ESRF	**Experiment title:** Analysis of silver nanoparticle transformations in a liver-like model upon exposure to non-toxic concentrations	**Experiment number**: LS-2967
Beamline: BM30	**Date of experiment**: from: 01/04/2021 to: 06/04/2021	**Date of report**: 13/12/2021
Shifts: 15	**Local contact(s)**: Isabelle Kieffer	*Received at ESRF:*

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Report:

We performed the X-ray Absorption Spectroscopy experiment in cryogenic conditions in the He cryostat of BM30. The samples were prepared in our home laboratory: ~100 µl drops of solution were pipetted into the custom sample holder sealed with kapton tape and immediately frozen in LN2, then transferred to the ESRF in a LN2 deware. We measured silver K-edge absorption spectra by scanning the edge region between 25.30 keV and 26.48 keV ($k = 16$ Å^{-1}).

The samples were:

1. Reference samples in solution: $AgNO_3$, silver nanoparticles (AgNP), Ag(I)-glutathion (GSH) complex.
2. Four silver-substituted zinc-finger proteins. These samples are named after the aminoacids involved in the Zn-binding loop (CCCC; CCHH; CCCH; CCHC), and metalated with Ag equivalent corresponding to the number of cysteines (C) in the loop.
3. 3D cultures of hepatic cells exposed to AgNP or to an Ag(I) salt. We used NPs with two different coatings: PVP or citrate. The 3D cell cultures were obtained either with the hanging-drop or with the organ-on-chip method (samples labelled "drop" or "chip", respectively).[1] The exposure to AgNPs lasted for 2, 4 or 7 days.

We could acquire high quality data for all samples. In silver-substistuted Zn-fingers, the oscillations of the exctracted EXAFS spectra extend till k=14 Å^{-1}. The data were Fourier-transformed in the k-range [2.2 - 12] Å^{-1} and compared to the AgGSH reference compound, to which they show a clear similarity (**Figure 1**). The *ab initio* analysis of the spectra reveals that Ag binds two S atoms in digonal coordination in all compounds; the second-shell contribution around 2.8 Å in the FT spectrum is attributed to a variable number of non-bonding Ag···Ag interactions, as previously observed in the Ag-GSH complex.[2,3] The structural characterization of the Ag binding motifs in these peptides is the subject of a publication that we recently submitted (K. Kluska et al. *Structures of silver fingers and a pathway to their genotoxicity*, submitted to Angew. Chem. Int. Ed.).

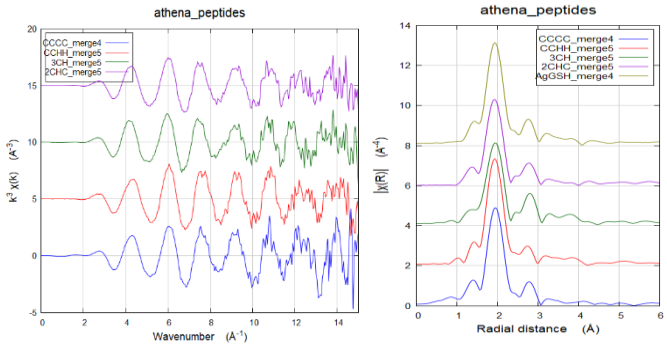
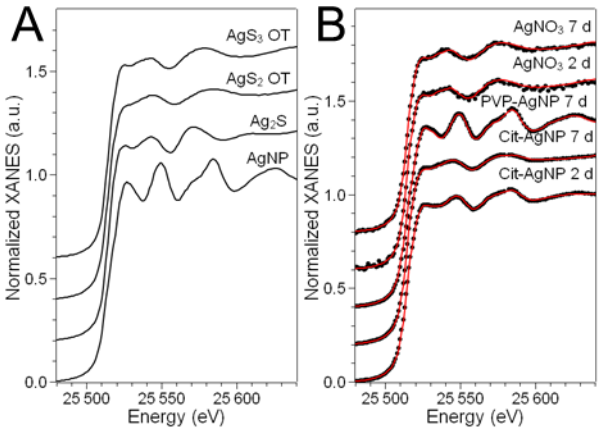


Figure 1. *Experimental EXAFS spectra of Ag-substituted Zn-finger proteins in the reciprocal (left) and in the real space (right). For comparison, the spectrum of a AgGSH reference compound is reported (right panel, khaki yellow curve).*

According to the cytotoxicity assays performed in our lab, we exposed 3D cultures of hepatic cells to sub-toxic doses of silver NPs and salt, resulting in a low but detectable amount of Ag in cells. We could measure the XANES spectra of all samples, and the EXAFS spectra of the samples prepared with the organ-on-chip method, which gave a higher density of cells with respect to the hanging-drop. The XANES region can be interpreted as a linear combination of reference compounds, and provide the purcentage of dissoution of the NPs *in cellulo* in the different exposure scenarii. In particular, by making use of different Ag-S compounds measured in this experiment and in previous experiments,[2] we could distinguish between the formation of inorganic Ag_2S crystals or Ag(I)-organothiol (OT) complexes.



The use of two OT reference compounds with different coordination geometry is crucial in the linear combination fitting of the Ag K-edge spectra of 3D hepatic cells exposed to silver NPs and salt (**Figure 2**). This is the first time that XAFS data are collected on a 3D functional liver-like model, capable to perform excretion. These results, combined with the results of XRF nano-imaging (LS-2710 on ID16B) that allowed for the sub-cellular localization of the Ag species, are the subject of a publication in preparation (V. Tardillo-Suarez et al. *Silver nanoparticle transformations, trafficking and excretion in hepatocyte spheroids mimic the fate of silver species in liver.*)

Figure 2. *(A) XANES spectra of AgNP and of three Ag-S reference compounds: Ag-organothiol (OT) in trigonal and digonal coordination (AgS_3 and AgS_2, respectively), and mineral acanthite (Ag_2S). (B) Experimental XANES spectra (black dots) of selected samples measured in this experiment:3D cultures of hepatic cells exposed to Ag nanoparticles or salt for 2 or 7 days, and best fitting curves (red) obtained as linear combination of the reference coumpounds reported in panel A.*

References

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