Dissecting the Intracellular Fate of Indium Phosphide Quantum Dots in vivo Using Synchrotron XRF and XANES

G. Veronesi,[1] M. Moros,[2] H. Castillo-Michel,[3] L. Mattera,[4] G. Onorato[2], K. D. Wegner,[4] P. Reiss,[4] C. Tortiglione [2]

[1]University Grenoble Alpes, CNRS, CEA, Laboratory CBM, Grenoble (France), [2]Istituto di scienze applicate e sistemi intelligenti ″E.Caianiello″, CNR, Pozzuoli (Italy), [3] ESRF, the European Synchrotron, Grenoble (France), [4] University Grenoble Alpes, CEA, CNRS, INAC/SyMMES/STEP, Grenoble (France),
claudia.tortiglione@cnr.it

The syntheses of environmental friendly semiconductor nanocrystals (QDs) such as Indium Phosphide (InP) QDs are nowadays well-established and they possess high-quality optical properties. Despite these synthetic advances, gaps in knowledge of their intracellular fate, persistence, and excretion from the targeted cell/organism still exist, preventing clinical applications. In this study by using a simple model organism having a tissue grade organization, we determined the toxicological impact of InP QDs [1]. Moreover, we analysed their biodistribution by X-ray fluorescence and complemented these information by mapping the single elements with X-ray absorption near edge structure spectroscopy, achieving unique information on *in situ* chemical speciation. We observed an unexpectedly fast dynamics of QD degradation, occurring within the first hour post incubation [2]. Our study brought new insights into the intracellular fate of photoluminescent nanocrystals after the loss of their optical properties and pave the way for the design of more biological stable InP QDs for biomedical purpose.

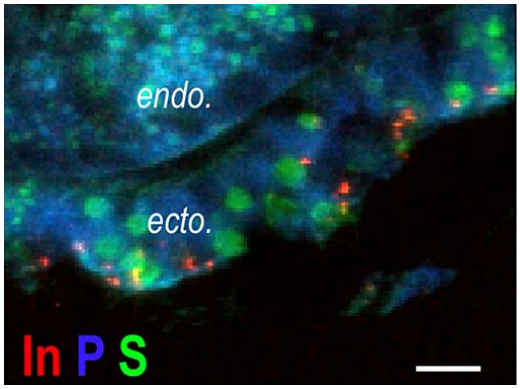

Figure 1. RGB representation ofindium (red), sulfur (green), and phosphorus (blue) distribution in transversal sections of Hydra exposed to core shell InP-QDs

References

[1] – M. Allocca , L. Mattera, A. Bauduin, B. Miedziak , M. Moros , L. De Trizio , A. Tino , P. Reiss, A. Ambrosone , C. Tortiglione. Env Sci Technol, **53**, 3938-3947 (2019).
[2] – G. Veronesi G, M. Moros, H. Castillo-Michel, L. Mattera , G. Onorato , K.D.D Wegner, W.L. Ling, P. Reiss P, C. Tortiglione. ACS Appl Mater Interfaces, **11**, 35630-35640 (2019).

Advanced magnetic spectroscopies for the fine characterization of bimagnetic nanoparticles and ferrofluids

A. Juhin[1], N. Daffé[1,2,3], M. Sikora[4], J. Zecevic[5], K. Trohidou[6], J. Nogués[7], N. Bouldi[1,2], V. Gavrilov[3], S. Neveu[3], F. Choueikani[2], V. Dupuis[3], P. Ohresser[2], M.-A. Arrio[1], M. Rovezzi[8,9], P. Glatzel[8] and Ph. Sainctavit[1,2]

[1]IMPMC, Sorbonne Université, CNRS, 75005 Paris, France- amelie.juhin@upmc.fr, [2] Synchrotron SOLEIL, L'Orme des Merisiers, Saint-Aubin – BP48, 91192 Gif-sur-Yvette, France, [3] PHENIX, Sorbonne Université, CNRS, 75005 Paris, France, [4] AGH University of Science and Technology, Al. Mickiewicza 30, 30-059 Krakow, Poland, [5]Debye Institute, Utrecht, Netherlands, [6] NCSR "Demokritos", Athens, Greece,, [7]Catalan Institute of Nanoscience and Nanotechnology (ICN2), Barcelona, Spain, [8] ESRF, BP220, 38043 Grenoble Cedex 9, France, [9]Université Grenoble Alpes, CNRS, OSUG, FAME, 38000 Grenoble, France

We will show how the combination of advanced magnetic spectroscopies (XMCD and hard x-ray RIXS-MCD [1,2]) can allow a profound understanding of the electronic and magnetic structures in complex magnetic nanomaterials such as bimagnetic nanoparticles and ferrofluids, and reveal emergent properties.

First, we have investigated **bimagnetic core-shell nanoparticles** that currently focus high interest owing to their applications in biomedicine and technology. The fine tailoring of particles requires a deep knowledge of their internal structure and morphology, from which the properties are directly inherited. In nominally γ-Fe_2O_3/Mn_3O_4 nanoparticles, RIXS-MCD gives the smoking gun evidence for the existence of a magnetic interdiffused inner shell growing from a γ-Fe_2O_3core and a Mn_3O_4 shell. Combined with TEM-EELS experiments, a quantitative multilayered structure is proposed, which allows understanding the influence of the interface quality on the measured magnetic properties [3].

Second, we have studied the magnetic anisotropies in a **ferrofluid of monodispersed $MnFe_2O_4$@$CoFe_2O_4$ nanoparticles** dispersed in heptane. Ferrofluids are well-known for their applications in optical waveguides, medicine or in fine arts. Their magnetic properties arise from both magnetic anisotropies of individual particles and interparticle interactions that are mediated by the liquid carrier. Using a dedicated liquid cell, developped in collaboration with ID26 beamline of the ESRF, we have measured element-selective magnetic properties in the liquid phase and in the frozen phase. This allowed investigating separately the cationic distribution and magnetic anisotropies in the core and those in the shell, as well as their mutual influence [4].

