Supplementary information

Speciation-dependent molecular mechanism of electron transfer from the *c*-type cytochrome MtrC to U(VI)-ligand complexes

Margaux Molinas1, Karin Meibom1, Ashley Brown1, Luciano Abriata2, Tim Prüßmann3, Rizlan Bernier-Latmani1\*.

1 Environmental Microbiology Laboratory, Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

2 Protein Production and Structure Core Facility, Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

3 Karlsruhe Institute of Technology (KIT), Institute for Nuclear Waste Disposal (INE), P.O. 3640, D-76021 Karlsruhe, Germany

\*Corresponding author

Rizlan Bernier-Latmani

[rizlan.bernier-latmani@epfl.ch](mailto:rizlan.bernier-latmani@epfl.ch)

This supporting information contains: 35 pages, 7 texts, 14 figures, 14 tables

**Text S1:** Terrific broth medium preparation for MtrC purification from *Shewanella* species

**Text S2:** SDS-Page experimental details

**Text S3:** Experimental method for the size-exclusion chromatography (SEC) tests in oxic conditions

**Text S4:** Experimental method for the size-exclusion chromatography (SEC) tests in reduced conditions

**Text S5:** Results of the SEC test in oxic and reduced conditions between MtrC MR-1 and the U(VI)-ligand complexes

**Text S6:** Alignment of MtrC baltica and MtrC MR-1 sequences

**Text S7:** Comparison of the two-references vs three-references models to describe the HR-XANES timelines of U(VI)-ligand complexes reacted with reduced MtrC

**Fig. S1**: On the left, crystal structure of MtrC baltica (6QYC in PDB), showing electron entrance and exit, interaction sites with other proteins or electron shuttles, when associated to the MtrCAB porin complex

**Fig. S2**: On the left, molecular structures of the selected ligands hydroxo, carbonate, citrate, NTA, and EDTA. On the right, structure of prevalent U(VI)-ligand complexes determined via Mineql software

**Fig. S3**: SDS-page gel of the pooled fractions of the different MtrC baltica mutants after gel filtration

**Fig. S4**: Distribution of U in the fractions recovered from 40kDa size-exclusion columns in the absence of protein or after reaction with oxidized MtrC

**Fig. S5**: Distribution of U in the fractions recovered from 40kDa size-exclusion columns under anoxic conditions

**Fig. S6**: Paramagnetic region of the 1H-1D-NMR spectra of MtrC baltica in presence of (i) 30 mM EDTA (pink) and (ii) 30mM EDTA + 120µM of U(VI) (red)

**Fig. S7**: Paramagnetic region of the 1H-1D-NMR spectra of MtrC baltica H499M, carrying a mutation in the vicinity of heme 7

**Fig. S8**: Superposition of MtrC MR-1 (blue) and MtrC baltica (green) structures fetched form PDB using the software ChimeraX. Superposition of the heme chains, for both MtrC MR-1 (blue) and MtrC baltica (green)

**Fig. S9**: Timeline of the redox reactions between reduced MtrC MR-1 and U(VI)-carbonate (turquoise), U(VI)-hydroxo (green), U(VI)-citrate (purple), U(VI)-NTA (orange) and U(VI)-EDTA (red)

**Fig. S10**: Second order kinetics for the two first phases observed on the kinetics timelines described in **Fig. S9** between reduced MtrC MR-1 and U(VI)-carbonate (turquoise), U(VI)-citrate (purple), U(VI)-NTA (orange), U(VI)-EDTA (red), and U(VI)-DTPA (green)

**Fig. S11**: LCF fitting results (red) compared to the data (dotted black) for the M4-edge HR-XANES spectra obtained after 30s, 1min, 2min, 5min and 20min of reaction between 300 μM reduced MtrC MR-1 and 600 μM U(VI)-carbonate at pH 7.5

**Fig. S12**: M4-edge HR-XANES spectra obtained after reacting 300 μM reduced MtrC baltica and 600 μM U(VI)-EDTA at pH 7.5 for 15s, 30s and 60s. LCF fitting are displayed in dotted black line on the top of the M4-edge HR-XANES spectra obtained

**Fig. S13**: LCF fitting results (red) compared to the data (dotted black) for the M4-edge HR-XANES spectra obtained after 15s, 30s and 60s of reaction between 300 μM reduced MtrC baltica and 600 μM U(VI)-NTA at pH 7.5. The fits were obtained by considering contributions from 2 references only, U(VI)-NTA and UO2

**Fig. S14**: LCF fitting results (red) compared to the data (dotted black) for the M4-edge HR-XANES spectra obtained after 15s, 30s and 60s of reaction between 300 μM reduced MtrC baltica and 600 μM U(VI)-EDTA at pH 7.5. The fits were obtained by considering contributions from 2 references only, U(VI)-NTA and UO2

**Table S1**: Primers used to introduce point mutations in *mtrCsol* by site-directed mutagenesis. The mutated triplets of bases are underlined

**Table S2:** PCR reaction details.

**Table S3**: Primers used for sequencing of plasmids encoding *mtrCsol* with point mutations.

**Table S4**: Concentrations in U and MtrC MR-1, and the ratio between both for the reactions between MtrC MR-1 and U-ligand (ligand = NTA, EDTA, DTPA, carbonate) under oxic (MtrC oxidized) and reduced (MtrC reduced) conditions

**Table S5**: Percentages of U eluted in the different fractions from 40kDa size-exclusion desalting **in oxic conditions**

**Table S6**: Percentages of U eluted from 40 kDa size-exclusion desalting columns in the different fractions **in reduced conditions**

**Table S7**: Percentages of the U oxidation obtained for each reaction mixture in fraction F1 (in which MtrC elutes) and in fraction F2 to F7 between reduced MtrC MR-1 and U-ligand complexes under **reduced conditions**

**Table S8**: Ratios between the concentration of U and MtrC (displayed in red) in the kinetics experiment reported on **Fig. 1**

**Table S9**: U speciation calculated using Mineql for U(VI)-ligand complexes at pH 7.5, with 50 mM NaCl, and 100 mM HEPES

**Table S10**: LogK of aqueous complexes of interest in this work

**Table S11**: Percentage of U(VI) and U(IV) obtained by ion exchange chromatography corresponding to the reaction between U(VI)-carbonate ([U(VI)-carbonate] = 600 µM) and reduced MtrC MR-1 ([MtrC MR-1] = 300 µM), analysed by M4-edge HR-XANES (**Fig. 5**, **Table S12.a**) after 30s, 60s, 2min, 5min and 20min of reaction

**Table S12**: Summary of the whitelines of the M4-edge HR-XANES spectra described in this work. Four HR-XANES experiments are presented here and were not necessarily performed during the same beamtime

**Table S13**: Experimental data illustrating the HR-XANES experiment reported **Fig. 6** and **Table S7.d**.

**Table S14**: Percentage of U(IV) obtained by ion exchange chromatography corresponding to the reaction between U(VI)-NTA or U(VI)-EDTA and reduced MtrC baltica after 2.5min, analysed by M4-edge HR-XANES

**Text S1: Terrific broth medium preparation for MtrC purification from *Shewanella* species**

12 g of tryptone, 64 g of yeast extract, and 4 mL of glycerol were added to 900 mL of deionized water. After autoclaving, 100mL of a 0.17 M of potassium phosphate monobasic (KH2PO4) and 0.72 M of potassium phosphate dibasic (K2HPO4) buffer (0.22 µm filtered) were added to the autoclaved 900 mL. Ampicillin was supplemented to the medium to a final concentration of 50 µg/mL.

