated with PEGylated chelators were synthesized and characterized. The PEGylated chelators are composed of an anchoring site, a thioctic acid moiety used for the immobilization onto the gold cores and a macrocyclic chelator, DOTA or DOTAGA, well known for their ability to form stable complexes with gadolinium ions ( $T_1$ -weighted MRI) or radioisotopes (nuclear imaging). In between, a polyethylene glycol (PEG) chain with various length (0, 4, 11 ethylene glycol units) was inserted to study their influence on biodistribution. The reduction of gold salts in presence of PEGylated chelators provides ultra-small nanoparticles Au@TAPEG<sub>n</sub>DOTA and Au@TAPEG<sub>n</sub>DOTAGA ( $\phi_{\text{core}} < 3$  nm). This strategy which rests on the use of PEGylated macrocycles appears attractive because it does not require, in contrast to the classical route of PEGylation, the post-functionalization of the nanoparticles by PEG chains. Preliminary results showed the potential of Au@TAPEG<sub>4</sub>DOTA to improve the treatment of 9L Gliosarcoma bearing mice by radiotherapy in comparison to non-PEGylated nanoparticles.



**Thematic Session:** Nano for imaging, diagnosis & theranostics

**Keywords:** HPMA polymers, antibody conjugates, nanoprobe, fluorescence

### Antibody conjugates with fluorescently labeled HPMA polymers for cell analysis

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The direct coupling of fluorescent dyes to monoclonal antibody is widely used in various diagnostic methods in biomedical sciences as well as clinical medicine. Commercially available labeling kits are provided with protocols for optimal labeling of the antibody, where the type of coupling reaction and optimal fluorophore:protein ratio are the most important factors in the preparation of efficient fluorophore-antibody conjugates with sufficient fluorescent signal.

The aim of this work was the preparation of new type of diagnostic probes with improved spectral properties and versatility of used antibody for sensitive and easier detection of different types of cells by FACS (fluorescence-activated cell sorting). For this purpose, the linear semitelechelic HPMA (*N*-(2-hydroxypropyl)methacrylamide) copolymers were synthesized and used as carriers of fluorescent dyes. After reaction with monoclonal antibody these conjugates can serve as new fluorescent nanoprobe. Different methods and procedures were used and compared for optimization of these systems. The results showed that the length of polymer precursor, number and type of used dyes, as well as reaction conditions affected the fluorescence of final antibody conjugate and subsequently also sensitivity and resolution of FACS analysis. Our new nanoprobe showed comparable fluorescent yield with commercial kits and significantly improved stability and versatility of these kits.

This work was financially supported by grant of the Ministry of Industry and Trade (Project FV10370) and grant of the Ministry of Education, Youth and Sports of CR within the National Sustainability Program II (Project BIOCEV-FAR LQ1604).



**Thematic Session:** Nano for imaging, diagnosis & theranostics

**Keywords:** nanoparticles, polymer, fluorescence, size, bioimaging

## Controlling Size and Fluorescence of Dye-Loaded Polymer Nanoparticles through Polymer Design

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Nanoprecipitation is a straightforward yet powerful technique to synthesize polymer nanoparticles loaded with various biologically active compounds or contrast agents.<sup>[1,2]</sup> Particle formation in this approach is kinetically controlled, and various assembly parameters have been used to control the size and properties of the formed nanoparticles. Here, the influence of the nature of the polymer on the formation of nanoparticles in nanoprecipitation was studied systematically by varying the hydrophobicity and charge over a broad range. For this, methacrylate copolymers with different types and fractions of hydrophobic, hydrophilic, and charged side groups were synthesized. Nanoprecipitation of these polymers showed that particle size increases with increasing global hydrophobicity of the polymers, while hydrophilic and charged groups<sup>[3]</sup> reduce it. In this way, we achieved control over particle size from ~10 to 200 nm.

