Metalloprotein Engineering

DOI: 10.1002/ange.200805262

Engineering A Uranyl-Specific Binding Protein from NikR**

*Seraphine V. Wegner, Hande Boyaci, Hao Chen, Mark P. Jensen, and Chuan He**

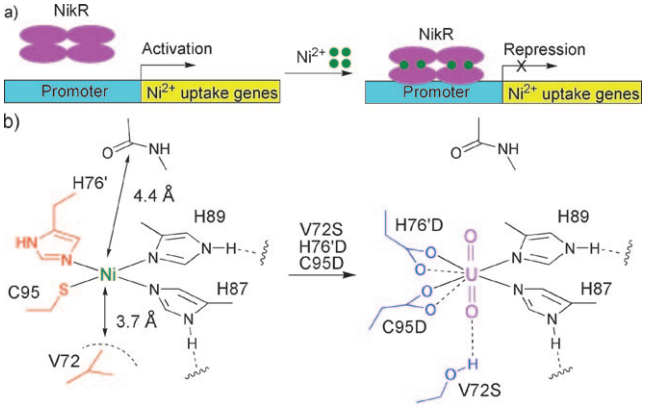
Uranium should not be considered rare, as it is the 49th most abundant element in the earth's crust. The use of uranium as a nuclear fuel and for weapons increases the risk of human exposure, and the storage of radioactive uranium wastes is also a potential environmental problem. Although all uranium isotopes are radioactive, uranium's chemical toxicity generally poses the greater health risk. In contrast to the d-block transition metals, which have crucial biological functions, actinides such as uranium have limited biological activity despite a rich chemistry. Current research on biological aspects of uranium focuses on developing chelation therapies to treat exposure,[1] sensitive detection of uranyl species,[2,3] and remediation of radioactive waste.[4] Some bacteria are known to reduce uranium in salts from its soluble +6 oxidation state to its less soluble +4 oxidation state, which has been proposed as a promising method for bioremediation by decreasing bioavailability of uranium.[5–7]

We sought to design actinide-specific binding proteins using known principles of actinide coordination chemistry. We envision that bacterial systems with actinide-specific transporters, chaperons, storage proteins, and regulators can be engineered, as protein design has been done for d-block metals.[8–11] As a first step we present herein the construction of a uranyl-selective DNA-binding protein using *E. coli* NikR as the template.

Uranium can be present in a number of oxidation states, of which the +6 oxidation state, as the uranyl cation (UO_2^{2+}) and its complexes, is the most stable under aerobic and aqueous conditions. Uranyl is a linear dioxo cation that prefers to coordinate up to six hard donor ligands in the equatorial plane. A number of serum proteins,[12] designed peptides,[13] and DNA[14] have been identified to interact with uranyl, but only a few cases (including transferrin[15,16] and serum albumin[17]) have been studied in any detail. Nevertheless, there are a variety of reported crystal structures of uranyl-containing proteins arising from the use of uranyl as a heavy-metal soaking reagent. These structures reveal that uranyl binds to proteins mainly through carboxylic acid groups such as aspartate, glutamate, and C-terminal carboxylic acid groups.[14,18] Furthermore, hydrogen bonds between the uranyl oxo groups and backbone amide groups have been suggested to enhance interactions between the uranyl cation and proteins.

NikR is a Ni^{2+}-dependent transcriptional repressor of the *nikABCDE* Ni^{2+} uptake system. It is a tetramer in solution and does not bind DNA in the absence of metal.[19–22] Binding of Ni^{2+} ions leads to NikR recognition of a specific promoter DNA and repression of the downstream Ni^{2+} uptake genes[23–25] (Scheme 1a). In the crystal structure of holo-NikR, Ni^{2+} is recognized in a square-planar geometry at the tetramerization interface, with His76 from one monomer and His87, His89, and Cys95 from the other monomer serving as the ligands (Scheme 1b).[26]

In our design, the square-planar coordination geometry of the Ni^{2+} ion in NikR was used as the starting point to construct an equatorial coordination plane for the uranyl core. To achieve a favorable uranyl coordination environment, His76 and Cys95 were mutated to aspartic acid (H76D C95D), which can coordinate either in a monodentate or a bidentate fashion. To accommodate the uranyl oxo groups, Val72 was mutated to serine (V72S), which has the potential to form a hydrogen bond to one of the oxo groups of the uranyl cation (Scheme 1b).