Third, a **binary ferrofluid of $MnFe_2O_4$ and $CoFe_2O_4$ nanoparticles** dispersed in heptane was investigated. The measurement of element-selective hysteresis curves, supported by cryogenic TEM experiments and Monte-Carlo simulations of the magnetic properties, has allowed quantifying the effect of interparticle interactions on the magnetic properties for each of both magnetic phases [5].

References

[1]- M. Sikora, A. Juhin, T.-C. Weng, P. Sainctavit, C. Detlefs, F. de Groot and P. Glatzel *Physical Review Letters*, **105**,037202 (2010).

[2]- P. Glatzel, and A.Juhin,. X-ray Absorption and Emission Spectroscopy in *Local Structural Characterisation*, (eds D. W. Bruce, D. O'Hare and R. I. Walton), John Wiley & Sons, 89-171 (2013).

[3]- A. Juhin, A. López-Ortega, M. Sikora, C. Carvallo, M. Estrader, S. Estradé, F. Peiró, M. D. Baró, P. Sainctavit, P. Glatzel, and J. Nogués, Nanoscale, **6**, 11911-11920 (2014).

[4]- N. Daffé, M. Sikora, M. Rovezzi, N. Bouldi, V. Gavrilov, S. Neveu, F. Choueikani, Ph. Ohresser, V. Dupuis, D. Taverna, A. Gloter, M.-A. Arrio, Ph. Sainctavit, and A. Juhin. Advanced Materials Interfaces **4**, 1700590 (2017).

[5]- N. Daffé, J. Zecevic, M. Sikora, K. Trohidou, M.Vasilakaki, M. Rovezzi, N. Bouldi, V. Gavrilov, S. Neveu, F. Choueikani, V. Dupuis, Ph. Sainctavit, and A. Juhin. In preparation.

Structural evolution of supported lipid bilayers intercalated with quantum dots

M. Wlodek[1], M. Kolasinska-Sojka[1], P. Warszynski[1], W.H. Briscoe[2]

[1]Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Krakow, Poland, **ncwlodek@cyf-kr.edu.pl, nckolasi@cyf-kr.edu.pl, ncwarszy@cyf-kr.edu.pl**
[2]School of Chemistry, University of Bristol, United Kingdom, **Wuge.Briscoe@bristol.ac.uk**

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Understanding interactions between functional nanoparticles and lipid bilayers is important to many emerging biomedical and bioanalytical applications. In particular, quantum dots (QDs; semiconductor nanoparticles) are promising components of functional systems for medical imaging, theranostics, targeted therapy, drug delivery and biosensing. Increasing use of QDs in biological applications also motivates the investigation of their physical interactions with biological systems, particularly the cell membrane. However, little is known about the influence of embedded hydrophobic QDs on the formation process of supported lipid bilayers (SLBs) *via* vesicle fusion, and how the presence of hydrophobic QDs influences the morphology and structure of the SLBs. Such an understanding is important to their bioanalytical applications and potential cytotoxicity.

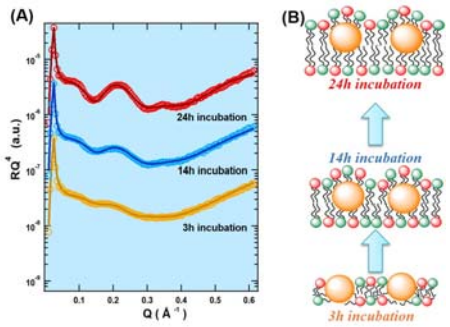


Figure 1. XRR curves of POPC/POPE lipid bilayers with 4.9nm CdS QDs at 3h, 14h and 24h incubation time (A) with schematic of the structural evolution and QD rearrangement (B).

The main goal of this study was to investigate the structural changes of negatively charged POPC/POPE supported lipid bilayers intercalated with QDs on a PEI monolayer as a function of incubation time, using *in situ* synchrotron XRR, with the resulting morphology imaged by atomic force microscopy (AFM) [1]. The structural properties such as thickness, roughness and surface coverage were observed over a period of 3-24 h. Our results show time-dependent perturbations in the SLB structure due to the interaction upon QD incorporation. Compared to the SLB without QDs, at 3 h incubation time, there was a measurable decrease in the bilayer thickness and a concurrent increase in the scattering length density (SLD) of the QD-SLB. The QD-SLB then became progressively thicker with increasing incubation time, which – along with the fitted SLD profile – was attributed to the structural rearrangement due to the QDs being expelled from the inner leaflet to the outer leaflet of the bilayer (Figure 1B) [2]. These findings will provide valuable information on the structure of QD-containing fluorescent SLBs, giving unprecedented mechanistic insights, which could have potential implications for drug delivery, cell toxicity, and related aspects of NP-cell interactions.

References

[1] – M. Wlodek *et al.*, Nanoscale 10, 17965-17974 (2018)
[2] – M. Wlodek *et al.*, Journal Colloid and Interface Science, accepted

Multi-modal scanning microscopy for nanomaterials

Michael E. Stuckelberger[1]

[1]Deutsches Elektronen-Synchrotron (DESY), Hamburg, Germany, **michael.stuckelberger@desy.de**

Nanomaterials pose unique challenges to X-ray microscopy for their characterization with respect to alignment, beam damage, and signal-to-noise ratio. At the same time, relevant information about nanomaterials is typically extracted from the point-by-point correlation of different properties, which requires the same spot being in the same condition for all measurements. Particularly for *in-situ* and *operando* measurements, this is not possible without the simultaneous evaluation of the critical measurement modes.

At various synchrotrons worldwide (APS, PETRA III, NSLS II, CLS, ESRF), we have set up experiments for multi-modal measurements involving up to 5 different modalities of nanomaterials and devices such as contacted nanowires or solar cells as depicted in Fig. 1. They allow the simultaneous evaluation of composition, structure, and performance.

