Wednesday, December 11th

Session: NANO-OBJECTS IN BIOLOGICAL FLUIDS: FROM DETECTION OF ENDOGENOUS VESICLES TO NANOTOXICITY OF EXOGENOUS NANOPARTICLES

Romanée-Conti Amphitheater

10h45 - 13h00

Keynote speaker: Lea Ann DAILEY

Nanomedicine Toxicology for Sensitive Administration Routes

Keynote speaker: Wilfrid BOIREAU

Some keys for exploring nanocosmos in biofluids: other exoplanets?

Abstracts

The SFNano C'Nano joint meeting 2019

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

Keywords: silica nanoparticles, intestinal permeability, actin cytoskeleton, tight junctions, Caco-2 cells

Small Silica Nanoparticles Transiently Modulate the Intestinal Permeability by Actin Cytoskeleton Disruption

Raphaël Cornu[1], Claire Chrétien[1], Yann Pellequer[1], Hélène Martin[1], Arnaud Béduneau[1]

1. PEPITE EA4267, FHU INCREASE, Univ. Bourgogne Franche-Comté, F-25000 Besançon, France

Amorphous silica nanoparticles are used in various application fields including pharmaceutical and food industries (E551). Recent findings emphasized the potential human health risk induced by food additives composed of nanoparticles. However, very few data regarding the oral toxicity of the silica nanoparticles additive (E551) are available. This study aims to evaluate the oral toxicity of E551 and silica nanoparticles with sizes ranging from 10 nm to 200 nm using two *in vitro* model of human intestinal barrier, a Caco-2 monolayer and a Caco-2/HT29-MTX co-culture. A size- and concentration-dependent reversible increase of the paracellular permeability is identified after a short-term exposure to silica nanoparticles. Nanoparticles of 30 nm induce the highest transepithelial electrical resistance drop whereas no effect is observed with the larger particles (200 nm). Additive E551 affect the Caco-2 monolayer permeability. In co-culture, mucus layer reduces the permeability modulation by limiting the cellular uptake of silica. After nanoparticle exposure, tight junction expression including Zonula-occludens 1 (ZO-1) and Claudin 2 is not affected, whereas the actin cytoskeleton disruption in enterocytes and the widening of ZO-1 staining bands are observed. A complete permeability recovery is concomitant with the *de novo* filament actin assembly and the reduction of ZO-1 bands. These findings suggest the paracellular modulation by small silica particles is directly correlated to the alteration of the ZO/actin binding strongly involved in the stability of the tight junction network. It demonstrates the toxicity of the E551 is clearly dependent of the nanoparticle/microparticle ratio varying according to the batch and the manufacturer.

The SFNano C'Nano joint meeting 2019

Thematic Session: (Nano-objects in biological fluids: from detection of endogenous vesicles to nanotoxicity)
Keywords: (pulmonary surfactant – nanoparticles – biomolecular corona – magnetic wires – microrheology)

Effect of Nanoparticles on the Bulk Shear Viscosity of A Biomimetic Lung Surfactant

L.P.A. Thai[1], F. Mousseau[1], E.K. Oikonomou[1], M. Radiom[1] and J.-F. Berret[1*]

1. *Matière et Systèmes Complexes, UMR 7057 CNRS Université Denis Diderot Paris-VII, Bâtiment Condorcet, 10 rue Alice Domon et Léonie Duquet, 75205 Paris, France.*

Abstract
Inhaled nanoparticles (< 100 nm) reaching the deep lung region first interact with the pulmonary surfactant, a thin lipid film lining the alveolar epithelium. To date, most biophysical studies have focused on particle induced modifications of the film interfacial properties. In comparison, there is less work on the surfactant bulk properties, and on their changes upon particle exposure. Here we study the viscoelastic properties of a biomimetic pulmonary surfactant in the presence of various engineered nanoparticles [1,2]. The microrheology technique used is based on the remote actuation of micron-sized wires *via* the application of a rotating magnetic field and on time-lapse optical microscopy [1,2]. It is found that particles strongly interacting with lipid vesicles, such as cationic silica (SiO_2, 42 nm) and alumina (Al_2O_3, 40 nm) induce profound modifications of the surfactant flow properties, even at low concentrations. In particular, we find that silica causes fluidification, while alumina induces a liquid-to-soft solid transition. Both phenomena are described quantitatively and accounted for in the context of colloidal physics models. It is finally suggested that the structure and viscosity changes could impair the fluid reorganization and recirculation occurring during breathing.