Scheme 1. Nickel(II)-responsive transcriptional regulator (NikR) in *E. coli*. a) NikR binds to its promoter DNA in the presence of Ni^{2+} to repress transcription of the downstream Ni^{2+} uptake genes. b) The Ni^{2+} binding pocket in wild-type NikR and the design of the UO_2^{2+} binding site by a series of mutations (V72S H76D C95D) that provide extra hard ligands in the equatorial plane to favor binding of UO_2^{2+}.

[*] S. V. Wegner, H. Boyaci, Dr. H. Chen, Prof. C. He
Department of Chemistry, The University of Chicago
929 East 57th Street, Chicago, IL 60637 (USA)
Fax: (+1) 773-702-0805
E-mail: chuanhe@uchicago.edu
Dr. M. P. Jensen
Chemical Sciences and Engineering Division
Argonne National Laboratory
9700 S. Cass Avenue, Argonne, IL 60439 (USA)

[**] This work and use of the Advanced Photon Source, was supported by the US Department of Energy, Office of Science, Office of Basic Energy Sciences under contract numbers DE-FG02-07ER15865 (C.H.) and DE-AC02-06CH11357 (M.P.J. and APS use), and a UChicago–Argonne Strategic Collaborative Initiative grant (C.H. and M.P.J.). We thank P. T. Chivers for the *nikR* encoding plasmid and are grateful for the use of XOR/BESSRC-CAT and the Actinide Facility. NikR is a Ni^{2+}-dependent transcriptional repressor protein.

Supporting information for this article, including experimental details, is available on the WWW under http://dx.doi.org/10.1002/anie.200805262.

The triple mutant NikR V72S H76D C95D (NikR′) was expressed and purified (Supporting Information, Figure S1), and the binding of the uranyl cation to NikR′ was tested through dialysis with a uranyl-containing solution and washing away of unbound uranyl. The uranyl content of the resulting protein complex was quantified to be (1.03 ± 0.09) equivalents uranyl per protein monomer by inductively coupled plasma mass spectrometry (ICP-MS) and was confirmed with the colorimetric dye arsenazo III (Supporting Information, Figure S2).

The uranyl-binding affinity of NikR′ was measured by competition experiments between NikR′ and diglycolic acid (dga). Diglycolic acid has a moderate affinity for uranyl and forms two major complexes, $[UO_2(dga)]$ and $[UO_2(dga)_2]^{2-}$, with an overall conditional dissociation constant of $10^{-5.53}$ M at pH 6.5 and 300 mM NaCl after ionic-strength correction.[27] In our experiment, 10 μM protein and 10 μM uranyl were incubated in the presence of different concentrations of diglycolic acid. After separating the uranyl diglycolate complexes from the protein using a concentrating tube, the protein and flow-through were analyzed for uranyl content using the colorimetric reagent arsenazo III (Figure 1 and Supporting Information, Figure S3–S5). The binding curve shows that 10 μM NikR′ has an affinity for uranyl equal to that of 553 μM diglycolic acid, from which a dissociation constant for the uranyl NikR′ complex is calculated to be $10^{-7.27 \pm 0.20}$ M (or 53 nM).

To probe the uranyl binding site in NikR′, uranium L_3 edge extended X-ray absorption fine structure (EXAFS) measurements of the uranyl-loaded protein were made at the Advanced Photon Source (see the Supporting Information). EXAFS has been used previously to elucidate the coordination environment of actinyl ions bound to bacterial surfaces or plants.[28–30] The structural model that best reproduces the EXAFS spectra (Figure 2) is composed of six nitrogen or oxygen donors coordinated at two different distances in the uranyl equatorial plane. These donors come from (1.8 ± 0.3) imidazole ligands and (1.9 ± 0.3) bidentate carboxylate ligands (Table 1). Other models that included coordinated water or monodentate carboxylate ligands in place of the imidazole ligands were unable to reproduce all of the features of the experimental data. The resulting structural model is fully consistent with the coordination of two histidines and two aspartate residues, as expected from the design of the NikR′ binding site. In modelling the EXAFS spectra, it was not possible to differentiate between the *cis* and *trans* arrangements of the ligands, but considering the constraints in the NikR′ metal binding pocket, a *cis* arrangement of these ligands is most plausible. Furthermore, wild-type NikR and the single-point mutants V72S, H76D, and C95D do not bind the uranyl cation (Suporting Information, Figure S6), which supports binding of uranyl to the designed site in NikR′.