In this contribution, we will demonstrate the application of multi-modal X-ray microscopy to nanoscale semiconductors and electronic devices, and discuss detector arrangement and compatibility with different scan modes and samples. Beyond state-of-the-art measurements such as shown in Fig. 2, we will give an outlook to new opportunities and challenges at nanoprobe endstations of 4th generation synchrotrons such as ID-16-B at ESRF-EBS.

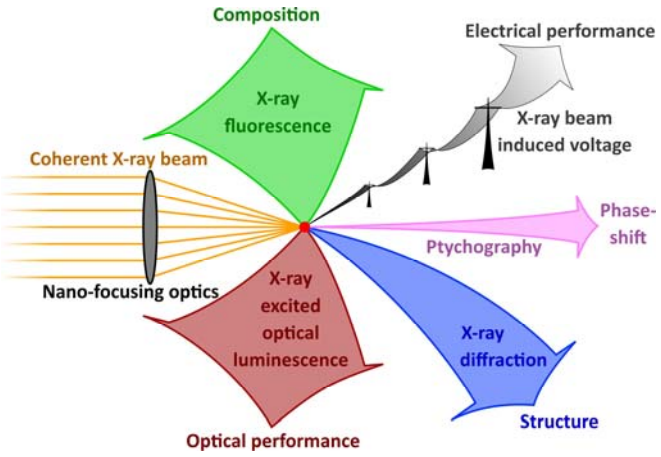


Figure 1: A “dream experiment” involving five-fold multi-modality in scanning X-ray microscopy [1]

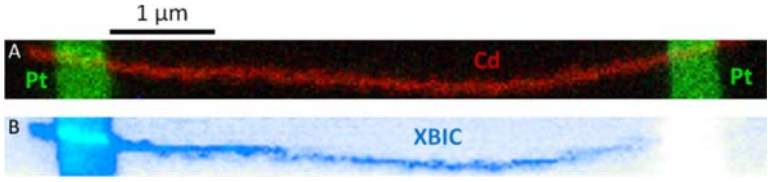


Figure 2: Multimodal measurement of the composition (top) and the performance (bottom) of a nanowire [2].

References

[1] - M. Stuckelberger, Spectroscopy 34(7), 2019
[2] - C. Ossig, et al., J. Vis. Exp. 150, e60001, 2019

Nanowire technology and toxicity

L. Charlet, B. Gilbert[2]

, [1]ISTerre, UGA-CNRS, Grenoble, France, [2]LBNL, Berkeley, California, USA,
charlet38@gmail.com, bgilbert@lbl.gov

Fibrous particles interact with cells and organisms in complex ways that can lead to cellular dysfunction, cell death, inflammation, and disease. The development of conductive transparent networks (CTNs) composed of metallic silver nanowires (AgNWsfor flexible touchscreen displays raises new possibilities for the intimate contact between novel fibers and human skin. Here, we report that a material property, nanowire-bending stiffness that is a function of diameter, controls the cytotoxicity of AgNWs to nonimmune cells from humans, mice, and fish without deterioration of critical CTN performance parameters: electrical conductivity and optical transparency. As shown by ID 16A holographic X-ray phase contrast maps and 2D elemental maps, completed by ID21 Ag LIII-edge X-ray absorption spectra, both 30- and 90-nm-diameter AgNWs are readily internalized by cells, but thinner NWs are mechanically crumpled by the forces imposed during or after endocytosis, while thicker nanowires puncture the enclosing membrane and release silver ions and lysosomal contents to the cytoplasm, thereby initiating oxidative stress. This finding extends the fiber pathology paradigm and will enable the manufacture of safer products incorporating AgNWs.

References

[1] - Lehmann, S.G., Toybou D., Pradas del Real, A.E., Arndt, D., Tagmount, A., Viau, M., Pacureanu, A., Cloetens, P., Bohic, S., Salome, M., Castillo, H., Omana, B., Hofmann, A., Vulpe, C., Simonato, J.P., Celle, C., Charlet, L., and Gilbert B. 2019 Crumpling versus Puncturing: A Biomechanical Threshold for the Acute Cellular Toxicity of Silver Nanowires *Proc. Nat. Academy of Sciences* (2019) 116 (30): 14893–14898.

[2] - Lehmann, S.G., Gilbert, B., Maffeis, T., Grichine, A., Pignot-Paintrand, I., Clavaguera, S., Rachidi, W., Seve, M., **Charlet** L. In Vitro Dermal Safety Assessment of Silver Nanowires after Acute Exposure: Tissue vs Cell Models *Nanomaterials* 8, 232; doi:10.3390/nano8040232

Asbestos bodies in human lung tissue: toward a definitive characterization

F. Bardelli, F. Brun[1], G. Veronesi[2], A. Cedola, P. Cloetens[3]

CNR-Nanotec, Rome (Italy), [1]University of Trieste (Italy), [2]CNRS/CEA/Université Grenoble Alpes, CBM-UMR 5249, Grenoble (France), [3]ESRF, Grenoble (France). **fabrizio.bardelli@gmail.com**

Thanks to their dimensions, 10-100μm in length and 200-300nm in diameter, and elevated bio-persistency, asbestos fibers can penetrate the lungs by inhalation and remain there for long time (even decades). In the lungs, alveolar macrophages are in charge of removing bad particles. They can do an excellent job while dealing with the majority of particulate matter, but when it comes to high aspect ratio materials, as asbestos (>20), macrophages are no more able to completely engulf them (frustrated phagocytosis). Organic and inorganic material start to deposit on the foreign fibers giving rise to an *in vivo* biomineralization process that leads to the formation of peculiar structures, consisting of the original asbestos fibers plus an Fe-rich layer. These structures are commonly referred as Asbestos Bodies (AB, Fig. 1a).