[1] F. Mousseau, J.-F. Berret *et al.*, *Nanoscale*, **9** (2017) 14967-14978
[2] F. Mousseau, J.-F. Berret, *Soft Matter*, **14** (2018) 5764-5774
[3] J.-F. Berret, *Nature Communications*, **7** (2016) 10134
[4] L.P.A. Thai, J.-F. Berret *et al.*, *Colloids and Surfaces B: Biointerfaces*, **178** (2019) 337-345

The SFNano C'Nano joint meeting 2019

Thematic Session: Nano-objects in biological fluids
Keywords: nanoparticles; metal; thiol; chelator; silver; metalloprotein

Nanoparticles dissolution induced by bio-relevant molecules

Marianne Marchioni[1], Jérôme Laisney[1],Thomas Gallon[1,2], Giulia Veronesi[1,3], Isabelle Worms[1], Mireille Chevallet[1], Pierre-Henri Jouneau[4], Delphine Truffier-Boutry[5], Colette Lebrun[2], Pascale Delangle[2], Aurélien Deniaud[1]* and Isabelle Michaud-Soret[1]*

1. *Univ. Grenoble Alpes, CNRS, CEA, IRIG, Laboratoire de Chimie et Biologie des Métaux, Grenoble, France;*
2. *Univ. Grenoble Alpes, CEA, CNRS, INAC-SyMMES, Grenoble, France;*
3. *ESRF, The European Synchrotron. Grenoble, France;*
4. *Univ. Grenoble Alpes, CEA, INAC-MEM, Grenoble, France;*
5. *LRN, LITEN/CEA-Grenoble*

The widespread use of silver nanoparticles (AgNPs) as biocides in consumer products raises concerns about their toxicity to humans and their environmental impact. The biocidal activity is mediated by the release of Ag(I). This metal ion is highly toxic to all living organisms and Ag(I) tightly binds to thiol functional groups that are abundant and essential to any cell type. The first intracellular source of thiol, glutathione, is crucial for the control of the redox balance. Dissolution studies using monothiol-containing biomolecules such as glutathione or cysteine provided controversial results, while the impact of polythiol molecules on AgNP behavior remains unexplored.
In order to gain insights into polythiol-assisted AgNP dissolution at constant and equal thiol : Ag molarity, we studied the impact of glutathione, phytochelatins with 2, 3 or 6 thiols, and chaperone Atx1 and and its metal site mimic P2, containing 2 pre-oriented thiols to chelate Cu(I). Metallothionein involved in metal chelation was also studied. The AgNP behavior was monitored by various physicochemical approaches. We demonstrated unambiguously that, these molecules favor AgNP dissolution into Ag(I) ions with a rate that increases with the number of thiols as well as with their pre-orientation. We also observed that AgNP dissolution into Ag(I) soluble species occurs progressively for the whole AgNP population. This work highlights how transformations of AgNPs are triggered by biomolecules and lays the basis for a deeper understanding of their fate in biological systems. Our results are discussed in regards of recent results showing the bio-assisted-dissolution of other metallic NPs.

Our publications : Liu *et al* (2017) Interaction of silver nanoparticles with metallothionein and ceruloplasmin: impact on metal substitution by Ag(I), corona formation and enzymatic activity. *Nanoscale*, 9, 6581.
Marchioni *et al* (2018) Insights into polythiol-assisted AgNP dissolution induced by bio-relevant molecules. *Environmental science.Nano*, 5, 1911.

The SFNano C'NANO joint meeting 2019

Thematic Session: nanomedicine
Keywords: nanodrug, serum albumin, interaction, complexation, disassembly

The fate of therapeutic nanoparticles in a model biological medium: interactions with serum albumin

F. Gobeaux[1]*, J. Bizeau[1], F. Samson[1], L. Marichal[1,2], I. Grillo[3], F. Wien[4], S. O. Yesylevsky[5,6], C. Ramseyer[6], M. Rouquette[7], S. Lepêtre-Mouelhi[7], D. Desmaële[7], P. Couvreur[7], P. Guenoun[1], J-P.Renault[1], F. Testard[1]

1. LIONS - NIMBE CEA, CNRS, Université Paris-Saclay, CEA Saclay, 91191 Gif-sur-Yvette Cedex, France
2. I2BC, JOLIOT, DRF, CEA, CNRS, Université Paris-Saclay, Gif-sur-Yvette, France
3. Institut Laue Langevin, 71 avenue des martyrs, B.P. 156, 38042 Grenoble Cedex 9, France
4. SOLEIL Synchrotron, Saint Aubin, France
5. Department of Physics of Biological Systems, Institute of Physics of the National Academy of Sciences of Ukraine, Prospect Nauky 46, 03028 Kyiv, Ukraine
6. Laboratoire Chrono Environnement UMR CNRS 6249, Université de Bourgogne Franche-Comté, 16 route de Gray, 25030 Besançon Cedex, France.
7. Institut Galien Paris-Sud, UMR 8612, CNRS, Université Paris-Sud, Université Paris-Saclay, Faculté de Pharmacie, 5 rue Jean-Baptiste Clément, F-92296 Châtenay-Malabry Cedex, France.

