ESRF	**Experiment title:** Characterization of superparamagnetic iron oxide nanoparticles degradation process inside macrophages by Fe K-edge XANES	**Experiment number**: LS-2562
Beamline: ID21	**Date of experiment**: from: 16th November 2016 to: 20th November 2016	**Date of report**: 07/03/2017
Shifts: 18	**Local contact(s)**: Murielle Salome	*Received at ESRF:*

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Report:

One of the most promising tools in nano-biomedicine is the use of superparamagnetic nanoparticles (SPION) as they can be used as contrast agents for magnetic resonance imaging, heating mediators for magnetic hyperthermia in cancer treatment and drug delivery systems. Although all this approaches take advantage of the good cellular tolerance of SPION, nanoparticles can also be modified by the organism and little is known about the residence time of the particles and the metabolic routes activated during SPION degradation. We decide Fe K-edge XANES to tackle the degradation process. With this purpose, we incubated RAW 264.7 (ATCC® TIB-71™), a murine leukemic macrophage-monocyte cell line, with 100 mM and 250 mM APS-SPION 12 nm iron oxide core (Aminopropylsilane-SPION) during 1 and 3 days, as a control we incubate the cells in the same conditions but without SPION. Once the different treatments were finished, we incubated the cells with 100 nM of LysoTracker® Red DND-99 to fluorescently-label acidic organelles, potentially where the nanoparticles are accumulated. The cells, treated with the fluorophores and grown on top of silicon nitride windows of 1.5 mm x 1.5 mm, 5 mm x 5 mm Si frame and membrane thickness of 200 nm, were cryo-preserved by plunge freezing in liquid nitrogen chilled ethane. Cryo-preserved samples were analysed by cryo-epifluorescence in order to select the better preserved samples and register the cellular and the acidic organelles fluorescently-labelled coordinates.

The selected samples were analysed at id21 beamline by X-ray fluorescence microscopy (XRF) at a fixed energy (7.3 keV) and Fluo-XANES between 7.0 and 7.3 keV with the intention of identifying different iron species product of the degradation of the internalized nanoparticles. The information obtained by cryo-epifluorescence was used for sample registration at the id21 microscope (fig. 1). Rough XRF maps (1 µm steps) of multiple cells were recorded to determine precisely the regions of interest. Higher resolution XRF maps (500 nm steps) of multiple cells were recorded in the same areas as the rough maps (fig. 1).

