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## What do we know about actinides-protein interactions?

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**Abstract.** Since the early 40s when the first research related to the development of the atomic bomb began for the Manhattan Project, actinides (An) and their association with the use of nuclear energy for civil applications, such as in the generation of electricity, have been a constant source of interest and fear. In 1962, the first Society of Toxicology (SOT), led by H. Hodge, was established at the University of Rochester (USA). It was commissioned as part of the Manhattan Project to assess the impact of nuclear weapons production on workers' health. As a result of this initiative, the retention and excretion rates of radioactive heavy metals, their physiological impact in the event of acute exposure and their main biological targets were assessed. In this context, the scientific community began to focus on the role of proteins in the transportation and *in vivo* accumulation of An. The first studies focused on the identification of these proteins. Thereafter, the continuous development of physico-chemical characterization techniques made it possible to go further and specify the modes of interaction with proteins from both a thermodynamic and structural point of view, as well as from a their biological activity. This article reviews the work performed in this area since the Manhattan Project. It is divided into three parts: first, the identification of the most affine proteins; second, the study of the affinity and structure of protein-An complexes; and third, the impact of actinide ligation on protein conformation and function.

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**Key words:** Actinides, Osteopontin, Calmodulin, Transferrin, Ferritin, Albumin, Fetuin, Phosvitin, Metallothionein, Hemoglobin, Centrin, spectroscopies, FTIR, EXAFS, TRLFS

## Introduction

Some metal ions, constituting what is called the "metallome", are essential and ubiquitous in biological systems. The occurrence of metal ion complexes in biology is further increased by the diversity of potential biological ligands contained in the cell machinery. Some metal elements are essential for life, others are or may be essential at trace levels, and others are non-essential. But in any case, toxicity depends on their concentration. As a general rule, most essential metals are located in the upper part of the periodic table (mainly the first row), although there are some exceptions. Actinide elements (An) are located at the very bottom of the periodic table and are non-essential elements for life. The actinide family is composed of 15 metallic elements, corresponding to the filling of the 5f layer (from actinium, n°89, to lawrencium, n°103). All actinide isotopes are radioactive, have a high tendency for hydrolysis and present a wide range of redox states (+III, IV, V and VI for the most commons). These radioelements are hard acids, according to Pearson classification, having a particular affinity for hard bases: hydroxide, carbonates, carboxylates, phosphates etc. Uranium (U) and thorium (Th) occupy a specific position within this family because they are the only elements at the origin of the three natural decay chains present today in the earth crust. They were discovered in 1789 by the German chemist Martin Heinrich Klaproth for U and in 1828 by the Swedish chemist Jöns Jakob Berzelius for Th. However, the toxicity of these elements was considered much later, a few decades after the discovery of radioactivity in 1896 by Henry Becquerel and shortly after the discovery of the first transuranic elements from anthropogenic origin, which are neptunium (Np), in 1940, by Edwin Mac Millan and plutonium (Pu), in 1941, by Glenn T. Seaborg (both from Berkeley, CA, USA). Thus, actinide toxicity research, including biokinetics and biochemistry, started in 1942 together with the expansion of the nuclear industry during the second half of the Second World War.

In the framework of the Manhattan Project (1942 – 1946), investigation of the health effects associated with radiochemistry and weapons production was initiated. In 1943, the same year

as the construction of the full-scale plutonium production reactor (Hanford B reactor, WA, USA), the University of Rochester was commissioned by the Atomic Energy Commission to assess the potentially deleterious effects of atomic energy production on human health. Many researchers, from various scientific disciplines, worked on the toxicity of U as well as fluorides, mercury and other chemicals used in the production of nuclear weapons. In 1962, Professor Harold C. Hodge created the first Society of Toxicology (SOT). **([1] Morrow, 2000)**

Early studies described the retention as well as the excretion rates of actinides depending on the element itself and its redox state, but also as a function of its mode of administration (injection, ingestion or inhalation). **([2] Durbin, 2010)** The main target organs of these elements were identified and the toxic impact according to exposure or injection dose was assessed. The sensitivity of various model living organisms was also explored: mice, rats, rabbits, dogs and monkeys (juveniles and mature, males and females). Based on those works, international committees such as the International Commission on Radiological Protection (ICRP) have defined exposure limits for nuclear workers and for the public. **(ICRP reports, 1959 [3], 1979 [4], 1980 [5], 1986 [6], 1993 [7], 1995 [8])**

It is interesting to note that lanthanide elements (Ln) corresponding to the filling of the electronic layer 4f (therefore 1 row before the actinides in the periodic table), have often been used as non-radioactive (thus, more easily manipulated) “analogues” of the actinides. **([9] Choppin, 1983; [10] Vidaud, 2012)** Although they may present some chemical similitudes with their unstable 5f neighbors, their chemistry is mostly limited to oxidation state +III and therefore often differs from that of the actinides. This is particularly true for early actinides for which the most stable oxidation states span from +IV to +VI. This considerably limits the validity of the analogy to oxidation state +III. Nonetheless, lanthanides have often been considered as actinide(III) analogues, “in a first approximation”, because they are all considered as hard acids and large cations with high coordination numbers. For both families, they all have a strong tendency for hydrolysis at physiological pH, although this is modulated by oxidation state.

80 Uranium is the most widely studied actinide to date, certainly because of its natural abundance in the earth's crust and hydrosphere, but also because of its privileged position in the nuclear cycle of energy production. In biological redox media ( $O_2$ ,  $H_2O$ ), independent from its physico-chemical form and from its mode of internalization (solid via the epidermis, particles via inhalation or dissolved in drinking water, etc.) uranium is easily dissolved, oxidized and then  
85 predominantly exists under its main redox state +VI, corresponding to the oxocationic form  $UO_2^{2+}$  (uranyl). Unlike other actinides, U is the most easily excreted element (about 70%, 6 days after injection). ([11] Hamilton, 1947; [12] Durbin, 1957) The 30% fraction retained in the body is shared between the bone (10-15%) and the kidneys (12-25%). ([13] Hamilton, 1948) For all other actinides, regardless of their redox state, the liver and skeleton are mainly  
90 targeted. Np(V) (in its oxocationic form  $NpO_2^+$ , neptunyl) is mainly present in bone (between 30-50%). It is also present in the liver, but to a lesser extent (2-10%). Its excretion rate is quite high, around 40%, after one day. ([14] Durbin, 1987) An(III) (americium, Am and curium, Cm) are retained between 30 and 50% in the skeleton, and up to 50% in the liver. Their excretion rate is very low, being less than 1%, one day after injection. ([15] Ansoborlo, 2006; ICRP  
95 reports 1993 [7], 1995 [8], 1998 [16]) An(IV) (mainly Th, Pu and Np) have the highest degree of affinity for the skeleton with retention rates up to 70%, 50% and 30-50%, respectively and low urinary excretion rates (as An(III)), from 0.4% for Pu(IV) to 6% for Th(IV) after 5 days. ([15] Ansoborlo, 2006) From this short summary, biokinetic data appear to be highly dependent on the element itself and its oxidation state. Another important parameter to assess toxicity is  
100 isotopy because actinides have a double toxicity: a radiological toxicity that dominates for short half-lives (depending also on the decay mode) and chemical toxicity that becomes predominant for the largest half-lives (as for  $^{238}U$  for instance, 4.5 billion years). During the years following the Manhattan Project (1942-1946), biokinetic and toxicological data were considered sufficient for radiation protection purposes, and less attention was given to the  
105 understanding of actinides biochemistry in blood, tissue fluids, and more generally in the mammalian body. During that time, mechanistic studies have been rather scarce. Knowing

that the chemical reactions of actinide ions in warm (37°C), nearly neutral (pH 7.4), dilute saline medium of the mammalian body are dominated by hydrolysis and complexation by metabolites, vectorization and accumulation mechanisms are very difficult to describe.

110 Proteins, large assemblies of amino acids chains, are ubiquitous macromolecules present in any eukaryotic cell and whose functions are extremely diversified. Among them, some could be found in both animal and plants cells (like calmodulin, ferritin, albumin and centrin), while others are specific to the animal cells (like some hyperphosphorylated proteins such as phosphotyrosin or osteopontin). Metalloproteins (which represent more than one third of all proteins)

115 have attracted the attention of chemists and biochemists, particularly since the 1950s when John Kendrew and his collaborators elucidated the first X-ray crystal 3D-structure of a protein, sperm whale myoglobin, indicating the presence of an iron atom. **([17] Kendrew, 1958)** Since (metallo)proteins are involved in the various biological processes of any living system (as enzymatic processes, transportation, electron transfer, oxygen fixation and transportation,

120 transcriptional activation, chelation or metal regulation...) and hold a key position in metabolism, they occupy a privileged position in the investigation of the mechanisms of actinide toxicology. Among the possible metal co-factors, iron, copper and manganese, with varying redox states, zinc, calcium and magnesium, at oxidation state +II, are the most common metal cofactors. To a lesser extent, nickel, cobalt, molybdenum, vanadium or

125 tungsten are also found in the active site of the proteins. These metal co-factors can have a structural or a catalytic role. **([18] Degtyarenko, 2005)** Amino acids are the basic building blocks of proteins controlling primary, secondary, tertiary and even quaternary structures of the protein that are the keys of their activity. The imidazole ring of histidines, the thiolate group of cysteines, the carboxylate group of aspartates and glutamates and the phenolate function

130 of tyrosines are the main groups involved in the chelation of metallic ionic species. However, in view of the diversity of metalloproteins, most amino acid side chains and the carbon skeleton (with oxygen from amide groups or deprotonated amines) can be involved in the chelation of

metal ions. In addition to these donor groups present on the protein itself, many organic cofactors can have a coordinating or chelating function (as porphyrin). Water and inorganic carbonate anions may also be present as ligands of metal ions.

As for many heavy metals (a loosely defined group of elements that include transition metals and some metalloids like arsenic, cadmium, mercury, lead and chromium) ([19] Appenroth, 2010), the molecular mechanisms underlying the chemical toxicity of actinides are poorly understood. It is admitted that heavy atoms interfere with the physiological activity of specific, particularly susceptible proteins, either by displacing essential metal ions or by forming a complex with different functional side chain groups of the metalloprotein. ([20] Lemire, 2013) Recent studies have revealed an additional mode of metal action targeted on unfolded proteins. Particular heavy metals and metalloids have been found to inhibit the *in vitro* refolding of chemically denatured proteins, to interfere with protein folding *in vivo* and to cause aggregation of nascent proteins in living cells. ([21] Tamas, 2014) By interfering with the folding of the nascent or non-native (unfolded) proteins, heavy metals in general may profoundly affect protein homeostasis and cell viability.

