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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:**

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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.

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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.

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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;
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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:** **Uptake of neptunium and plutonium by topologically constraint peptides**	**Experiment number**: CH-2539
Beamline: BM20	**Date of experiment**: from: 23/01/2008 to: 28/01/2008	**Date of report**: 21/02/2008
Shifts: 15	**Local contact(s)**: C. Hennig	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

***C. Den Auwer, *A. Jeanson, Ph. Moisy, *S. Petit, *S. Dahou**

CEA Marcoule DEN/DRCP/SCPS, 30207 Bagnols sur Cèze

S. Coantic

Université Montpellier 1, IBMM, LAPP, Montpellier

In the field of human toxicology, internal contamination with actinides can induce both radiological and chemical toxicity. Although there is a tremendous volume of data available on the interaction of plutonium with living organisms as plants, nearly all the studies are limited to macroscopic or physiological measurements with no specific information at the molecular level. In order to mimic the metal binding site of potential actinide carriers in the human body (metalloproteins, enzymes etc…) amino-acids known to bear high affinity donor groups such as carboxylates have been selected [1]. Such amino acids have been used as molecular bricks in order to design specific peptides of small molecular weight. We have combined here a screening approach of the possible peptide candidates with a structural investigation of one model peptide-cation complex. With such small peptides, one should however keep in mind that the cooperative effects originating from the macromolecule tertiary structure as in metalloproteins are not taken into account. For instance the tertiary structure of metalloproteins dramatically modifies the chelation properties of each functional group of the metal binding site.

The "split mix" method has been used to produce a library of over 1000 supported pentapeptides for which Fe(III) has been selected as a screening "test cation". Iron has been selected because colorimetric tests are easily available (for instance with phenantroline) and Fe(III) can be considered as a biological surrogate of Pu(IV). Among the sequences present in the library, those able to complex Fe(III) with the largest affinity have been detected by the colorimetric test and identified by mass spectrometry. Not surprisingly, most of the sequences that do complex Fe(III) contain several aspartic units because carboxylate groups have a high affinity for hard acid cations. On the other hand, one of the targeted peptide, AcAsp-Asp-Pro-Asp-AspNH_2 (also noted DDPDD, shown in Figure 1) has been selected as a model and synthesized in weighted amounts for aqueous complexation studies. Characterization of the peptide-metal complex by UV-Vis spectroscopy has suggested that 1:1 complexes are formed in the case of Fe(III), Th(IV), Np(IV) and Pu(IV). Complexation is carried out in aqueous/HEPES solution at pH around 4.

In our previous report (20-01-659) we have presented our first EXAFS data with Th, Np, Pu suggesting that all three cations yield similar complexes. The typical shortening of the coordination sphere from Th to Pu is observed in the present data. In the first coordination sphere, at least two contributions are present. Complementary NMR data have suggested that the oxygen atoms of the amide functions play a major role in the complexation. The EXAFS spectrum of the well characterized $Np(NTA)_2^{2-}$ complex (NTA =

nitrilotriacetic acid) is presented in Figure 2. NTA is a small aminocarboxylate acid with three available carboxylic groups. With this ligand, the actinide cation (from Th to Pu) is surrounded by the 6 carboxylate oxygens plus the 2 amine nitrogens [2]. In that sense, NTA and DDPDD (D = asp, P = pro) bear very similar functional groups although the topology is radically different. Figure 2 suggests that the peptide ligand dramatically modifies the actinide environment. Since our last report, we have prepared a series of slightly modified peptides in order to better understand the role of the side chain functional groups in the structure of the actinide coordination sphere. In order to identify the binding mode of the peptide (bridging, terminal….) we have varied the positioned of the aspartic acid (with lateral carboxylate groups) or the length of the peptide : pentapeptide with 4 aspartic acids (DDPDD) or two aspartic acids (ADPDA) (A = ala); decapeptide identical to two times the pentapeptide (DDPDDDDPDD). The use of larger peptides is expected to produce chains or helices around the actinide cations. Figure 2 compares the neptunium L_{III} edge of this series of Np(IV)-peptide complexes. At this point, similar spectra have been obtained as shown in Figure 2.