**Text S2: SDS-Page experimental details**

The SDS-PAGE was set up using the pre-casted NuPage 4-12% Bis-Tris gels (Invitrogen). Prior loading, the samples were mixed with 4X LDS Sample Buffer (Genscript), reduced using DDT and heated up 5 min at 95°C. The gels were run in Mini Gel Tank (Invitrogen) with Tris-MES-SDS Running Buffer prepared using a powder mix (Genscript). The gels were revealed using QuickBlue Protein Stain (Lubio).

**Text S3: Experimental method for the size-exclusion chromatography (SEC) tests *in oxic conditions* for the reaction between MtrC MR-1 and the four U(VI)-ligand complexes (carbonate, NTA, EDTA, DTPA)**

40kDa size-exclusion desalting columns (Zeba® Spin Desalting Columns, Thermofisher Scientific, Waltham MA USA) were conditioned by 3 washes with buffer A (100 mM HEPES and 50 mM NaCl). Working solutions of oxidized MtrC and U(VI)-ligand were prepared to a concentration of 300µM each. The U(VI)-ligand working solutions were prepared by diluting the appropriate volume of U(VI)-chloride in the freshly prepared oxic ligand stock solutions. The reaction was initiated by mixing equal volumes of oxidized MtrC and the U-ligand complex solutions, and allowing the reaction to proceed for 30min. No protein controls were set up by mixing equal volumes of the U-ligand working solution with buffer A. Then, 100µl of the reaction mixtures were loaded onto the size-exclusion columns and spun for 1.5min at 3500xg. The first fraction eluted, fraction F1, contains the protein. Then, 6 successive washes were performed by adding 350µL of 6M HCl and spinning for 1.5min at 3500xg and fractions F2 to F7 recovered. U was quantified by ICP-MS and the concentration of MtrC evaluated in both the reaction mixtures and the fractions F1 by the BCA assay.

**Text S4: Experimental method for the size-exclusion chromatography (SEC) tests in *reduced conditions* for the reaction between MtrC MR-1 and the four U(VI)-ligand complexes (carbonate, NTA, EDTA, DTPA)**

A week ahead of the reaction, the 40kDa size-exclusion resins were degassed and introduced in the glovebox. They were conditioned as described above, in anoxic buffer A. Working solutions of reduced MtrC and U-ligand were prepared as mentioned above but using anoxic ligand stock solutions. The experimental steps are similar to those followed for oxidized MtrC. Once the 7 fractions were recovered from the no-protein controls and the reaction mixtures with reduced MtrC, ion-exchange chromatography was performed on fraction F1 and on a combined mixture of F2 to F7 to separate U(VI) and U(IV). U was measured by ICP-MS and the concentration of MtrC was evaluated by BCA in the whole reaction mixture and in the fraction F1.

**Text S5: Results of the SEC test for the reaction between MtrC MR-1 and the U(VI)-ligand complexes (carbonate, NTA, EDTA, DTPA) in oxic or reduced conditions**

We investigated whether U complexes bind to the purified protein by using size-exclusion columns. MtrC reacted with one of the U complexes was placed on a size-exclusion column (40 kDA pore size) to trap unbound U and to collect MtrC along with any U bound to the protein. These experiments were carried out with oxidized MtrC under oxic conditions to establish the binding of U(VI) to the protein and with reduced MtrC under anoxic conditions to quantify the binding of remaining U(VI) and the reduction product(s) to the protein. The concentrations of U and MtrC MR-1 for both set of conditions are reported **Table S4**.

*Binding to oxidized MtrC.* The results for the oxic binding tests are summarized in **Fig. S4** and **Table S5**. Fraction F1 includes the protein and subsequent fractions do not. In the experiment without MtrC, little U was eluted in the first fraction through the size exclusion columns, suggesting that the majority of free U was efficiently trapped into the pores of the resin beads (**Fig. S4.a.**). The six following washes allowed the recovery of >95% of the total U initially loaded. This confirmed that we can use this size-exclusion technique to quantify U associated with MtrC. In the case with MtrC, limited binding was observed with around 11% U(VI) bound for NTA, EDTA, and DTPA and 14% for carbonate. (**Fig. S4.b.**).

*Binding to reduced MtrC.* Reactions identical to the ones described with oxidized MtrC were performed under anoxic conditions with reduced MtrC. In addition to probing the U concentration eluted in each fraction, ion-exchange chromatography was performed on fraction F1 (the one corresponding to MtrC) and on combined fractions F2 to F7. This allowed the determination of the U speciation upon reaction with MtrC, and of the oxidation state of U bound to MtrC.

The tests without protein gave similar results to those performed with oxidized MtrC (**Fig. S5**, **Table S6** and **S7**). Indeed, most of the U was retained in the size exclusion column and eluted with the six following washes (**Fig. S5.a.)**. However, a small amount of U was eluted in the first fraction, particularly for DTPA and carbonate, probably due to negative charge repulsion with the resin beads, as DTPA and carbonate complexes have charges -3 and -4, respectively. In the presence of reduced protein, we observed that, with carbonate, 72% of the U was eluted in F1, suggesting that it was associated with the protein (**Fig. S5.b.**). In contrast, the U recovered upon reaction of U aminocarboxylate ligand complexes with reduced MtrC was not bound to the protein and corresponded to U(IV) (**Fig. S5.c.**, **Table S7**). For the carbonate system, the MtrC-associated U consists of 84% U(IV) (60.5% of the total U) and 16% U(VI) (11.5% of the total U) (**Table S7**). We hypothesize that U(VI) found in association with MtrC actually corresponds to U(V) that is disproportionated due to acidification prior to ion-exchange chromatography separation. If that is correct, U(V) would actually represent 32% of MtrC-associated U (23% of total U) and U(IV) ~68% of MtrC-associated U (49% of total U). Additionally, ~24% of U recovered in fraction F2 to F7 was U(VI), which can be interpreted as a combination of incomplete reduction of U(VI) and stabilization of U(V)-ligand complexes that disproportionate upon acidification.

**Text S6: Alignment of MtrC baltica and MtrC MR-1 sequences**

The online tool SIM from Expasy (<https://web.expasy.org/sim/>) was used to align the protein sequences.