Furthermore, the effect of the polymer nature on the photophysical properties of nanoparticles loaded with a fluorescent dye, a rhodamine B derivative with a bulky hydrophobic counterion (R18-F5-TPB),<sup>[4]</sup> was studied. The hydrophobic/hydrophilic balance of the polymer modulated to a large extent the spectral properties and fluorescence quantum yield of the dye encapsulated at high concentration, reflecting changes in the dye aggregation within the polymer matrix. Thus, we showed how polymer chemistry can tune kinetically controlled formation of nanoparticles and encapsulation of the load. The concepts introduced here should be valuable tools for the design of nanoparticles for imaging and drug-delivery applications.<sup>[5]</sup>

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**Thematic Session:** Nano for imaging, diagnosis & theranostics

**Keywords:** *In vitro* and *in vivo* imaging, detection, instrumentation, biodistribution, theranostics

### Subcellular-resolved mouse organ distribution of fluorescent nanodiamonds covalently functionalized with a cationic copolymer for siRNA delivery

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Nanodiamond (size 5-50 nm) has been shown to be an efficient drug delivery agent. In early studies, we have used fluorescent nanodiamonds (FND) coated with cationic polymers to deliver siRNA to cultured cancer cells, that reduced significantly the expression of the oncogene responsible of the proliferation (1). FND perfectly stable fluorescence was instrumental in measuring siRNA release kinetics.

We have extended these experiments to mice bearing a xenografted tumor. To this aim, we have improved the FND-based siRNA delivery vehicle, by covalently grafting a cationic copolymer from the FND surface (Cop-FND). After having validated its ability to deliver siRNA with high inhibition activity into cultured cancer cells, we have studied its distribution in mice, after injection.

To this aim we have developed a new method relying on a time-gated wide-field fluorescence microscope. After sacrifice, mice organ sections were prepared for subcellular-resolution imaging. We took advantage of FND long fluorescence lifetime ( $\approx 30$  ns) to filter them from tissue autofluorescence by time-gated detection (2). The automatization of bi-modal (FND fluorescence and regular brightfield) image acquisition and processing allowed us to reliably quantify the FND distribution with single particle resolution and to determine which cell types were able to interact with nanodiamonds, and whether they end-up aggregated or isolated.

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**Thematic Session:** Nano for (bio-)imaging, diagnosis and theranostics

**Keywords:** DNA detection, Surface plasmon, Gold nanoparticle, Biosensor

## Femtomolar detection of nucleic acid based on functionalized gold nanoparticles

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### Abstract

Deoxyribonucleic acid (DNA) detection is essential for accurate and early diagnosis of disease. In this study, a femtomolar DNA detection method based on the exploitation of the localized surface plasmon (LSP) resonance of gold nanoparticles (AuNPs) was developed. We prepared Poly Ethylen Glycol (PEG) functionalized AuNPs with specific DNA capture probe (CP) directly modified on the gold surface. Two strategies are proposed using different kinds of CP to detect the target DNA (tDNA). In the first strategy, CP is the complementary of the complete sequence of the DNA (CCP method). For the second strategy, we used two CPs which were half complementary to tDNA respectively and hybridized with tDNA to form sandwich structures (MIX method). Our results showed that our detection methods are highly sensitive and limits of detection of 124aM and 2.54fM tDNA can be reached for CCP and MIX methods respectively. In addition, the specificity of our two strategies was also demonstrated with mismatch DNAs. The proposed method provides a simple, fast, sensitive and specific DNA biosensor which has the potential to be used for point of care test (POCT).

This work was supported by the National Key Basic Research and Development Plan 973 Project (2015CB755400); the National Natural Sciences Foundation of China (81430054, 81371641, 81601832) and Priority projects of Military Sciences Fund (BWS13C013) (SWH2017ZDCX4210).

J. Huang, Y. Zhang, Z. Lin, W. Liu, X. Chen, Y. Liu, H. Tian, Q. Liu, R. Gillibert, J. Spadavecchia, N. Djaker, M. Lamy de la Chapelle, Y. Xiang, W. Fu, *Nanophotonics*, 8(9), 1495, 2019

**Thematic Session:** Nano for imaging, diagnosis & theranostics

**Keywords:** Contrast Agents, MRI, PycLen, Polymersomes

### Novel manganese complexes based on a pycLen derivative: synthesis and relaxometric characterization

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In medicine, medical imaging has a leading place in the diagnosis setting. This is why some researches are continually carried out to improve the available techniques. One of the most used techniques to obtain anatomical information is magnetic resonance imaging (MRI). The commercially available contrast agents are based on gadolinium complexes. Recently, it has been shown that gadolinium can lead, mainly for patients with renal disfunctions, to a pathology named NSF (nephrogenic systemic fibrosis). It is thus interesting to develop efficient contrast agents based on another paramagnetic ion such as manganese. The macrocycle used in this work is a pycLen derivative which is functionalized to respect protection and deprotection steps. Their efficacy as contrast agents for MRI was evaluated by relaxometry and <sup>17</sup>O NMR and even if the results are encouraging, the manganese ion is, as expected, less effective than the gadolinium ion. So, it could be interesting to encapsulate the Mn-complexes in nanostructure like polymersomes which allow a fast exchange of the water molecules through the pores. The complexes could thus be encapsulated inside the polymersomes or in the membrane in order to improve the relaxivity through an increase of the rotational correlation time.