This report reviews current knowledge on actinide-protein interaction. In the first section, the identification of actinide's target proteins, mostly in serum, but also in target organs will be presented. In the second part of the paper, the thermodynamic and structural determination of the actinide's complexation sites will be summarized. Finally a third section, smaller, deals with recent works on the impact of actinides on protein conformation and function.

## I. Identification of actinide's target proteins

Among the potential actinide target proteins, the proteins involved in metals transport (in serum) and homeostasis and the proteins present in the main target organs (liver, kidneys and skeleton) have attracted a great deal of attention from the scientific community. We can see

160 that the vast majority of studies devoted to the identification of target proteins are limited to the major actinides U(VI) and Pu(IV), although some studies have also focused on An(III).

- *Serum proteins:*

Serum proteins are of particular interest since they play an essential role in the transportation  
165 of metals and thus actinides, to their different target organs. These proteins have therefore attracted most of the interest from the scientific community. They have been classified into two groups, according to electrophoresis experiments developed by Arne Tiselius ([22] Tiselius, 1937), Nobel laureate in 1948: albumin (60%) and globulins ( $\alpha_1$ ,  $\alpha_2$  including fetuin A,  $\beta$  including transferrin,  $\gamma$  or immunoglobulins, hiding more than 10 000 proteins). Electrophoresis  
170 is a technique that remains very commonly used in medical diagnosis.

The first published data on the interaction of actinides with serum proteins dates back to the 40s. After the Manhattan Project, Muntz and Beliaev ([23] Muntz, 1947; [24] Beliayev, 1959) showed, through electrophoresis, that Pu(VI) is mainly associated with proteins from the  $\beta$ -globulin group. In their study published in 1965 in *Nature*, Boocock and his collaborators also  
175 identified transferrin (Tf) as the main target of Pu(IV) (the main redox state of Pu *in vivo*) in serum *in vivo* after 0.5 hours of exposure to this actinide. ([25] Boocock, 1965) This single-chain glycoprotein has a molecular weight around 79 kDa and consists of 679 amino acids. In “normal” functioning, Tf can complex up to two Fe(III) cations in its two different lobes, namely C and N, leading to the lobes’closure. This conformation change ensures its recognition by the  
180 Tf membrane receptor Tf-R and then its cellular internalization (schematized in Figure 1). Turner et al. confirmed this preliminary result in 1968, with an *in vitro* study of the complexation of Pu(IV) in equine serum. ([26] Turner, 1968)

**Figure 1.** TfR-mediated intracellular iron uptake. (a) Once bound to two Fe(III) ions, the complex Fe<sub>2</sub>Tf  
185 is recognized by the TfR membrane receptor and internalized via endocytosis; metal release occurs at



endosomal pH. This is the proposed pathway for intracellular uptake of exogenous metals such as Cm(III). (b) Metal binding site of the Tf N- Lobe, with Tyr 188, Tyr 95, His 249, Asp 63 and a synergistic carbonate anion participating in the coordination of Fe(III).<sup>14</sup> (c) Structure of the (Tf) –TfR complex, with TfR in green, Tf N-lobes in 2 red) and Tf C-lobes in blue. **([90] Sturzbecher-Hoehne, 2013)**

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They showed not only that Tf is the main target protein in serum, but also highlighted the role of bicarbonate as synergistic anions in the complexation of Pu(IV) by the protein. Later, Taylor and colleagues **([27] Taylor, 1987)** suggested that Pa(V), Np(V) and Th(IV) are also mainly transported by Tf in blood. The authors suggested the internalization of these actinides into

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the liver cells and also their complexation with ferritin (F), another cellular protein involved in Fe(III) storage in mitochondria. They argued that the high affinity of Pa(V) and Np(V) for Tf can be explained by a reduction into Pa(IV) and Np(IV) in physiological conditions. **([28] Taylor, 1998)**

This hypothesis is thermodynamically sounded since those redox potentials fall within the range of redox potentials expected in aqueous media, respectively -0.1V for Pa(V)/Pa(IV) **([29] Fried, 1954)** and +0,596V for Np(V)/Np(IV). **([30] Kihara, 1999)** Wirth came to the same conclusion after having demonstrated a predominant complexation of Np(V) by Tf. **([31] Wirth, 1985)**

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The affinity of An(III) such as Ac, Am, Cm and californium (Cf) for Tf has been less clearly established **([32] Duffield, 1986)**, but only 30% of Am(III) and Cm(III) appears to be associated with Tf. **([33] Cooper, 1981)**

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The case of U(VI) is more ambiguous. In blood, it forms various complexes, mainly with carbonate ions (60%) and proteins (30%). Among the main serum proteins presenting an affinity for U(VI), one finds fetuin A (FETUA) and albumin (HSA) although this latter has a lower affinity than Tf. In the case of HSA, its huge concentration in serum (about 40 g/L) explains its

210 binding capacity for U(VI), simply by displacing the thermodynamic equilibrium in favor of complexation. FETUA protein has a relatively low concentration in serum (40 times lower than HSA) but an apparent affinity constant  $K_D$  ( $K_D = [\text{Ligand}_{\text{free}}]$  when  $[\text{UO}_2^{2+}] = [\text{UO}_2^{2+}\text{-Ligand}]$ )

meaning that  $K_D$  equals  $[Ligand_{free}]$  leading 50 % of  $[UO_2^{2+}]$  occupancy, **([34] Abergel, 2017)** for U(VI) around 30 nM, far higher than the Tf affinity constant equal to 2.80  $\mu$ M. **([35] Basset,**  
215 **2013)** Among a cocktail composed of 15 proteins selected both for their relative abundance in blood and for their potential affinity to U(VI), a calculation shows that FETUA could bind 80% of the U(VI) present in the serum, while HSA, Tf,  $\alpha_2$  macroglobulin and haptoglobin bind only 15% of the remaining oxocation. These results complete previous work where the U(VI) target serum proteins were screened by coupling two-dimensional chromatography of serum proteins  
220 with Time Resolved Fluorescence Spectroscopy (TRFS) of U(VI)-complexes and proteomic analysis. **([36] Vidaud, 2005)** Ten major target proteins were primarily identified, FETUA was identified later. This protein is secreted into the blood at around 1 mg/mL by liver cells. It is known to bind strongly to "young" apatite, to inhibit apatite precipitation and to transport calcium phosphate particles to the bone surface, thus contributing to bone formation and  
225 accretion, and constituting 25% of the fraction of non-collagenous proteins in the bone matrix. It is not excluded that FETUA acts, not only as the major carrier of U(VI) in serum, but also as an agent promoting the biomineralization of U(VI) in bone. **([37] Bourgeois, 2015)** More recently, isoelectric focusing coupled with inductively coupled plasma mass spectrometry (CE-ICP-MS) has been successfully used to characterize the interaction of U(VI) with FETUA, Tf  
230 and Alb along competitions between these proteins, confirming FETUA as the preferred U(VI)-target in serum. **([38] Huynh, 2015)**

*In silico* approaches were also used to identify U(VI) protein targets, in particular by gathering structural information of the uranyl-first shell atoms in proteins from two structural data bases (the Protein Data Bank (PDB) and the Cambridge Structural Databank (CSD)). **([39] Pible,**  
235 **2006)** This approach allowed to screen the potential U(VI)-binding sites in proteins of known crystallographic structures. For instance for the C-reactive protein (serum protein appearing during acute inflammation in the body), this screening methodology highlighted the fact that the U(VI)-binding site is very similar to the Ca(II)-binding site, with a 100 times greater affinity

for U(VI) than for Ca(II). **([40] Pible, 2010)** In 2014, Zhou et al., based on theoretical screening  
240 of U(VI) affine proteins (as illustrated in Figure 2), proposed the use of a U(VI) super-affine  
mutant protein (SUP) with an affinity constant of about 7.4 fM to recover U(VI) from seawater.  
**([41] Zhou, 2014)**

**Figure 2. The main steps in computational screening and design of uranyl-binding proteins.**

245 Primary steps in the computational screening and subsequent design of super-uranyl-binding proteins.  
The desired coordination features are identified (step 1), the protein database is surveyed and a protein  
selected for further study (step 2), possible mutations for the protein are considered (step 3), potential  
uranyl binding sites are sought (step 4), and uranyl coordination geometries for the selective potential  
binding sites are evaluated and scored (step 5). If no hits are identified, the computational algorithm  
250 returns to step 2. **([41] Zhou, 2014)**

*- Proteins involved in bone remodeling:*

After *in vivo* contamination, uranium has been found to be mostly located in calcifying zones  
of bones in exposed rats. It accumulates in the endosteal and periosteal area, in calcifying  
255 cartilage and in recently formed bone tissue along trabecular bone. **([37] Bourgeois, 2015)**  
Milgram and its co-authors showed that osteocalcin protein (synthesized by the osteoblast  
cells and involved in calcium binding) and sialoprotein, two non-collagenous proteins involved  
in bone remodeling, are inhibited following exposure to U(VI). **([42] Milgram, 2008)** Also, the  
excretion level of osteopontin (OPN), a phosphorylated protein secreted by bone cells and  
260 involved in mineralization processes, decreases dramatically in the case of uranium exposure.  
**([43] Prat, 2011)** This protein, together with FETUA have been identified as bone protein  
actinide targets. **([44] Safi, 2013; [45] Qi, 2014; [35] Basset, 2013)** These results were later  
confirmed by using capillary electrophoresis techniques coupled to inductively-coupled mass  
spectrometry. **([38] Huynh, 2015; [46] Huynh, 2016)**

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- *Kidney proteins:*

Kidneys are the main target organ of uranium which is a well-known nephrotoxic agent. The renal function is altered by the uptake of small uranyl particles that are filtered through renal glomeruli and present at the level of the proximal tubule epithelium. The carbonate, citrate and also phosphate complexes of uranyl were suspected to be responsible for the observed cytotoxic effects. ([47] Chevari, 1968; [48] Cooper, 1982; [49] Carrière, 2004)

Transcriptomics and proteomics are two powerful approaches for identifying the molecular events (modulation of gene and protein expressions respectively) involved in the cellular response to U(VI) exposure. Such transcriptomic studies on human kidney cells have shown that the expression of several genes were up- or down-regulated after U(VI) exposure which is also dependent on its concentration. Combined with transcriptomic analyses, it has led to the identification of the U(VI) modulated proteins. ([50] Taulan, 2006; [51] Prat, 2006) But having a modulated expression does not imply that those proteins are U(VI) targets. To this purpose, the implementation of an original chromatography setup using the immobilization of U(VI) by aminophosphonate groups grafted on a chromatographic phase led to the capture of U(VI) affine proteins. ([52] Basset, 2008) Using this technique, Dedieu *et al.* identified 64 U(VI)-affine proteins in soluble extracts of human kidney cells (HK-2). ([53] Dedieu, 2009) Also in 2009, Frelon *et al.* explored rat kidney proteins-U(VI) interactions using isoelectric focusing gel electrophoresis and by laser ablation ICPMS. ([54] Frelon, 2009) They evidenced that U(VI) can bind few proteins and that most of the uranium-protein complexes in this organ are non-covalent but electrostatic ones.

