EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

	Experiment title: **Stability of Ag_2S quantum dots for integrated imaging and therapy**	**Experiment number**: MA-4831
Beamline: BM23	**Date of experiment**: from: 24/06/2021 to: 28/06/2021	**Date of report**: 07/12/2021
Shifts: 12	**Local contact(s)**: Thomas Buslaps	*Received at ESRF:*

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Report:

Experimental Methods:

We performed the X-ray Absorption Spectroscopy experiment in cryogenic conditions in the He cryostat of BM23. Samples were prepared in our home laboratory: ~50 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. The first batch of samples was deposited in a holder made of a black plastic in a 3D printer: we realized that the black plastic breaks in LN2, so we had to trasfer the samples in **peek sample holders**. This will have to be kept in mind for future preparations.

We measured silver K-edge absorption spectra by scanning the edge region between 25.30 keV and 26.49 keV ($k = 16$ Å^{-1}) with the **Si(311)** monochromator, with constant steps in k in the exafs region. The continuous scanning mode was tested, but in this mode the glitches of the Si(311) monochromator could not be corrected through the normalization by the incoming current, in contrast with what was previously observed with the Si(111). Therefore, the scans were run in **step-by-step mode**.

The samples were two different preparations of Ag_2S quantum dots (QD) synthesized using the same precursors but different thiolate capping agents: glutahtion (GSH) or D-Penicillamine (Pen). The reaction time during the synthesis was chosen in order to produce nanocystals of different sizes, ranging from 2 to 6 nm diameter,

according to a TEM characterization. The size is proportional to the reaction time, which was 1h or 2h for GSH-Ag_2S, and 30m or 1h30m for Pen-Ag_2S.
The four QD were measured as synthesized in water (samples labeled "wat"), after 24h in cell culture medium (labeled "med"), or after 24h in hepatic cells (labeled "cell"), in order to probe the stability of these engineered nanomaterials in biological media. As reference samples we used a previously characterized Ag(I)-GSH complex (5 mM) in solution, [1] and a bulk Ag_2S powder diluted in BN and pressed in a pellet (5 mm diameter).

The concentration of the "wat" samples was in the range 1-4 mM (~100-400 ppm): the EXAFS spectra could be measured on all "wat" samples, but the least concentrated ones required a long integration time (up to 9 scans to be merged in the final spectrum). In some "med" and "cell" samples that were not concentrated enough to acquire the EXAFS spectra, only the XANES region was scanned up to 25.70 keV.

Results:
The experimental spectra of the "wat" samples are reported in **Fig.1**, together with the spectrum of the bulk form of Ag_2S.

