



## 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:**  
**Interaction of actinide cations with metalloprotein**

**Experiment number:**  
20-01-644

<b>Beamline:</b> BM20	<b>Date of experiment:</b> from: 12/06/2005 to: 14/06/2005	<b>Date of report:</b> 06/09/2005  <i>Received at ESRF:</i>
<b>Shifts:</b> 6	<b>Local contact(s):</b> A. Rossberg	

**Names and affiliations of applicants (\* indicates experimentalists):**

**\*C. Den Auwer, CEA Marcoule DEN/DRCP/SCPS, 30207 Bagnols sur Cèze**

**Ph. Moisy, CEA Marcoule DEN/DRCP/SCPS**

**C. Vidaud, CEA Marcoule DSV/SBTN**

## **Report:**

When tightly bound to protein ligands, metal ions are critical to the function, structure, and stability of proteins, by only allowing specific interactions to take place and/or selective chemistry to occur. Metallobiomolecules are thus considered as elaborated inorganic complexes with well-designed metal active site structures. Although the various interaction processes between the metallic cation and the protein are widely studied in all the fields of biochemistry, focus on the specific actinide family is more seldom [1]. In particular, the knowledge of transportation, fixation and interaction mechanisms of these cations in the biologically active sites are only poorly understood. Plutonium and neptunium have been generated by the industrial nuclear activity. Oxidation state (IV) has been of particular concern for its relative stability in physiological conditions and reactivity similarities with Fe(III). For instance Pu(IV) as well as most of the transition metal cations have been reported to be complexed by transferrin [2-3].

In order to avoid actinide hydrolysis at physiological pH, the Th(IV), Np(IV) and Pu(IV) aquo species were complexed by NTA (nitrilotriacetic acid). Note that U(IV) is unstable under atmospheric conditions and is not included in the series. NTA is an interesting protecting and synergistic anion towards transferrin uptake that has been used in the complex of known crystal structure TlFe(III)NTA [4].

In a first step, the 1:2 complexes of An:NTA (An = Th, Np, Pu at oxidation state IV) have been investigated combining spectrophotometry and EXAFS data. These results are currently being correlated to quantum chemical calculations (manuscript in preparation including the spectrophotometric data). In all cases, the NTA ligand is tetradentate with 3 coordinating carboxylate functions and one nitrogen atom. The overall coordination number is 8. An additional water molecule may enter the first coordination sphere (most probably for the larger actinide cation, i.e. Th(IV)) but no definite conclusion can be drawn from the EXAFS data. Figure 1 summarizes the interatomic distances obtained from the EXAFS data fitting and compared to preliminary quantum chemical calculations (ADF-Zora).

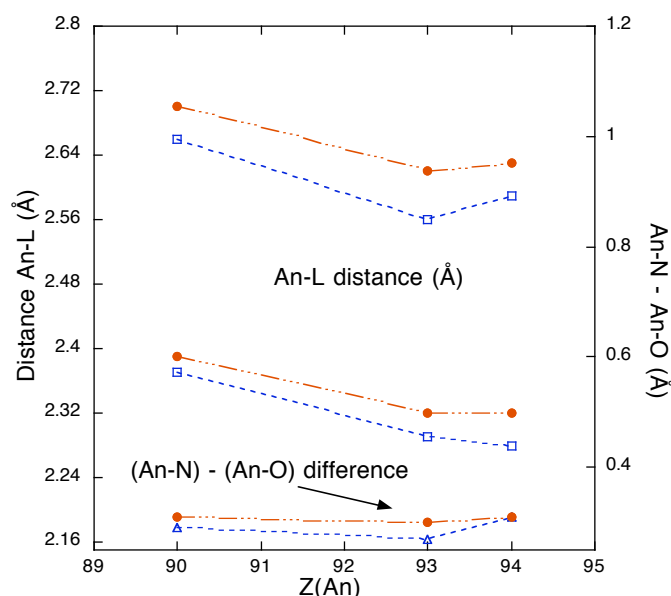


Figure 1 : An-N (top graph) and An-O (middle graph) distances obtained from EXAFS data (●-●-●-) and quantum chemical calculations (□-□-□-). The difference between these two distances is reported in the bottom graph from EXAFS data (●-●-●-) and quantum chemical calculations (△-△-△-).

Although the calculation is always c.a. 0.03 Å shorter than the EXAFS data, both methods agree well on the distance difference between oxygen and nitrogen ligation.

In a second step, the Np(IV) and Pu(IV) transferrin complexes have been investigated with NTA as a protecting/synergistic ligand. A former XAS investigation of the 1/2 Tf:Np(IV) complex lead to a putative coordination pattern including the NTA anion in the iron binding site [5] (*cf* also ESRF report of CH-1764). Such model must be refined by combining the EXAFS data with models obtained from molecular mechanics. Figure 2 compares the EXAFS data at the Np and Pu  $L_{III}$  edge of 1:2 An:NTA and 2:1 An:NTA:Tf (An = Np(IV), Pu(IV)).

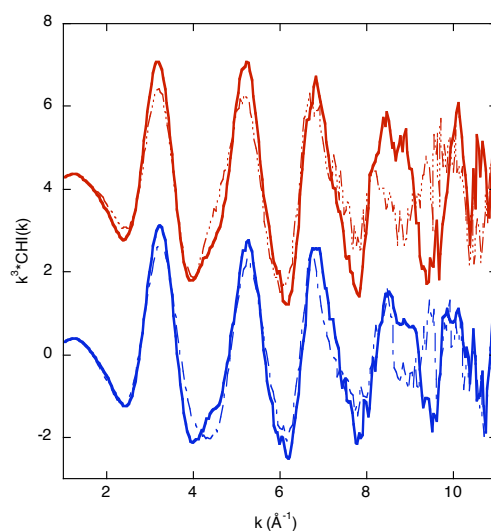


Figure 2 : EXAFS LIII edge of 1:2 An:NTA (\_\_\_\_) and 2:1 An:NTA:Tf (-----). An = Np(IV) (top set) and Pu(IV) (bottom set)

From a qualitative point of view there is a clear structural modification of the actinide coordination sphere upon uptake by transferrin. Furthermore, this modification is similar for Np and Pu. Simulations are currently undertaken in order to refine the putative Fe site as the preferred actinide binding site.

- [1] : Gorden, A. E. V., Xu, J., Raymond, K. N., Durbin P. W.: *Chem. Rev.* **103**, 4207 (2003).
- [2] : Harris, W. R.: *Structure and Bonding* (Springer Verlag, Heidelberg) **92**, 121 (1998).
- [3] : Taylor, D. M.: *J. Alloys Comp.* **271**, 10 (1998).
- [4] : K. Mizutani, H. Yamashita, H. Kurokawa, B. Mikami, M. Hirose : *J. Biol. Chem.* **274**, 10190 (1999).
- [5] : I. Llorens, C. Den Auwer, P. Moisy, E. Ansoborlo, C. Vidaud, H. Funke : *FEBS J.* **272**, 1739 (2005).