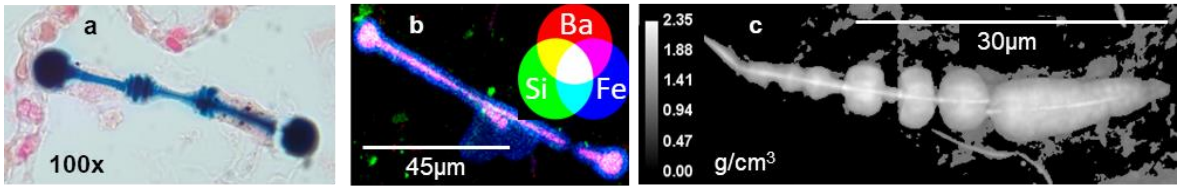


Figure 1: a) Optical microscopy image of an AB in lung tissue; b) μXRF map of an AB (ID21@ESRF, pixel size 0.5x0.5μm^2); c) Density map of an AB in lung tissue (ID16A@ESRF, pixel size 0.07x0.07μm^2).

Asbestos can lead to mesothelioma, an aggressive cancer of the lung lining. Despite the link between asbestos exposure and mesothelioma has long been demonstrated, little advancement has been made in anti-cancer therapies and, still, only one person over twenty is alive five years after the diagnosis. In fact, since the latency period from the first exposure to the development of mesothelioma can be very long (20-40 years, on average), while AB start to form soon after asbestos reaches the lungs, it is reasonable to believe that they may play a major role in the pathogenesis. Indeed, AB have been shown to induce the formation of reactive oxygen species and DNA damage. One of the open questions that prevent developing a sound model to explain the carcinogenic mechanism is that a full knowledge of the growth mechanism and composition of the AB is still lacking, preventing determining their role in the pathogenesis. The common methods exploited to study AB require invasive sample preparation, such as recovering them after removing the biological tissue by chemical digestion. Nevertheless, this could alter their composition and the spatial information is usually lost. Conversely, synchrotron-based imaging and micro-probe techniques available at the ESRF, allowed studying single AB without altering the original lung tissue.

Scanning micro X-ray fluorescence (μXRF) and Fe K-edge micro X-ray absorption spectroscopy (μXAS) have been performed at the ID21 beamline, allowing for the determination of the elemental distribution and of the Fe speciation [1] (Fig. 1b).

Phase-contrast and fluorescence x-ray nano-tomography have been performed at the ID16A beamline to reveal the 3D morphology, and obtain a reliable elemental quantification. The latter was achieved by combining elemental distribution, thickness, and mass density high resolution maps (down to 25nm, Fig. 1c). The above techniques can be successfully exploited also to study other types of health-threatening micro- and nano-materials, such as, for example, those that are believed to be able to cross the brain barrier or the placenta.

References

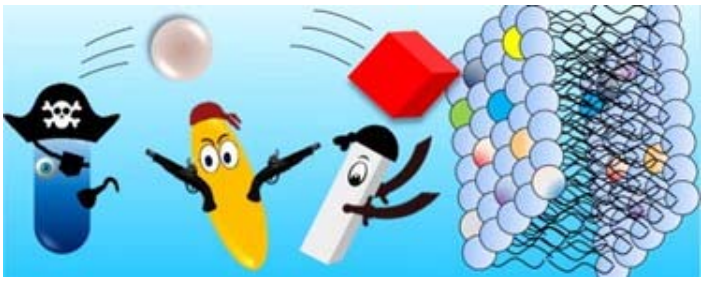
[1] – F. Bardelli *et al.* Scientific Reports 7, 44862; doi: 10.1038/srep44862 (2017).

Understanding nanoparticle cellular entry: A physicochemical perspective

Wuge H. Briscoe

School of Chemistry, University of Bristol, Bristol BS8 1TS, UK; **wuge.briscoe@bristol.ac.uk**

A key mechanism for nanoparticles (NPs) to impart toxicity is to gain cellular entry directly. Many parameters affect the interactions of nanomaterials in a cellular environment with cell membranes, including their size, shape and surface chemistry as well as factors such as the cell type, location and external environment (e.g. other surrounding materials, temperature, pH and pressure) [1, 2].



In addition to *in vitro* and *in vivo* experiments, model cell membrane systems have been used in both computer simulations and physicochemical experiments. We have used model membrane systems and physicochemical methodologies to study nanoparticle-membrane interactions. Our results from high pressure small angle X-ray scattering (HP-SAXS) show that *hydrophobic* nanoparticles could encourage the lamellar to inverted hexagonal phase transition [3], whereas the effect of *hydrophilic* nanoparticles depends on their concentration [4], with more recent work showing that dendritic polymer nanoparticles could cause membrane thinning and structural disorder in lipid mesophases [5]. In addition, using X-ray reflectivity (XRR), we have observed structural re-organisation in supported lipid bilayers intercalated with quantum dots [6, 7] and dendritic nanoparticles [8].

These results shed light on how the fundamental energetic process of NP cellular entry can be evaluated by studying the effects of nanoparticles on lipid mesophase transitions and structural disorder. This highlights both the challenge and the opportunity in this interdisciplinary area, where collaborative efforts from the insights and expertise of biological and physical scientists are urgently needed for future progress.

References

[1] C.M. Beddoes, C.P. Case, W.H. Briscoe, Understanding nanoparticle cellular entry: A physicochemical perspective, Adv Colloid Interface Sci 218 (2015) 48-68.

[2] L.J. Fox, R.M. Richardson, W.H. Briscoe, PAMAM dendrimer - cell membrane interactions, Adv Colloid Interface Sci 257 (2018) 1-18.

[3] J.M. Bulpett, T. Snow, B. Quignon, C.M. Beddoes, T.Y. Tang, S. Mann, O. Shebanova, C.L. Pizzey, N.J. Terrill, S.A. Davis, W.H. Briscoe, Hydrophobic nanoparticles promote lamellar to inverted hexagonal transition in phospholipid mesophases, Soft Matter 11(45) (2015) 8789-800.

[4] C.M. Beddoes, J. Berge, J.E. Bartenstein, K. Lange, A.J. Smith, R.K. Heenan, W.H. Briscoe, Hydrophilic nanoparticles stabilising mesophase curvature at low concentration but disrupting mesophase order at higher concentrations, Soft Matter 12(28) (2016) 6049-57.