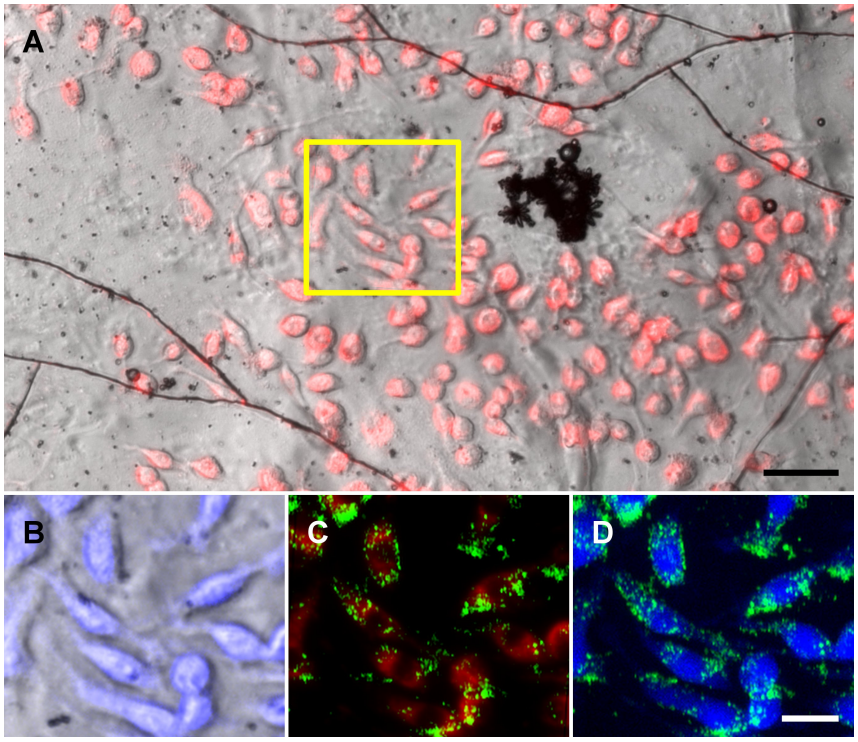

Figure 1. Correlative workflow between cryo-epifluorescence and XRF on MCF-7 cells treated with 100 mM of APS-SPION during 72 h . **A**, cells treated with APS-SPION imaged by cryo-epifluorescence, in gray scale color a bright-field image, in red the signal coming from acidic organelles (Lysotracker), where the nanoparticles are accumulated. The yellow square points at the area of XRF map acquisition shown in B, C and D. **B**, overlay image of sulfur XRF map signal and bright field image. **C**, overlay image of lysotracker in red and iron XRF map signal in green. **D**, overlay image of sulfur (in blue) and iron XRF map signals (in green). Scale bar in A, 50 µm. Scale bar in B, C and D, 20 µm.

Fluo-XANES data series were collected in the same areas as the XRF maps in order to identify different iron species and their proportion by comparison with different references. The references recorded at id21 were representing the potential different states of the nanoparticle degradadtion process. The initial or non-degraded step: APS-SPION and maghemite (iron specie composing the SPION core); possible intermediate products of the nanoparticle degradation: iron citrate; The final intracellular product of nanoparticles degradation: ferrihydryte (iron specie present inside the ferritin protein complex). Unfortunatelly, the SPION concentrations employed for this experiment, 100 mM and 250 mM (same SPION concentrations used to determine the SPION amount in different organelles by ICP-MS and magnetic susceptibility), was too high, preventing the identification of iron species inside the cells different from APS-SPION (fig. 2). Nevertheless, the XRF maps acquired for the different treatments are going to be use to perform a semi-quantification of the intracellular nanoparticle accumulation.

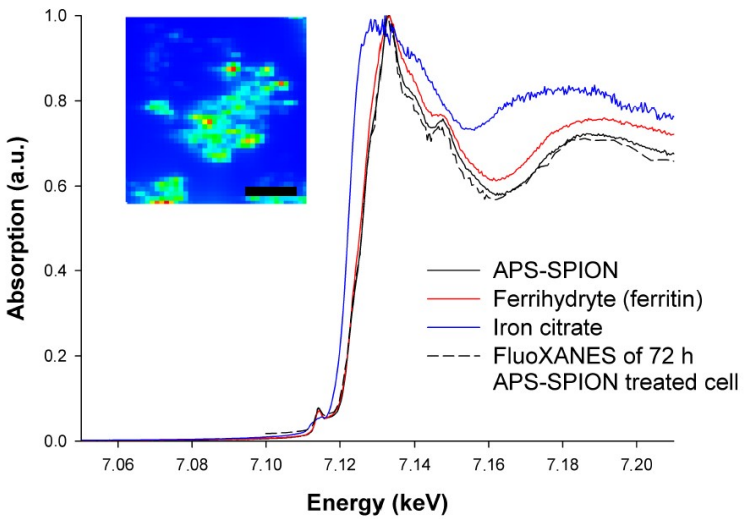

Figure 2. Fluo-XANES spectras of iron species references and a MCF-7 cell treated with 100 mM of APS-SPION during 72 h. The spectra of the cell Fluo-XANES fits with the APS-SPION reference. No signal different from APS-SPION was detected. Inset image shows a XRF map at 7.14 keV of the Fluo-XANES data series. Inset scale bar 20 µm.