EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Intracellular transformations of Ce oxidation state after CeO_2 internalisation by micro-XANES pulse-chase experiments.	**Experiment number**: CH-5096
Beamline: ID21	**Date of experiment**: from: 12-04-2017 to: 18-04-2017	**Date of report**: 27/07/2017
Shifts: 18	**Local contact(s)**: Hiram Castillo-michel Giulia Veronesi	*Received at ESRF:*

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Report:

Background CeO2 nanoparticles (CNP) are actively investigated as promising antioxidant agent with possible applications in the therapy of a number of pathologies associated to free radicals or oxidative stress. The proposed mechanisms by which CeO2 displays its activity is the scavenging of free radicals, associated to the Ce3+/Ce4+ redox equilibrium. However, up to now, a direct proof of this rationale is still lacking. In a previous XANES experiment, carried out at BM08 (CH4478), we have been able to observe an the ncrease in the amount of Ce(III) in cell internalized CNP. A more recent experiment, carried out at ID21 (CH4716) using μXRF and Ce-L_{III}-edge μ-XANES, we evidenced for the first time a dependence of CNP oxidation state on their intracellular distribution [1]. Aim of the proposed experiment is to further analyze the CeO_2 intracellular transformations by following over time and *in situ* the Ce oxidation state by performing pulse-chase experiments.

Experimental description

Sample preparation

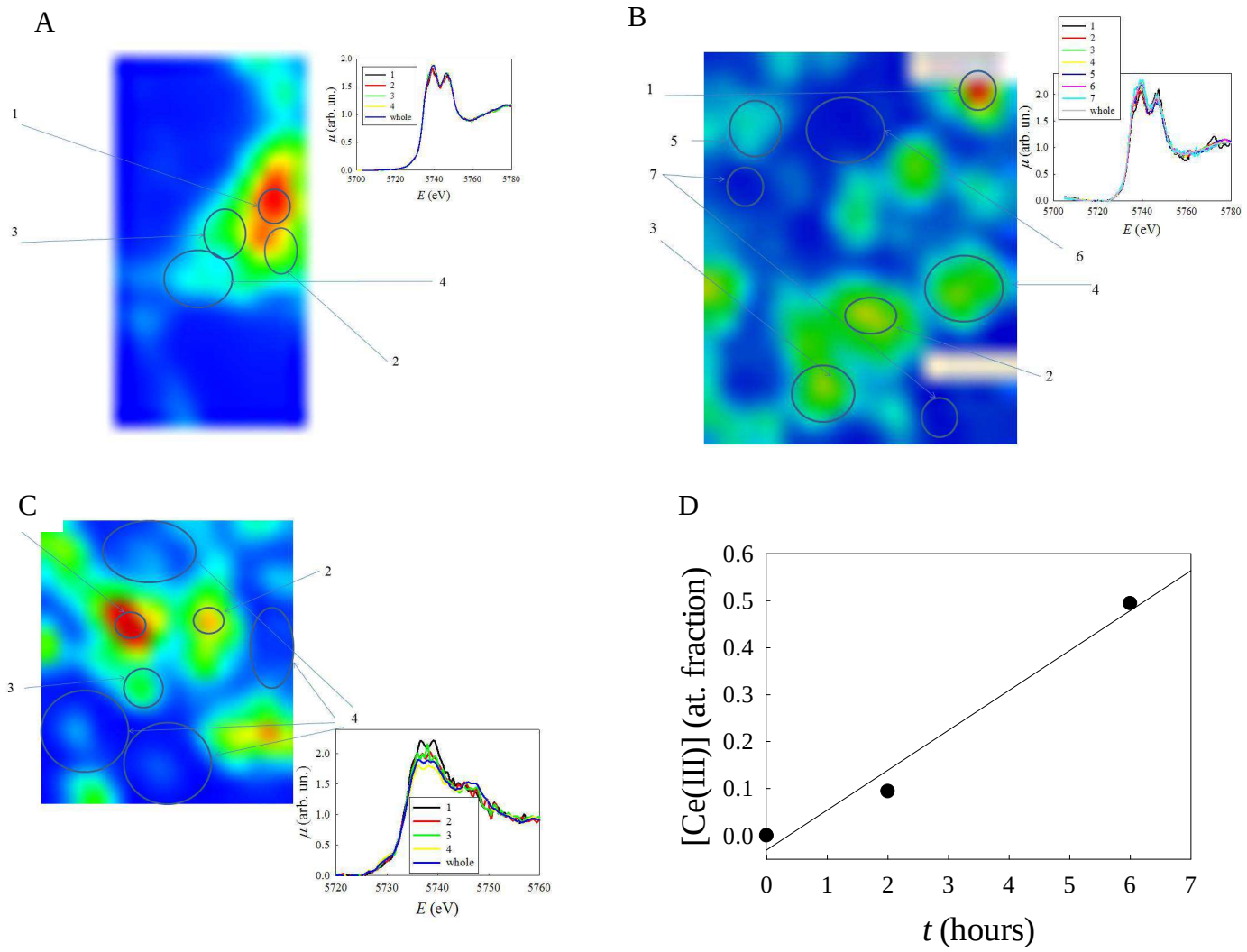
HeLa cells were grown on Si3N4 membranes and treated with CNP. After 1h of incubation (pulse), cells were washed to remove the unattached CNP and the internalized CNP were left for the following 2 and 6 hours (chase). The samples were then freeze-dryed and stored at -80°C. For the X-ray synchrotron analysis, the samples were mounted in the pre cooled sample holder under LN2-vapors and then immediately inserted into the cryostat. The entire transfer process took less than 2 minutes.

