Supporting Information

Impact of Haloarchaea on speciation of uranium – a multi-spectroscopic approach

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Supplementary section Material and Methods

Batch experiments to study bioassociation of uranium. Batch experiments to study the bioassociation of uranium were also performed with dead cells. The dead cells were prepared by incubation at 3 M NaCl, pC_{H+} 2 for half an hour at room temperature. Afterwards the cells were stained with LIVE/DEAD® BacLight™ Bacterial Viability Kit L7012, Molecular Probes™ to check its viability.

Cell viability. To proof cell viability, cell suspensions (1 ml) treated under different experimental conditions were centrifuged at 18 °C and 10 000 g for 10 min. For Live/Dead staining the Kit LIVE/DEAD® BacLight™ Bacterial Viability Kit L7012, Molecular Probes™, Inc., Eugene, OR, USA was used according to the manufacturer's instructions. The suitability of this kit for halophilic microorganisms was demonstrated by (Leuko *et al.*, 2004). The samples were analyzed with an Olympus BX-61 (Olympus Europa Holding GmbH, Hamburg, Germany) microscope using the imaging software "cellSense Dimension 1.11". Fluorescence was generated by light with excitation wavelengths between 420 and 460 nm using a super wideband filter mirror unit.

X-ray absorption spectroscopy. In addition to the eight samples shown in Fig. 3 in the main text, two additional samples were included in the data analysis to improve the statistics for the mathematical analyses on the acquired XAS data. Hence, in total ten samples were included in the analysis. Up to eleven scans were performed for a single sample and the data were averaged. XANES is often utilized to acquire information about the oxidation states of the target element, while EXAFS is sensitive to the local structural environment of the target atom. In case that several oxidation states and/or several structural environments are present at the same time, the measured XANES and EXAFS spectrum consists of the sum of the contributions from all the co-existing species, hence a spectral mixture is obtained. Pattern recognition methods such as factor analysis can be utilized to deconvolute such spectral mixtures into their single

components, i.e. to calculate the spectral components of pure (i.e. individual) species and their corresponding fractions. In this study, we used ITFA (Malinowski, 2002; Rossberg *et al.*, 2003) and TFA (Malinowski, 2002) for analyzing the XANES and EXAFS spectral mixtures. In the first step of ITFA, the spectral mixtures are decomposed into a set of eigenvectors which allows a reproduction of the data by their linear combination. In this step, the number of spectral components is also estimated based on the impact of the resulting eigenvectors on the reproduction of the data by their linear combination. By using constraints, such as reference spectra identified by TFA and/or fractions of the components, which might be known for some of the samples, the eigenvectors are transformed into the physico-chemical meaningful component spectra and their corresponding fractions for each spectral mixture in the second step of ITFA by the iterative target test (Brayden *et al.*, 1988). Additionally, TFA (or target transformation) is a valuable tool to evaluate possible reference spectra, i.e. target spectra, which are suitable for the reproduction of the spectral mixtures spectra by their linear combination. The applicability of a target spectrum as a suitable reference is estimated by the use of the SPOIL value. According to Malinowski's definition a target spectrum with SPOIL = 1 – 3 is acceptable, 3 – 6 moderately acceptable, and > 6 not acceptable. Moreover, in the case a sufficient number of acceptable reference spectra is obtained, TFA allows the direct calculation of the fractions of the references in the spectral mixtures without linear combination fitting. ITFA was performed on all the collected ten XAS data to have the maximum statistics on the calculations of eigenvectors, while only eight of them are shown in Fig. 3 in the main text mainly because of a clearer comparison with the results from other methods. The ITFA reproduction given in Fig. 3 in the main text is, therefore, based on the eigenvectors calculated from the ten sample data. All the collected ten XAS data and relevant analysis results are provided in Figs. S5 and S7. Both algorithms (ITFA and TFA) can calculate the fractions of the species, although the mathematical treatment differs. An agreement of the resulting fractions from the two different algorithms would, therefore, enhance the reliability of the results.

The XANES data were analyzed independent of the EXAFS data due to the different physical origins of the phenomena. This means that two independent sources of experimental data can be acquired from XAS measurements and the acquired data are complement to one another.

Based on the indicator values (IND_n) calculated from the initial ITFA running on the ten experimental XANES spectra, up to four Eigenvectors can be considered to reproduce the experimental data. When we calculated the principal components based on the four Eigenvectors, we obtained the four XANES spectra for the four principal components, as shown in Fig. S4b. It is obvious from the edge position (i.e. first inflection point) that component 2 corresponds to U(IV), whilst the rest of three components 1, 3 and 4 correspond to U(VI). It is also clear from Fig. S4b that there is no U(V) species in the ten samples. Hence, at least in terms of oxidation states, we need to consider U(IV) and U(VI) to reproduce the XANES spectra of the ten samples. When taking a careful look at the components 1, 3 and 4, one may realize that component 1 is different from the other two components, but components 3 and 4 are similar to one another. This indicates that there are possibly two independent U(VI) species in the samples. However, when we calculate the fractions of three components (i.e. one U(IV) and two U(VI) species), the fraction of U(IV) stays the same as shown in Fig. 3c in the main text, and the fraction of U(VI) in Fig. 3c is just split into two fractions. Therefore, the overall tendency of U(IV) and U(VI) fractions are unchanged, even if we assume three principal components (Eigenvectors) to reproduce the experimental spectra. Given their physical origins, we employed XANES mainly to obtain the information about the oxidation states, whilst EXAFS is employed mainly to obtain the speciation information. In this context, we conclude that two Eigenvectors are sufficient to reproduce the experimental XANES spectra. In fact, the experimental XANES spectra can be reproduced reasonably by assuming only two Eigenvectors (Fig. 3a in the main text and Fig. S5 in SI), supporting the validity of our XANES analysis assuming two Eigenvectors.

