	Experiment title: Bioaccumulation and physico-chemical transformation of safer-by-design InP quantum dots	**Experiment number**: A30-2 1156
Beamline:	**Date of experiment**: from: 10/11/2021 to: 16/11/2021	**Date of report**: 20/12/2021
Shifts:	**Local contact(s)**: Isabelle Kieffer	*Received at ESRF:*
Names and affiliations of applicants (* indicates experimentalists): Marie Carriere (*), Jacques Pecaut (*), Sarah Hostachy (*)		

Report:

Introduction:

Quantum dots (QDs) are semi-conductor nanocrystals with exceptional optical properties; for this reason they are used in a variety of high tech products, as well as for medical imaging and diagnostic. Presently, QDs that are used in the highest number of commercial products are CdSe QDs. Their intensive use poses the question of their impact on human health, which is largely dependent on their dissolution. Emerging alternative QDs which are potentially less toxic are composed of InP. The aim of the present proposal was to analyse In speciation in samples consisting of human keratinocytes exposed to InP QDs or In ions to decipher any intracellular dissolution of QDs, which would lead to their toxicity.

Materials and methods:

HaCat human keratinocyte cell line was exposed to In salts (dissolved in a citrate buffer, phosphate buffer or in water) or to InP QDs, consisting of an InZnP core, coated with a ZnS shell. After their synthesis, these QDs had been transferred to aqueous solution via ligand exchange using penicillamin. After cell exposure for 24 h or 48 h, cells were washed, harvested and flash frozen as a cell pellet in liquid nitrogen, then stored in liquid nitrogen until analysis. Cell exposure medium containing In-citrate, In-phosphate, In dissolved in water or InZnP/ZnS QDs was also prepared for its analysis. All samples were analysed at the FAME beamline at the In K-edge (27.94 keV), at 15 K in a He cryostat.

Preliminary results:

We first analysed reference samples, i.e., In-citrate, In-phosphate, In-bicarbonate, In dissolved in water (supposedly In-OH) and In dissolved in water and diluted in cell culture medium. We already had the reference spectrum for InZnP QDs from a previous experiment. The spectra for In-bicarbonate, In dissolved in water and In dissolved in water and diluted in cell exposure medium were similar, while in the spectrum for In-phosphate the white line maximum was slightly shifted towards higher energies. The spectrum for In-citrate reference differred from the other ones; it lacked the shoulder after the white line and the shape of the first oscillation differed from that of the other compounds (Fig. 1A). In keratinocytes exposed to In-citrate, the obtained spectrum differed from that of the In-citrate reference (Fig. 1B). It was more similar to that of In-phosphate, but deeper analysis would be necessary to firmly identify the In speciation in this condition. In keratinocytes exposed to In dissolved in water, or In-phosphate, the obtained spectra were very similar to that

of In-phosphate. When keratinocytes were exposed to QDs and analysed, the obtained spectrum was similar to that of the formerly acquired QD reference spectrum, suggesting that QDs had not dissolved intracellularly.