**Thematic Session: (Bio-)imaging, diagnosis and theranostics**

**Keywords: PLGA, fluorescent labels, the blood-brain barrier, FRET, biodistribution**

## **Old story, new results: properties of fluorescent labels and visualization of PLGA nanoparticles**

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Fluorescent labeling is a useful tool for investigation of the nanoparticle (NP) distribution in vitro and in vivo. However, the impact of the physicochemical properties of the fluorescent labels on the imaging quality is often underestimated. In the present study, we evaluated the dyes used commonly for labeling of the PLGA NP (Dil, coumarin 6, rhodamine 123, Cy5.5) and their influence on the imaging results. Cy5.5 was covalently bound to the PLGA polymer; other dyes were encapsulated in the polymeric core of the NP (100-140 nm). Aggregative stability, polydispersity, brightness of fluorescence, dye release and its interaction with liposomes as a cell membrane model were the key parameters for evaluation of these labels. The PLGA-PEG-Cou6 NP exhibited the highest brightness ( $Br = 10^8 \text{ M}^{-1} \times \text{cm}^{-1}$ ); however, the drawback of coumarin 6 is its tendency to partitioning in the cell membrane (> 60% as shown in the experiment using liposomes). The Dil-PLGA NP appeared to be much more stable in the bioenvironment but had lower brightness. The disadvantage of the NP labeled with rhodamine 123 is the quick release of the dye (>50%) upon dilution. The best result in vitro and in vivo was exhibited by the NP double labeled with the encapsulated Dil and Cy5.5 bound to PLGA. Employment of these NP enabled visualization of their transport across the BBB in mice with chronic intracranial windows using two-photon microscopy. Moreover, in vitro imaging of these NP showed the presence of FRET between Dil and Cy5.5 with the process efficiency of 65%.

**Thematic Session:** Nano for imaging, diagnosis & theranostics

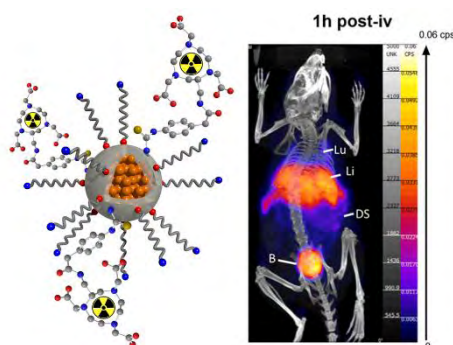
**Keywords:** Iron oxide, contrast agent, MRI, PET, cardiovascular disease

## Iron oxide nanoparticle-based MRI-PET double imaging contrast agents

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SuperParamagnetic Iron Oxide Nanoparticles (SPIONs) are widely used in biomedical applications (hyperthermia, drug delivery and T<sub>2</sub> contrast agents for MRI) [1-3]. These probes are thus a great tool for theranostics applications. For many years, our lab has developed nanoparticles with a narrow size distribution by means of a continuous hydrothermal process [4]. These particles can be coated *in situ* by organic molecules like catechols, in particular 3,4-dihydroxy-L-phenylalanine (L-DOPA) [5,6]. This type of ligand leads to an anti-oxidizing effect and an improvement of nanoparticle stability at physiological pH [5-6].



In this study, Fe<sub>3</sub>O<sub>4</sub> nanoparticles used as contrast agent for MRI/PET double imaging have been developed. These multimodal agents are easily synthesized by a step-by-step aqueous protocol, with the successive grafting of MeO-PEG<sub>2000</sub>-NH<sub>2</sub> to confer both good colloidal stability and stealth properties in physiological conditions followed by the grafting of *p*-NCS-Bz-MANOTA to ensure the stable chelation of copper (<sup>64</sup>Cu) for PET imaging [7].