[5] L. Fox, J., Matthews, L., Stockdale, H., Pichai, S., Snow, T., Richardson, R. M., Briscoe, W. H., Structure Changes in Lipid Mesophases due to Intercalation of Dendritic Polymer Nanoparticles: Swollen Lamellae, Suppressed Curvature, and Augmented Structural Disorder, Acta Biomater, under review (2019).

[6] M. Wlodek, M. Kolasinska-Sojka, M. Szuwarzynski, S. Kereiche, L. Kovacik, L.Z. Zhou, L. Islas, P. Warszynski, W.H. Briscoe, Supported lipid bilayers with encapsulated quantum dots (QDs) via liposome fusion: effect of QD size on bilayer formation and structure, Nanoscale 10(37) (2018) 17965-17974.

[7] M. Wlodek, Slastanova, A., Fox, L., Taylor, N., Bikondoa, O., Szuwarzynski, M., Kolasinska-Sojka, M., Warszynski, P., Briscoe, W.H., Structural evolution of supported POPC/POPE lipid bilayers intercalated with quantum dots, J Colloid Interface Sci Accepted (2019).

[8] L. Fox, J., Slastanova, A., Taylor, N., Wlodek, M., Bikondoa, O., Richardson, R. M., Briscoe, W. H., Interactions between PAMAM dendrimers and DOPC lipid multilayers: Membrane thinning and structural disorder, Biochimica et Biophysica Acta (BBA) - General Subjects accepted (2019).

Synchrotron-based imaging reveals silver ions trafficking within hepatocytes exposed to silver nanoparticles

A. Deniaud[1], G. Veronesi[1,2], V. Tardillo Suàrez[2], B. Gallet[3], M. Chevallet[1], V. Raj Sharma[1,4], P. Charbonnier[1], R. Tucoulou[2], P.-H. Jouneau[4], I. Michaud-Soret[1]

Affiliation: *[1] CEA - CNRS - Univ. Grenoble Alpes, Laboratoire de Chimie et Biologie des Métaux (LCBM) UMR 5249, 17 Rue des Martyrs F-38054 Grenoble, France ; [2] ESRF, The European Synchrotron. 71, Avenue des Martyrs, 38043 Grenoble, France ; [3] CEA – CNRS – Univ. Grenoble Alpes – IBS, 71, Avenue des Martyrs, 38043 Grenoble, France; [4] INAC-MEM, CEA - Université Grenoble Alpes, 17 Rue des Martyrs F-38054 Grenoble, France ; email :* ***aurelien.deniaud@cea.fr***

The widespread use of silver nanoparticles (AgNP) in consumer goods raises concerns about their toxicity to humans and their impact on environment [1]. AgNP toxicity in cells and animals has been extensively studied and it has been shown that the toxicity depends upon the release of Ag(I) ions from the NP [2,3]. Besides, Ag accumulates in liver following AgNP exposure [4]. In this context, we studied AgNP internalization and fate into hepatocytes. We made use of a synchrotron nanoprobe to visualize the subcellular distribution of silver. The combined use of X-ray fluorescence (XRF) microscopy on whole cells and electron microscopy allowed the discrimination between the nanoparticle form located inside endosomes and lysosomes and the ionic species that distribute throughout the cell [5]. Besides, synchrotron X-ray absorption spectroscopy showed that Ag(I) recombines with sulphur in hepatocytes in the form of AgS_2 and AgS_3 complexes [5,6].

More recently, we developed a nano-XRF method performed on cell sections (Figure 1) that can be correlated with electron microscopy to reveal Ag(I) species distribution at the organelle level under long-term exposure to non-toxic concentration of AgNPs. We thus observed Ag(I) species in different organelles including in the nucleus [7]. This approach was also used on sections from 3D hepatic cell cultures that mimic liver architecture including bile canaliculi. XRF allowed to visualize Ag(I) excretion into these intercellular structures. To get more insights into the fate and effects of AgNPs, these data were completed with 3D electron microscopy, STEM-EDX and physiology assays. The later revealed, for the first time, that Ag(I) species translocating into the nucleus can trigger an endocrine disruptor-like effect. Overall, synchrotron-based imaging was central in our studies that aim at understanding the fate of nanomaterials in cells and organisms.

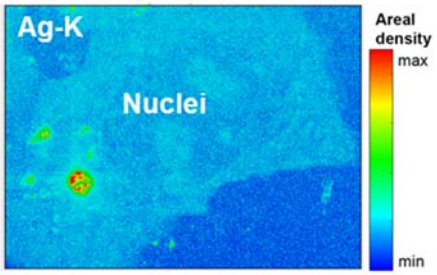


Figure 1: Ag XRF map of a hepatocyte section exposed to AgNPs.

References

[1] - S. Chernousova, and M. Epple, *Angewandte Chemie,* 2013, **52**, 1636-1653.
[2] - F. Herzog, et al. *Particle and Fibre Toxicology,* 2013, **10**, 1-14.
[3] - V. De Matteis, et al. *Nanomedecine: Nanotechnology, Biol and Med,* 2015, ***11***, 731-39.
[4] - M. Van der Zande, et al. *ACS Nano,* 2012, **6**, 7427-7442.
[5] - Veronesi, G. et al., *Nanoscale,* 2016, **8**, 17012-21.
[6] - Veronesi, G. et al., *Inorg Chem,* 2015, **54**, 11688-96.
[7] - Tardillo Suàrez V. et al., *BioRχiv,* doi: 10.1101/825919.

Synchrotron imaging of nano-particles released from implants in human and an outlook on new tools to investigate the associated tissue response in 4D

J. Schoon[1], B.Hesse[2]

[1]Charité - Universitätsmedizin Berlin; [2] Xploraytion GmbH & ESRF, hesse@xploraytion.com;

The exposure to toxic elements in humans is an increasing concern. Sources of exposure are multifold: food, cosmetics, dust in air, tattoo colorants, pharmaceutical substances or medical implants, to only name a few. For medical implants the balance between patient benefit and associated risk can be delicate. The doctors decision crucially depends on the awareness and understanding of the exposure scenario and its impact on the cellular level and thus also on the patient's health. Regulations for the approval and market surveillance of applied biomaterials have recently been challenged on a cross-national level.