- *Liver proteins:*

Because the liver is not the main target organ of U(VI), data on specific U(VI) affine proteins in this tissue are rare. Surprisingly, Pu(IV) and Am(III) affine liver proteins have not been described in detail, although the liver represents one of the main target organs for An(III) and

An(IV). ([55] Taylor, 1989) However, similar to the aforementioned chromatographic methodology, Aryal *et al.* used the immobilization of Pu(IV) on nitrilotriacetate (NTA) groups to capture Pu(IV)-affine proteins from PC12 cell lines from the rat adrenal gland, another target organ of plutonium. The authors identified 7 potential Pu(IV)-binding proteins ([56] Aryal, 2011): the 78 kDa glucose-regulated protein precursor, the nucleoside diphosphate kinase, the B. actin, the gamma 1 propeptide-like, the galectin-1, a pyruvate kinase and the complement component 1 Q, most of which are calcium binding proteins.

300 - *Other target organs:*

Proteomic approaches on the tumor cell line PC12 from the adrenal gland of rats have led to the identification of affine proteins of the Pu(IV) cation in this organ. ([58] Aryal, 2012) The proteins involved in the transportation of Ca(II), and more generally of divalent transition metals which also have anti-apoptosis functions, have a proven affinity for Pu(IV). This suggests that Pu(IV) could selectively stimulate these proteins and promote the development of cancers. Calmodulin (CaM) is a good candidate among the target proteins of Pu involved in Ca(II) binding. In a study published in 1997, Seeger *et al.* used small angle neutron scattering (SANS) to show that the complexation site of Pu(III) corresponds to that of Ca(II) within the protein. ([59] Seeger, 1997) Calmodulins with a specifically tuned affinity for U(VI) have also been recently successfully engineered in order to recover U(VI) from seawater which represents an important (in volume) uranium reserve. ([60] Le Clainche, 2006, [61] Pardoux, 2012)

Quite recently, some authors have started to take interest in phosphorylated protein binding sites ([44] Safi, 2013, [45] Qi, 2014, [62] Creff, 2015) which, surprisingly, were almost absent from the actinide-protein literature. In a work on the identification of U(VI) target proteins in the ovaries of zebrafish, Eb-Levadoux highlighted the importance of phosphorylations in the ability of proteins to bind U(VI). ([63] Eb-Levadoux, 2017) He showed that the 20 potential ovary target proteins of U(VI) are also proteins involved in Fe(III) homeostasis and that they are

involved in three main biological processes: regulation of oxidative stress, cytoskeleton structure and primary embryonic development.

320 Finally U(VI), in the form of U(VI) acetate, also appears to affect DNA-binding proteins such as "zinc finger" proteins, with an inhibition of the protein function in the presence of the exogenous metal, most certainly *via* a direct but non-specific binding of the metal to the protein. **([57] Hartsock, 2007)**

325 - *Other proteins non-involved in target organs metabolism:*

If the very large majority of research works published up to now concerns the interaction of actinides with proteins involved in the transport *via* the serum and in the metabolisms in the main target organs (skeleton, kidney and liver), few studies have focused on proteins involved in trans-membranous transport and in the penetration pathways of these toxic metals. For  
330 example, Barkleit was interested in the proteins involved in digestion and more specifically in alpha amylase, an enzyme found in saliva. Using spectroscopic and thermodynamic modelling tools, he explored the interaction of this protein with uranium (VI) **([64] Barkleit, 2018)** as well as with curium (III) and its non-radioactive surrogate. (Eu (III)) **([65] Barkleit, 2016, [66] Wilke, 2017, [67] Barkleit, 2017)** On the other hand, Malard and his collaborators identified by  
335 proteomic analysis the response of human lung cells exposed to uranium. **([68] Malard, 2005)** Among 81 proteins they were able to show that cytokeratins CK8 and CK18 are involved in the toxicity mechanisms associated with uranium exposition in lung cells. Still in the family of proteins involved in the mechanisms of respiration, Wan identified the impact of uranium on the structure and function of cytochrome b5, a heme protein capable to bind cell membranes  
340 and involved in electron exchanges. **([69] Wan, 2012a, [70] Wan, 2012b)** Finally the role of glycophorin A (GpA), a trans-membranous protein, was highlighted in the aggregation and hemolysis phenomena observed in human erythrocytes exposed to natural thorium. **([71] Kumar, 2010)**

345 **II. Characterization of complexation sites using modelling, thermodynamics and spectroscopy**

The identification of actinide major target proteins opened the door to a new field of research focusing on thermodynamic and structural characterization of the binding sites of these  
350 macromolecules. This is essential for defining and understanding the impact of An on protein structure and function. Overall, this is the entryway into the understanding of An transport and accumulation mechanisms in the cells and tissues. While the development of spectroscopic techniques has contributed significantly to the latest advances in this field, the results remain incomplete. For thermodynamic data in particular, values are condition dependent and often  
355 not even comparable. The systematic determination of comparable affinity constants remains difficult until now. In addition, the choice of the model system or medium is essential and strongly influences data determination. Composition, pH and concentration are all factors that influence metal speciation and will induce deviations from *in vivo* conditions. Nevertheless, trends in An affinity have been drawn and chelating groups, or families most often involved in  
360 protein complexing sites, have been identified.

Most of the proteins involved in the complexation of U(VI) have structural similarities. They contain carboxylic acids of the aspartate or glutamate amino acids. **([72] Van Horn, 2006)** Amide groups also seem to contribute significantly to the first coordination sphere of the metal.  
365 Very recently, Carugo *et al.* pointed out that U(VI) tends to interact with carboxylic acids from aspartic and glutamic residues, most often via monodentate bonding. **([73] Carugo, 2018)** Water molecules may also complete the metal's coordinating sphere.

- *Transferrin (Tf)*:

370 In the case of Tf, by far the most studied protein for its role in actinide transfer and all-body diffusion, the combination of various spectroscopic tools has been implemented. For this

protein, Fe(III) binding sites were already well-defined and reported in the Protein Data Bank.

**([74] Princiotta, 1975)** According to Yang's work, two Fe(III) cations are bound to apotransferrin via two tyrosine residues (Tyr95 and Tyr188) in the N2-subdomain (a bidentate sulfate and a bidentate carbonate, as synergistic ion, complete the coordination sphere) and four residues two tyrosine, one aspartate and one histidine (Tyr426, Tyr517, Asp392 and His585) (a bidentate carbonate complete the coordination sphere), as schematized in Figure 3. **([75] Yang, 2012)**

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380 Figure 3: (a) Representation of the Fe<sub>N</sub>Fe<sub>C</sub>-hTF structure with subdomains N1 in blue, N2 in green, C<sub>1</sub> in yellow, C<sub>2</sub> in red, and peptide linker in purple. Fe(III) ions are represented as sphere models in brown. Two N-acetylglucosamine moieties (NAG and NAG') are represented as sphere models, (b) Coordination of the Fe(III) in the N-lobe of Fe<sub>N</sub>Fe<sub>C</sub>-hTF. (c) Iron binding center in the C-lobe of Fe<sub>N</sub>Fe<sub>C</sub>-hTF. **([75] Yang, 2012)**

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Boocock and Turner investigated the similarity between Fe(III) and Pu(IV) coordination by observing the displacement of Pu(IV) outside the Tf binding site, in the presence of an excess of Fe(III). **([25] Boocock, 1965; [26] Turner, 1968)** On the other hand, in 1968 Stevens *et al.* underlined the importance of the conformation of the protein complexation site for its ability to bind Pu(IV). **([76] Stevens, 1968)** By eliminating the glycopeptide chain from Tf, they highlighted the fact that Pu(IV) is not able to bind the protein while Fe(III) does not seem to be affected by this modification. Duffield and his co-authors also showed in a preliminary approach which used UV-visible absorption spectroscopy that the interaction of Tf with Fe(III) is similar to that of Pu(IV), Th(IV) and Hf(IV). **([77] Duffield, 1987)** They suggested that the metabolic differences observed in the case of Pu(IV) are more likely to be related to a conformational modification of the protein in the presence of exogenous metals. Harris showed

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that Th(IV) binds Tf as a di-Th(IV)-Tf complex, involving three tyrosine groups, two in the C lobe and one in the N lobe.

But the complexing site of the N lobe appears slightly smaller than the site located within the C lobe. This suggests that Th(IV) has steric limitation for the N site while Pu(IV) could be easily inserted into both sites (the ionic radius of Pu(IV) is  $\sim 0.07 \text{ \AA}$  smaller than Th(IV)). ([78] Harris, 1981) Combining X-Ray Absorption spectroscopy and spectrophotometry, C. Den Auwer and collaborators obtained preliminary results on the possible Tf uptake sites when the protein is exposed to U(VI), Np(IV) and Pu(IV). ([79] Den Auwer, 2005) In a comparative study between the interaction of An(IV) (Pu, Np and Th) with Tf by X-ray Absorption Spectroscopy (XAS), Jeanson *et al.* found some similarities between the behavior of Fe(III) and that of Np(IV), Pu(IV). ([80] Jeanson, 2010) In addition, they suggested that the complexation of actinide(IV) by apo-Tf is quantitative at physiological pH for Np and Pu, while at  $\text{pH} < 7$  complexation is impossible. These results are in agreement with the "cycle" of Tf *in vivo* which complexes Fe(III) at  $\text{pH} = 7.4$ , before release it in cells at  $\text{pH} \sim 5$ . For Np and Pu, complexation may occur via a tyrosine group of the protein with the possible formation of a mixed hydroxo complex. The synergistic anion NTA (trinitrioloacetate) used in the experiment to protect actinides against hydrolysis might also be involved in the metal coordination sphere as a synergistic anion, as suggested by Llorens *et al.* ([81] Llorens, 2005) The authors confirmed a common complexation site for Pu(IV), Np(IV) and Fe(III) while Th(IV) does not seem to interact with the protein under the experimental conditions studied. From a thermodynamic point of view, the complexation constants of An(IV) with Tf were initially assessed on the basis of UV spectroscopic measurements. Yule *et al.* give the complexation constant of Pu(IV)-Tf:  $\text{Log } K_{\text{Pu(IV)-Tf}} = 21.75 \pm 0.75$ . ([82] Yule, 1991) Very recently, Sauge-Merle and collaborators revisited the affinity constants of Pu(IV) and Fe(III) for Tf with an original approach using CE-ICP-MS. ([83] Sauge-Merle, 2017a) Using the NTA anion as a synergistic anion and as a competitor of Tf and working at concentrations low enough to be outside the hydrolysis zone of Pu(IV), they showed that under physiological conditions, the affinity constant of the protein