Supplementary Figures and Tables

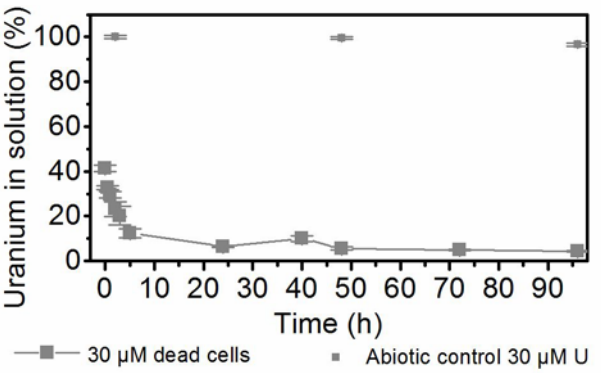


Fig. S1. Uranium content in supernatant after contact with dead cells of *Halobacterium noricense* DSM 15987^T (pC_{H+} = 6, DBM = 0.5 mg/mL, [NaCl] = 3 M, [U(VI)] = 30 µM).

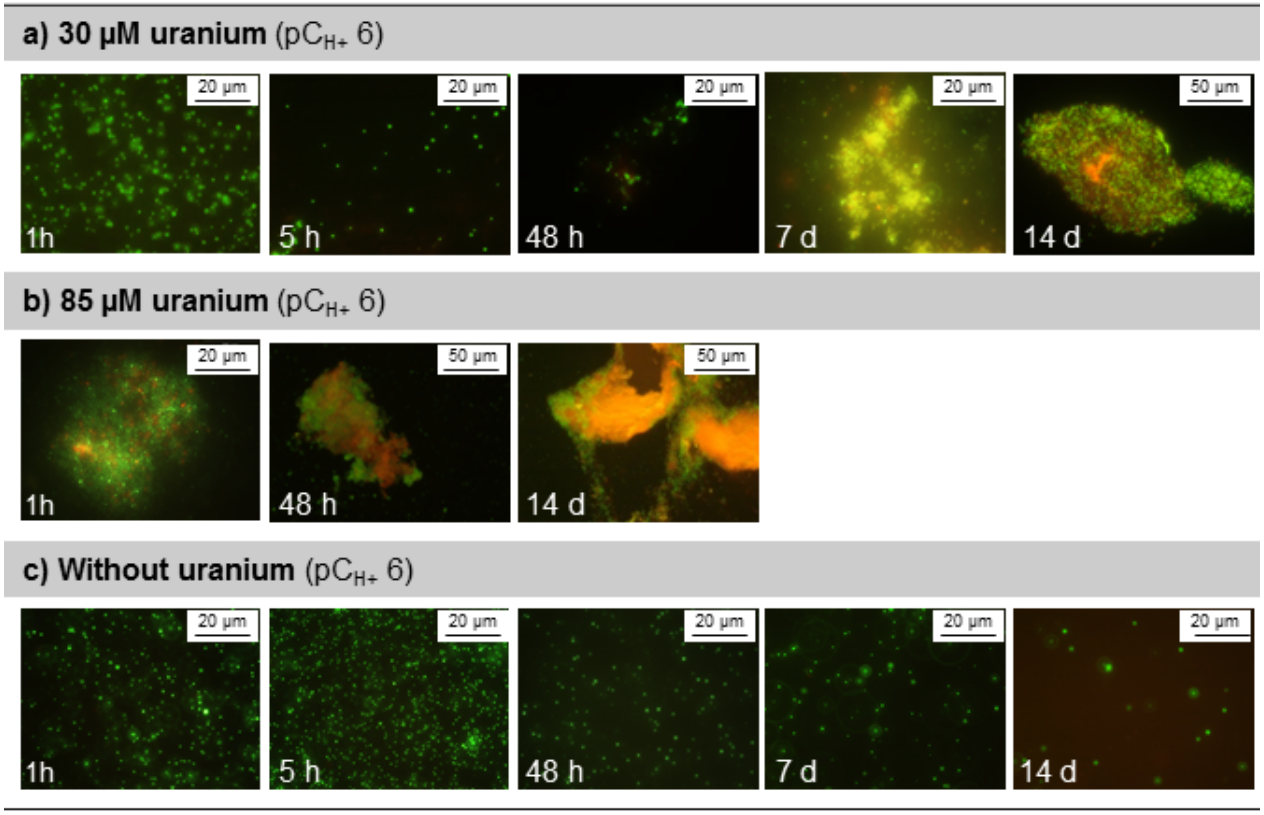


Fig. S2. Fluorescence micrographs of *Halobacterium noricense* DSM 15987^T cells stained with SYTO® 9 (green, viable cells) and propidium iodide (red, non-viable cells) incubated with a) [U(VI)] = 30 µM; b) [U(VI)] = 85 µM and c) without uranium for different incubation times.

