ESRF	**Experiment title:** Characterization of superparamagnetic iron oxide nanoparticles degradation process inside macrophages by Fe K-edge XANES	**Experiment number**: LS-2562
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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 to use 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 10 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 5 mm x 5 mm frame and membrane thickness of 200 nm, were cryo-preserved by plunge freezing in liquid nitrogen chilled ethane. 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. Cryo-preserved samples were freeze dryed since the cryo-sample environment was not abailable at id16b.

Dryed samples were analysed at id16b beamline by X-ray fluorescence microscopy (XRF) at fixed energy (7.2 keV) and Fluo-XANES between 7.05 keV and 7.2 keV energies to identify different iron species product of the degradation of the internalized nanoparticles. The information obtained by cryo-epifluorescence was used for sample registration at the id16b microscope (fig. 1). We worked with a beam focus of 90 nm. Rough XRF maps at 500 nm steps were recorded to precisely locate regions of interest. Higher resolution XRF maps (100 nm steps) were also recorded in the same areas than the rough maps (fig. 1).

Fluo-XANES data series of small areas (1 µm x 1 µm) around high XRF iron signal identified in high resolution XRF maps were collected. Beam instabilities in addition to a drift of the region of interest prevented doing fluo-XANES for the gathering of useful information related to the identification of different iron species product of the degradation of intracellular nanoparticles. Since fluo-XANES data could not be collected, we acquired single point scans between 7.05 and 7.2 keV energies in different areas where the iron XRF signal was high as well as in the surrounding region. We compare.these single point spectras to single point reference spectras of potential iron species product of the nanoparticle degradation process (fig. 2). The initial or non-degraded step: APS-SPION and maghemite (iron specie compossing the SPION core); possible intermediate products of the nanoparticle degradation: iron citrate, iron chloride and ferric sulfate; and the final intracellular product of nanoparticles degradation: ferrihydryte (iron specie present inside the ferritin protein complex). We could identify the initial APS-SPION (fig. 1 b) and an absorption profile that can not be assigned to any of the acquired references at both incubation times (fig. 2d). This may hinder reaching a clear conclusion on the degradation process, as the nanoparticles have an average size of 12 nm and the focus used was 90 nm. In addition, during the energy scan, there was a vertical drift of the focus of 50 nm, which implies that the XANES "point" spectra are in reality an average over a bigger vertical area of 140 nm. Single point spectras have to be analysed further in order to be able to identify intermediate iron species and to elicit a more detailed view of the intracellular SPION degradation process.

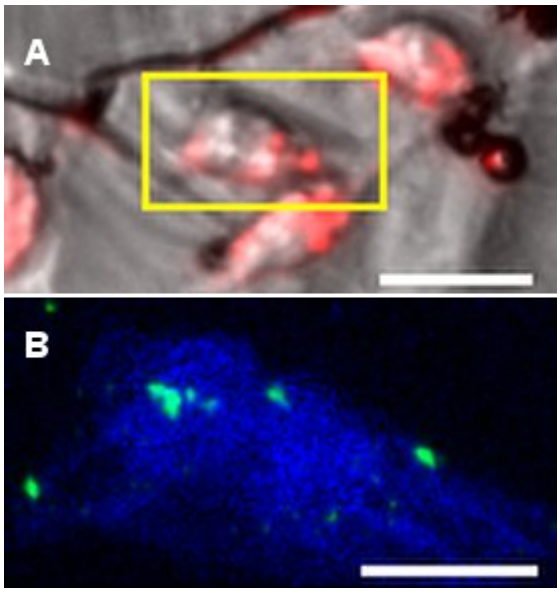

Figure 1. XRF maps of MCF-7 cells incubated with 10 mM APS-SPION during 72 h. A, overlay of bright field (gray scale color) and cryo-epifluorescence Lysotracker red signal (acidic organelles) images. Yellow square point at XRF map acquisition area in B. **B**, XRF map, sulfur signal (blue) and iron signal (green). A scale bar 20 µm. B scale bar 5 µm.

