



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 using the **Electronic Report Submission Application:**

<http://193.49.43.2:8080/smis/servlet/UserUtils?start>

Reports supporting requests for additional beam time

Reports can now 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.



Experiment title: Actinide selective recognition by biomimetic molecules		Experiment number: 20-01-659
Beamline: BM20	Date of experiment: from: 13/12/2006 to: 17/12/2006	Date of report: 01/02/2007 <i>Received at ESRF:</i>
Shifts: 12	Local contact(s): H. Funke	

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Report:

“Metallomics” stands at the interdisciplinary research field for the promotion of bio-metal science, with emphasis on recent development of chemical speciation of elements in biological samples. It is now well known that the speciation of an element drives its bioavailability and toxicity. Generally, the speciation analysis of a metallic cation in living organisms involves the determination of its chemical forms. However, the structure of these latter remains very often elusive as well as their structure/function relationship. In this field, XAS is an ideally suited probe for the understanding of metallobiomolecules structure-functions relationship at the molecular level and for the speciation of a metallic cation in a biological medium. One of the strategy to understand selective complexation and transport of actinide elements is to observe metallobiomolecules acting as elaborated inorganic complexes with well-designed metal active sites. Among the large variety of metalloproteins involved in carriage processes, transferrin has been shown to bind a wide variety of d-block transition metals as well as actinides like plutonium [1] and lanthanides [2]. Transferrin is a regulator of Fe(III) carriage in blood. It is a glycoprotein of 670 amino acids (81 000 Da) with a tertiary structure made of two equivalent lobes (C and N) with one possible complexation site each. The chemical behavior similarity between Pu(IV) and Fe(III) has already been observed for instance in the siderophore-mediated uptake of Pu by *Microbacterium flavescens* [3]. However the specific mechanisms that lead to actinide complexation (Th(IV), Np(IV) and Pu(IV); U(IV) is excluded because it is unstable under atmospheric conditions) are still unknown, as well as the structural modifications of the protein metal binding site. In our search to better understand the uptake mechanisms of actinide(IV) (An(IV)) by metalloproteins as transferrin, several approaches have been considered [4]. A “global approach” related to the characterization of the actinide coordination sphere upon complexation by the apo- metalloprotein itself and a “biomimetic approach” involving simplified biomimetic peptides have been proposed.

In this proposal we used the biomimetic approach and investigated the complexation of actinide(IV) (Th, Np, Pu) by linear pentapeptides. Three pentapeptides with a given functional group (Tyr, Asp and Glu) have been synthesized. As explained in the proposal report 30-02-759, a comparison between Fe(III) and An(IV) is also under study. These peptides are partially “biomimetic” of part of the transferrin MBD with phenolate and

carboxylate groups. All the complexes were prepared in aqueous HEPES solution with a careful control of pH to avoid hydrolysis.

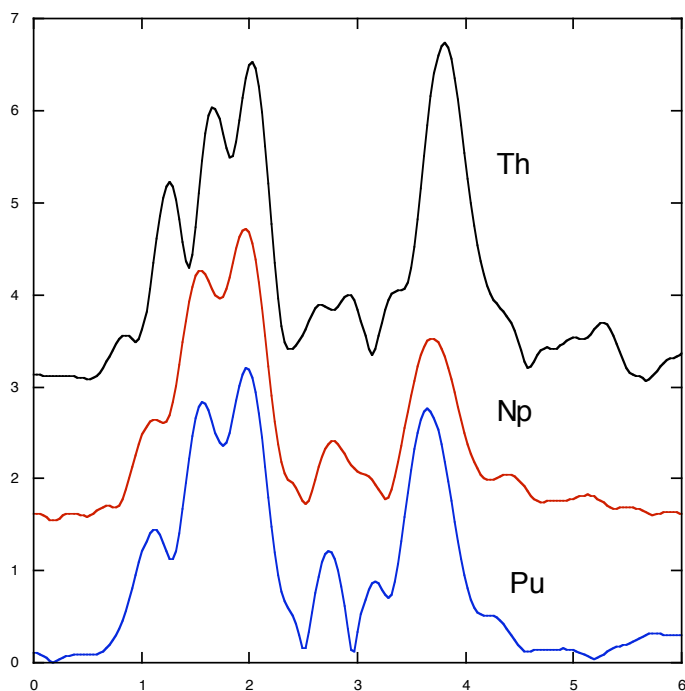


Figure 1 : Fourier Transform of the EXAFS spectra the three complexes Th(IV)/AcAspAspProAspAspNH₂, Np(IV)/AcAspAspProAspAspNH₂ and Pu(IV)/AcAspAspProAspAspNH₂ at the actinide L_{III} edge. Ligand to cation ratio = 1.5.

In Figure 1, the Fourier transform of the EXAFS spectra of the three complexes in aqueous solution are presented. Several ligand/actinide ratios have been tested and they all exhibit the same spectrum. Therefore and only the 1.5 ratio is presented here. Qualitatively, all the spectra are identical and the typical shortening of the coordination sphere from Th to Pu is visible. In the first coordination sphere, at least two contributions are present. According to the literature they can be attributed to An-water, An-carboxylate(monodentate) and/or An-carboxylate(bidentate) ligation. In their study of Th(IV) acetate, Rao *et al.* also observe two distinct distances with most probably water molecules at 2.37 Å and bidentate acetates at 2.52 Å. Our results differ significantly :

Th(IV)/AcAspAspProAspAspNH ₂	3 O at 2.30 Å, 6 O at 2.49 Å, 1 Th at 3.92 Å
Np(IV)/AcAspAspProAspAspNH ₂	3 O at 2.21 Å, 6 O at 2.41 Å, 1 Np at 3.81 Å
Pu(IV)/AcAspAspProAspAspNH ₂	3 O at 2.20 Å, 6 O at 2.40 Å, 1 Pu at 3.79 Å

This is the first time to our knowledge that diatomic actinide molecules are prepared with such type of ligands. However the unusual short distances of the first coordination sphere suggest the formation of hydroxo species although the UV-Vis data do not confirm the formation of colloidal hydrolyzed species. Mixed hydroxo-carboxylato adducts might also be formed. Mass spectroscopic and ¹³C NMR measurements are also being carried out.

To our surprise, we did not succeed in preparing the glutamate adducts. It suggests that the length of the peptide side chains is of significant influence on the complexation ability.

[1] : H. Lee, P. J. Sadler, H. Sun, *Eur. J. Biochem.* (1996), **242**, 387.

[2] : Raymond K. N., Pecoraro, V. L., Harris, W. R., Carrano, C. J.: *Proc. Int. Symp. (Environ. Migr. Long-Lived Radionuclides, 1981, IAEA-SM) 571* (1982).

[3] : M. P. Neu, in *Advances in Plutonium Chemistry 1967-2000*, Ed. D. C. Hohhman, Am. Nucl. Soc. (2002), 169.

[4] : A. Jeanson, C. Den Auwer, P. Moisy, C. Vidaud, OECD-NEA Proceedings on Speciation, Techniques and Facilities for Radioactive Materials at Synchrotron Light Sources, Karlsruhe, September 2006