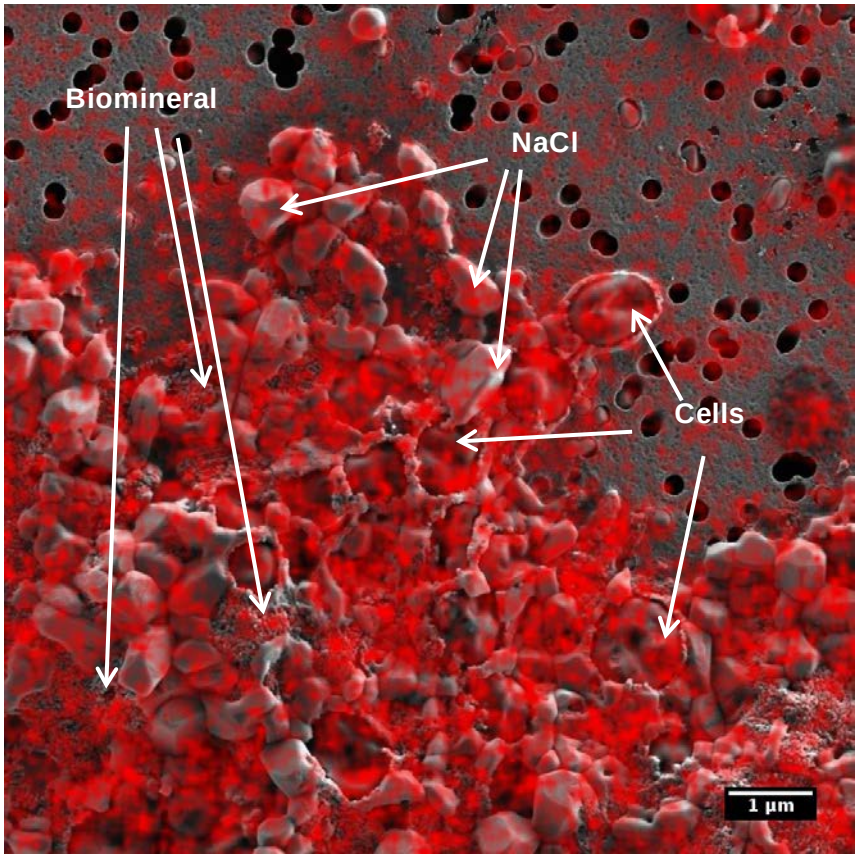


Fig. S3. Scanning electron microscopy image of *Halobacterium noricense* DSM 15987^{T} cells treated with uranium ([U(VI)] = 30 µM, pC_{H+} 6, [NaCl] = 3 M) for 96 h. The red colored image is an overlay of SEM and mapping data for uranium from EDX.

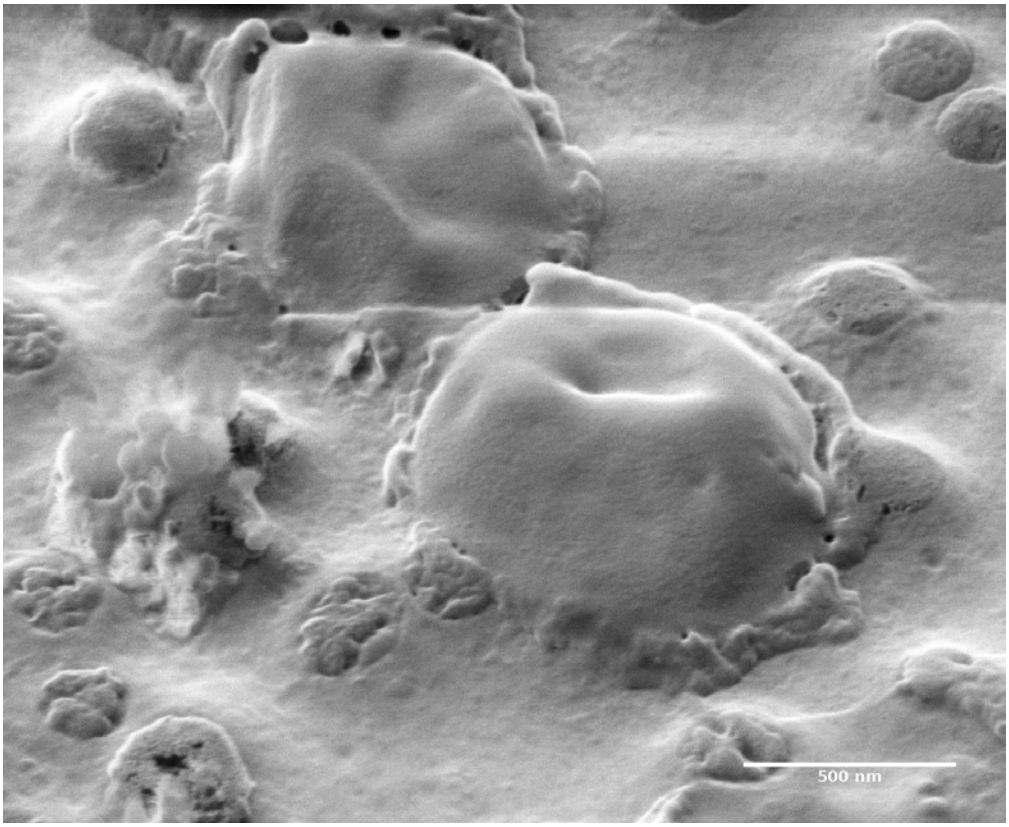


Fig. S4. High resolution microscopy image showing that the cell surface is still intact after 96 h of incubation in the presence of uranium ([U(VI)] = 30 µM, pC_{H+} 6, [NaCl] = 3 M NaCl). For high-resolution images a Zeiss Orion NanoFab Helium Ion Microscope (Carl Zeiss, MA, USA) was used.