C lobe (higher affinity site) for Pu(IV) is  $10^4$  times higher than for Fe(III) ( $\text{Log } K_{\text{C Pu(IV)-Tf}} = 22.5 \pm 0.19$  and  $\text{Log } K_{\text{C Fe(III)-Tf}} = 18.06$ , respectively). Based on the same approach, Brulfert determined very recently the complexation constant of Th(IV)-Tf,  $\text{log } K_{\text{C Th(IV)-Tf}} = 18.65 \pm 0.19$  at pH=7, comparable to that of Fe(III) ( $\text{Log } K_{\text{1 Fe(III)-Tf}} = 18.06$ ). **([84] Brulfert, 2018)**

As already mentioned in the introduction, lanthanides (Ln(III)) have often been used as stable analogues of An(III). For example, it has been suggested that only one Gd(III) atom is complexed by Tf via the C lobe. **([85] Frausto da Silva, 1991)** Various studies involving Nd(III), Pm(III), Sm(III), Eu(III), Y(III), Ho(III) and Tb(III) ions lead to the same conclusion. **([86] Luk, 1971; [87] Harris, 1986; [88] Unalkat, 1992)** It is therefore reasonable to consider the same type of interaction with An(III). Like Jeanson **([80] Jeanson, 2010)** with An(IV), Bauer and her co-authors explored in a work combining TRLS and XAS, the coordination modes of Cm(III) and Am(III) with Tf under variable pH conditions. **([89] Bauer, 2014)** They showed that for pH > 7.4, 30% of Cm(III) binds Tf at the same complexing site as Fe(III) (in the C lobe), while at lower pH, a partially coordinated Cm(III) transferrin species is present (possibly at a nonspecific site). At physiological pH, the complexing site of Cm(III) (in agreement with that of Am(III)) is composed of two tyrosine groups (Tyr-188 and Tyr-95), a histidine group (His-249) and an aspartate group (Asp-63) as shown in Figure 4. Three carbonate, bicarbonate or hydroxide ligands may complete the coordination sphere as well as two water molecules, thus increasing the coordination number of An(III) to 9. **([90] Bauer, 2015a; [91] Bauer, 2015b)**

Figure 4. Proposed structure of the Cm(III) transferrin species; X represents additional ligands, such as carbonate, bicarbonate and/or hydroxide (coordinating water molecules are not shown). **([90,91] Bauer, 2015)**

Based on the determination of the complexation constants of Nd(III) and Sm(III), Harris  
450 proposed the affinity constants of Am(III) and Cm(III) ( $\text{Log } K_{\text{Am(III)-Tf}} = 6.3 \pm 0.7$  and  $\text{Log } K_{\text{Cm(III)-Tf}} = 6.5 \pm 0.8$  respectively). ([87] Harris, 1986) But those values are not described as site specific. They are significantly lower than for An(IV), in agreement with the preliminary studies conducted by Duffield indicating a relatively moderate affinity of An(III) for Tf. This might be explained by the large ionic radius of these cations compared to that of Fe(III) or Pu(IV). More  
455 recently, Sturzbecher-Hoene confirmed this tendency with Cm(III). He proposed two complexation constants corresponding to the two complexation sites,  $\text{Log } K_{\text{C Cm(III)-Tf}} = 8.8$  and  $\text{Log } K_{\text{N Cm(III)-Tf}} = 7.0$ , respectively within the C and N lobes of the protein. ([92] Sturzbecher-Hoene, 2013) He also confirmed by TRLS the involvement of the tyrosine group in the metal coordination sphere, leading to an exaltation of the luminescence of Cm(III) (an effect well  
460 known for lanthanides as the antenna effect). Finally, the affinity of the Cm(III)-Tf complex for the Tf receptor (Tf-R) may explain the rapid elimination of An(III) from the blood and its high retention rate by the liver. ([92] Sturzbecher-Hoene, 2013) Another recent study using high-performance liquid chromatography allowed the determination of the complexation constants of different Ln(III), An(IV, VI), as well as Fe(III) with the Tf-R, the Tf receptor. ([93] Deblonde,  
465 2013) The authors confirmed the highest affinity for the receptor with Fe(III), while the Pu(IV)<sub>2</sub>-Tf complex has the lowest affinity to the receptor (see table 1). Th(IV) and U(VI) are very well "recognized" by the receptor, while Ln(III), taken here as analogs of An(III), have an intermediate affinity.

470 Although Tf does not appear to be the major target protein for U(VI) in plasma, some studies have described the interaction between U(VI) and Tf. Back in the late 90's, Scapolan characterized the U(VI)-Tf complex by TRLS. ([94] Scapolan, 1998) For low concentrations of U(VI) ( $[\text{U}] = 10^{-6} \text{ M}$ ), he showed the formation of a 2:1 (U : Tf) complex with a global complexation constant of  $\text{Log } K_{\text{U(VI)-Tf}} = 16$ . More recently, Vidaud *et al.* ([95] Vidaud, 2007)  
475 by combining Fourier Transform Infrared (FTIR) and UV-visible (UV-vis) spectroscopies,

showed that two tyrosine groups are involved in the oxocation coordination plane, while histidines do not seem to participate. This unsuitability of the Fe(III) ligands to bind U(VI) may be explained by the particular size and shape of the oxocation. Therefore, the lobes' closure cannot be complete and the U(VI)-Tf complex cannot be recognized by the transferrin receptor, 480 inhibiting/limiting thus its internalization into the cells. Montavon combined measurements in UV-vis and TRLS spectroscopies and highlighted the formation of ternary complexes with carbonate ions acting as synergistic anions. **([96] Montavon, 2009)** In these conditions, the complexation constants were also assessed:  $\log K_{C-U(VI)-Tf} = 13$  (high affinity site) and  $\log K_{N-U(VI)-Tf} = 12$  (low affinity site). During this same time, the complexing constants of U(VI) with the 485 proteins Tf, HSA, F and metallothionein (MT) have been reassessed on the basis of TRLS measurements. **([97] Michon, 2010)** The complexation constants for U(VI)-Apo-Tf appear to be slightly higher than those observed for U(VI)-HSA, U(VI)-MT and U(VI)-F (see Table 1). More recently, Wang used density functional theory to explore the mechanisms of U(VI) binding to Tf and precised the role of the carbonate ligand. **([98] Wang, 2017)** Benavides- 490 Garcia proposed an *ab initio* quantum mechanical computational approach to model the IR spectrum of U(VI) complex with human Tf and brought structural insights into the U(VI)-Tf binding. **([99] Benavides-Garcia, 2009)**

The case of Np(V) oxocation has been relatively underexplored since it shows a tendency to 495 reduce to Np(IV) *in vivo*. Racine *et al.* have shown that it binds relatively weakly to apo-Tf and that citrate and carbonate anions may act as competing ligands with the metal. **([100] Racine, 2003)** These results are in line with the early work published by Durbin in 1998. **([101] Durbin, 1998)** The geometry of the oxocation itself (as for U(VI)) is highlighted in order to explain its relatively low interaction with the protein. One can also mention that as a general rule of thumb, 500 Np(V) has less ability for complexation than U(VI) simply because its net positive charge is decreased.

*Other serum proteins : Albumin(HAS), hemoglobin*

Although HSA is not the preferred target of actinides in blood plasma, its interaction with U(VI) and Th(IV) has been explored using conventional tools: UV-vis, FTIR, TRLS and circular dichroism (CD) spectroscopies. ([102] Ali, 2016) In the case of HSA, the carbonyl and the amide groups are the main groups involved in the interaction with the metal. Moreover, using the strong fluorescence of the protein (due to the presence of tryptophan residues), the authors showed that U(VI) affinity is slightly higher than that of Th(IV) at 298K. The secondary structure of HSA seems also to be affected by the presence of the actinides since there is a decrease in  $\alpha$  helices and an increase in  $\beta$  sheets indicating partial protein unfolding.

Hemoglobin, one of the most abundant proteins present in red blood cells and composed of 4 hemic groups complexing Fe(II), is not an proven target of actinides *in vivo*. However, its mode of interaction with U(VI) and Th(IV) has recently been investigated. ([103] Kumar, 2016) In a way quite comparable to albumin, the authors showed that both cations interact with the protein, mainly via carbonyl and amide groups, resulting in a loss of alpha helix conformation.

*Calcium binding protein : calmodulin (CaM):*

As already mentioned in the previous paragraph, CaM, an ubiquitous protein found in all life, is responsible for the regulation of Ca(II), with 4 Ca binding sites and a modulator of other protein activities, by modifying its conformation when interacting with the metal. CaM is an interesting example since it has been tuned for uranyl affinity. ([104] Le Clainche, 2005) The structural environment of U(VI) and Np(V) oxocations in interaction with CaM was reported by Brulfert *et al.* using a combination of XAS and DFT calculations. ([105] Brulfert, 2016) Coordination occurs via two carboxyl groups (with mono- and bidentate interactions) and one carbonyl group. The coordination sphere is completed by a water molecule (at pH=3) or a hydroxyl group (at pH=6). The coordination sphere of the Np(V) is similar to that of the U(VI). The study revealed the importance of the role of little explored small ligands, such as water

molecules or hydroxo groups in the stabilization of the actinide binding site. Pardoux and his  
530 co-authors also explored the influence of phosphorylation on the ability of proteins to bind  
uranyl. **([61] Pardoux, 2012)** They modified the sequence of the CaM complexation site I  
(already showing a 1000-fold higher affinity for U(VI) than for Ca(II)), by phosphorylating the  
threonine residue in position 9 and by including a tyrosine group in position 7. They showed  
that the affinity of U(VI) is increased from  $25 \pm 6$  nM to  $5 \pm 1$  nM at pH = 6 and to  $0.25 \pm 0.06$   
535 nM at pH = 7. FTIR data are in agreement with this increase because they show that the  
phosphate groups are directly involved in cation complexation and that the band corresponding  
to the antisymmetric vibration of the oxocation undergoes a bathochromic displacement of 923  
to 908  $\text{cm}^{-1}$ , characteristic of an interaction with phosphoryl groups.