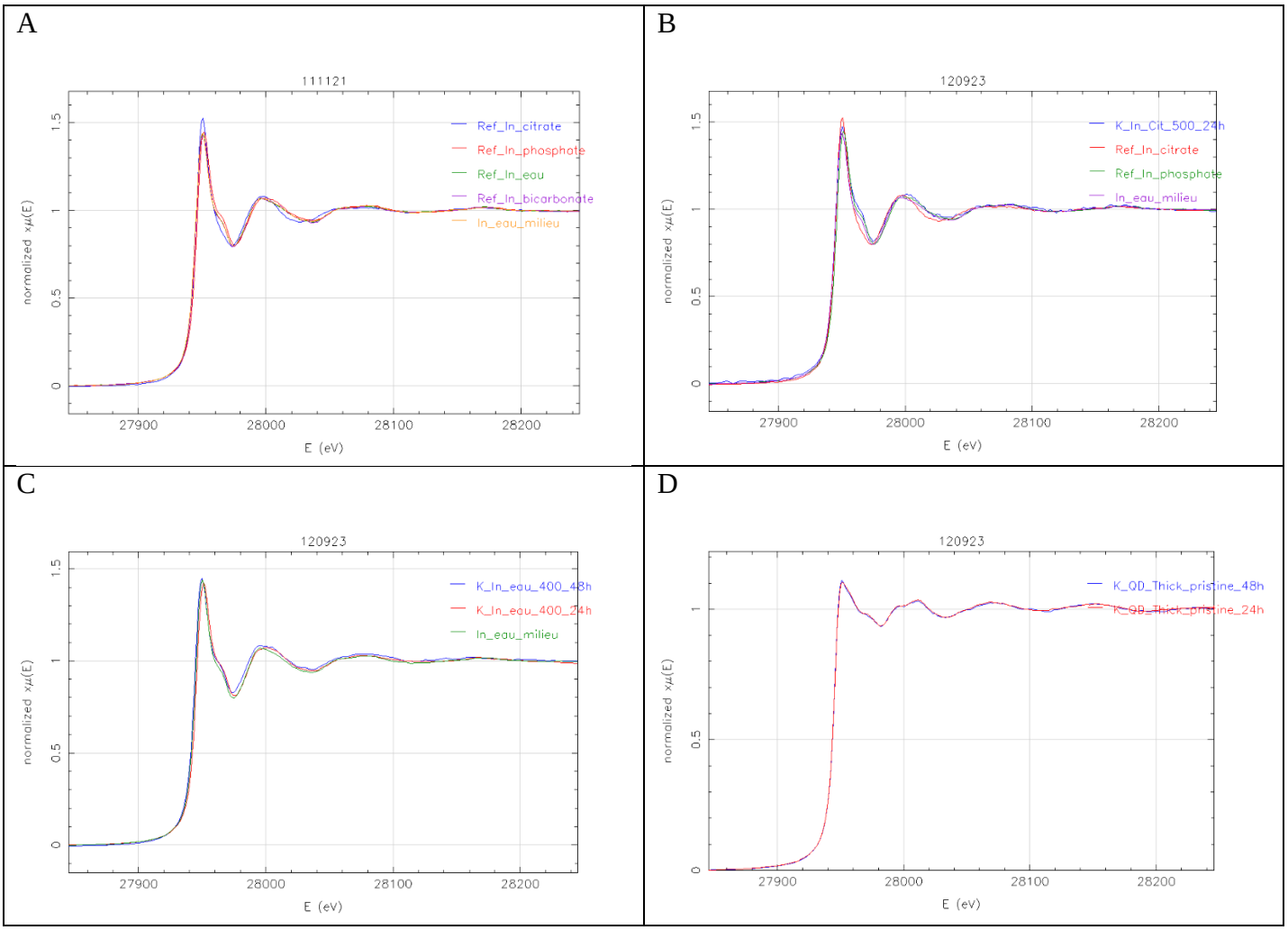

Figure 1. X-ray absorption spectroscopy spectra of In compounds. A: spectra obtained for reference compounds; B: spectra obtained from keratinocytes exposed to In-citrate at 500 µM for 24 h compared to In-citrate, In-phosphate and In dissolved in water reference spectra; C: spectra obtained from keratinocytes exposed to In dissolved in water at 400 µM for 24 h or 48 h compared to the reference spectrum of In dissolved in water and diluted in exposure medium; D: spectra obtained from keratinocytes exposed to InZnP/ZnS quantum dots for 24 h or 48 h.

Conclusions:

This experiment allowed successful recording of In K-edge spectra on human keratinocytes exposed to In salts or InZnP/ZnS QDs. The analysis now needs be deepened in order to identify the exact speciation of In in this context, and/or to identify any mixture of In compounds in each tested condition.