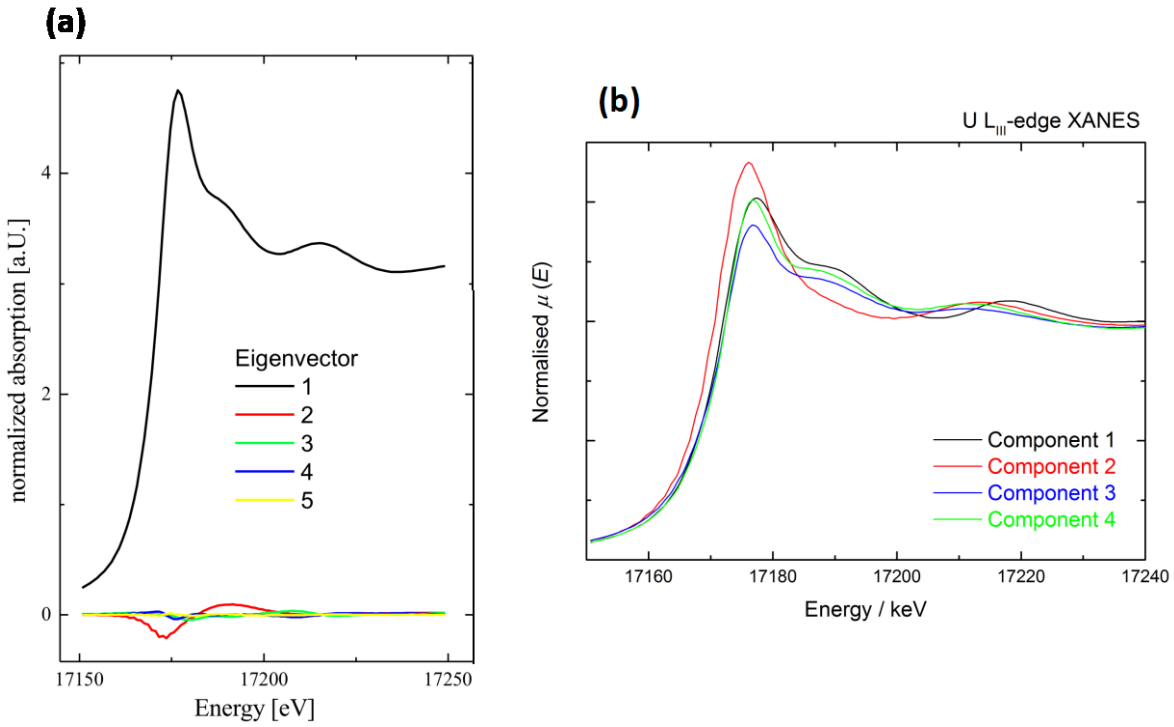


Fig. S5. (a) The first five eigenvectors extracted from the ten XANES spectra from the samples with different uranium concentrations and different incubation times and (b) the first four principal components calculated by ITT based on the first four eigenvectors (see the text in Page S3).