46.6% identity in 652 residues overlap was calculated. The stars indicate common amino acids between both sequences.

Sequence1: MtrC from *S. oneidensis* MR-1 (PBD 4LM8)

Sequence2: MtrC from *S. baltica* (PBD 6QYC)

Sequence1 42 AGSIQTLNLDITKVSYENGAPMVTVFATNEADMPVIGLANLEIKKALQLIPEGATGPGNS

Sequence2 1 APAIQILNFTFDKSVITNGVPSVEFTVTNENDLPVVGLQKMRFA-AAQLIPQGATGAGNA

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Sequence1 102 ANWQGLGSSK---------SYVDNKNG--SYTFKFDAFDSNKV-FNAQLTQRFNVVSAAG

Sequence2 60 SQWQYFGDETCDVAATCPGTFVDQKNGHYSYTFNMNLTANAKITYNDQLAQRVLIRAYNT

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Sequence1 150 KLADGTTVPVAEMVEDFDGQ-GNAPQYTKNIVSHEVCASCHVEGEKIYHQA--TEVETCI

Sequence2 120 PLPDGTQVPNSNAFVDFTADTGAAPTYSRKIVATESCNTCHQDLANVKHGGAYSDVNYCA

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Sequence1 207 SCHTQEFADGRGKPHVAFSHLIHNVHNANKAWGKDNKIPTVAQNIVQDNCQVCHVESDML

Sequence2 180 TCHTA----GKVGVGKEFNVLVHAKH-------KDLTLGSL------ESCQSCHAANDAA

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Sequence1 267 TEAKNWSRIPTMEVCSSCHVDIDFAAGKGHSQQLDNSNCIACHNSDWTAELHTAKTTATK

Sequence2 223 PDWGNWSRIPTAATCGSCHSTVDFAAGKGHSQQLDNSNCIACHNSDWTAELHTGKTADKK

\*\*\*\*\*\*\* \* \*\*\* \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\* \*\* \*

Sequence1 327 NLINQYGIETTSTINTETKAATISVQVVDANGTAVDLKTILPKVQRLEIITNVGPNNATL

Sequence2 283 AVIAQLGMQATLVGQTDD-TAVLTVSILDKDGNAIDAATVQDKIKRLETVTNVGPNFPIM

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Sequence1 387 GYSGKDSIFA-------IKNGALDPKATINDAGKLVYTTTKDLKLGQNGADSDTAFSFVG

Sequence2 342 GYNKSPGSGAAKIAKDLVKDGALQAGVTLVD-GKLVFTTPA---LPFGTGDTDTAFTFIG

\*\* \* \* \*\*\* \* \* \*\*\*\* \*\* \* \* \*\*\*\* \* \*

Sequence1 440 WSMCSSEGKFVDCADPAFDGVDVTKYTGMKADLAFATLSGKAPSTRHVDSVNMTACANCH

Sequence2 398 LEMCSTGTSLTACT------VD-SATTSMKAELAFGTKSGNAPSMRHVNSVNFSTCQGCH

\*\*\* \* \*\* \* \*\*\* \*\*\* \* \*\* \*\*\* \*\*\* \*\*\* \* \*\*

Sequence1 500 TAEFEIHKGKQHAGFVMTEQLSHTQDANGKAIVGLDACVTCHTPDGTY-SFANRGALELK

Sequence2 451 SDTFEIHKG-HHSGFVMTEQVSHAKDANGKAIVGVDGCVACHTPDGTYASGANKGAFEMK

\*\*\*\*\*\* \* \*\*\*\*\*\*\* \*\* \*\*\*\*\*\*\*\*\* \* \*\* \*\*\*\*\*\*\*\* \* \*\* \*\* \* \*

Sequence1 559 LHKKHVEDAYGLIGGNCASCHSDFNLESFKKKGALNTAAAADKTGLYSTPITATCTTCHT

Sequence2 510 LHVIHGEQ--GVIK-ECTQCHNDFNLDAFKVKGALATSA-----GKYTTPITATCTSCHA

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Sequence1 619 VGSQYMVHTKETLESFGAVVDGTKDDATSAAQSETCFYCHTPTVADHTKVKM

Sequence2 562 PES-----IGHGLENMGAIVNGDYVQANQAAQSETCFYCHKPTPTDHTQVKM

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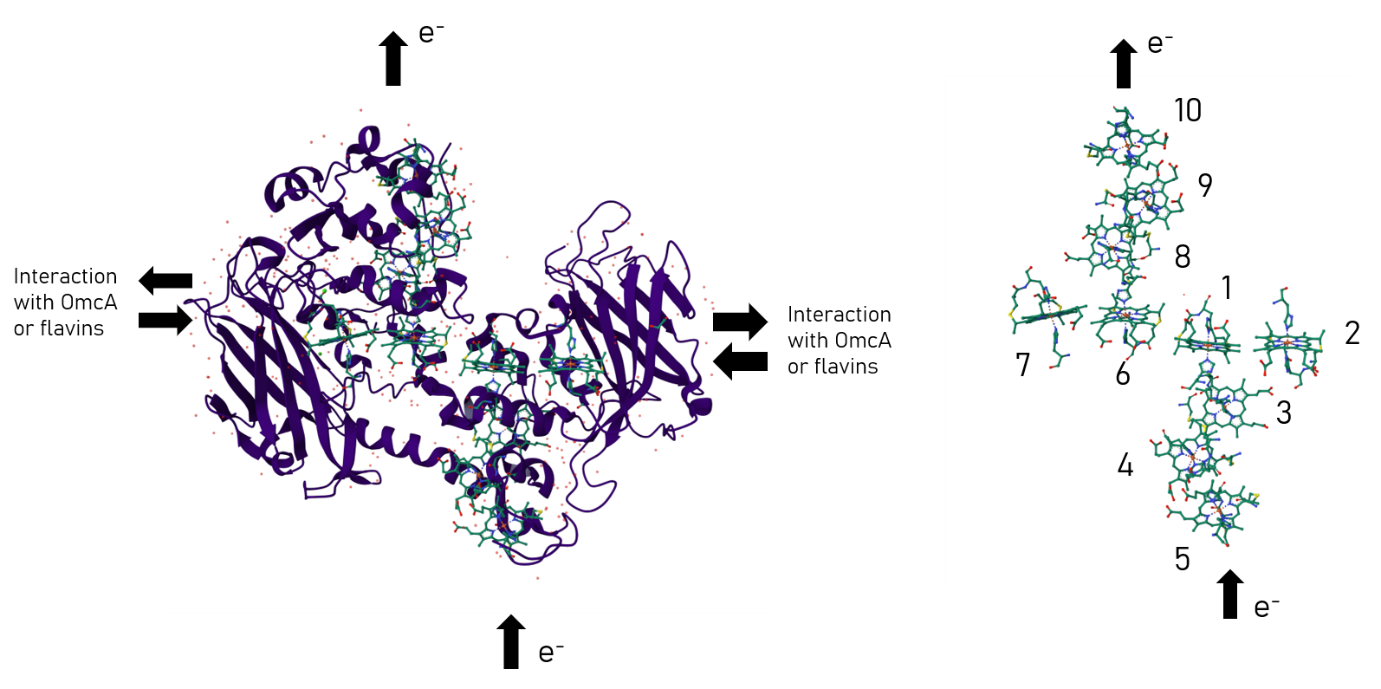
**Text S7: Comparison of the two-reference vs three-reference models to describe the HR-XANES timelines of U(VI)-ligand complexes reacted with reduced MtrC**