540 **Figure 5.** Structural models obtained by MD simulations for the CaM1P–U complex: A) with  
monodentate coordination of the deprotonated phosphoryl group, B) with bidentate coordination of the  
deprotonated phosphoryl group, and C) with a protonated phosphoryl group. D) Structural model of the  
CaM–U complex. **([106] Sauge-Merle, 2017b)**

Later, Sauge-Merle *et al.* showed that the coordination sphere is composed of a phosphoryl  
545 group linked through a monodentate interaction to U(VI) **([106] Sauge-Merle, 2017b)** As it is  
the case for the native protein, a monodentate bound carboxyl group and a bidentate bound  
carboxyl group complete the first coordination sphere of the oxocation (see figure 4).

- *Hyperphosphorylated proteins: vitellogenin (Vtg), phosvitin and osteopontin*  
550 *(OPN).*

Very recently, the importance of phosphorylations in the complexation of actinides has  
attracted increasing interest from the scientific community. In particular, the interaction of  
osteopontin (OPN) and phosvitin proteins with Th(IV) and U(VI) has been explored. OPN was  
first identified in 2011 as a potential biomarker of worker exposure in uranium mining. **([43]**

555 **Prat, 2011)** OPN is a hyperphosphorylated protein, intrinsically disordered, involved in the metabolism of mineralization. It is secreted by several organs including the bone cells and the kidneys. Its main role is related to bone remodeling. Because the skeleton represents one of the preferred target organs for all actinides, whatever their oxidation state, the properties of OPN are important for the delineation of the mechanisms underlying the chemical toxicity of actinides in bone. The secretion of OPN by the Human Kidney 2 (HK2) cells decreases following exposure to U(VI) but on another side, the suppression of the protein does not lead to an increase in the cell sensitivity to U(VI). Following this study, the interaction of OPN with U(VI) ([45] Qi, 2014), first, and then Th(IV) ([62] Creff, 2015), was described. In the case of U(VI), Safi *et al.* worked with a His-pSer-Asp-Glu-pSer-Asp-Glu-Val peptide which belongs to 565 the OPN sequence. They used a combination of spectroscopic (XAS, FTIR, TRLS), thermodynamic (Isothermal Titration Calorimetry, ITC) and theoretical (DFT calculations) tools to determine the structuration of the peptide around the U(VI). ([44] Safi, 2013) The coordination sphere of U(VI) in peptides includes a bidentate carboxylate group and a phosphate group bound in a monodentate mode. Two water molecules may complete the 570 coordination sphere, bringing the number of coordination to 5 in the equatorial plane (see Figure 6). The similarity of the EXAFS spectra of OPN and this peptide is important and proves that a simplified peptide model could be used to mimic the protein binding site.

**Figure 6.** Theoretical model based on DFT calculation, for the possible U(VI) interaction site on the peptide mimicking OPN protein possible binding site. ([44] Safi, 2013) 575

In the case of Th(IV), because the typical coordination polyhedron of U(VI) and Th(IV) are radically different, the coordination sphere involves two monodentate phosphate groups and one bidentate carboxylate function. Four water molecules complete the Th(IV) coordination 580 sphere, bringing the number of coordination to 8. ([62] Creff, 2015) In both cases, the

complexation site of the actinide, therefore, seems to be different from that of Ca(II), which only involves carboxylate groups. Moreover, this work confirmed the high affinity of An(IV) for phosphonate groups, perhaps explaining the very high affinity of the latter for the bone matrix itself (retention rates of 70%, 50% and 10-15%, respectively for Th(IV), Pu(IV) and U(VI)). Qi and collaborators confirmed also the importance of phosphorylation in U(VI) complexation and suggested that the protein forms stable complexes with 6 to 9 U(VI) cations. **([45] Qi, 2014)** They also highlighted by CD a partial folding of the disordered protein when interacting with U(VI).

Some research groups have thus developed the design of phosphorylated peptides in order to rationalize the role of phosphorylated amino acids in their interaction with actinides, and more particularly U(VI). **([107] Starck, 2015; [108] Starck, 2017)**

Quite recently, Bourrachot *et al.* highlighted a significant decrease in the production of vitellogenin (Vtg) in zebrafish (*Danio rerio*) upon U(VI) exposition. **([109] Bourrachot, 2014)** Vtg is one of the most phosphorylated proteins of the living cells. At the same time, Coppin *et al.* defined, by TRLS, the affinity constant of the Vtg protein with U(VI) within the two protein sites: see Table 1). **([110] Coppin, 2015)** He highlighted the strong affinity of U(VI) for Vtg in comparison to the other proteins: Vtg > Apo-Tf > Apo-F > HSA > MT, thus confirming the essential role of the phosphate groups. In a vibrational spectroscopy study, Li and her co-authors have already shown the increasing importance of phosphate groups in the U(VI) coordination sphere of phosvitin, a fragment of Vtg, for increasing P/U ratios. **([111] Li, 2010)**

**Table 1.** Compilation of apparent affinity constants and complexation constants for various cations and proteins. Values are reported as published without any further correction.

### 605 **III. Impact of actinides on protein conformation and function:**



Metals in metalloproteins are known for their role in electron exchange, catalysis, or coordination, but they may also modulate the protein structure (locally), or protein folding. When exogenous metals are involved, the question on how these metals will affect folding and  
610 conformation (also in a dynamic way) has become a central topic.

In this field, Tf has time and again been the most explored protein by far. In 1993, using Small Angle X-ray scattering Spectroscopy (SAXS), Grosseman's investigated the conformation modification of Tf in the presence of exogenous Hf(IV) used as a non-radioactive analog of Pu(IV). He suggested that Hf(IV), and by extension all An(IV) cations (including Pu(IV)), are  
615 unable to induce closure of the C and N lobes of Tf, unlike Fe(III). **([112] Grosseman; 1992, [113] Grosseman, 1993)** This first approach to the conformational modification of Tf in the presence of an exogenous metal converges with Planas-Bohne's work which highlights the inability of cells to internalize the Pu(IV)-Tf complex via the Tf-R receptor. **([114] Planas-Bohne, 1990)** But more recently, Jensen demonstrated the cellular (adrenal gland cells, PC12)  
620 internalization of a Pu(IV)-Tf complex **([115] Jensen, 2011)** by combining SAXS and X-ray fluorescence microscopy. In his work, Jensen showed that only the mixed Pu<sub>C</sub>Fe<sub>N</sub>Tf complex adopts a conformation that can be recognized by the Tf-R receptor (see Figure 7). If Tf does not seem to completely block the cellular internalization of Pu, as suggested by Grosseman's early work, it nevertheless restricts access to it.

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**Figure 7.** Metal-dependent conformations of transferrin. (a) Reconstruction of Fe<sub>2</sub>Tf bound to the Tf-R (not shown). The N-terminal lobe is shown in green, and the C-terminal lobe is rendered in blue. The two Fe(III) atoms are shown in red. Adapted from PDB entry code 1SUV5. (b) Conformation of apo-Tf and the mixed Pu<sub>C</sub>Fe<sub>N</sub>Tf complex, reconstructed from small-angle X-ray scattering. Of all the  
630 metallated Tf complexes tested, only the Pu<sub>C</sub>Fe<sub>N</sub>Tf form binds strongly to the TfR in vitro and facilitates cellular Pu accumulation in PC12 cells via TfR-mediated endocytosis. **([115] Jensen, 2011)**

More recently, Brulfert and co-authors showed, using the original method of CE-ICP-MS, that the protein conformation is not modified in the presence of An(IV) (Pu(IV) and Th(IV)). On the  
635 contrary, Fe(III) and In(III) ( $\text{Fe}_2\text{Tf}$  and  $\text{In}_2\text{Tf}$ ) cations induce a closure of the protein lobes and thus a significant modification of the protein structure. **([84] Brulfert, 2018)**

In her work on the interaction of U(VI) with apo-Tf, Vidaud *et al.* combined CD and ITC to highlight the conformational modification of the protein in the presence of uranyl. **([93] Vidaud, 2007)** As presented above, the protein adopts a semi-open conformation of the N lobe (unlike  
640 Fe(III)) which excludes the interaction of the complex with the Tf-R receptor and prevents its internalization into the cell. Again, this comparison underlines the fundamental differences between the actinyl binding mode and the An(IV) binding mode.

Despite the fundamental importance of folding and, more generally, of the tertiary structure modification upon actinide complexation, experimental and computational works are still very  
645 rare. In the future, such studies based on multi-scale approaches will be essential in order to assess the function of the metalloproteins.

## **Conclusion.**

Although early studies describing the retention as well as the excretion rates of actinides go  
650 back as early as the second half of the XX<sup>th</sup> century, little is known about their biochemical pathways. Although researchers have been interested in the identification of the actinide target proteins as early as the 40's, in concomitance with the first biokinetics and toxicological studies, efforts in this field have been quite limited. Moreover, among the actinides, Pu(IV) and U(VI) represent the most studied elements because of their particular place in the history of  
655 the nuclear industry. However, to date, because of the increase in mining activities associated with lanthanide elements, there is growing concern about thorium exposition, as thorium is co-extracted as a by-product of lanthanide mining. **([116] Lu, 2016)**

While the identification of major actinide target proteins represents an essential step towards a better understanding of their accumulation, storage and, more generally, their toxicity, 660 through extensive mechanistic approaches are still lacking.

Affinity (a thermodynamic approach) and structure of the possible binding sites (a spectroscopic approach) is the information needed in order to interpret the biokinetic data. In this sense, transferrin, the main protein targeted by actinides in the serum, has been relatively well documented in the literature. Some studies have also focused on albumin, although it is 665 known to have a lower affinity for actinides. Some other proteins, known for their important role in the function of major target organs, have also been explored (OPN and FETA, in bone and serum for example). Their role in the homeostasis of abundant elements in biology (like Ca or Fe for instance) has also garnered attention. More recently, hyperphosphorylated proteins (OPN, Vtg, phosvitin) have also been gaining notice from the scientific community, particularly 670 because of the affinity of the phosphate groups for actinides but also because of the importance of phosphorylation in all biological systems.

On another note, the impact of actinides on the modulation of metalloprotein conformation and function remains relatively unexplored to date. This is surprising if one considers that the description of those modifications is essential for better understanding the biochemical 675 mechanisms associated with the chemical toxicology of actinides.

There is still, therefore, a wide-open field for investigation on the interaction of proteins with actinides.

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Figure 1.