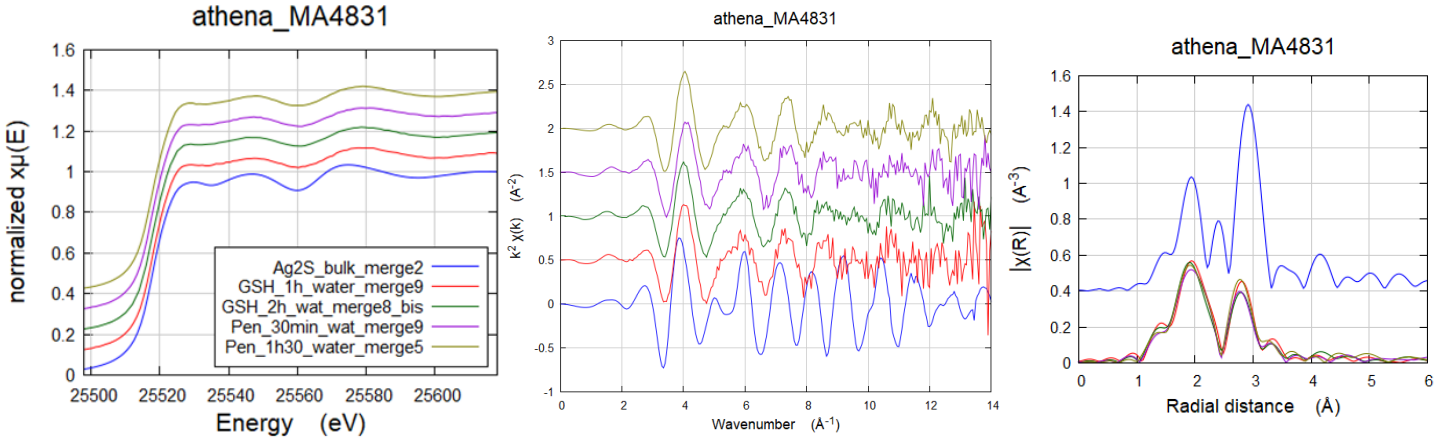

Figure 1. *Experimental XANES (left) and EXAFS spectra in the reciprocal space (central panel) and in the real space (right) on Ag_2S in the bulk (blue) and nanocrystal form. Ag_2S nanocrystals were synthesized in water in the presence of glutathion for 1h (red) or 2h (green), or in the presence of D-Penicillamine for 30m (purple) or 1h30m (khaki yellow).*

The EXAFS spectra of the QD, as extracted in the reciprocal space (**Fig.1**, central panel), show a quick damping of the oscillations, indicative of highly disordered systems. When the sample is concentrated enough, weak but significant oscillations are observable up to k = 14 $Å^{-1}$ (*e.g.* Pen 1h30 sample, khaki curve. Concentration ~ 4 mM). All spectra could be fourier-transformed in the range [2.3 - 11] $Å^{-1}$ and compared (**Fig. 1**, right) with the bulk. The spectra of the Ag_2S QDs show two main peaks attributable to Ag-S and a Ag-Ag single scattering contributions, as in the bulk sample (blue curve). However, the second shell Ag-Ag contribution is much weaker in the QDs than in the bulk Ag_2S sample.
The EXAFS spectra were fitted *ab initio* in order to obtain the number of S and Ag neighbors for the Ag absorber. The data were fitted in the real space, in the [1 – 3.2] Å range. A very simple fitting model based on two single-scattering paths accounts for the observed spectral features, as shown in **Figure 2**, where the best fitting curve and the relative single scattering Ag-S and Ag-Ag contributions are shown for the Pen-Ag_2S "wat" 1h30 sample. The fit results indicate that, in all QD samples, the Ag absorber is surrounded by 2 S neighbours at ~ 2.48 Å, and by ~4 Ag atoms at 2.97 Å. The values obtained for the first S shell do not differ (within the error) from the ones relative to the bulk sample. The second shell, instead, differes dramatically in the number of neighbors (4 *vs* 8) and significantly in their distance from the absorber (0.1 Å shorter in QDs than in bulk Ag_2S). Again, this suggests a strong disorder in the QDs, and a poor crystallinity in their Ag_2S core.

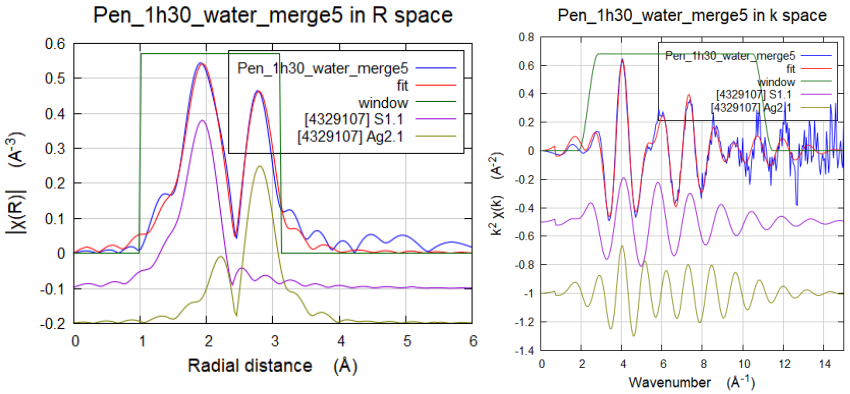

Figure 2. *Experimental spectrum (blue) relative to the Pen-Ag_2S 1h30m sample in water, and relative best-fitting curve (red) based on a 2 shells single scattering model. The individual contributions of the first S shell and second Ag shell are reported (purple and khaki yellow, respectively). The fits are performed in the real space (left), then back-transformed into the reciprocal space (right).*