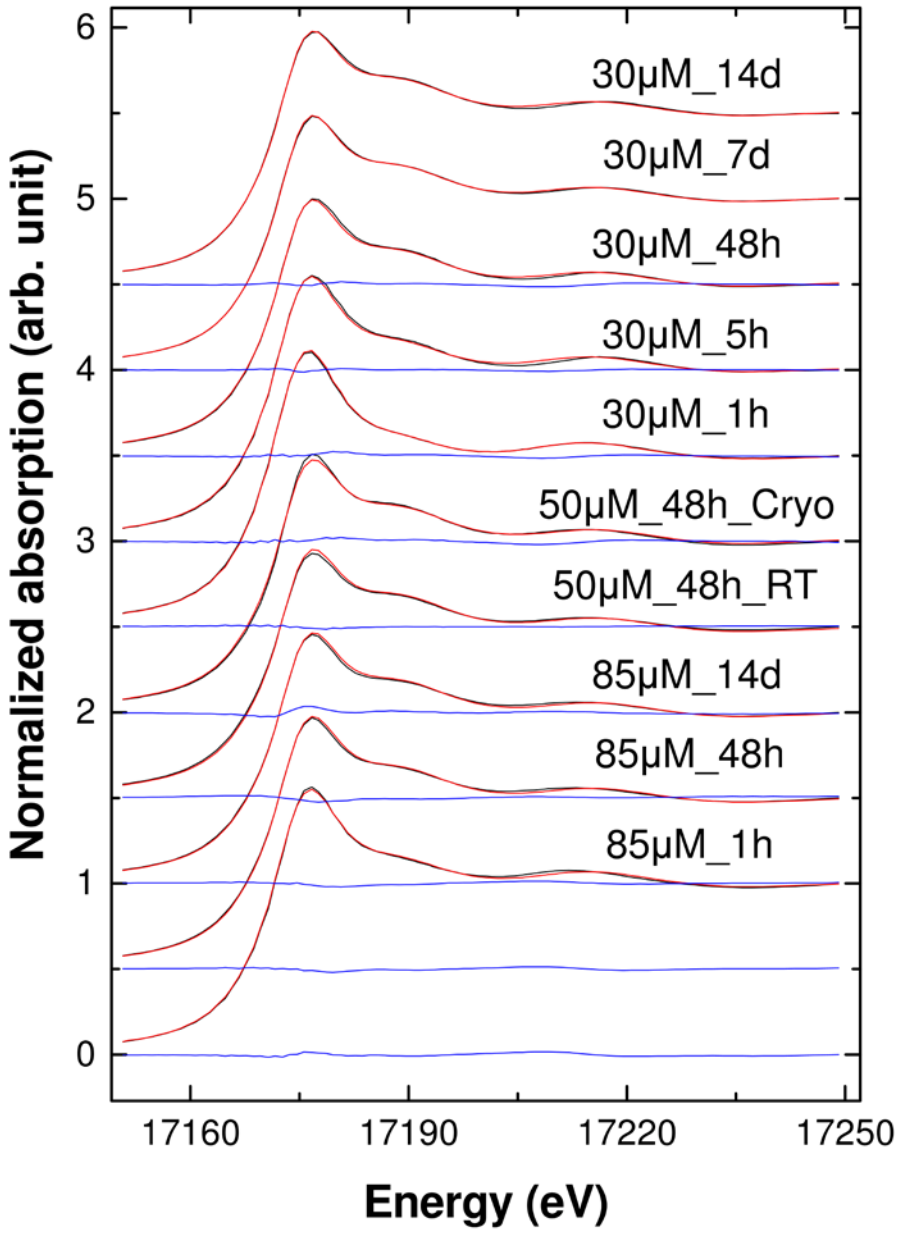


Fig. S6. ITFA reproduction of the normalized U L_{III}-edge XANES spectra of the ten samples with different uranium concentrations and different incubation times by using the first two eigenvectors (see Fig. S5). Experimental data (black), reproduction (red), residual (blue).

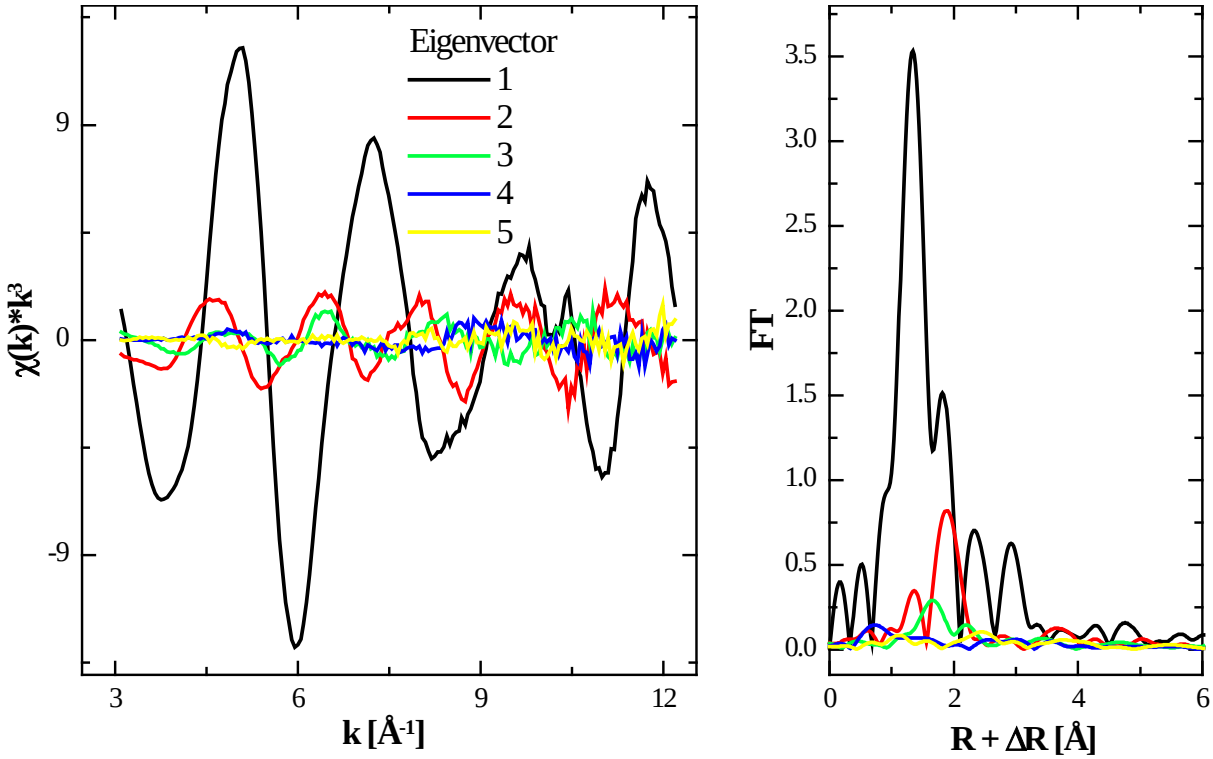


Fig. S7. The first five eigenvectors extracted from the EXAFS spectra from the ten samples with different uranium concentrations and different incubation times. The FT magnitude of the eigenvectors 4 and 5 is in the noise level, suggesting that the eigenvectors higher than 4 are insignificant components to reproduce the experimental spectra.