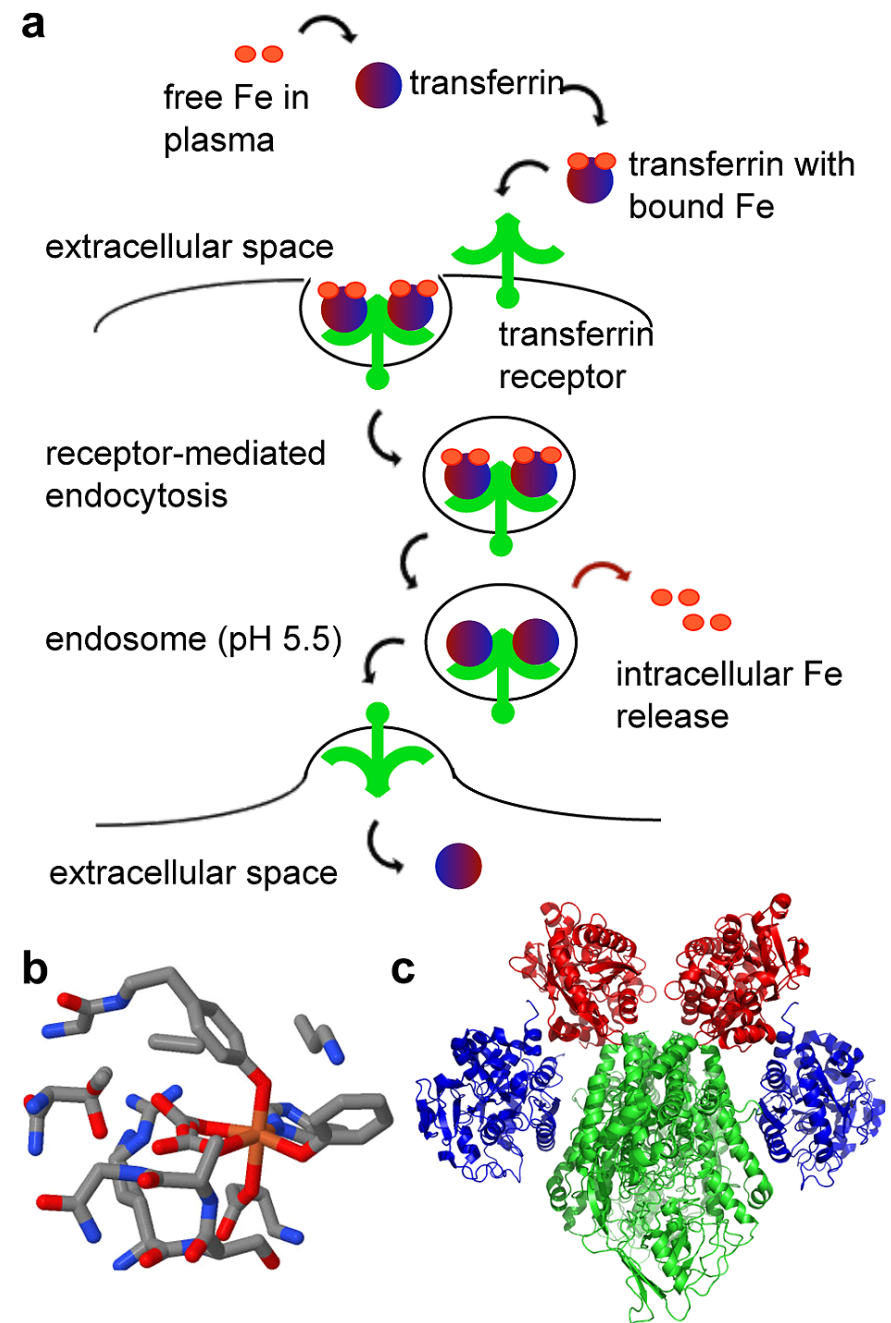




Figure 2.

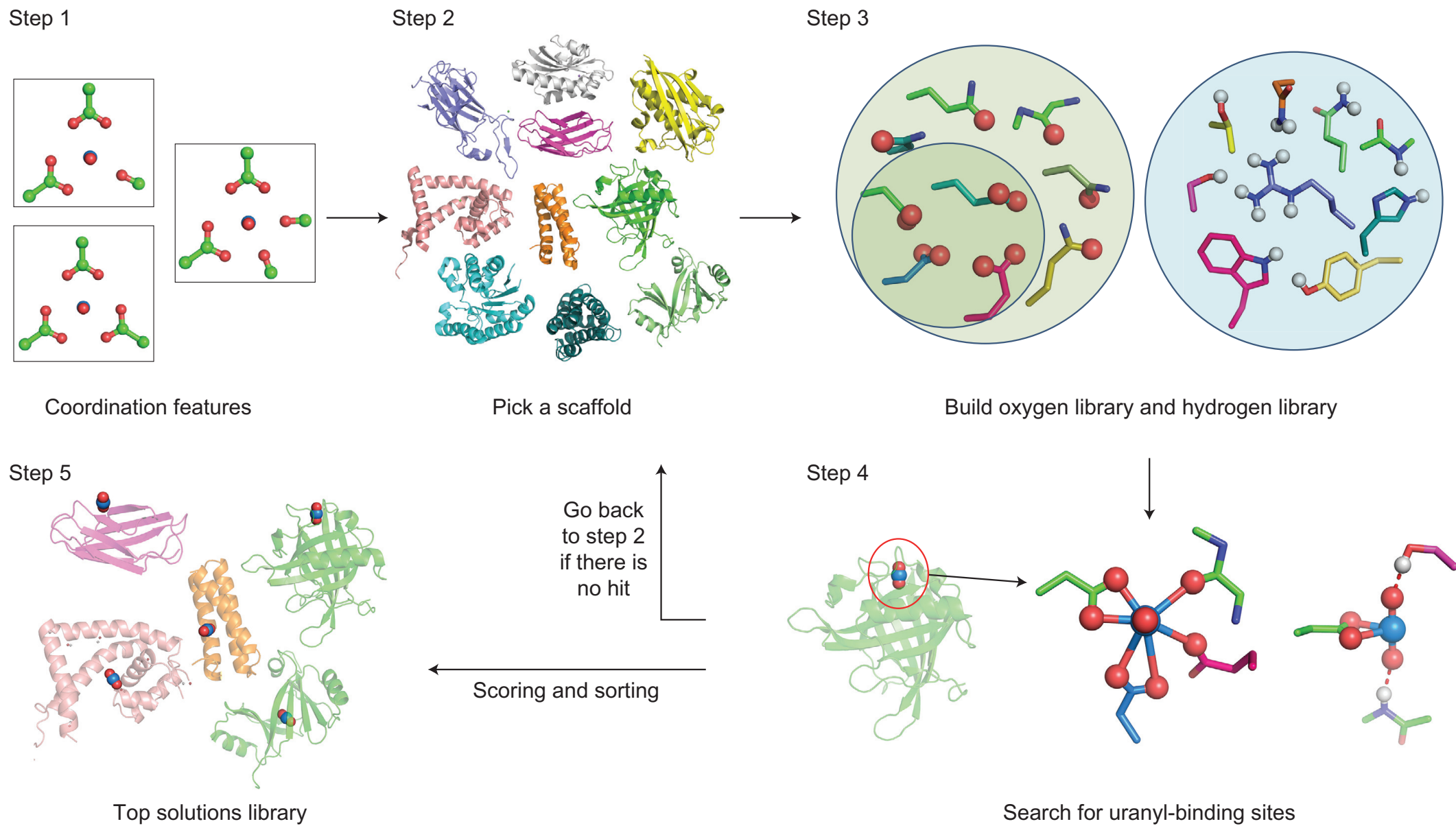


Figure 3.

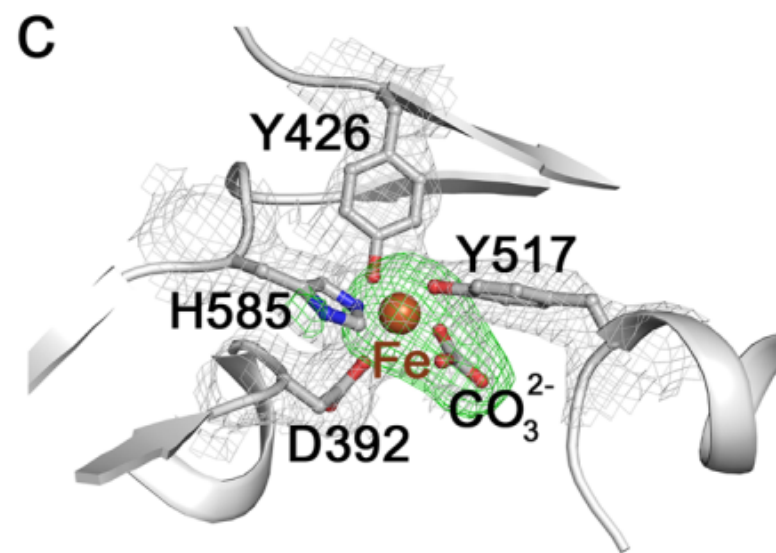
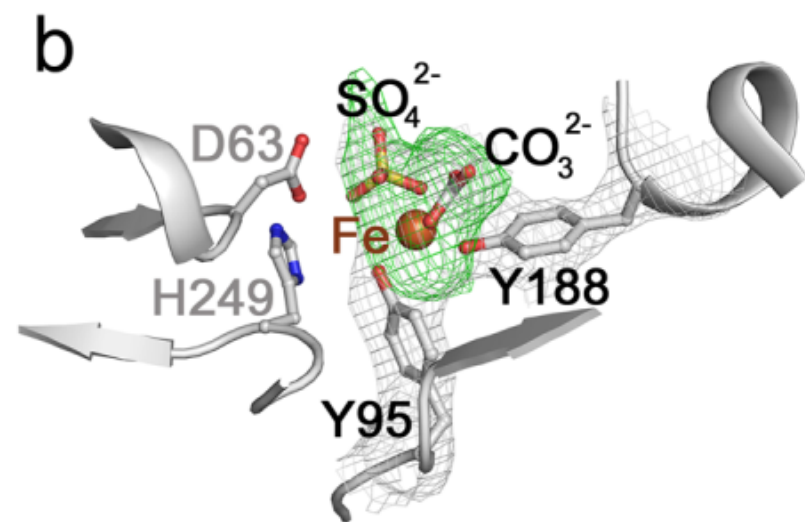
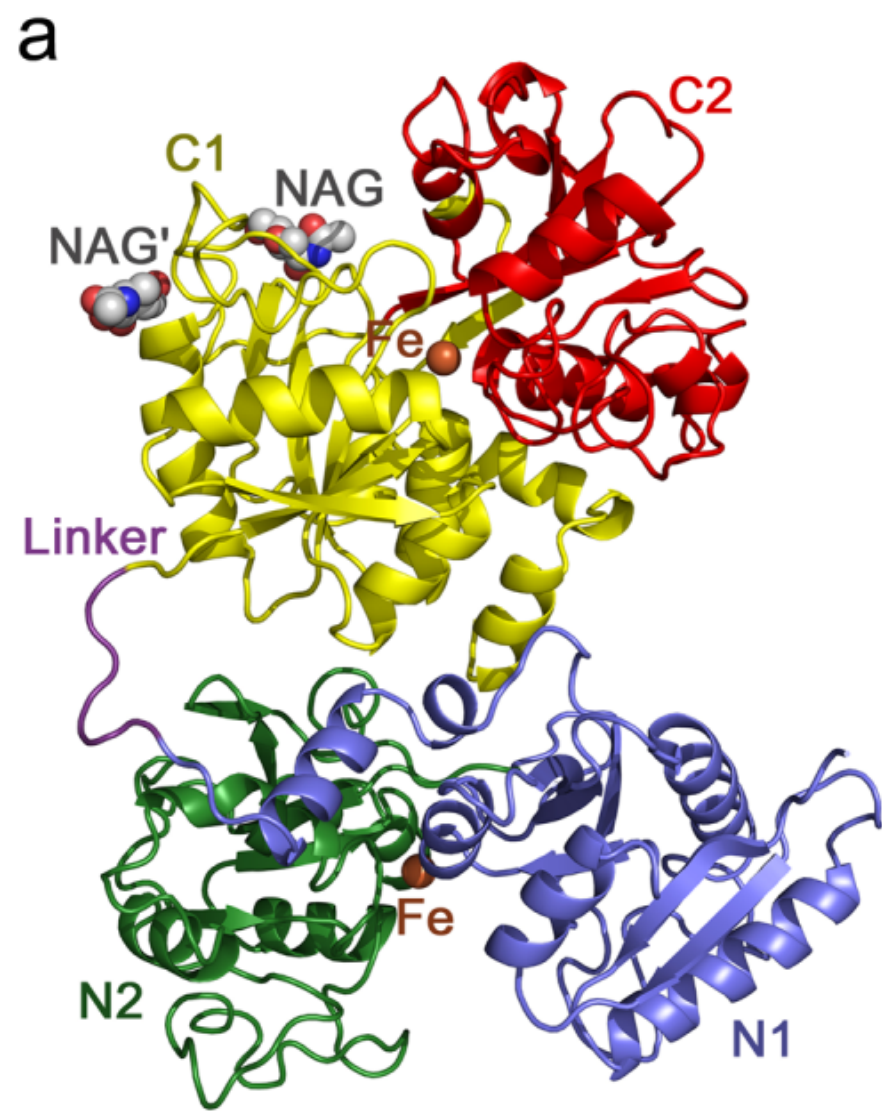