We ensured that the three-reference fits (**Fig. 5, Fig. 7, Fig. S12)** were robust by comparing them to a two-reference model that uses only U(VI) and U(IV) as references (**Fig. S11, Fig. S13, Fig. S14**). For instance, for the U(VI)-carbonate system the model with U(VI) and U(IV) as references for the 30s timepoint does not properly reproduce the data, as the edge of the fit is shifted to lower energies and the uranyl characteristic feature at 3.7324 keV is accentuated compared to the data. (**Fig. S11.b.**). In addition, the reduced χ2 of the U(VI) and U(IV)-only model was calculated to be 0.3110, whereas that with U(V) and U(IV) had a reduced χ2 of 0.0895, pointing to a better fit (**Fig. 5**). Similar observations can be derived from the fits of 1min, 2min 5min and 20min timepoints, for which results are presented **Fig. S11**.

As for U-NTA, when modelled with two references, at 15s, 56.56% U(VI) and 43.44% U(IV) are observed (**Fig. S13.a.**), and similarly, at 60s, 51.50% U(VI) and 48.50% U(IV) (**Fig. S13.c.**). However, 32.4% U(VI) and 67.6% U(IV) were measured after 2.5min by ion-exchange chromatography (**Table S14**). If any U(V) is present, then total amount of reduced species would reach 78.8% (the acidification step prior to the IEC forces U(V) to disproportionate into half U(VI) and half U(IV)). A better correspondence was achieved with the three-references model (**Fig. 7**) which totalizes between 80% to 90% of reduced species. In addition, the R-factor and reduced χ2 values were 2x lower for the three-references model.

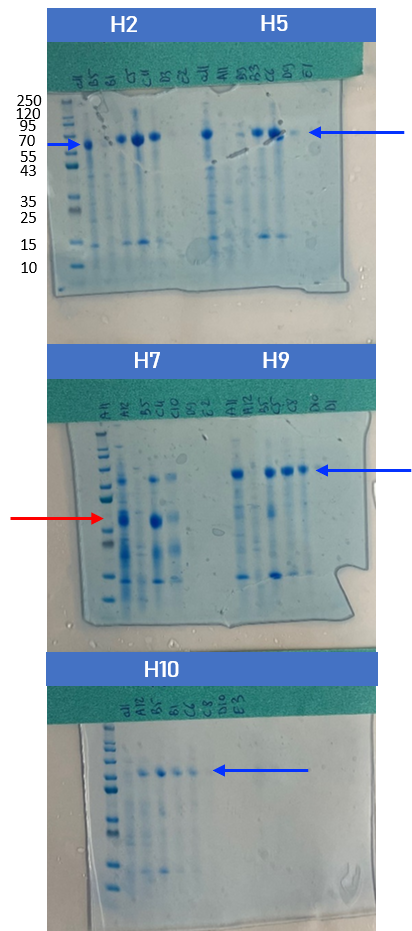
Regarding U-EDTA, χ2 for the two-reference models are 2x higher than those observed for the three-reference models (**Fig. S14**). The amount of U(IV) with the two-references models ranges between 24% and 37% (**Fig. S14**). However, 18.6% U(VI) and 81.4% U(IV) were measured by ion exchange chromatography (**Table S14**). If any U(V) is present, the total amount of reduced species would reach about 90%. Hence, similarly to the U-NTA system, the three-reference models better accounts for the amount of reduced species present in the system.

These considerations support the persistence of U(V) in the U-carbonate system, up to 20min, and the transient presence of U(V) in the U-NTA and U-EDTA systems.



**Fig. S1**: On the left, crystal structure of MtrC from *S. baltica*, 6QYC in PDB (Edwards *et al.* 2020), showing electron entrance and exit, interaction sites with other proteins or electron shuttles, when associated to the MtrCAB porin complex. On the right, the heme chain embedded in the MtrC structure. The electrons flow from heme 5 to hemes 10, 2 and 7.

**Fig. S2**: On the left, molecular structures of the selected ligands hydroxo, carbonate, citrate, NTA, and EDTA. On the right, structures of prevalent U(VI)-ligand complexes determined via Mineql software. The structures of UO2(H2O)NTA- was evidenced by Teleb et al.2004, and that of UO2EDTA2- by Kim et al. 2021.

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**Fig. S3**: SDS-page gel of the pooled fractions of the different MtrC baltica mutants after gel filtration. Blue arrows indicate the expected size while the red arrow indicates a much smaller size than expected for the MtrC protein. H2 refers to the heme 2 mutant, etc. H7 shows a smaller size, pointing to possible degradation or structural issues with that mutant protein.



**Fig. S4**: Distribution of U in the fractions recovered from 40kDa size-exclusion columns in the absence of protein or after reaction with oxidized MtrC *under oxic conditions*. **A**. In control reactions without protein with U(VI)-NTA, U(VI)-EDTA, U(VI)-DTPA, and U(VI)-carbonate (**Table S5**); and **B**. In reactions between oxidized MtrC and U(VI)-NTA, U(VI)-EDTA, U(VI)-DTPA, and U(VI)-carbonate for 30min (**Table S5**). If present, proteins elute in the first fraction F1 (purple). Fractions F2 to F7 (green) corresponds to successive washes with 6M HCl aiming at washing remaining U out of the size exclusion columns.