In the field of nanomedicine, nanostructured nanoparticles (NPs) made of self-assembling prodrugs emerged in the recent years. In particular, the squalenoylation concept has been applied to several therapeutic agents with promising results.[1,2] These nanoparticles allow a high encapsulation rate of the active principle, the protection from quick degradation, and a good control of the targeting and release. Beyond the high potential of these NPs, there is still a need for a better understanding of their evolution in biological media. The colloidal stability of the NPs, their interaction with proteins and the impact of their internal nanostructure on their efficacy are essential questions to go towards a better understanding of the mechanism of their fate in the organism (nanoparticle disassembly, targeting etc…).

We chose to investigate these questions on the particular case of Squalene-Adenosine (SQAd) nanoparticles,[3] whose neuroprotective effect has already been demonstrated in murine models and model biological media.[4] From the combination of multiple techniques (neutron and x-ray scattering, cryogenic transmission electron microscopy, circular dichroism, fluorescence spectroscopy, isothermal titration calorimetry and DFT calculations) we have investigated the interactions between the SQAd NPs and the serum albumin, one of the main proteic components of blood plasma. We show that albumin affects the colloidal stability of the nanoparticles but also partially disassembles the nanoparticles by forming SQAd-albumin complexes. Albumin should thus play a crucial role in the transport of the prodrug, while the nanoparticles would act as a circulating reservoir in the blood stream.[5]

References: [1] Couvreur et al. Nano Lett. **2006**, 6 (11), 2544–2548. [2] Couvreur et al. Small **2008**, 4 (2), 247–253. [3] Rouquette M. et al, J Drug Target. **2019**, DOI: 10.1080/1061186X.2019.1566340 [3] Gaudin et al. Nat. Nanotechnol. **2014**, 9 (12), 1054–1062. [4] Gobeaux et al, submitted

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The SFNano C'NANO joint meeting 2019

Thematic Session: Nano for (bio-)imaging, diagnosis and theranostics
Keywords: SERS, biomolecules, biosensor, structure, diagnosis

Detection, identification and structural study of biomolecules by quantitative analysis of SERS spectra

Ryohei Yasukuni[1], Raymond Gillibert[2], Mohamed N. Triba[3], Wafa Safar[4], Qiqian Liu[3], Monica Potara[5], Andra-Sorina Tatar[5], Mathieu Edely[4], Aymeric Leray[6], P. Gucciardi[3], Eric Finot[6], Simion Astilean[5], Marc Lamy de la Chapelle[4]

1. Graduate school of science and technology, Nara Institute of Science and Technology, 8916-5, Takayama-cho, Ikoma, Nara, 630-0192, Japan
2. CNR-IPCF, Viale F. Stagno D'Alcontres 37, I98168, Messina, Italy
3. Université Paris 13, Sorbonne Paris Cité, Laboratoire CSPBAT, CNRS UMR 7244, 74 rue Marcel Cachin, Bobigny F-93017, France
4. IMMM - UMR 6283 CNRS, Le Mans Université, Avenue Olivier Messiaen, 72085 Le Mans, Cedex 9, France
5. Babes-Bolyai University, Institute for Interdisciplinary Research in Bio-Nanosciences and Faculty of Physics, Nanobiophotonics and Laser Microspectroscopy Center, 1 Str. M Kogalniceanu, RO-400084 Cluj-Napoca, Romania
6. Lab. Interdisciplinaire Carnot de Bourgogne (ICB), UMR CNRS 6303 Université Bourgogne Franche-Comté, BP 47870, 9 avenue Alain Savary, 21078 Dijon cedex, France

Surface Enhanced Raman Scattering (SERS) is a powerful tool to detect and study the structure of molecules. It is extensively used as sensor to observe biomolecules. The SERS substrate is then covered by a bioreceptor specific to the targeted analyte. One of the most used bioreceptor is aptamer, a single strand DNA, that exhibits a high affinity to the molecule to be detected through the formation of a specific secondary structure. The detection often faces to a difficulty of quantitative analysis because of fluctuated background signals that can be related to instable structures of the aptamer. In this communication, we addressed quantitative analysis of SERS spectra of the aptamer in interaction with its targeted analytes (manganese superoxide dismutase (MnSOD)[1] known as a cancer biomarker and ochratoxin A (OTA)[2] a mycotoxin in our case). Using multivariate statistical analysis (Principal Component Analysis and Partial Least Square methods) for different concentrations of the analyte we are able to detect the analyte at very low concentration (down to 10 pM) and to observe the analyte/aptamer interaction. Moreover, by mapping the interaction on a 2D SERS substrate or by temporal SERS measurements, we are able to observe strong spectral fluctuations that can be correlated to aptamer conformation modifications and to its stability as a function of its interaction with the analyte. These results would open new detection and analytical strategy of SERS based bio-sensors using aptamers.

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[1] M. Cottat et al., J. Phys. Chem. C, 2015, 119, 15532 ; R. Yasukuni et al., Nanophotonics, 2019, 8(9), 1477.
[2] R. Gillibert et al., Analyst, 2018, 143, 339.