EUROPEAN SYNCHROTRON RADIATION FACILITY

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Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

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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.

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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:** **Complexation of Hf(IV) and iron(III) by cyclic peptides**	**Experiment number**: 30-02-915
Beamline: BM29	**Date of experiment**: from: 18/09/2009 to: 22/09/2009	**Date of report**: 28/10/2009
Shifts: 12	**Local contact(s)**: I. Alliot	*Received at ESRF:*

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Report:

Most data available on the interaction of actinides with biological systems are based on macroscopic measurements, with very few structural information at the molecular level. However, in case of accidental release of radionuclide, internal contamination with actinides (Th, U, Np, Pu, Am) under either acute or chronic conditions has the potential to induce both radiological and chemical toxicity. 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 can thus be considered as elaborated inorganic complexes with well-designed metal active site structures. Besides these functional aspects, metallic interactions strongly influence the folding of a peptide or protein into a stable tertiary and/or quaternary structure. Conversely, cooperative interactions originating from the tertiary structure of the protein and mostly not well understood to date may be at the origin of the selectivity of the binding site. 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. Actinide elements display a very rich chemistry because of the specific properties of their 5f and 6d's valence electrons. At oxidation state +IV, actinides behave mostly as hard acids in the Pearson classification, resulting in strong interactions with hard donor groups as carboxylates. In biological media, surrogates of Pu(IV) have been often considered as a first approach. Fe(III) and Hf(IV) are two examples that have been often considered for instance for their hydrolysis ability comparable to that of Pu(IV)[1].

Since fours years, our approach has been to consider the complexation of **actinide(IV)** on the one hand, surrogates cations and in particular **Fe(III)** and **Hf(IV)** on the other hand with simple synthetic peptides that bear some of the functional groups of a protein binding site without the intricacy of tertiary structure properties. Three years ago, we have selected one model **pentapeptide** with four aspartic amino acids that can mimic part of a protein binding site with hard base groups (called DDPDD). This peptide bears 4 carboxylic functional groups (D = aspartate) with enough chain flexibility to ensure partial folding. It also contains a proline residue (P = proline) that defines a symmetric peptide with some allowed flexibility and potential turn[2,3] facilitating the interaction of aspartate side chain with cations albeit the short length might moderate tertiary effects in a rough approximation. Parallel actinide measurements have been carried on BM20 (ROBL) at ESRF, Fe(III) and Hf(IV) have been carried out on BM30b (FAME). This experimental report focuses on our last results of September 2009 as well as full analysis of previous results of October 2006 (see also report of 30-02-759).

We have investigated the role of this pentapeptide in actinide complexation in buffered aqueous solution with Th(IV), Np(IV) and Pu(IV) in comparison with Fe(III).. The EXAFS data have revealed a long range cation-cation contribution

as well as two distances in the actinide coordination sphere. Combination of these data with spectrophotometric and NMR data strongly suggests that the interaction of the peptide with Th(IV), Np(IV) and Pu(IV) cations relies on a original type of peptidic complexes. For Fe(III), a dimmer has been most probably obtained as suggested by the EXAFS data and complementary molecular dynamics simulation. A paper describing these results has recently been accepted[4]. Molecular dynamics trajectories obtained for the peptide with all the aspartic carboxylates that are protonated first indicated that the peptide was able to adopt two different conformations around the ionic system : i) a mostly extended conformation close to polyproline and ii) a folded β-turn conformation encompassing the Asp2-Pro3-Asp4-Asp5 residues (Figure 6a). Examining the internal energy of the peptide along the obtained trajectories argued for a better stability of the β-turn conformation. After energy minimization of the most representative structures extracted from these trajectories, it was observed that the β-turn conformation was the same and independent of the choice of the two Asp residues complexing both Fe^{3+}. This suggests a preferred orientation of the peptide around the ionic system (Figure 6b).

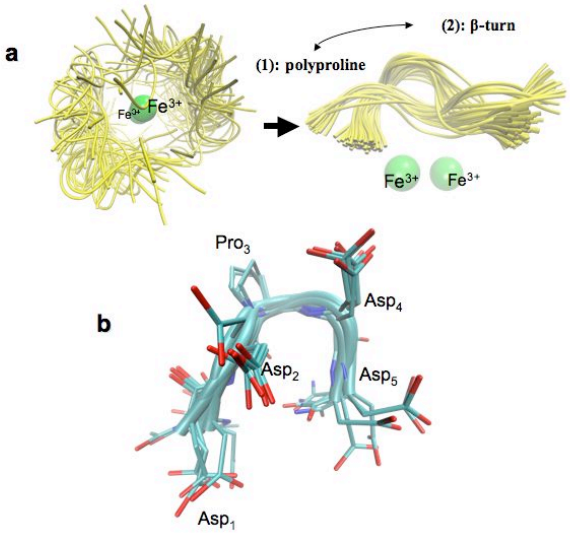

Fig. 6 : Analysis of the molecular dynamics simulations performed for the PP1-2Fe^{3+} system, Asp2/Asp5 complexing Fe^{3+}. a) After a structural fit on the peptide backbone atoms, models compatible with the experimental distances constraints included a quasi-extended polyproline like conformation and a Asp2-Pro3-Asp4-Asp5 β-turn; b) the same β-turn conformation was retrieved in all simulations except for the Asp1/Asp4 couple.

I order to complement this approach and to better understand the complexation ability of this type of peptides, we have produced macroscale quantities of ligand with various sequences : AAPAA, ADPAA and ADPDA (A = alanine) as well as the cyclic analogue c-DDPDDDDPDD. For Hf(IV), we have failed to stabilize a complex with any of the above peptides because of strong competition against hydrolysis[5]. We were however able to measure the complex in acidic solution with nitrilotiacetic acid, an amino carboxylic acid that we have often used as a protecting ligand against hydrolysis of the actinide(IV)[6]. In that complex, the average Hf-O distances are 0.18 Å shorter than the corresponding Pu-O distances in the same complex, in agreement with the values of r_{Hf}(CN=8) =0.97 Å and r_{Pu}(CN=8) =1.10 Å. For Fe(III), two types of results have been obtained. The cyclic decapeptide analogue gives comparable fitting parameters as for DDPDD. This suggests that there is no encapsulation of the cation inside the cycle as expected but complexation similar to that described for DDPDD. For AAPAA and ADPAA, no complexation occurs (as expected for AAPAA when no carboxylate group is present) yielding fitting parameters similar to that of aqueous Fe(III). For ADPDA, a mixing of the aqueous and complexed forms are present in the solution, suggesting that the number and position of the D residue is critical.
In a coming proposal we propose to modify the functional group itself by working i) with peptoides (carboxylic groups on the amide function) in order to test the role of the amide in complexation; ii) with hydroxamic analogues since the hydroxamates are known to play a key role in siderophore complexation. The comparison between actinide(IV) and surrogates as Fe(III) will also be pursued.

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