To investigate if the engineered uranyl-binding NikR′ retains the ability to function as a metal-dependent DNA-binding protein, gel mobility shift assays were performed. Wild-type NikR binds to its promoter DNA in the presence of

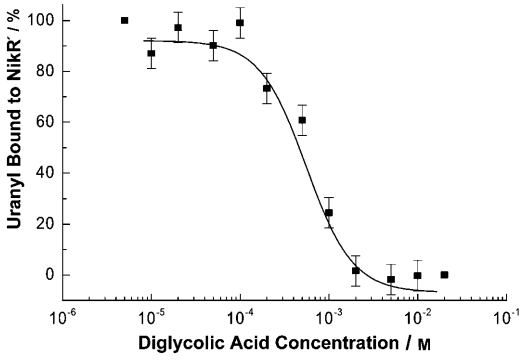


Figure 1. Competition of uranyl (10 μM) binding to NikR′ (10 μM) and diglycolic acid. Extreme points are the control samples: the higher value is protein without competitor and the lower one is uranyl with neither protein nor diglycolic acid.

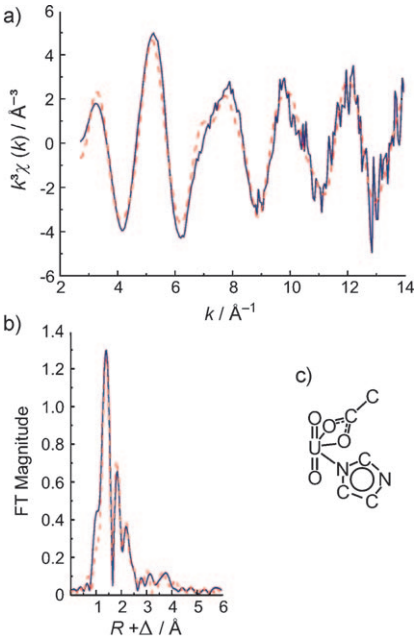


Figure 2. a) Uranium L_3 edge EXAFS of NikR′ loaded with uranyl (data in blue and best-fit model in red) and b) its Fourier transformation, where k is the photoelectron wavenumber, $k^3\chi(k)$ is the k^3-weighted EXAFS function, and $R+\Delta$ is half the scattering pathlength without phase-shift correction. c) Cluster of atoms used to generate the phase and amplitude functions for the best-fitting model.

Table 1: Structural parameters from the EXAFS study of uranyl NikR′.[a]

Scattering shell	N	R [Å]	σ^2 [10^{-3} Å^{-2}]
U=O_{yl}	2[b]	1.782(4)	1.8[b]
U–$O_{carbox,short}$	1.9(3)	2.31(1)	3.0[b]
U–N	1.8(3)[c]	2.47(2)[c]	5.8[b]
U–$O_{carbox,long}$	1.8(3)[c]	2.47(2)[c]	5.8[b]
U–C_{carbox}	1.9[d]	2.90(2)	4(3)
U–C_{imid}	3.6[d]	3.43(6)	13(10)

[a] Uncertainties in the last digit of the varied parameters at 95% confidence are given in parentheses. $S_0^2 = 1.0$, $\Delta E_0 = (0.9 \pm 1.2)$ eV, $\chi^2_{red} = 8.3$. S_0^2 is the amplitude reduction factor, ΔE_0 is the energy shift, χ^2_{red} is the reduced chi-square statistic, N is the number of scattering interactions of a given type, R is the corresponding separation, and σ^2 is the Debye-Waller factor. [b] Fixed parameter. [c] $N_{U-Ocarbox,long} = N_{U-N}$ and $R_{U-Ocarbox,long} = R_{U-N}$. [d] $N_{Ccarbox} = N_{Ocarbox}/2$, $N_{Cimid} = 2N_{Nimid}$.

