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<tr><td> </td><td>Experiment title:
Biodistribution and intracellular fate of colloidal semiconductor nanocrystals in an invertebrate model organism</td><td>Experiment number:
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Report:

The aim of the experiment was to follow InP Quantum Dots (QDs) uptake, translocation and transformations in a model organism, *Hydra vulgaris*, in order to predict their toxicity *in vivo* and their potential biomedical applications.

Methods. We used two types of InP/ZnS QDs designed and synthesized at the INAC laboratories: “core” and “core-shell”, the latter being characterized by an extra ZnSe protective layer. Hydra Vulgaris was exposed to the two types of QDs for 1h, 3h, 24h or 48h. Exposed samples were embedded in OCT resin and snap-freezed in isopentane cooled in LN_2, than shipped to the ESRF where they were cut in thin slices in the cryo-microtome of ID21. We obtained 20 µm-thick slices with good tissue preservation (see Fig.1a).

We intended to acquire µXRF elemental images to localize the QDs, and In L-edge µXAS spectra to probe their transformations within the tissue.

A monochromatic beam of 4.00 keV was used for mapping. The beam was focused to 0.4x1.0 μm^2 by means of KB mirrors, providing a flux at sample of 10^{10} ph/s. XRF hyperspectral images with 0.5x0.5 μm^2 or 1x1 μm^2 step size and 500 ms integration time were acquired, and In from the QDs was unambiguously detected (Fig. 1b). In order to improve the count rate and the detection limit, two undulators were used in a second time. This provided a flux of $3*10^{10}$ ph/s, allowing us to reduce the integration time to 200 ms/pt. An SDD detector from SGX, with 80 mm^2 active area and Be window was used to collect fluorescence from the sample.

Indium was detected through its L_{II} emission lines at 3.49 keV, while the main L_{III} emission line at 3.29 keV could not be separated by the potassium K-line at 3.31 keV (Fig.1b). For the same reason, In L_{II}–edge XANES spectra were acquired on internalized QDs.

The experiment was performed at LN2 temperature in the ID21 cryotome. The system did not show any failure throughout the whole experiment.

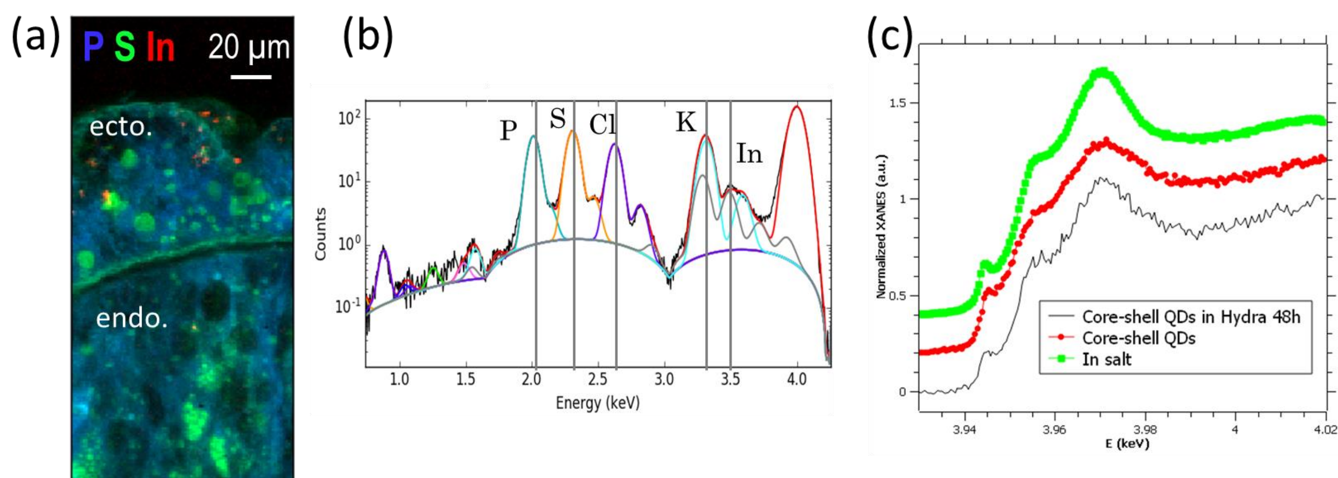

Fig.1. (a) P (blue), S (green) and In (red) distribution in a transversal section of Hydra vulgaris exposed to "core-shell" InP/ZnS/ZnSe quantum dots, extracted from an XRF hyperspectral image. (b) XRF sum spectrum of In-rich pixels in map (a). (c) XANES spectra of core-shell QDs in solution (red) and after 48h in *Hydra* (black), and of an In-myristate solution (green).

Results. We obtained high quality elemental images of *Hydra* tissue sections under the several exposure conditions, for the two QDs. These images provide not only the expected information on QDs biodistribution, but also novel information about *Hydra* elemental physiology (*e.g.* S in Fig. 1a). The entry of QDs through Hydra's ectoderm was highlighted, as well as their diffusion towards the inner part of the animal (endoderm, Fig.1). XANES spectra of InP QDs in Hydra could be acquired, and compared with the spectra of QDs in solution and of an In salt (Fig. 1c). This comparison suggests a degradation of the QDs in *Hydra* cells. We are currently treating the data in order to understand how this degradation progresses with exposure time, and the influence of the coating on it.

These results will be merged with the bioassays performed at the CNR-ISASI laboratory and be the core of a publication to be submitted to a high impact factor journal. Remarkably, this is the first elemental imaging study on *Hydra vulgaris*.

Perspectives

Over the last years research performed at CNR-ISASI used *Hydra* as a unique model system to investigate the mechanisms of internalization of several nanocrystals into biological tissues. The simple structure of Hydra body, indeed, resembles the epithelial tissue of higher organism, allowing to investigate the mechanism of interaction between nanoparticles and cells, controlling biocompatibility and toxicity. Transmission electron microscopy may allow studying cell localization of electron dense nanoparticles, but it does not provide informations on the chemical state of the nanoparticle, resulting from trafficking and interaction with cell compounds. XRF imaging coupled with Xanes spectroscopy provide unique details on the stability, permanence, degradation and fate of inorganic nanoparticles within biological tissues. For this reason further analysis on nanoparticles of different composition or surface coating may provide important data for the safe design of advanced tools for diagnosis and therapy applications.