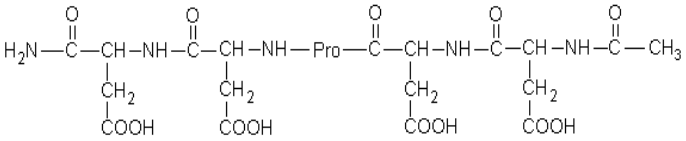

Fig. 1 : Formula of the DDPDD pentapeptide

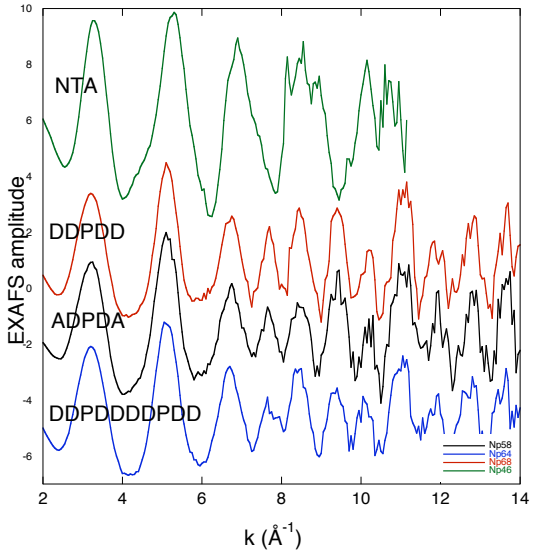

Fig. 2 : EXAFS spectra at the neptunium L_{III} edge of the following complexes (from top to bottom) : $[Np^{(IV)}(NTA)_2]^{2-}$, $Np^{(IV)}$:DDPDD, $Np^{(IV)}$:ADPDA, $Np^{(IV)}$:DDPDDDDPDD.

Fitting in the EXAFS data shows that the actinide coordination sphere is split in two distances, one short corresponding to hydroxo bridges as in actinide colloids [3] and one long corresponding to either carboxylate and/or water coordination [4]. Remarkably, a strong additional metal contribution indicates the presence of actinide neighbors. Overall, the NTA complex has been adjusted with *6* O at 2.33 Å (σ^2 = 0.0066 Å^2) (the two additional N are invisible with the EXAFS probe) and the DDPDD complex with 2-3 O at 2.21 Å (σ^2 = 0.0063 Å^2), 6-7 O at 2.41 Å (σ^2 = 0.0071 Å^2) (6+3 fixed to *9*) and 1-2 Np at 3.81 Å (σ^2 = 0.0041 Å^2). Very similar results have also been obtained with Th(IV) and Pu(IV) (EXAFS spectra not shown). The model proposed to date is an actinide chain with hydroxo bridges and bridging or terminal peptides. The similarity between all the peptide complexes, whatever the position of the D chain supports the NMR results suggesting that only the amide groups of the principal chain play a role in the complexation. On should add that in the same conditions without any peptide, the neptunium cations precipitates and forms colloids. This result confirms the essential role of the peptide in the complexation process.

These results suggest that unlike the NTA case, oligomeric species are formed with peptide ligands. However surprisingly enough, the sequence or length of the peptide does not seem to have any effect on the structure of the complex. The corresponding cyclic peptides are being synthesized. Cyclic decapeptides are topologically constraint and this should induce a major modification of the actinide coordination sphere by disabling the hydroxo bridging chain.

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[1] 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, NEA n° 6288 (2007), 235-247.

[2] L. Bonin, A. Jeanson, D. Guillaumont, M. Grigoriev, S. Coantic, C. Den Auwer, J-C. Berthet, P. Thuéry and P. Moisy, to be submitted.

[3] J. Rothe, C. Walter, M. Denecke T. Fanghänel, Inorg. Chem. (2004), 43, 4708.

[4] L. F. Rao, Z. C. Zhang, P. L. Zanonato, P. Di Bernardo, A. Bismondo, S. B. Clark, Daton Trans. (2004), 2867.