ESRF	**Experiment title:** Probing the structure of photoluminescent Ag_2S-based quantum dots in hepatic cells	**Experiment number**: MA-5469
Beamline: BM23	**Date of experiment**: from: 04/10/2022 to: 12/10/2022	**Date of report**: 04/07/2023
Shifts: 15 allocated + 5	**Local contact(s)**: Olivier Mathon	*Received at ESRF:*

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Report:

Experimental Methods:

We performed a silver K-edge X-ray Absorption Spectroscopy experiment in cryogenic conditions on BM23. The samples were prepared in our home laboratory and consisted in Ag_2S quantum dots (QD) coated with different thiolate molecules: glutathione (GSH) and D-Penicillamine (DPen). For GSH, we tested two Ag/S ratios in the synthesis (1/1 and 1/2), resulting in three formulations of Ag_2S QD. For each formulation, we prepared the samples in different media:

1. In water, after synthesis and purification. Ag concentration 10 mM (~ 1000 ppm)
2. In cell culture medium (CCM) for 24h. Ag concentration 2.5 mM (~ 250 ppm).
3. In cell culture medium (CCM) for 72h. Ag concentration 2.5 mM (~ 250 ppm).
4. In hepatic cells (HepG2-C3A) for 24h. Ag concentration dictated by the cellular uptake.
5. In hepatic cells (HepG2-C3A) for 72h. Ag concentration dictated by the cellular uptake.

Additionally, we prepared the complexes formed by silver with GSH (ratio 1/1 and 1/2) and with DPen (1/1) at pH 9, corresponding to an intermediate step of the heat up synthesis approach used for the QD.
As reference samples we used a previously characterized Ag(I)-GSH complex (5 mM) in solution at pH 7,[1,2] and a bulk Ag_2S powder diluted in BN and pressed in a pellet (5 mm diameter).

We used custom, screw-less sample holders produced with a 3D printer at the ESRF, sealed with kapton foils on both sides. 100 µl drops of solutions were deposited in the sample holders and immediately frozen in LN2 in our home laboratory. The samples were transferred to the ESRF in LN2, then mounted in the He cryostat of BM23 for XAS measurements.

We measured Ag 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 scans were run in the step-by-step mode. The slits were opened to provide a beam size at sample of ~3 × 1 mm^2 and minimize the

photon density, in order to avoid radiation damage. All solutions were measured in the fluorescence mode, with a Vortex SDD detector. The Ag_2S reference powder was prepared as a pellet diluted in BN and measured in transmission. 3 to 14 scans per sample were acquired and merged, depending on the Ag concentration.

Results:

We could acquire high-quality data for all conditions. The data reduction was performed with standard methods using the Athena software. The extracted EXAFS spectra were Fourier-Transformed in the k range [2.3, 12.3] $Å^{-1}$, then fitted in the real space in the R range [1, 3.2] Å, with the XAS viewer GUI implemented in Larch.[3] An example of the acquired experimental EXAFS signals and of the relative best-fitting curves is given in Figure 1 for GSH-coated Ag_2S QDs (Ag/GSH = 1/2).

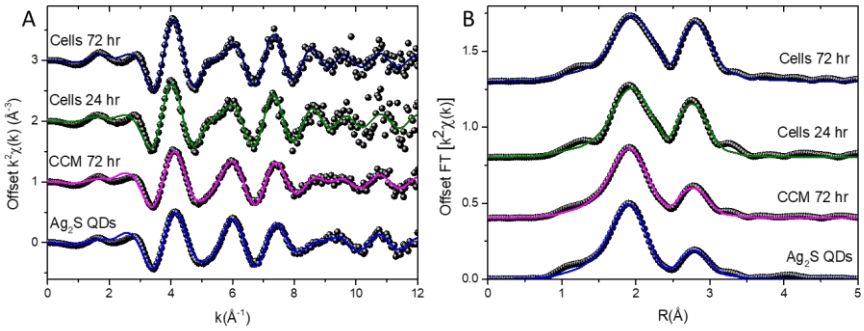
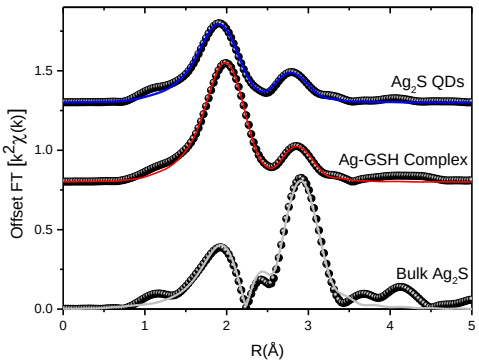


Figure 1. *Experimental (circles) and theoretical (solid lines) Ag K-edge EXAFS signals in the reciprocal space (A) and Fourier-Transformed into the real space (B). All data were acquired on Ag_2S QD coated with GSH, synthesized using a S/Ag ratio of 2. The QDs were measured in different media, from bottom to top: in water, in cell culture medium for 72h, in hepatic cells for 24h or for 72h.*



Moreover, by comparing the spectra of each QD with the spectrum of the corresponding silver complex formed at pH 9, we could follow the evolution of the coordination sphere of Ag during the synthesis (Fig 2 red vs blue curve). A comparison of the QDs with the spectra of bulk Ag_2S (Fig 2, grey) reveals a very low crystallinity in the nanocrystals, which explains why XRD measurments failed on these samples. The structural information retrived with XAFS and the photophysical characterization of the same samples are the core of a publication currently in preparation.

Figure 1. *Experimental (circles) and theoretical (solid lines) Ag K-edge EXAFS signals of Ag_2S QD coated with GSH, of the Ag(I)-GSH complex formed at pH 9, and of bulk Ag_2S .*

Publication in preparation: Omar El-Dahshan, Louise Poutot,….. Aurélien Deniaud, Peter Reiss*, Giulia Veronesi* *Evolution of the Ag local environment during the synthesis of Ag_2S nanocystals and in biological media revealed by XAFS.*

References

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(2) Veronesi, G.; Gallon, T.; Deniaud, A.; Boff, B.; Gateau, C.; Lebrun, C.; Vidaud, C.; Rollin-Genetet, F.; Carrière, M.; Kieffer, I.; Mintz, E.; Delangle, P.; Michaud-Soret, I. XAS Investigation of Silver(I) Coordination in Copper(I) Biological Binding Sites. *Inorg. Chem.* **2015**, *54* (24), 11688–11696.

(3) Newville, M. Larch: An Analysis Package for XAFS and Related Spectroscopies. *J. Phys. Conf. Ser.* **2013**, *430*, 012007.