Ni^{2+} ions (Scheme 1a), and a number of other divalent metal ions such as Cu^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, and Cd^{2+} can also induce protein–DNA complex formation.[31] However, NikR does not bind to DNA in the presence of 50 μM UO_2^{2+} (Figure 3a).

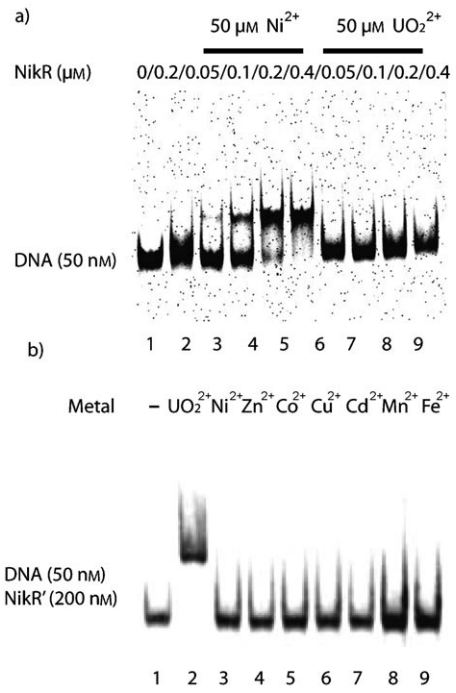


Figure 3. Gel mobility shift assay. a) DNA binding by wild-type NikR in the absence of metal, with 50 μM Ni^{2+}, and with 50 μM UO_2^{2+}. b) DNA binding of engineered NikR′ in the presence of uranyl and various other metal ions (250 μM).

The mutant NikR′ binds to DNA neither in the absence of metal ions (Figure 3b, lane 1) nor in the presence of Ni^{2+} ions (lane 3), but it forms a protein–DNA complex in the presence of UO_2^{2+} (lane 2). In comparison to NikR, the metal selectivity of NikR′ has been altered. Experiments with other metal ions show that the mutant protein only forms the protein–DNA complex in the presence of the uranyl cation while Ni^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+}, Cd^{2+}, Mn^{2+}, and Fe^{2+} ions do not result in any observable complex formation (Figure 3b). Attempts to load NikR with uranyl or NikR′ with Ni^{2+} did not yield any observable metal binding. Thus, this mutant NikR′ shows a uranyl-specific DNA-binding ability.

Small proteins or peptides that recognize lanthanides have been studied.[32–34] Herein we report the design of a uranyl-responsive DNA-binding protein by reengineering the nickel(II)-responsive NikR protein. This study demonstrates that basic coordination principles unique to actinyl ions such as uranyl can be applied in a protein framework to achieve selective uranyl binding. Future work will be devoted to improving the affinity of this or other designed actinide-binding proteins and peptides through further protein engineering or protein evolution.

Received: October 28, 2008
Published online: February 6, 2009

Keywords: bioinorganic chemistry · metalloproteins · nickel · protein engineering · uranium