Results

μXRF and Ce-L_{III} edge μXANES measurements were performed using the scanning X-ray microscope of beamline ID21 at ESRF, equipped with a cryostage, keeping the sample stable at −170 ± 5 °C. The μXRF acquisition was done in hyperspectral mode for which the XRF spectrum for each pixel in the image was registered. All maps were fitted using PyMCA software to obtain the intensity distribution of the elements. The μXANES spectra were acquired in fluorescence mapping mode by scanning the beam with a 0.6 × 0.6 μm^2 step size and a 100 ms dwell time per pixel, using a region of interest selective for Ce L3M4 and L3M5 emission lines, corrected for the detector dead time and normalized by I0. Each map was processed using

PyMCA for XANES spectra extraction. The Athena software then was used for background removal and normalization: the pre-edge background was fitted by a straight line and subtracted. The spectra were normalized at the unit absorption coefficient at 200 eV above the edge. Fig. 1 shows the Ce concentration maps of the sample incubated for 1(pulse), plus 2 or 6 hours (chase). Hot spots correspond to regions where the concentration of Ce is high. However, the presence of Ce is detected everywhere in the region of interest. Ce-L_{III} XANES spectra were extracted from the zones indicated by numbers and are shown together with the maps. It is quite apparent that the spectral shape of the XANES manifold for the sample incubated for 1 hours does not change with position, and is always representative of almost pure CeO_2 with no Ce(III). On the contrary, the sample incubated for the additional 2 or 6 hours shows considerable variations of the spectral shape with position. In particular, the region around 5738 eV shows a significant increase in amplitude if compared to the spectrum of CeO_2, the increase being larger approaching the hot spot. In addition, a shift in the edge energy positions (as detected from the first maximum in the derivative spectra, not shown here for space reasons) is also detected: these evidence points towards the formation of a considerable amount of Ce(III) in this sample. Assuming that the edge energy position varies linearly with Ce(III) content and averaging the spectra over all the map area, a linear trend of Ce(III) amount vs. time is obtained as shown in Fig. 1. In summary, our result inequivocally demonstrate that CeO_2 are processed by cells with the formation of Ce(III).

Figure 1. (A-C) Ce concentration map and Ce-L_{III} XANES spectra at selected regions for cells incubated with CNP for 1 h (pulse) (A); 1 h incubation with CNP (pulse) plus 2 h (chase) (B) or plus 6 h (chase) (C); (D) Ce(III) amount vs. incubation time as derived from the Ce-L_{III} edge position.



References

[1] D. Ferraro, I. G. Tredici, P. Ghigna, H. Castillo-Michel, A. Falqui, C. Di Benedetto, G. Alberti, V. Ricci, U. Anselmi-Tamburini and Patrizia Sommi Dependence of the Ce(III)/Ce(IV) ratio on intracellular localization in ceria nanoparticles internalized by human cells *Nanoscale* **2017**, 9 1527-1538