Our current findings revealed that human peri-implant bone and bone marrow are distinctively exposed to micron- and nanoparticles consisting of cobalt, chromium and titanium. The detected metal quantities and their element- and tissue-specific distribution provide evidence that the peri-implant membrane does not chemically isolate implant components. Our work reveals toxico-kinetic mechanisms and a novel view on the long-term effects of metallic degradation products. Our findings prompt for a paradigm shift towards a consideration of bone and bone marrow being the most relevant organs for pre-clinical testing and post-clinical risk-benefit evaluation of orthopedic biomaterials.

By proving that metal accumulation occurs in peri-implant bone and bone marrow, our findings add significance to in vitro studies using cells of bone and bone marrow origin as models for evaluating functional and cytotoxic effects of acute metal exposure.

To extend the meaningfulness of existing *in vitro* studies based on a bone-on-chip model we currently develop a sample holder that allows for a controlled liquid environment, usable in Synchrotron CT setups, exploiting the high sensitivity and spatial resolution at very high scanning speeds. The aim is to have a time-resolved 3D bone tissue response after controlled metal particle exposure as previously assessed in real human conditions. By that we will not only have assessed the exposure scenario but also its impact on living tissue helping for saver materials and thus better patient benefit. Existing methods rely on animal experiments. Bringing organ-on-a-chip to the Synchrotron will thus help to reduce use of animals which has not only an ethical but also a commercial advantage for R&D of pharmaceutical or medical device industry. Eventually, combining data obtained through high resolution chemical imaging at ID16B and ID21 with time-resolved 3D in-vitro imaging such as at ID19 will draw a picture that could not be generated without sophisticated facilities like the ESRF.

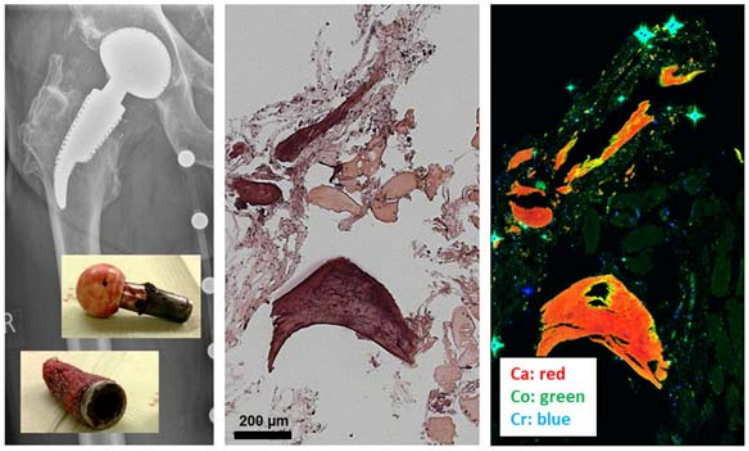


Figure 1: Left: Radiography of a hip joint and corroded implant pieces after explantation. Middle: Histology image showing bone and bone marrow. Right: XRF map collected at ID21 showing Co and Cr deposition into the bone marrow tissue regions.

X-ray imaging and X-Ray Absorption Spectroscopy applied to Environmental Nanotechnologies

M. Auffan[1], J. Rose[1], O. Proux[2,3], I. Kieffer[3], J-L Hazemann[4,3]

[1] CEREGE, CNRS, Aix Marseille Univ, IRD, INRA, Coll France, Aix-en-Provence, France, [2] OSUG UMR832 UGA, Grenoble, France, [3] FAME-UHD, FAME, ESRF, Grenoble, France, [4] Institut Néel CNRS UGA, Grenoble, France, **auffan@cerege.fr**

Engineered nanomaterials (ENMs) have become a fast growing economic sector. As a consequence of the many debates concerning their safety, efforts are developed at international and national levels to develop a code of ethics for a safe and responsible development of ENMs. A sustained growth of the nanotechnology industry will rely heavily on the characterization of risks to the environment (water and soil resources, trophic transfers, biodiversity) and human health that may be posed by ENMs in relevant exposure conditions (low doses, mid-/long-term, trophic and transgenerational transfers, etc.)

In this regard, physical-chemists, (micro)biologists, and ecologists need to conduct meaningful experiments to study the environmental risk of ENMs with access to relevant mechanistic data across several spatial and temporal scales (Auffan et al. 2019). Experimental devices as mesocosms that can be tailored to virtually mimic any ecosystem appear as particularly well-suited (Auffan et al. 2014) for the determination of the (bio)degradability, (bio)distribution, (bio)transformation, and impacts of ENMs. However, adhering to environmentally relevant exposure scenarios implicitly represents a technical challenge since it requires to explore the localization and the speciation of a target chemical element at relevant and consequently low doses in complex matrices, which is critical in the fields of environmental and biogeochemistry sciences.

These past few years, the significant improvement of X-ray imaging (2D and 3D) and X-Ray Absorption Spectroscopy techniques in term of detection limit and resolution (spectroscopic and spatial) helped us to determine unambiguously and with greater precision the speciation and distribution of the probed metal composing ENMs in sediment, biota, nanomaterials... The positive impact of these techniques will be discussed based on examples dealing with the behavior and fate of TiO2-, CeO2- and Ag-based ENMs in ecologically relevant conditions (Tella et al. 2014, 2015) and obtained both on synchrotron beamlines and laboratory apparatus.

References

[1] - Auffan M, Masion A, Mouneyrac C, de Garidel-Thoron C, Hendren CO, Thiery A, Santaella C, Giamberini L, Bottero J-Y, Wiesner MR, Rose J NanoImpact **2019** 13: 66-69.

[2] - Auffan M, Tella M, Santaella C, Brousset L, Pailles C, Barakat M, Espinasse B, Artells E, Issartel J, Masion A, Rose J, Wiesner M, Achouak W, Thiery A, Bottero J-Y Scientific reports **2014** 4: 5608.