The elaborated imaging agents were fully characterized and are stable under physiological conditions. Yields of radiolabeling are good and indicate that the radioactive tracer is efficiently chelated by the grafted macrocycles. *In vitro*, *in vivo* and biodistribution results on the constituting elements of the final nanohybrid will be presented. Considering these preliminary results, the elaborated nanohybrids appear as promising contrast agents for MRI/PET double imaging with a view to cardiovascular diseases imaging.

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**Thematic Session:** Nano for imaging, diagnosis and theranostics

**Keywords:** nanoparticles, fluorescence, zwitterion, polymer, intracellular imaging

## Zwitterionic Stealth Dye-loaded Polymer Nanoparticles for Intracellular Imaging

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Imaging individual biomolecules at work inside cells can give a wealth of information on biological processes. Achieving this with high speed and precision requires very bright fluorescent probes capable to function in the crowded and complex cytoplasmic environment [1]. Dye-loaded polymer nanoparticles (NPs) have achieved very high brightness through encapsulation of salts of a cationic dye with a bulky hydrophobic counterion (R18/F5-TPB) [2]. However, their intracellular applications also need non-interacting and protein-sized fluorescent NPs [1, 3]. Here, we synthesized and optimized zwitterionic dye-loaded polymer NPs for their use in intracellular imaging. For this purpose, we synthesized poly (alkyl methacrylates) bearing zwitterionic (ZI) and sulfonate groups and used them to assemble NPs loaded with R18/F5-TPB by nanoprecipitation. Depending on the hydrophobicity of the alkyl methacrylate NPs with sizes from 11 to 35 nm were obtained. Fluorescence correlation spectroscopy (FCS) measurements indicated a good resistance of ZI NPs against protein adsorption. The combination of very small size with the non-fouling nature of these NPs enabled their spreading in the whole cytosol after microinjection in living cells. In addition, the study of their trajectory showed a diffusion 4 fold faster than PEGylated NPs of similar size.

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[2] Reisch *et al. Nat. Commun.* 5, (2014), 4089.

[3] Reisch *et al. Adv. Funct. Mater.* 28, (2018) 1805157.

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# The SFNano C'Nano joint meeting 2019

**Thematic Session:** Nano for imaging, diagnosis & theranostics

**Keywords:** Quantum dots; Functional imaging; pH-sensitive probes; SWIR

## Shortwave infrared emitting QD-gold nano-assemblies for *in vivo* pH imaging

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Intracellular and extracellular pH are key parameters in many physiological processes and diseases. For example, the extracellular pH of the tumor micro-environment is slightly more acidic than in healthy tissue. *In vivo* mapping of the extracellular pH within the tumor would therefore improve our understanding of the tumor physiology. Fluorescent quantum dots (QDs) emitting in the shortwave infrared (SWIR) range are promising probes for *in vivo* imaging in depth. Here, we present two strategies to design pH-sensitive QDs in order to image pH *in vivo*. In the first one, central fluorescent QDs are coated with a copolymer ligand and conjugated to gold nanoparticle (AuNP) quenchers. As the pH decreases from physiological (7.5) to slightly acidic (5.5–6), the copolymer reversibly shrinks, which increases the energy transfer between the QDs and the AuNPs and modulates the QD fluorescence signal. In a second design, QDs and AuNPs are encapsulated into micelles using pH-sensitive copolymer surfactants, providing stability in serum, sharp pH transitions and high contrast. We demonstrate the design of SWIR QD ratiometric probes. In addition, these probes can be easily encapsulated and remain functional within ghost erythrocyte membranes, which facilitate their *in vivo* application.