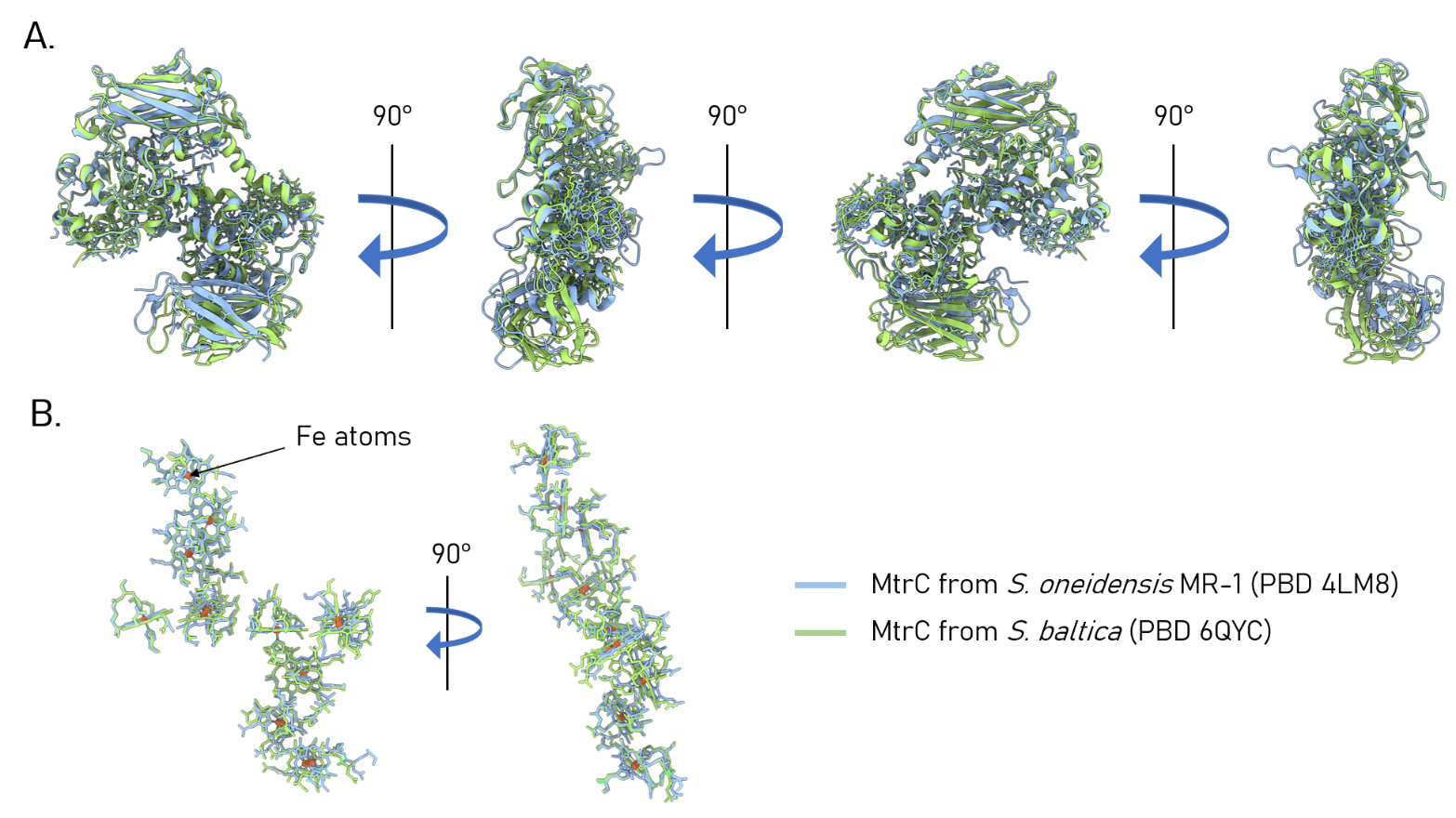
**Fig. S5**: Distribution of U in the fractions recovered from 40kDa size-exclusion columns *under anoxic conditions*. **A**. Control reactions without protein with U(VI)-NTA, U(VI)-EDTA, U(VI)-DTPA, or U(VI)-carbonate (**Table S6**); and **B**. Reaction of reduced MtrC with U(VI)-NTA, U(VI)-EDTA, U(VI)-DTPA, or U(VI)-carbonate for 30min. If present, proteins elute in the first fraction F1 (purple). Fractions F2 to F7 (green) corresponds to successive washes with 6M HCl aiming at washing the remaining U out of the size exclusion columns (**Table S6**). **C**. represents the oxidation states of U in fraction F1 and in the combined fractions F2 to F7 gathered for the reaction shown in B (**Table S7**). with MtrC. The U(VI) and U(IV) fractions were obtained by ion exchange chromatography.



**Fig. S6**: Paramagnetic region of the 1H-1D-NMR spectra of MtrC baltica H499M, carrying a mutation in the vicinity of heme 7. The bottom plain line (black) represents MtrC baltica wild type (WT), and the top line (yellow) represents MtrC baltica H499M, superposed on the WT in dotted line to facilitate the identification of potential shifted or missing signals. The spectra were recorded with 30 µM MtrC in 50 mM NaCl and 100 mM HEPES at pH 7.5.



**Fig. S7**: Paramagnetic region of the 1H-1D-NMR spectra of MtrC baltica in presence of (i) 30 mM EDTA (pink) and (ii) 30mM EDTA + 120µM of U(VI) (red). The bottom plain line (black) represents MtrC baltica wild type and provide a standard for chemical shifts perturbations in the reaction where ligand and U were added. The table below the figure summarizes the chemical shifts observed for MtrC baltica wild type (first line). The chemical shifts highlighted in grey correspond to signals which appeared to be perturbed upon interaction of oxidized MtrC baltica and the U(VI)-ligand complexes. The bottom part of the table reports ∆δ between the signals of the wild type and that of the reaction mixture in presence of EDTA or U(VI)-EDTA. The color code indicates the extend of ∆δ as described in the legend below the table. The threshold for a relevant shift was set to |0.05| ppm and above. Hence, the highlighted ∆δ values inform on regions where the interaction may take place.

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**Fig. S8**: A. Superposition of MtrC MR-1 (blue) and MtrC baltica (green) structures fetched form PDB using the software ChimeraX. B. Superposition of the heme chains, for both MtrC MR-1 (blue) and MtrC baltica (green), inside the protein scaffold of both. The position of the hemes and the central iron atom (colored in orange) is conserved between the two proteins.



**Fig. S9**: Redox reactions between reduced MtrC MR-1 and U(VI)-carbonate (turquoise), U(VI)-hydroxo (green), U(VI)-citrate (purple), U(VI)-NTA (orange) and U(VI)-EDTA (red). Time points were obtained at 5s, 15s, 30s and 120s. Reaction progress is characterized by the percentage of the reduction product U(IV) (%U(IV) reported on the y-axis) formed over time, determined by ion exchange chromatography. Ion exchange chromatography allows to separate U(VI) and U(IV) oxidation states. The concentrations of reactants were: [U(VI)] = 100 μM and [MtrC baltica] = 100 μM. Ligands concentrations are the following: [carbonate] = [NTA] = [EDTA] = [DTPA] = 30 mM and [citrate] = 5 mM. The buffer contained [NaCl] = 50 mM and [HEPES] = 100 mM at pH 7.5. Three phases were identified to describe the reaction kinetics, a first phase (blue), second phase (red) and third phase (green). This is repeat of Figure 1 (replicate experiment).