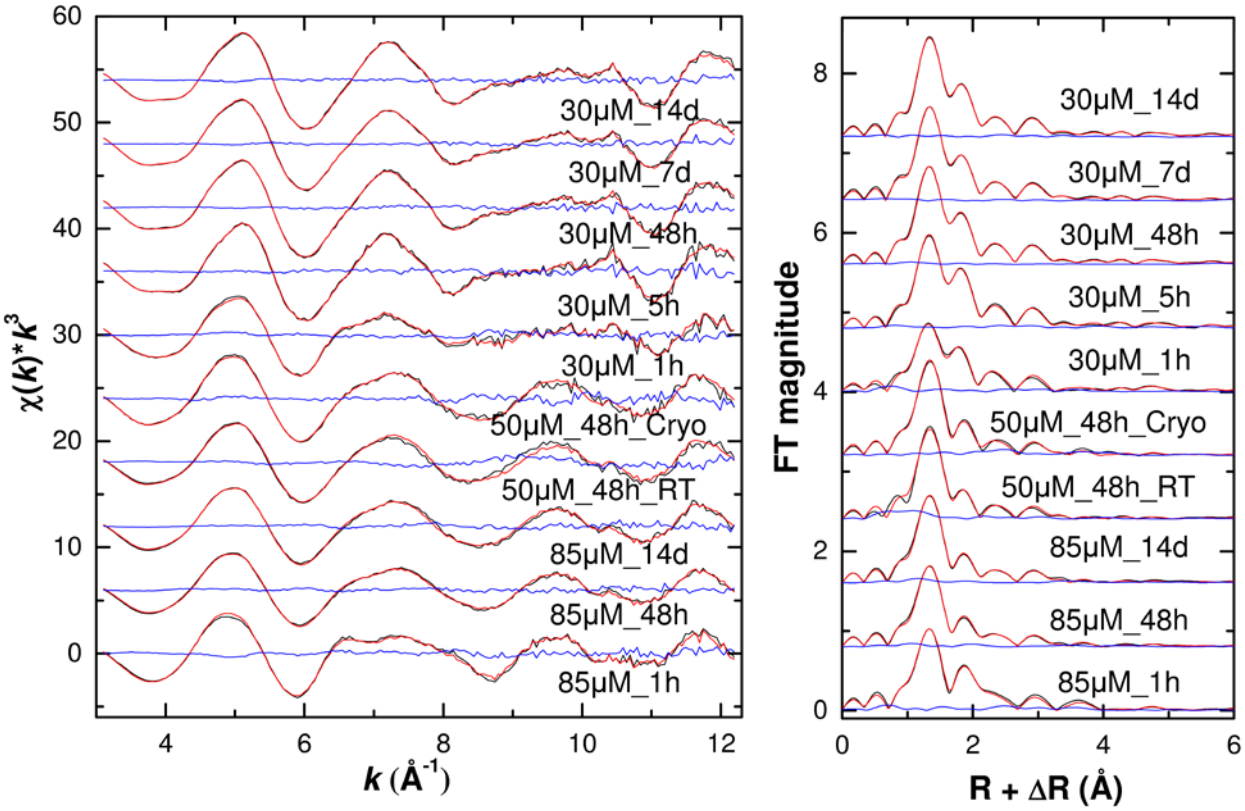
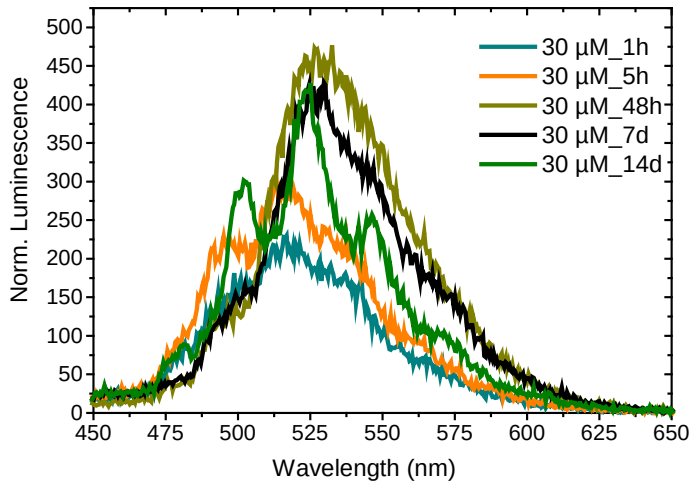


Fig. S8. ITFA reproduction of the U L_{III}-edge EXAFS spectra of the ten samples with different uranium concentrations and different incubation times by using the first three eigenvectors (see Fig. S7). Experimental data (black), reproduction (red), residual (blue).

a)



b)

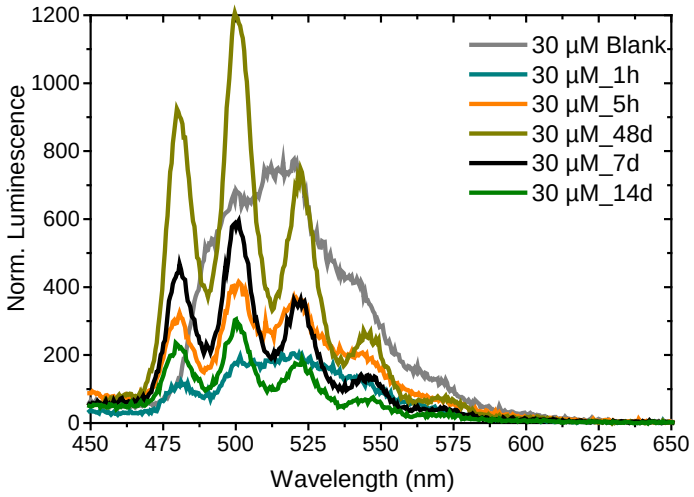


Fig. S9. Orginal luminescence spectra a) for 30 µM U(VI) from cell pellets and b) from the supernatants. The spectra were collected by cryo time-resolved laser-induced spectroscopy measurements.