Figure 4.

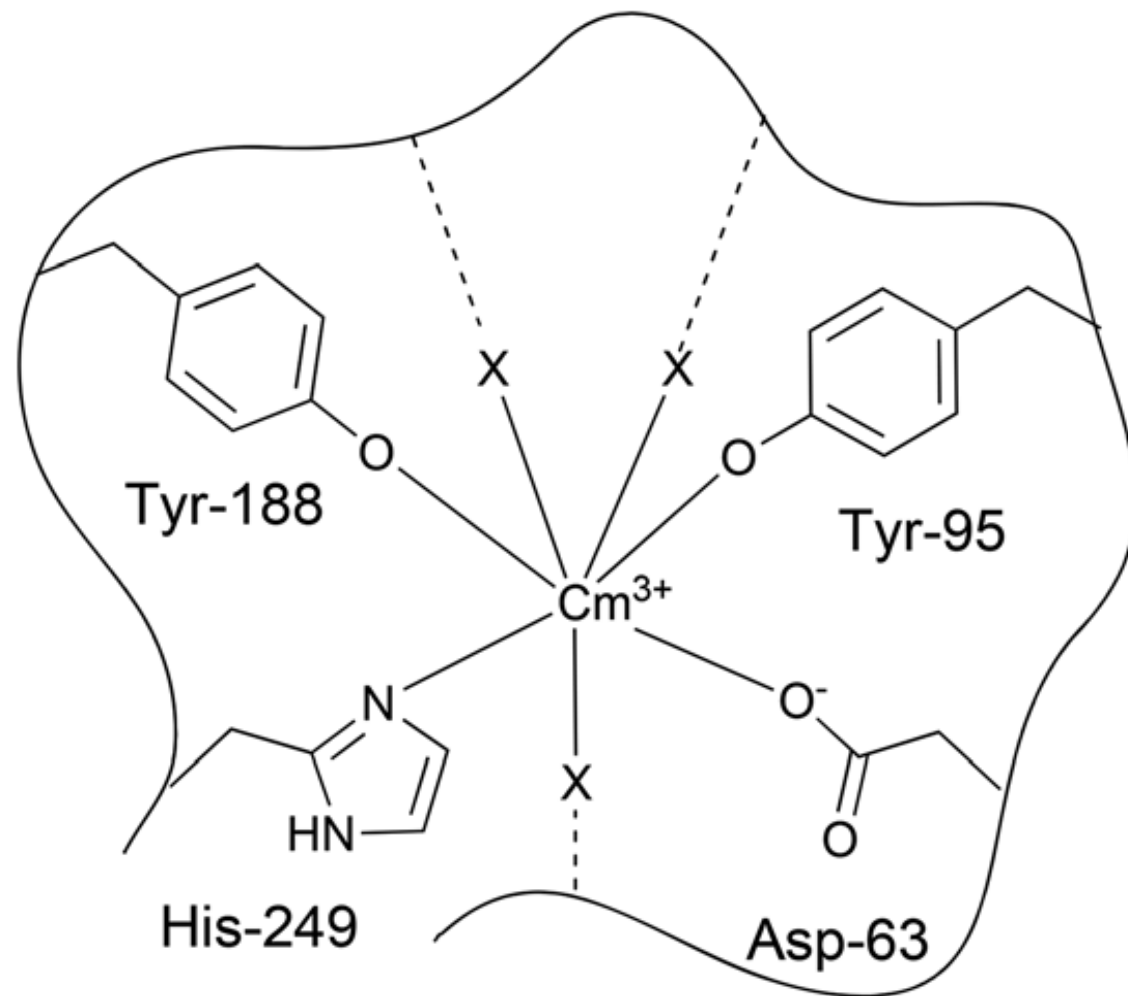


Figure 5.

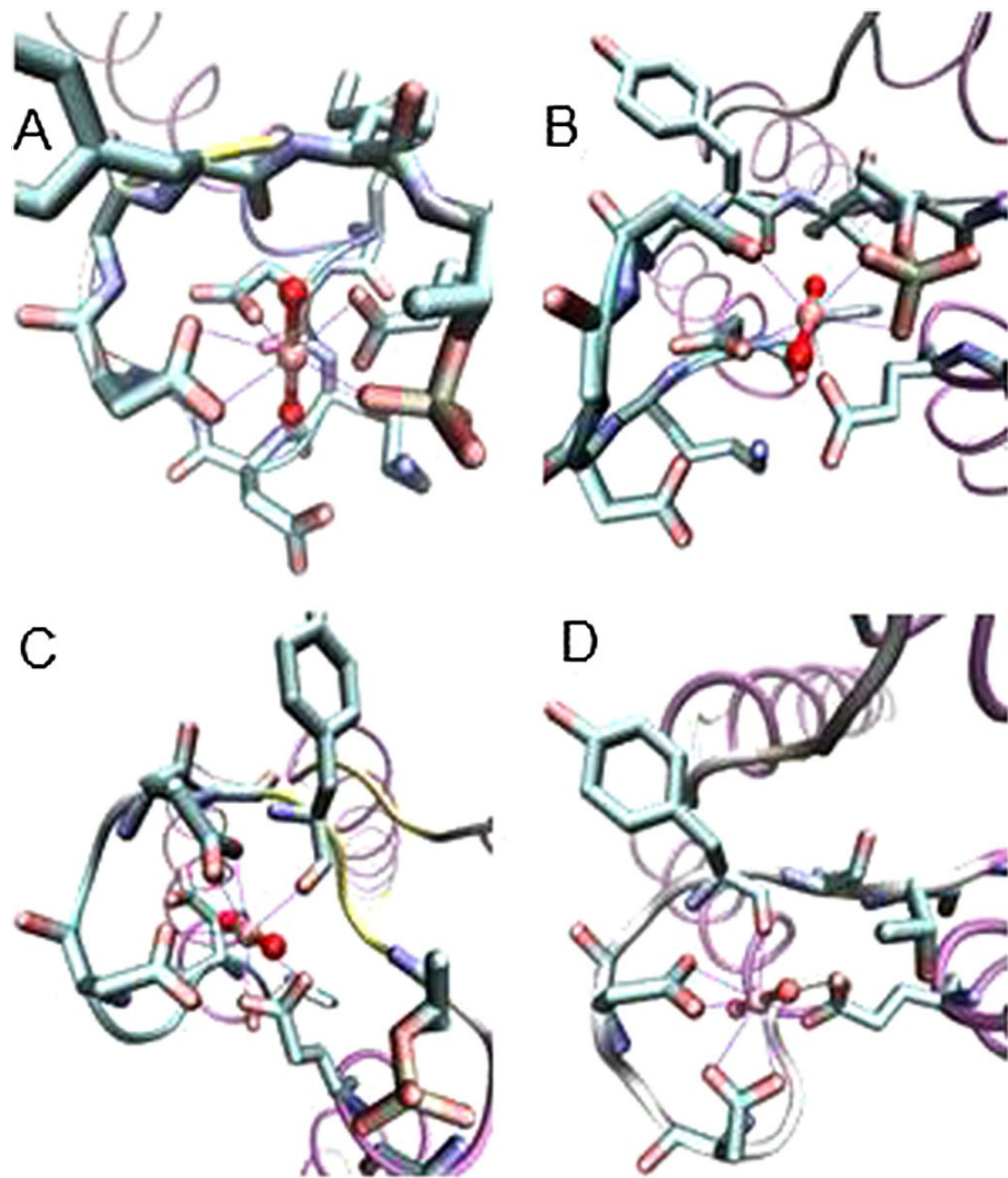


Figure 6.