[3] - Tella M, Auffan M, Brousset L, Issartel J, Kieffer I, Pailles C, Morel E, Santaella C, Angeletti B, Artells E, Rose J, Thiery A, Bottero J-Y Environmental Science & Technology **2014** 48: 9004–9013.

[4] - Tella M, Auffan M, Brousset L, Morel E, Proux O, Chaneac C, Angeletti B, Pailles C, Artells E, Santaella C, Rose J, Thiery A, Bottero J-Y Environmental Science: Nano **2015** 2: 653-663.

Investigating the fate of TiO_2 nanoparticles in soils

G. Sarret[1], A.E. Pradas del Real[1,2,3], M. Bakshi[4], C. Larue[4], J. Reyes-Herrera[2], R. Tucoulou[2], R. Kaegi[5] and H. Castillo-Michel[2]

ISTerre (Institut des Sciences de la Terre), Université Grenoble Alpes and CNRS, Grenoble, France[1], ESRF-The European Synchrotron, CS40220, 38043 Grenoble Cedex 9, France[2], IMIDRA (Madrid Institute for Agroenvironmental Research), 28800 Alcalá de Henares. Spain [3], EcoLab, Université de Toulouse, CNRS, Toulouse, France[4], Eawag, Particle Laboratory, Dübendorf 8600, Switzerland[5],
geraldine.sarret@univ-grenoble-alpes.fr

Nanosized TiO_2 is one of the most produced nanomaterials, and there is an intense debate at the EU level on the ban of TiO_2 additive (E171) in food products. TiO_2 nanoparticles (TiO_2-NPs) released from consumer products end up into the sewer system, and are accumulated in sewage sludge. Agricultural soils are a major compartment of accumulation due to the use of sewage sludge as a soil amendment. TiO_2 is naturally present in soils, and distinguishing between anthropogenic and natural TiO_2 in soils is thus important to measure anthropogenic inputs and to assess the risks associated with the use of nano-TiO_2.

Methods to identify these materials in complex matrices such as soils are currently lacking. In this study, the potential of physical techniques (micro and nano X-ray fluorescence (XRF) performed on ID21 and ID16b, X-ray absorption spectroscopy (XANES), X-ray diffraction (XRD) performed on BM25 and transmission electron microscopy coupled with X-ray microanalysis (TEM-EDX)) to distinguish natural versus anthropogenic particles has been investigated [1]. Three matrices were compared: sewage sludge, agricultural soil that had never received sewage sludge, and sludge-amended soil. Particle size and crystal structure were not specific of the source. The morphology of the TiO_2 particles proved to be different in the two matrices studied, with smooth faceted particles in the sludge and rough irregular ones in the soil. In addition, natural TiO_2 particles were included in micro and macroaggregates of the soil and formed intricate assemblages with minerals and organic compounds. In the sludge, TiO_2 formed homo and heteroaggregates of simpler structure, richer in organic matter. The observed differences may attenuate over time due to the weathering of TiO_2 minerals and to the progressive incorporation of anthropogenic TiO_2 within soil aggregates. So it is likely that with time, engineered TiO_2 becomes indistinguishable from the natural background in soils.

Then, the effects of sewage sludge containing TiO_2-NPs used as an amendment in agricultural soil were assessed on plants (tomato) during a full plant life cycle (until fruit ripening)[2]. The sewage sludge amendment increased plant growth without causing major changes in biochemical responses, except for a decrease in leaf tannin concentration. Changes in elemental concentrations (mainly Fe, B, P, Na, and Mn) of plant stem, leaves and, to a lesser extent fruits were observed. No significant Ti enrichment was detected in tomato fruits. Fourier-transformed infrared (FTIR) analysis performed on ID21 showed effects on plant leaves (decrease in tannins and lignins and increase in carbohydrates) but no effects on fruits. In conclusion, the sewage sludge amendment containing TiO_2-NPs improved plant yield probably due to its high organic matter and nutrient content, and did not lead to significant changes in the edible part of tomato. Effects on the long term, with increasing TiO_2 inputs should be evaluated, as well as effects on other crops and on soil bacterial communities.

References

[1] - A.E. Pradas del Real, H. Castillo-Michel, R. Kaegi, C. Larue, W. de Nolf, J. Reyes-Herrera, R. Tucoulou, N. Findling, E. Salas-Colera and G. Sarret, Environmental Science: Nano **5**, 2853 (2018).

[2] – M. Bakshi, C. Liné, D.E. Bedolla, R.J. Stein, R. Kaegi, G. Sarret, A.E. Pradas del Real, H. Castillo-Michel, P.C. Abhilash and C. Larue, Journal of Hazardous Materials **369**, 191-198 (2019).

Determining the influence of time, soil properties and the plant–soil interface on silver nanomaterial speciation using x-ray absorption spectroscopy

S. Harrison[1], E. Lahive[2], R. Sekine[3], W. W. Bennett[3], A. Robinson[2], C. L. Schultz[2], M. Matzke[2], D. Spurgeon[2], S. Lofts[1], C. Svendsen[2], A. Green Etxabe[2]

[1]UK Centre for Ecology & Hydrology, Lancaster Environment Centre, Lancaster University, LA1 4AP, UK.
[2]UK Centre for Ecology & Hydrology, Maclean Building, Benson Lane, Wallingford, OX10 8BB, UK.
[3]Griffith University, Gold Coast Campus, Southport, QLD 4215, Australia.

Silver nanomaterials (nano-Ag) are one of the most abundant engineered nanomaterials in the consumer market, with a plethora of uses largely based on exploiting their antimicrobial properties, such as textiles, medical applications and cleaning products [1]. A major pathway for nano-Ag release into the environment is the application of sewage sludge to agricultural soils. Their chemical form in soils affects the overall impact they have on the soil ecosystem, for example in the rate at which nano-Ag is taken up by organisms [2] and the toxicity of nano-Ag which is taken up [3]. While nano-Ag behaviour in soils has been studied in some detail, few have extended this to examining uptake across the plant–soil interface. Here, we detail studies into nano-Ag speciation in a range of cropped soils with different properties, including the role of the plant–soil interface in determining speciation, as well as speciation in the plant roots themselves. The work was undertaken using Ag K-edge XANES and EXAFS spectroscopy performed at Diamond Light Source (Oxford, UK) and The Australian Synchrotron (Melbourne, Australia).

