Engineering of actinide-chelating EF-hand proteins for bioremediation

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Proteins or peptides are very versatile metal ligands. We chose a protein engineering approach to analyze structural factors governing uranyl or cerium binding and thermodynamic stabilization in proteins and to develop affine and specific ligands for selective actinide uptake, that could be used for bioremediation.

We selected the recombinant N-terminal domain of calmodulin as a structured template that contains two EF-hand Ca^{2+} binding motifs to engineer peptide variants with increased uranyl affinity and specificity, as well as peptides with strong Ce(III)/Ce(IV) affinity. By combining site directed mutagenesis and/or the introduction of phosphoryl groups, we could modulate metal binding properties, as well as the U/Ca selectivity.

The protein variants are characterized by fluorescence spectroscopy or microcalorimetry to obtain thermodynamic parameters of the metal-protein complexation. A modeling approach based on molecular dynamics is used together with FTIR and EXAFS spectroscopies to identify structural characteristics of the protein-metal complexes.

We showed that the introduction of a phosphoryl group in the metal binding loop increased the affinity of the protein for uranyl by almost two orders of magnitude at pH 7.[1] We also obtained a uranyl binding motif with ($K \approx 5\ 10^9$) at pH 6 and pH 7 and a uranyl/calcium specificity of 10^7 without phosphorylation.[2] We also analyzed binding properties of Ce(III/IV) to the EF-hand motif as model for An(III/IV), by combining fluorescence and electrochemistry.

[1] Pardoux, Sauge-Merle, Lemaire, Delangle, Guilloreau, Adriano, Berthomieu (2012) *PLoS One* **7**, e41922 ; [2] Pardoux, Sauge-Merle, Lemaire, Berthomieu, Guilbaud, Delangle, Bremond, Beccia *Patent* WO2014155356 A8