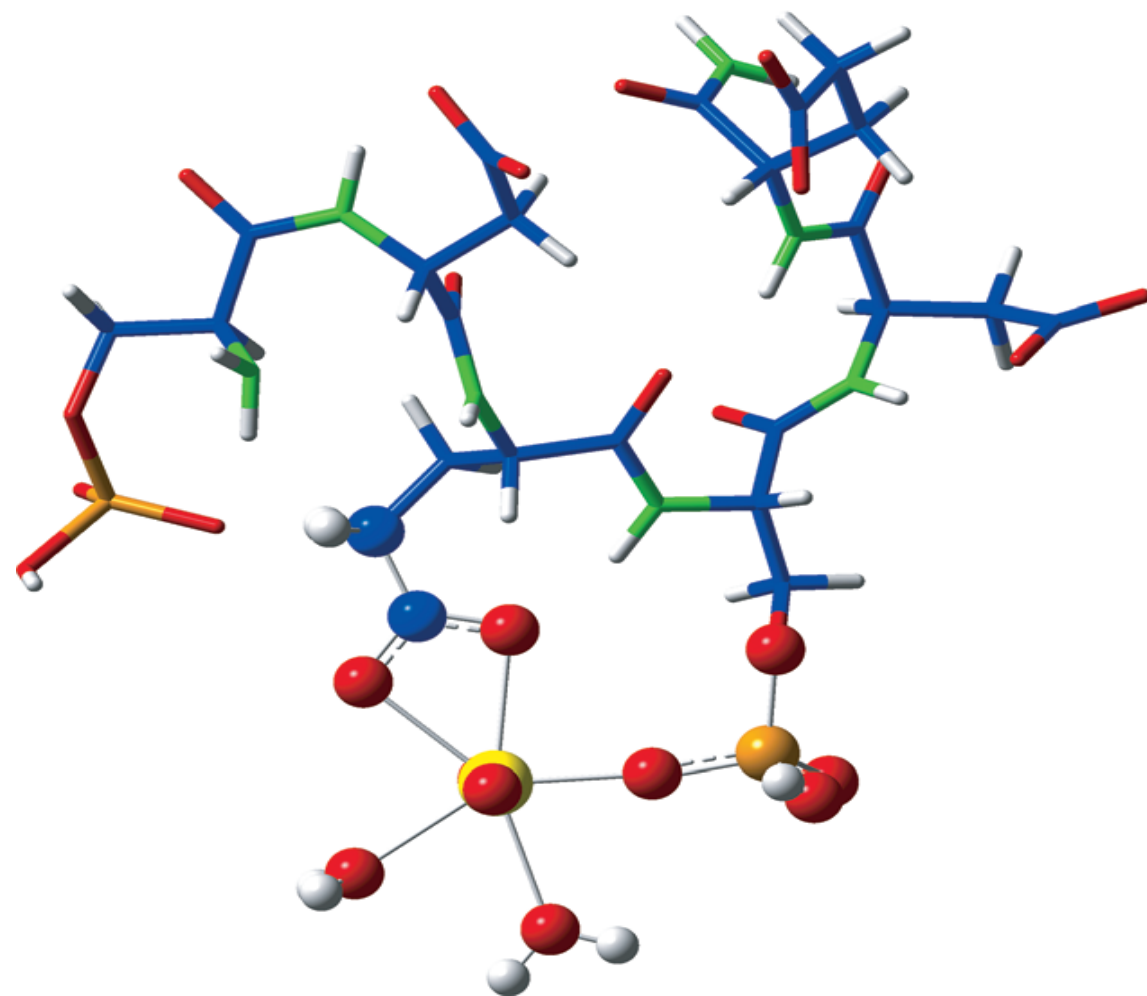


Figure 7.

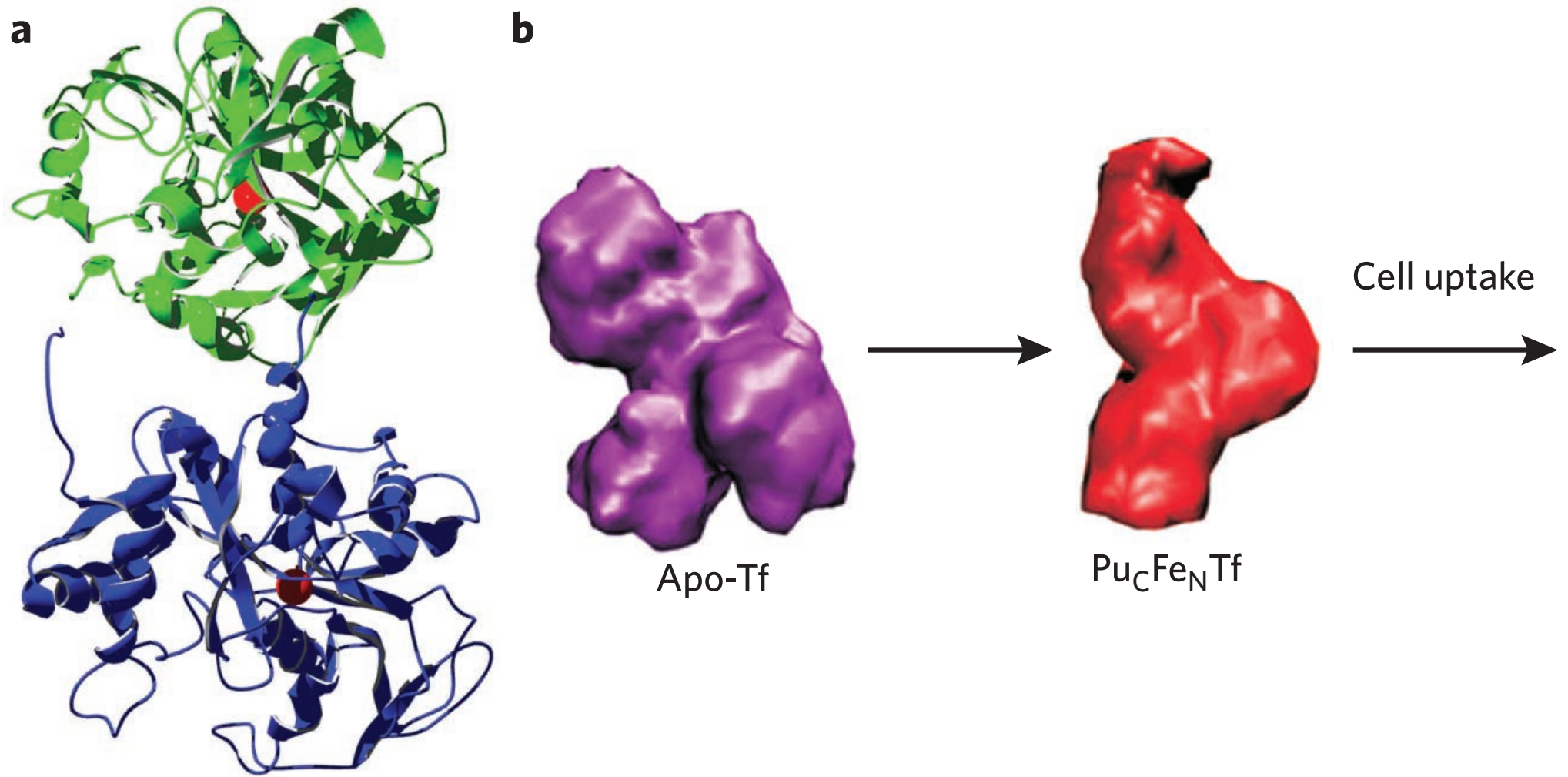


Table 1.

	Fe(III)	Pu(IV)	U(VI)	Th(IV)	Np(IV, V) Cm(III), Am (III)
Tf	$\log K_{C\text{Fe(III)-Tf}} = 18.06$ (Sauge-Merle, 2017)	$\log K_{\text{Pu(IV)-Tf}} = 21.75 \pm 0.75$ (Yule, 1991) $\log K_{C\text{Pu(IV)-Tf}} = 22.5 \pm 0.19$ (Sauge-Merle, 2017)	$K_d = 2.80 \mu\text{M}$ (Basset, 2013) $\log K_{U(VI)\text{-Tf}} = 16$ (Scapolan, 1998) $\log K_{C\text{U(VI)-Tf}} = 13$ , $\log K_{N\text{U(VI)-Tf}} = 12$ (Montavon, 2009) $\log K_{C\text{U(VI)-Tf}} = 7.7$ , $\log K_{N\text{U(VI)-Tf}} = 4.6$ (Michon, 2010)	$\log K_{\text{Th(IV)-Tf}} = 18.65 \pm 0.19$ (Brulfert, 2018)	$\log K_{\text{Am(III)-Tf}} = 6.3 \pm 0.7$ and $\log K_{C\text{m(III)-Tf}} = 6.5 \pm 0.8$ (Harris, 1986) $\log K_{C\text{m(III)-Tf}} = 8.8$ and $\log K_{N\text{Cm(III)-Tf}} = 7.0$ (Sturzbecher-Hoene, 2013)
TfR	$K_{d1\text{Fe(III)-Tf-R}} = 5 \text{ nM}$ and $K_{d2\text{Fe(III)-Tf-R}} = 20 \text{ nM}$ (Deblonde, 2013)	$K_{d1\text{Pu(IV)-Tf-R}} = 0.28 \mu\text{M}$ , $K_{d2\text{Pu(IV)-Tf-R}} = 1.8 \mu\text{M}$ (Deblonde, 2013)			
FETUA			$K_d = 30 \text{ nM}$ (Basset, 2013)		
CaM			$K_{d\text{U(VI)-CaM}} = 25 \pm 6 \text{ nM}$ to $K_{d\text{U(VI)-CaMP}} = 5 \pm 1 \text{ nM}$ pH 6 $K_{d\text{U(VI)-CaMP}} = 0.25 \pm 0.06 \text{ nM}$ pH 7 (Pardoux, 2016)		
HSA			$\log K_{1\text{U(VI)-HSA}} = 6.1$ , $\log K_{2\text{U(VI)-HSA}} = 4.8$ (Michon, 2010) $\log K_{\text{U(VI)-HSA}} = 4.27$ (Ali, 2016)	$\log K_{\text{Th(IV)-HSA}} = 4.27$ (Ali, 2016) $\log K_{\text{Th(IV)-HSA}} = 5.74$ (Kumar, 2016)	
SUP			$K_d = 7.4 \text{ fM}$ (Zhou, 2014)		
MT			$\log K_{1\text{U(VI)-MT}} = 6.5$ , $\log K_{2\text{U(VI)-MT}} = 5.6$ (Michon, 2010)		
F			$\log K_{1\text{U(VI)-F}} = 5.3$ , $\log K_{2\text{U(VI)-F}} = 3.9$ (Michon, 2010)		
VtG			$\log K_{1\text{U(VI)-VtG}} = 5.7 (\pm 1.0)$ , $\log K_{2\text{U(VI)-VtG}} = 4.9 (\pm 1.1)$ (Coppin, 2015)		