**Fig. S10**: Second order kinetics for the two first phases observed on the kinetics timelines described in **Fig. S9** between reduced MtrC MR-1 and U(VI)-carbonate (turquoise), U(VI)-citrate (purple), U(VI)-NTA (orange), U(VI)-EDTA (red), and U(VI)-DTPA (green): A. 0s to 5s; B. 5s to 30s. The linear regression for each U-ligand complex is displayed in dotted black line, along with R2 and the coefficient α of the regression line, corresponding to the kinetic constant of these reactions.



**Fig. S11**: LCF fitting results (red) compared to the data (dotted black) for the M4-edge HR-XANES spectra obtained after 30s, 1min, 2min, 5min and 20min of reaction between 300 μM reduced MtrC MR-1 and 600 μM U(VI)-carbonate at pH 7.5. The fits were obtained by considering contributions from 2 references U(VI)-carbonate and UO2.



**Fig. S12**: M4-edge HR-XANES spectra obtained after reacting 300 μM reduced MtrC baltica and 600 μM U(VI)-EDTA at pH 7.5 for 15s, 30s and 60s. Spectra of the references are displayed: U(VI)-NTA (red), U(V)-iodide (pink) and UO2 (blue). The two dotted lines represent the whitelines of the UO2 standard on the left, and the U(VI)-NTA standard on the right. LCF fitting are displayed in dotted black line on the top of the M4-edge HR-XANES spectra obtained. The fits were obtained by considering contributions from U(VI)-carbonate, U(V)-iodide and UO2 (top panel) or contributions from U(VI)-nta, U(V)-iodine and U(IV)O2 (bottom panel). Statistical parameters and results of the fit are summarized in the table below the figures.



**Fig. S13**: LCF fitting results (red) compared to the data (dotted black) for the M4-edge HR-XANES spectra obtained after 15s, 30s and 60s of reaction between 300 μM reduced MtrC baltica and 600 μM U(VI)-NTA at pH 7.5. The fits were obtained by considering contributions from 2 references only, U(VI)-NTA and UO2.



**Fig. S****14**: LCF fitting results (red) compared to the data (dotted black) for the M4-edge HR-XANES spectra obtained after 15s, 30s and 60s of reaction between 300 μM reduced MtrC baltica and 600 μM U(VI)-EDTA at pH 7.5. The fits were obtained by considering contributions from 2 references only, U(VI)-NTA and UO2.

|  |  |
| --- | --- |
| Primer name | Nucleotide sequence (5'-3') |
| H208M\_F | GAT CTA GCA AAC GTT AAA ATG GGT GGT GCC TAC TCT G |
| H208M\_R | CTG GTG GCA CGT ATT ACA GC |
| H292M\_F | GCG GCT GGT AAA GGT ATG TCG CAG CAG TTG GAC |
| H292M\_R | AAA ATC CAC GGT GGA ATG AC |
| H497M\_F | GAT ACG TTT GAG ATA ATG AAA GGT CAT CAT AGT G |
| H497M\_R | ACT ATG ACA ACC TTG ACA TGT A |
| H607M\_F | CCC GAA AGC ATC GGC ATG GGC CTG GAA AAT ATG |
| H607M\_R | GGC ATG GCA GCT AGT GCA GG |
| H643M\_F | CCA ACT CCA ACG GAC ATG ACA CAA GTT AAA ATG |
| H643M\_R | CTT GTG GCA ATA AAA GCA GG |

**Table S1**: Primers used to introduce point mutations in *mtrCsol* by site-directed mutagenesis. The mutated triplets of bases are underlined. The primers are phosphorylated at the 5’- end. The primer name is composed by the mutated histidine (H), the position in the gene sequence, and the amino acid of substitution methionine (M).

|  |  |  |
| --- | --- | --- |
| **Steps** | **Temperature** | **Time** |
| Initial denaturation | 95°C | 5min |
|  | 98°C | 5s | 30 cycles |
| Annealing | 55°C | 30s |
| Extension | 72°C | 1min |
| Final extension | 72°C | 5min |
| Hold | 4°C | ∞ |

|  |  |
| --- | --- |
| **Starting reagents** | **Concentration in PCR reaction** |
| 5x Phusion HF buffer | 1X |
| 10mM dNTPs | 200 µM |
| 10µM forward primer | 0.5 µM |
| 10µM reverse primer | 0.5 µM |
| Phusion DNA Polymerase | 1 unit per 50 µL PCR reaction |
| Template DNA | 100 to 500 ng |
| H2O | To dilute to 50 µL |

**Table S2:** PCR reaction details. Top table summarizes the different steps; times, temperature and number of cycles performed. Lower table lists the reagents used and their respective concentration in a PCR reaction mixture of 50µL.

|  |  |
| --- | --- |
| Primer name | Nucleotide sequence (5'-3') |
| pBAD-RO | GAT TTAATC TGT ATC AGG |
| MtrCbsol\_H2\_H5\_for | GGCGACGAAACCTGCGATGTCG |
| MtrCbsol\_H9\_H10\_for | GGCGCCGCCAAAATTGCAAAAG |

**Table S3**: Primers used for sequencing of plasmids encoding *mtrCsol* with point mutations. Plasmids with point mutations in heme 2 and 5 of *mtrC* were sequenced with the MtrCbsol\_H2\_H5\_for primer, whereas plasmids with mutations in heme 7, 9 and 10 with MtrCbsol\_H9\_H10\_for primer to verify the mutations after introduction into *S. oneidensis ∆*OMC.

|  |  |  |  |
| --- | --- | --- | --- |
| Ligand | [MtrC] (µM) | [U] (µM) | [U]/[MtrC] |
| **oxic conditions** | | | |
| NTA | 129.14 | 126.6 | 0.98 |
| EDTA | 123.4 | 120.84 | 0.98 |
| DTPA | 125.79 | 128.5 | 1.02 |
| carbonate | 135.38 | 124.40 | 0.92 |
| **reduced conditions** | | | |
| NTA | 220.71 | 171.69 | 0.78 |
| EDTA | 234.13 | 173 .42 | 0.74 |
| DTPA | 155.42 | 192.09 | 1.24 |
| carbonate | 147.74 | 171.69 | 1.16 |

**Table S4**: Concentrations in U and MtrC MR-1, and the ratio between both for the reactions between MtrC MR-1 and U-ligand (ligand = NTA, EDTA, DTPA, carbonate) under oxic (MtrC oxidized) and reduced (MtrC reduced) conditions described in **Fig. S4 and S5**, in the whole reaction mixtures.

|  |  |  |
| --- | --- | --- |
| **no protein control** | | |
| Ligand | **F1** | **F2 to F7** |
| NTA | 0.114 | 99.886 |
| EDTA | 0.325 | 99.675 |
| DTPA | 0.691 | 99.309 |
| carbonate | 0.693 | 99.307 |
|  |  |  |
| **oxidized MtrC MR-1** | | |
| Ligand | **F1** | **F2 to F7** |
| NTA | 10.663 | 89.337 |
| EDTA | 10.989 | 89.011 |
| DTPA | 10.814 | 89.186 |
| carbonate | 13.552 | 86.448 |