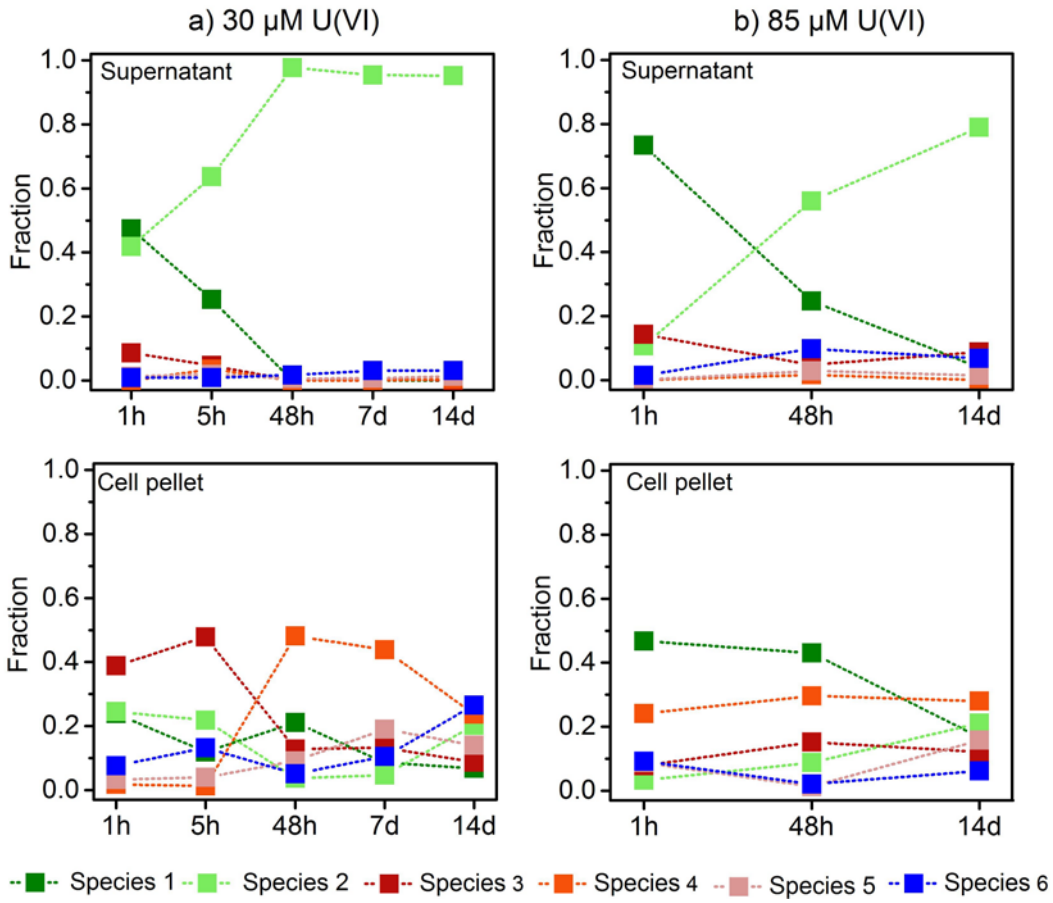


Fig. S10. Distribution of identified species in supernatants and in cell pellets based on parallel factor (PARAFAC) analysis on the cryo time-resolved laser-induced fluorescence spectroscopy data a) at 30 µM U(VI) and b) 85 µM U(VI).

Table S1: Summary of all samples including incubation time, uranium concentration, associated uranium and analysis method (pC_{H+} 6.0, [NaCl] = 3 M).

Incubation time (h)	[U(VI)] (µM)	U(VI) associated (%)	Analysis methods
0	30	19.6 ± 2.4	ICP-MS
0.5	30	27.2 ± 3.5	ICP-MS
1	30	24.2 ± 2.0	ICP-MS, XAS, TRLFS, L/D
3	30	3.6 ± 8.0	ICP-MS
5	30	1.2 ± 4.1	ICP-MS, XAS, TRLFS, L/D
16	30	26.4 ± 3.5	ICP-MS
24	30	53.7 ± 1.5	ICP-MS, SEM-EDX
40	30	78.7 ± 0.2	ICP-MS
48	30	75.3 ± 2.0	ICP-MS, XAS, TRLFS, L/D
72	30	77.6 ± 6	ICP-MS
96	30	74.2 ± 2,9	ICP-MS, SEM-EDX, HIM
168	30	87.8 ± 3.0	ICP-MS, XAS, TRLFS, CFM-LIFS, L/D
336	30	93.1 ± 0.1	ICP-MS, XAS, TRLFS, L/D
48	50	78.2 ± 3.0	ICP-MS, XAS
48	50	76.0 ± 0.5	ICP-MS, XAS
0	85	18.8 ± 1.7	ICP-MS
0.5	85	25.8 ± 0.4	ICP-MS
1	85	26.6 ± 0.7	ICP-MS, XAS, TRLFS, L/D
2	85	29.8 ± 1.2	ICP-MS
3	85	30.5 ± 1.2	ICP-MS
18	85	74.1 ± 2.9	ICP-MS
24	85	75.2 ± 3.4	ICP-MS
42	85	82.0 ± 1.8	ICP-MS
48	85	85.7 ± 0.7	ICP-MS, XAS, TRLFS, L/D
66	85	86.1 ±1.1	ICP-MS
89	85	88.6 ± 0.8	ICP-MS
168	85		CFM-LIFS, L/D
336	85	95.1 ± 1.0	ICP-MS, XAS, TRLFS, L/D