[1] A. E. Gorden, J. Xu, K. N. Raymond, P. Durbin, *Chem. Rev.* **2003**, *103*, 4207.
[2] J. Liu, A. K. Brown, X. Meng, D. M. Cropek, J. D. Istok, D. B. Watson, Y. Lu, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 2056.
[3] A. K. Burrell, G. Hemmi, V. Lynch, J. L. Sessler, *J. Am. Chem. Soc.* **1991**, *113*, 4690.
[4] G. M. Gadd, C. White, *Trends Biotechnol.* **1993**, *11*, 353.
[5] J. R. Lloyd, *FEMS Microbiol. Rev.* **2003**, *27*, 411.
[6] J. R. Lloyd, J. C. Renshaw, *Curr. Opin. Biotechnol.* **2005**, *16*, 254.
[7] J. D. Wall, L. R. Krumholz, *Annu. Rev. Microbiol.* **2006**, *60*, 149.
[8] H. Wade, S. E. Stayrook, W. F. Degrado, *Angew. Chem.* **2006**, *118*, 5073; *Angew. Chem. Int. Ed.* **2006**, *45*, 4951.
[9] Y. Lu, S. M. Berry, T. D. Pfister, *Chem. Rev.* **2001**, *101*, 3047.
[10] A. K. Jones, B. R. Lichtenstein, A. Dutta, G. Gordon, P. L. Dutton, *J. Am. Chem. Soc.* **2007**, *129*, 14844.
[11] C. Letondor, N. Humbert, T. R. Ward, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 4683.
[12] C. Vidaud, A. Dedieu, C. Basset, S. Plantevin, I. Dany, O. Pible, E. Quemeneur, *Chem. Res. Toxicol.* **2005**, *18*, 946.
[13] L. Le Clainche, C. Vita, *Environ. Chem. Lett.* **2006**, *4*, 45.
[14] J. D. H. Van Horn, H. Huang, *Coord. Chem. Rev.* **2006**, *250*, 765.
[15] S. Scapolan, E. Ansoborlo, C. Moulin, C. Madic, *Rad. Prot. Dosimetry* **1998**, *79*, 505.
[16] C. Vidaud, S. Gourion-Arsiquaud, F. Rollin-Genetet, C. Torne-Celer, S. Plantevin, O. Pible, C. Berthomieu, E. Quemeneur, *Biochemistry* **2007**, *46*, 2215.
[17] M. R. Duff, Jr., C. V. Kumar, *Angew. Chem.* **2006**, *118*, 143–145; *Angew. Chem. Int. Ed.* **2006**, *45*, 137.
[18] O. Pible, P. Guilbaud, J. L. Pellequer, C. Vidaud, E. Quemeneur, *Biochimie* **2006**, *88*, 1631.
[19] E. R. Schreiter, S. C. Wang, D. B. Zamble, C. L. Drennan, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 13676.
[20] P. T. Chivers, R. T. Sauer, *Chem. Biol.* **2002**, *9*, 1141.
[21] S. C. Wang, A. V. Dias, S. L. Bloom, D. B. Zamble, *Biochemistry* **2004**, *43*, 10018.
[22] P. E. Carrington, P. T. Chivers, F. Al-Mjeni, R. T. Sauer, M. J. Maroney, *Nat. Struct. Biol.* **2003**, *10*, 126.
[23] P. T. Chivers, R. T. Sauer, *J. Biol. Chem.* **2000**, *275*, 19735.
[24] S. Leitch, M. J. Bradley, J. L. Rowe, P. T. Chivers, M. J. Maroney, *J. Am. Chem. Soc.* **2007**, *129*, 5085.
[25] N. S. Dosanjh, S. L. J. Michel, *Curr. Opin. Chem. Biol.* **2006**, *10*, 123.
[26] E. R. Schreiter, M. D. Sintchak, Y. Guo, P. T. Chivers, R. T. Sauer, C. L. Drennan, *Nat. Struct. Biol.* **2003**, *10*, 794.
[27] J. Jiang, J. C. Renshaw, M. J. Sarsfield, F. R. Livens, D. Collison, J. M. Charnock, H. Eccles, *Inorg. Chem.* **2003**, *42*, 1233.
[28] M. Merroun, M. Nedelkova, A. Rossberg, C. Hennig, S. Selenska-Pobell, *Radiochim. Acta* **2006**, *94*, 723.
[29] A. Günther, G. Bernhard, G. Geipel, T. Reich, A. Roßberg, H. Nitsche, *Radiochim. Acta* **2003**, *91*, 319.
[30] S. D. Kelly, K. M. Kemner, J. B. Fein, D. A. Fowle, M. I. Boyanov, B. A. Bunker, N. Yee, *Geochim. Cosmochim. Acta* **2002**, *66*, 3855.
[31] S. L. Bloom, D. B. Zamble, *Biochemistry* **2004**, *43*, 10029.
[32] K. J. Franz, M. Nitz, B. Imperiali, *ChemBioChem* **2003**, *4*, 265.
[33] J. T. Welch, W. R. Kearney, S. J. Franklin, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 3725.
[34] L. Le Clainche, M. Figuet, V. Montiardet-Bas, S. Blanchard, C. Vita, *Biotechnol. Bioeng.* **2006**, *95*, 29.