**Table S5**: Percentages of U eluted in the different fractions from 40 kDa size-exclusion desalting ***under oxic conditions***, in no protein control (above part of the table) and in reaction between MtrC MR-1 and the different U-ligand complexes studied. The last column sums the U in the fractions F2 to F7. Data presented in **Fig. S4**.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **no protein control** | | | | | | | | |
| Ligand | **F1** | **F2** | **F3** | **F4** | **F5** | **F6** | **F7** | **F2 to F7** |
| NTA | 0.44 | 24.93 | 47.14 | 20.31 | 5.33 | 1.53 | 0.31 | 99.56 |
| EDTA | 0.75 | 24.56 | 40.57 | 26.26 | 6.54 | 1.09 | 0.23 | 99.25 |
| DTPA | 5.73 | 65.67 | 23.15 | 4.46 | 0.87 | 0.10 | 0.02 | 94.27 |
| carbonate | 4.80 | 57.32 | 26.49 | 9.18 | 1.87 | 0.34 | 0.02 | 95.20 |
|  |  |  |  |  |  |  |  |  |
| **reduced MtrC** | | | | | | | | |
| Ligand | **F1** | **F2** | **F3** | **F4** | **F5** | **F6** | **F7** | **F2 to F7** |
| NTA | 3.52 | 60.25 | 21.99 | 10.54 | 2.67 | 0.76 | 0.26 | 96.48 |
| EDTA | 1.39 | 33.25 | 26.06 | 23.42 | 12.18 | 3.08 | 0.63 | 98.61 |
| DTPA | 2.73 | 54.65 | 19.62 | 15.65 | 4.90 | 1.85 | 0.60 | 97.27 |
| carbonate | 71.97 | 11.35 | 10.08 | 4.88 | 1.29 | 0.31 | 0.11 | 28.03 |

**Table S6**: Percentages of U eluted from 40 kDa size-exclusion desalting columns in the different fractions ***under reduced conditions***, in no protein control (above part of the table) and in reaction between MtrC MR-1 and the U-ligand complexes studied. The last column sums the U in fractions F2 to F7. Data presented in **Fig.S5**.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | **F1** | |  | **F2 to F7** | |
| Ligand |  | %U(VI) | %U(IV) |  | %U(VI) | %U(IV) |
| NTA |  | 0.46 | 3.07 |  | 1.78 | 94.69 |
| EDTA |  | 0.22 | 1.17 |  | 1.63 | 96.97 |
| DTPA |  | 1.26 | 1.47 |  | 1.93 | 95.34 |
| carbonate |  | 11.51 | 60.46 |  | 23.69 | 4.34 |

**Table S7**: Percentages of the U oxidation obtained for each reaction between reduced MtrC MR-1 and U-ligand complexes ***under reduced conditions*** for fraction F1 (containing MtrC) and in the combined fractions F2 to F7. The U(VI) and U(IV) fractions were obtained by ion exchange chromatography. Data presented in **Fig. S5**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | [MtrC] (μM) | Standard deviation [MtrC] | [U] (μM) | Standard deviation [U] | [U]/[MtrC] | Standard deviation [U]/[MtrC] |
| carbonate | 75.79 | 5.52 | 85.57 | 3.19 | **1.17** | 0.11 |
| hydroxo | 74.11 | 2.36 | 88.32 | 7.27 | **1.15** | 0.05 |
| citrate | 75.79 | 4.74 | 91.26 | 2.12 | **1.15** | 0.06 |
| NTA | 74.67 | 0.78 | 87.53 | 1.53 | **1.17** | 0.02 |
| EDTA | 74.95 | 5.14 | 92.95 | 6.27 | **1.24** | 0.10 |

**Table S8**: Ratios between the concentration of U and MtrC (displayed in red) in the kinetics experiment reported on **Fig. 1**. The data from two replicate reactions were averaged here.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **carbonate - [U] = 100 µM, [HCO3-] = 30 mM** | | |
| Species | UO2(CO3)22- | UO2(CO3)34- |  |
| Speciation (%) | 33.3 | 66.7 |  |
|  |  |  |  |
|  | **NTA - [U] = 100 µM, [NTA] = 30 mM** | | |
| Species | UO2NTA | UO2(OH)NTA |  |
| Speciation (%) | 2.03 | 97.6 |  |
|  |  |  |  |
|  | **EDTA - [U] = 100 µM, [EDTA] = 30 mM with De Stefano et al. 2006** | | |
| Species | UO2EDTA2- | UO2(OH)EDTA3- | UO2 (H)EDTA- |
| Speciation (%) | 5.09 | 94.9 | 0.039 |
|  |  |  |  |
|  | **EDTA - [U] = 100µM, [EDTA] = 30 mM with Hummel et al. 2005** | | |
| Species | UO2EDTA2- | UO2 (HEDTA)- | (UO2)2EDTA(aq) |
| Speciation (%) | 100 | 0 | 0 |
|  |  |  |  |
|  | **citrate - [U] = 100 µM, [citrate] = 5 mM** | | |
| Species | UO2cit- | UO2(H)cit | (UO2)2cit22- |
| Speciation (%) | 73.8 | 0 | 26.2 |
|  |  |  |  |
|  | **hydroxo - [U] = 100 µM** | | |
| Species | UO2OH+ | UO2(OH)3- |  |
| Speciation (%) | 0.2 | 99.8 |  |

**Table S9**: U speciation calculated using Mineql for U(VI)-ligand complexes at pH 7.5, with 50 mM NaCl, and 100 mM HEPES.