Three different soils with varying characteristics were dosed with three different forms of Ag: pristine 20-nm Ag nanoparticles, aged 27-nm Ag_2S nanoparticles and the dissolved, ionic form (as $AgNO_3$), to concentrations of 10 mg Ag/kg soil dry weight. Wheat (*Triticum aestivum*) was germinated in the dosed soil and the bulk soil sampled at determined time points. In addition, to investigate the effect of the plant–soil interface, for one of the soils at 14 days post emergence of shoots, soil fractions were operationally separated by their proximity to the root: as bulk soil (soil unaffected by the roots), loosely-attached soil (which could be removed from the roots by shaking) and rhizosphere soil (closely adhered to roots).

In this presentation, we will highlight the key results, demonstrating the importance of soil properties, time and proximity to roots on Ag speciation. For example, we show sulphidation is the dominant ageing transformation, but the rate at which pristine nano-Ag are sulphidised depends on soil properties. We will discuss these results in the context of environmental exposure and risk, and detail how these results have been incorporated into multimedia nanomaterial exposure models for next-generation exposure assessment.

References

[1] – European Chemicals Agency, European Union Observatory for Nanomaterials website. https://euon.echa.europa.eu/. Accessed 03/12/2019.
[2] – M. Baccaro *et al*, Environmental Science: Nano **5**, 1107 (2018).
[3] – P. Wang *et al*, Nanotoxicology **9**, 1041 (2015).

Advanced techniques to investigate the internalization mechanism of TiO_2 NPs in the roots grown in a biosolid-amended agricultural soil

E. Tassi, L. Bellani[1,2], L. Giorgetti[2], F. Barbieri[1], M. Ruffini Castiglione[3], S. Muccifora[1]

Research Institute on Terrestrial Ecosystems, CNR Pisa, Italy, elianalanfranca.tassi@cnr.it, [1]Life Science Department, University of Siena, Italy, lorenza.bellani@unisi.it, barbierifrance88@gmail.com, simonetta.muccifora@unisi.it, [2]Institute of Agricultural Biology and Biotechnology, CNR Pisa, Italy, lucia.giorgetti@ibba.cnr.it, [3]Biology Department, University of Pisa, Italy, monica.ruffini.castiglione@unipi.it

Plants play an important role in introducing the engineered nanoparticles (ENPs) into the food chain. The pathway of ENPs uptake from soil, their distribution in the edible plant parts, and their impact in the food production are important issues to be investigated. In the present study, *Pisum sativum* plants were grown at microcosm scale under medium-term TiO_2 NPs exposure, to possibly mime environmental conditions in an agricultural soil amended with biosolids from a wastewater treatment plant in Pisa, Italy. TiO_2 NPs were applied as pure rutile, pure anatase and a mixture of both crystalline phases in the biosolid amended-soil.
Micro-XRF and μ-XANES from ID21 beamline were used for Ti elemental mapping and crystalline phase identification to indicate a relative distribution/localization of TiO_2 crystalline phases within a given cross-section of roots, as well as the possible speciation and preferential crystalline phase uptake in the roots. Titanium in roots showed a main localization in the rizoderma, independently of the crystalline phase. Fewer Ti spots were found localized in the cortex or in vessel, however the roots grown in presence of a mixture of both phases showed a main presence of anatase, suggesting a preferential adsorption and translocation of this crystalline form through the roots. Our data indicated also a reduced translocation of Ti to the aerial part of the plant, confirming the chemical analysis of shoots and roots separately, which showed that Ti concentration was about 40 times lower in the upper part than in the below ground tissues.
The TiO_2 NPs were characterized on the basis of their size and shape by TEM analysis. Moreover, observations on cell ultrastructure of control and of anatase, rutile and mixture of both crystalline phases treated roots were performed. The root cells of plant grown in the presence of all NPs treatments shared the same alterations of ultrastructure: mitochondria with swollen cristae, nuclei with condensed chromatin, and part of the cytoplasm degraded, probably in consequence of an autophagic process. As detected by μ-XRF and μ-XANES, electron dense prismatic or round profiled particles of about 30-40 nm were observed mainly in form of aggregates in the intercellular spaces or crossing the wall of the cells next to rizoderma and in the cortex cells. Furthermore, the anatase treated cells were mostly damaged in respect to control and rutile treated roots, and more frequently internalized NPs were observed in these samples.

Nanoscience Foundries and Fine Analysis (NFFA): the widest range of tools for research at the nanoscale

C. Girardot[1], E. Capria[1]

Affiliation: [1]ESRF, **cecile.girardot@esrf.fr**

NFFA•EUROPE is a distributed research infrastructure which offer access to state-of-the-art facilities and instruments for multidisciplinary research projects at the nanoscale. It deals with a large panel of research field from synthesis to nanocharacterization to theory and numerical simulation. In particular fine analysis with Synchrotron, FEL and Neutron radiation sources. Each institutes is integrated in a local node to offer transnational access and enable European and international researchers from diverse disciplines to carry out advanced proposals impacting science and innovation. NFFA enables coordinated access to infrastructures on different aspects of nanoscience research that is not currently available at single specialized ones and without duplicating their specific scopes.

Approved user projects have access to the best-suited instruments and support competences for performing the research. Their access includes several “installations” and is coordinated through a single entry point portal to build up a personalized access programme with an increasing return on science and innovation production. Until now, the majority of submitted proposal are linked with materials science, chemistry or physics, but there is a will to enlarging the community of NFFA’s user and especially increase proposal dealing with nanosafety research topics, nanosafe by desing and life cycle assessment of nanoparticles.

We will present NFFA project and collect every feedback for potential future enlargement of the catalogue, in particular to intercept the needs from the nanosafety community.

References

[1] - https://www.nffa.eu/