**Thematic Session:** Nano for imaging, diagnosis & theranostics

**Keywords:** *in vivo* imaging, biodistribution, theranostics, gold nanoparticles, PET, MRI

### Theranostic ultra-small gold nanoparticles for integrated PET/MR imaging

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Medical imaging plays a crucial role in diagnostic, therapy guidance and treatment monitoring. Although high resolution and high sensitivity are essential, no imaging modality exhibits both characteristics. To associate both during a same imaging session, the combination of magnetic resonance imaging (MRI) and positron emission tomography (PET) has been proposed. The implementation of integrated PET/MRI device encourages the development of original nanoprobe for plentifully exploited MRI and PET after a single intravenous injection. In this context we developed radiosensitizing gold nanoparticles which are designed for immobilizing in their organic shell both gadolinium ions (for MRI) and positron emitters ( $^{64}\text{Cu}^{2+}$ ,  $^{68}\text{Ga}^{3+}$  for PET). To that end, macrocyclic chelators (DOTAGA and NODAGA) were coupled with a dithiolated anchoring part and used to get a mixed layer on the surface of the nanoparticles. To overcome the complicated characterization of these nanoparticles, “two-in-one” platforms functionalized by two different macrocyclic chelators were also developed. The reduction of a gold salt in the presence of different dithiolated chelators provided ultra-small gold nanoparticles (core size: <3 nm, hydrodynamic diameter: 6-10 nm). After complexation of gadolinium, the nanoparticles were radiolabeled with  $^{68}\text{Ga}$ . With a single intravenous injection, the biodistribution of these nanoparticles was monitored simultaneously by  $T_1$ -weighted MRI (Gd) and by PET ( $^{68}\text{Ga}$ ) in mice using a PET/MR preclinical imaging device. As a result, gold nanoparticles with a mixed shell of macrocyclic chelators and gold nanoparticles decorated with the molecular platform can be followed up by PET and MRI and exhibit a safe behavior.

# Abstracts

## Poster Session

### P5 - NANO-OBJECTS IN BIOLOGICAL FLUIDS

# Exploring the sensitivity of NanoBioAnalytical platform for analysis of biological extracellular vesicles: How far we can push?

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## Background:

The comprehensive characterizations of extracellular vesicles (EVs) are mainly hindered by their heterogeneity in size and cell origin, low abundance and it is further complicated by the media containing EVs (blood, cell supernatant, urine...). A NanoBioAnalytical (NBA) platform has been implemented (Obeid *et al.*, 2017) as a label-free and multiplexed biophysical system for the qualitative and quantitative analysis of EVs. Indeed, EVs are immuno-captured and detected by using Surface Plasmon Resonance imaging (SPRi) and their size and chip surface density of EVs are studied by Atomic Force Microscopy (AFM). In this work, we present the results of the refinement carried out for determining the NBA platform dynamic range and limit of detection for EVs characterization.

## Material & Methods:

EVs derived and isolated from platelets are used in this study. After an estimation concentration by Tunable Resistive Pulse Sensing, SPRi experiments are carried out at varying EV concentrations.  $\alpha$ -CD41 is used as a ligand to capture specifically the platelet derived EVs, versus a negative IgG control. The sensorgram is analyzed with reference subtraction method. AFM imaging is performed in contact mode in air. Quantification of particles and metrological analysis is performed using SPIP software.

## Results:

The studies revealed that the NBA platform has an improved dynamic range up to four orders of magnitude and the lowest concentration detectable by the system was  $10^5$  particles/mL, on  $\alpha$ -CD41 grafted at  $100\mu\text{g}/\text{mL}$  after injection of  $180\mu\text{L}$  of sample. The system offers the possibility to optimize various parameters in the experiments such as ligand density, analyte injection time and volume to obtain the best performance in terms of detectable, specific and efficient on chip EVs immuno-capture.

## Conclusion

This study allows to determine the sensitivity and dynamic range of our platform for EVs qualification. Compared to other label free techniques (Kilic *et al.*, 2018), the NBA platform is unique, since leading to quantitative and qualitative analysis, in a multiplexed format, at a concentration of  $10^5$  EVs/mL in a complex biological sample.



**Thematic Session:** Nano-objects in biological fluids

**Keywords:** Parkinson disease– Alpha-synuclein – Aggregation– Coarse-grained MD simulations

## Deciphering the structure of multimers and nanostructures of $\alpha$ -synuclein using coarse grained molecular dynamics

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The hallmark of the neurodegeneration is the conversion of soluble proteins in insoluble aggregate in neurons. In the Parkinson disease, the largest structures of the protein aggregates, named amyloids, were identified as composed of  $\alpha$ -synuclein (aS). Deciphering the mechanism of aggregation is a prerequisite to develop identification methods of the early onset of the Parkinson disease. The aim of the present work is to identify the aggregation mechanism of aS using molecular dynamics simulations and high-performance computer resources. We used the coarse-grained model UNRES (UNited Residue Model) to represent the aS dynamics. The model allows us to simulate the dynamics at the atomic scale on an impressive time-scale of hundred of milliseconds relevant for the condensation mechanism. The monomer of aS is an intrinsically disordered protein composed of 140 amino-acids characterized by a repeat motif of 6 amino-acids (KTKEGV). The repeat motif in aS sequence plays a role in its aggregation and can disturb the equilibrium between monomers and multimers which seems to be a key to understand the disease.