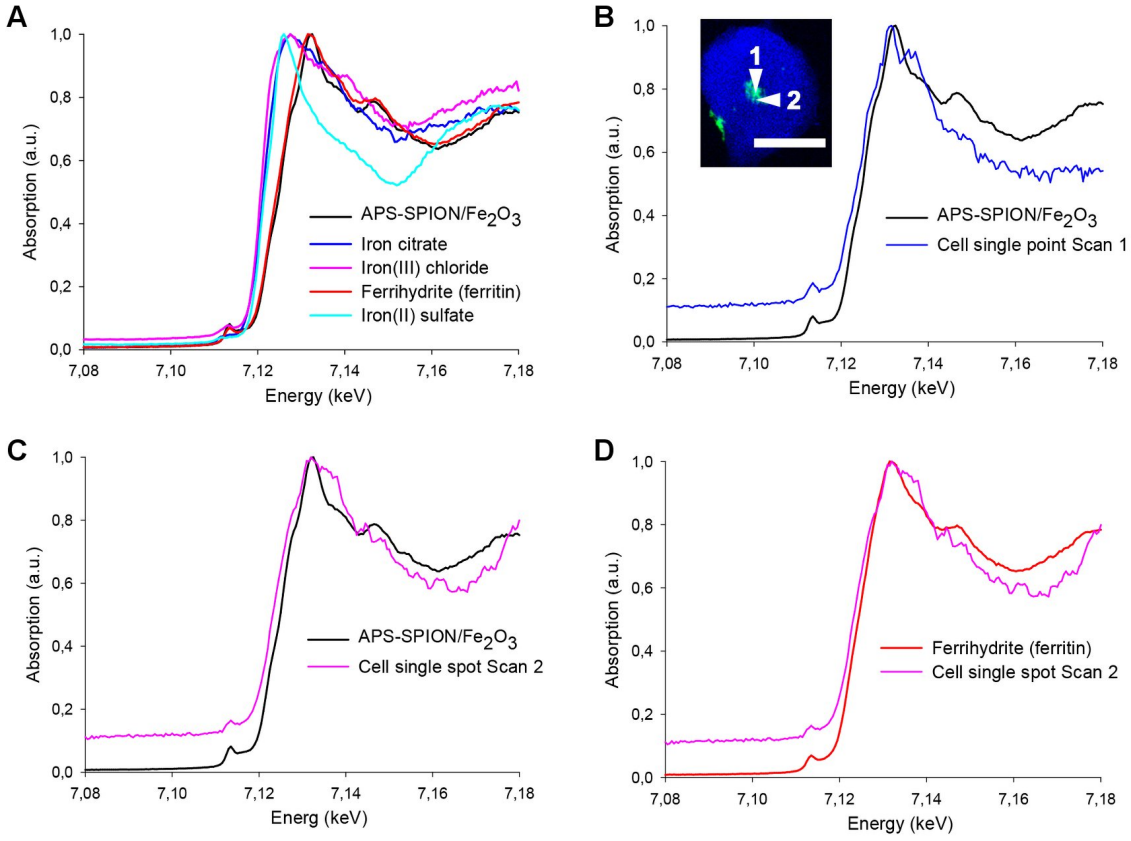

Figure 2. Single point scans of different iron species references and single point scans in a MCF-7 cell incubated with 10 mM APS-SPION during 24 h. A, absorption spectras of different iron species. **B**, the inset image shows a XRF map of a cell where single spot energy scans (pointed out with white arrow heads and numbers corresponding to the scan id) were acquired using a beam size of 90 nm. Single point spectra 1 compared to APS-SPION reference. **C**, comparison of single point spectra 2 and APS-SPION reference. **D**, comparison of single point spectra 2 and APS-SPION reference. Inset image scale bar 8 µm.