CFM: Cryo fluorescence microscopy, EDX: energy dispersive X-ray spectroscopy, HIM: Helium ion microscopy, LIFS: Laser-induced fluorescence spectroscopy, SEM: Scanning electron microscopy, TRLFS: Time-resolved laser-induced fluorescence spectroscopy, XAS: X-ray absorption spectroscopy

Table S2. Summary of TFA analysis for possible references.

Reference data	Form	Oxidation state of U	SPOIL
UO_2^{2+} in aq. $NaCO_3$, pH = 11	Solution	6	1.54
$[Na_6(U(CO_3)_5]$	Solid	4	1.60
U(IV) in aq. $HClO_4$	Solution	4	1.65
Meta-autunite (uranyl(VI) phosphate)	Solid	6	1.86
UO_2^{2+} in aq. $(NH_3)_2CO_3$, pH = 8	Solution	6	2.36
Andersonite (uranyl(VI) tricarbonate)	Solid	6	2.56
UO_2^{2+} in aq. lactic acid, pH = 7	Solid (precipitate)	6	2.87
UO_2-colloid	Solution (colloidal)	4	3.37
UO_2^{2+} in aq. $NaClO_4$, pH = 2	Solution	6	6.15

Based on the Malinowski's definition (Malinowski, 2002), the targets with SPOIL = 1 – 3 are acceptable, 3 – 6 moderately acceptable, and > 6 not acceptable. The TFA analysis for other U(VI) references, including U(VI) with fructose-1,6-biphosphate (as an organic phosphate species), with DNA, and with other carboxylates such as acetate) resulted in the SPOIL values higher than 4 and, hence, they were not considered as appropriate references.

Table S3. ITFA calculated fractions of the reference compounds in the experimental XANES spectra for the ten samples with different U concentrations and different incubation times (Fig. 3a).

Sample	Component 1 (U(VI))	Component 2 (U(IV))	Sum
85µM_1h	0.71	0.29	1.00
85µM_48h	0.84	0.14	0.98
85µM_14d	0.87	0.12	0.99
50µM_48h_RT	0.89	0.10	0.99
50µM_48h_Cryo	0.90	0.10	1.00
30µM_1h	0.57	0.43	1.00
30µM_5h	0.76	0.24	1.00
30µM_48h	0.88	0.13	1.01
30µM_7d	0.87	0.13	1.00
30µM_14d	0.90	0.10	1.00
Meta-autunite	1 (fixed)	0 (fixed)	1
U(IV)-carbonate	0 (fixed)	1 (fixed)	1

Root mean square error in fractions calculated by the method described in (Malinowski, 2002): 0.01.

Table S4. Summary of fractions calculated by ITFA and TFA on EXAFS spectra. TFA was performed based on the selected three references of meta-autunite, U(IV)-carbonate ($Na_6[U(CO_3)_5]$) and U(IV)-lactate (UO_2^{2+} in aq. lactic acid at pH = 7) shown in Fig. 3b.

	ITFA reproduction				TFA reproduction			
Sample	Component 1	Component 2	Component 3	Sum	Meta-autunite	U(IV)-carbonate	U(VI)-lactate	Sum
85µM_1h	0.20	0.31	0.58	1.09	0.21	0.35	0.56	1.12
85µM_48h	0.21	0.14	0.57	0.92	0.14	0.09	0.65	0.88
85µM_14d	0.26	0.11	0.58	0.95	0.17	0.02	0.70	0.89
50µM_48h_RT	0.31	0.05	0.56	0.93	0.24	-0.03	0.66	0.87
50µM_48h_Cryo	0.23	0.10	0.70	1.04	0.19	0.07	0.75	1.01
30µM_1h	0.57	0.27	0.10	0.94	0.76	0.45	-0.12	1.09
30µM_5h	0.89	0.14	0.01	1.04	0.95	0.17	-0.04	1.08
30µM_48h	0.76	0.09	0.19	1.05	0.79	0.10	0.18	1.07
30µM_7d	0.66	0.08	0.25	1.00	0.66	0.07	0.27	1.00
30µM_14d	0.77	0.04	0.20	1.01	0.77	0.02	0.22	1.01
Meta-autunite	0.98	0.00	0.00	0.98	-	-	-	-
U(IV)-carbonate	0.00	0.99	0.00	0.99	-	-	-	-
U(VI)-lactate	0.00	0.00	1.03	1.03	-	-	-	-

Root mean square error in fractions calculated by the method described in (Malinowski, 2002): 0.02 (component 1), 0.01 (component 2), 0.02 (component 3).

References

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