In the present work, we simulated the structural properties of aS monomer for the wild-type (WT) protein and toxic mutants and found agreement with the scarce experimental data. Simulations of aS dimers were performed and differences between WT and toxic mutants were identified. Preliminary data will be presented for the classification of the dimeric structures based on a statistical analysis. These preliminary results could be used in the future to identify relevant physical properties for early detection of the disease and to identify a possible way of inhibition.



**Thematic Session:** Nano for imaging, diagnosis & theranostics

**Keywords:** Nanoparticles, Quantum dots, Nanothermometry, Nanophotonics

## Towards a novel biocompatible probe allowing for real-time temperature measurements at cellular scale

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Biocompatible nanoheaters are of great interest as they can induce low and localized temperature gradients within malignant cells. Hyperthermia at cellular scale may significantly improve tumour response to current cancer-treatment gold standards, while reducing their invasiveness. To investigate this potential, a novel probe allowing for cellular scale temperature gradient measurements is required. Indeed, using currently available thermometers, thermal and spatial resolutions do not permit such measurements. In addition, abnormal temperature distribution at cellular scale is the first manifestation of health disorders. Thus, our novel temperature probe could surpass hyperthermia-therapy application and allow for early-stage diagnosis of diseases.

Silver sulphide quantum dots ( $\text{Ag}_2\text{S}$  Qds) were synthesized following two main routes and evaluated as potential nanothermometer to monitor temperature changes at cellular scale. Quantum dot's emission spectrum consists in a real-time fingerprint of its surrounding temperature. Indeed, temperature increase strongly quenches emission, allowing for concentration independent temperature sensing based on ratiometric measurements. Nevertheless, emission spectrum shape is strongly impacted by solvent or biological media optical behaviour (e.g photon reabsorption), preventing to perform one universal temperature probe calibration.

The talk details a method to figure out the best luminescent parameter for *in-vivo*- $\text{Ag}_2\text{S}$ -Qds-based temperature sensing. Luminescent parameter selection is of crucial importance to perform a robust probe calibration and to move towards low uncertainties on nanothermometer's measurements. Our experimental approach involves different  $\text{Ag}_2\text{S}$ -Qds-containing biological media and the study of corresponding emission spectra thermal dependency for *in-situ* temperature going from 25°C to 60°C.



**Thematic Session:** (Nano-objects in biological fluids: from detection of endogenous vesicles to nanotoxicity of exogenous nanoparticles)

**Keywords:** (Gold nanoparticles, Lipidots<sup>®</sup>, Nanotoxicology, Immunometabolism)

## Antigen Presenting Cells (APCs) functions after exposure to nanoparticles

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### Abstract

With the increasing usage of nano material for therapeutic and diagnostic applications, nanotoxicology has emerged to evaluate safety of nano material. Phagocytic capacity of Antigen Presenting Cells (APCs) leads to accumulation of exogenous materials like nanoparticles (NPs). Hence, we hypothesize that the accumulation of NPs could alter physiological and functional properties of APCs. Therefore, we exposed mouse Bone Marrow Derived Dendritic Cells (BMDCs) to 15 nm gold NPs (AuNP) and 50 nm neutral lipidic NPs (LNPs). As metabolic profile underpins all immune cell functions, we used an extracellular flux analyzer to assess the metabolic profile of stimulated (LPS and IL-4) and unstimulated BMDCs upon AuNPs and LNPs exposure. The results revealed neither AuNPs nor LNPs altered glycolysis in BMDCs. Analysis of mitochondrial metabolism showed that exposure to these NPs significantly reduced Spare Respiratory Capacity (SRC) of IL-4 stimulated BMDCs while not impacting basal respiration, ATP production, proton leak, non-mitochondrial oxygen consumption. As NO and ROS are closely related with metabolism, we also assessed the impact of these NPs on NO and ROS production. Our results revealed no impact of both these NPs on NO production in LPS stimulated BMDCs. Although different concentrations of AuNPs don't alter ROS production, 200 µg/mL of LNPs significantly reduced ROS production in LPS activated BMDCs. Altogether these results showed that AuNPs and LNPs have little effect on BMDCs. Reduced SRC suggest that both these NPs has indirect effect on electron transport chain which could have consequences in the development of specific immune responses.