Finally, we could compare the XAFS spectra of the four QDs formulations in water, after 24h in cell culture medium, and after 24h or 72h in hepatic cells. No difference is observed in the XANES spectra of the samples in the different conditions. When the EXAFS spectra could be acquired and extracted, small differences seem to arise in the first spectra after 72h in cells, indicating a possible small deformation of the average coordination sphere of silver (**Figure 3**). Overall, our data reveal that the core of the QDs is essentially stable and no significant release of metal ions and recombination with biomolecules is observed, in contrast with our previous observations on InP-based QDs.[2]

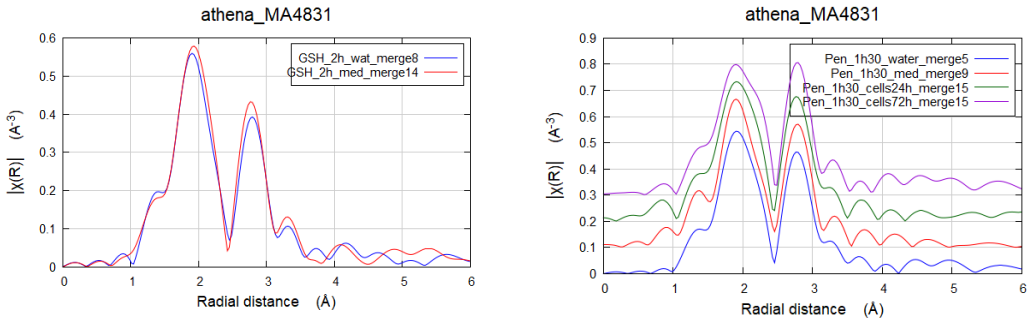

Figure 3. *Experimental EXAFS spectra of GSH-Ag_2S 2h (left) and of Pen- Ag_2S 1h30 QDs in different media. The QDs were measured as synthesized in water (blue curves), after 24h in cell culture medium (red), and in hepatic cells for 24h (green) or 72h (purple).*

We are currently merging these results with the photophysical measurements performed at the SyMESS laboratory, in order to understand wether a correlation exists between the observed structural disorder and the photoluminescence (PL) of the QDs. We are improving the formulations with the aim to enhance the PL quantum yield, in particular of the D-penicillamine-coated QDs. The latter showed no cytotoxicity in the assays performed at the LCBM, and the XAFS results proved their structural stability in biological media.

This study contributed to identify promising engineered nanomaterials for biomedical applications. The experimental protocol, consisting in the selective interrogation of the core atoms of the QDs in different biological media to probe teir stability, should be applied to the new formulations with improved PL properties that we are currently synthesizing.

References

[1] G. Veronesi, T. Gallon, A. Deniaud, B. Boff, C. Gateau, C. Lebrun, C. Vidaud, F. Rollin-Genetet, M. Carrière, I. Kieffer, E. Mintz, P. Delangle, I. Michaud-Soret. XAS investigation of silver(I) coordination in copper(I) biological binding sites. *Inorg. Chem.* **2015**, 54, 11688.

[2] G. Veronesi, M. Moros, H. A. Castillo-Michel, L. Mattera, G. Onorato, K. D. Wegner, W. Li Ling, P. Reiss, C. Tortiglione. *In vivo* biotransformations of indium phosphide quantum dots revealed by X-ray micro-spectroscopy. *ACS Appl. Mater. Interfaces* **2019**, 11, 35630.