|  |  |  |
| --- | --- | --- |
| Compound | LogK | Reference |
| **U(VI)** | | |
| U(VI)O2OH+ | -5.25 ± 0.24 | Guillaumont et al. 2003 |
| U(VI)O2(OH)2(aq) | -12.15 ± 0.07 | Guillaumont et al. 2003 |
| U(VI)O2(OH)3- | -20.25 ± 0.42 | Guillaumont et al. 2003 |
| UO2(OH)42- | -32.40 ± 0.68 | Guillaumont et al. 2003 |
| (U(VI)O2)2OH3+ | -2.70 ± 1.00 | Guillaumont et al. 2003 |
| (U(VI)O2)3(OH)42+ | -11.90 ± 0.30 | Guillaumont et al. 2003 |
| (U(VI)O2)3(OH)5+ | -15.55 ± 0.12 | Guillaumont et al. 2003 |
| (U(VI)O2)3(OH)7- | -32.20 ± 0.80 | Guillaumont et al. 2003 |
| (U(VI)O2)4(OH)7+ | -21.90 ± 1.00 | Guillaumont et al. 2003 |
|  |  |  |
| U(VI)O2(CO3)22- | 16.94 ± 0.12 \* | Grenthe et al. 1992 |
| U(VI)O2(CO3)34- | 21.60 ± 0.05 \* | Pashalidis et al 1997 |
|  |  |  |
| U(VI)O2NTA- | 8.21 ± 0.02 | De Stefano et al. 2006 |
| U(VI)O2(OH)NTA2- | 2.39 ± 0.04 | De Stefano et al. 2006 |
|  |  |  |
| U(VI)O2EDTA2- | 9.81 ± 0.015 | De Stefano et al. 2006 |
| U(VI)O2(OH)EDTA3- | 3.58 ± 0.03 | De Stefano et al. 2006 |
| U(VI)O2 (H)EDTA- | 15.19 ± 0.02 | De Stefano et al. 2006 |
| U(VI)O2 (H)EDTA- | 8.37 ± 0.1 | Hummel et al. 2007 |
| U(VI)O2EDTA2- | 13.7 ± 0.2 \* | Hummel et al. 2007 |
| (U(VI)O2)2EDTA(aq) | 20.6 ± 0.4 | Hummel et al. 2007 |
|  |  |  |
| U(VI)O2cit- | 8.96 ± 0.11 | Hummel et al. 2007 |
| U(VI)O2(H)cit | 5 ± 1.0 | Hummel et al. 2007 |
| (U(VI)O2)2cit22- | 21.3 ± 0.3 | Hummel et al. 2007 |
|  |  |  |
| **U(IV)** | | |
| U(IV)EDTA | 29.5 ± 0.2 \* | Hummel et al. 2007 |
|  |  |  |
| U(IV)NTA | 15.6 ± 0.8 | Bonin et al. 2009 |
| U(IV)NTA2 | 28.6 ± 1.6 | Bonin et al. 2009 |

|  |  |  |
| --- | --- | --- |
| U(IV)(cit)22- | 19.46 | Hummel et al. 2007 |

**Table S10**: LogK of aqueous complexes of interest in this work. The logK bearing the symbol \* are the recognized value of the Nuclear Energy Agency (NEA).

|  |  |  |  |
| --- | --- | --- | --- |
| U(VI)-carbonate + MtrC MR-1 | | | |
| Time | %U(VI) | %U(IV) | [U tot] (µM) |
| 30s | 19.5 | 80.5 | 518.5 |
| 60s | 21.2 | 78.8 | 563.4 |
| 2min | 20.8 | 79.2 | 558.5 |
| 5min | 26.7 | 73.3 | 613.3 |
| 20min | 17.8 | 82.2 | 608.7 |

**Table S11**: Percentage of U(VI) and U(IV) obtained by ion exchange chromatography corresponding to the reaction between U(VI)-carbonate ([U(VI)-carbonate] = 600 µM) and reduced MtrC MR-1 ([MtrC MR-1] = 300 µM), also analyzed by M4-edge HR-XANES (**Fig. 5**, **Table S12.a**) after 30s, 60s, 2min, 5min and 20min of reaction. The ratio between U and MtrC MR-1 concentrations was [U]/[MtrC MR-1] = 1.54. The ion exchange chromatography separation cannot directly identify U(V), because the samples are acidified prior to loading onto the column. Acid treatment is known to disproportionate uranyl(V) to produce equal proportions of U(V) and U(IV). Therefore, here, the equal proportions observed for U(VI) and U(IV) in the supernatant are a proxy for U(V) (result demonstrated by U M4-edge HR-XANES spectroscopy).

|  |  |  |
| --- | --- | --- |
| A - U(VI)-carbonate + MtrC MR-1 | | |
|  | Sample name | White line (keV) |
| standards | U(VI)-carbonate + MtrC oxidized | 3.72746 |
| U(V)-iodine | 3.72699 |
| U(IV)O2 | 3.72572 |
| timeline samples | U(VI)-carbonate + MtrC reduced at 30s | 3.72689 |
| U(VI)-carbonate + MtrC reduced at 1min | 3.72666 |
| U(VI)-carbonate + MtrC reduced at 2min | 3.72656 |
| U(VI)-carbonate + MtrC reduced at 5min | 3.72657 |
| U(VI)-carbonate + MtrC reduced at 20min | 3.72620 |
|  |  |  |
|  |  |  |
| B - U(VI)-NTA + MtrC baltica | | |
|  | Sample name | White line (keV) |
| standards | U(VI)-NTA | 3.72724 |
| U(V)-iodine | 3.72693 |
| U(IV)O2 | 3.72595 |
| timeline samples | U(VI)-NTA + MtrC reduced at 15s | 3.72680 |
| U(VI)-NTA + MtrC reduced at 30s | 3.72617 |
| U(VI)-NTA + MtrC reduced at 60s | 3.72650 |
|  |  |  |
|  |  |  |
| C - U(VI)-EDTA - MtrC baltica | | |
|  | Sample name | White line (keV) |
| standards | U(VI)-NTA | 3.727235 |
| U(V)-iodine | 3.72693 |
| U(IV)O2 | 3.72595 |
| timeline samples | U(VI)-EDTA + MtrC reduced at 15s | 3.72693 |
| U(VI)-EDTA + MtrC reduced at 30s | 3.72673 |
| U(VI)-EDTA + MtrC reduced at 60s | 3.7266 |
|  |  |  |
|  |  |  |
| D- Size-exclusion experiment - MtrC baltica | | |
|  | Sample name | White line (keV) |
| standards | U(VI)-NTA | 3727.32 |
| U(VI)-carbonate | 3727.28 |
| U(V)-iodine | 3726.63 |
| U(IV)O2 | 3725.58 |
| timeline samples | NTA - 15min | 3727.11 |
| carbonate -15min | 3727.19 |

**Table S12**: Summary of the whitelines of the M4-edge HR-XANES spectra described in this work. Four HR-XANES experiments are presented here and were not necessarily performed during the same beamtime. Hence for each set of samples, the set of associated standards used for linear combination fitting is also reported.

1. Timeline between U(VI)-carbonate and reduced MtrC MR-1 (from *S. oneidensis* MR-1). [U(VI)-carbonate] = 600 µM and [MtrC MR-1] = 300 µM, pH 7.5, buffer [NaCl] =50 mM and [HEPES] = 100 mM. Time points were taken and instantaneously frozen after 30s, 60s, 2min, 5min and 20min of reaction.
2. Timeline between U(VI)-NTA and reduced MtrC baltica (from *S. oneidensis* baltica). [U(VI)-NTA] = 600 µM and [MtrC baltica] = 300 µM, pH 7.5, buffer [NaCl] = 50 mM and [HEPES] = 100 mM. Time points were taken and instantaneously frozen after 15s, 30s, and 60s of reaction.
3. Timeline between U(VI)-EDTA and reduced MtrC baltica (from *S. oneidensis* baltica). [U(VI)-EDTA] = 600 µM and [MtrC baltica] = 300 µM, pH 7.5, buffer [NaCl] = 50 mM and [HEPES] = 100 mM. Time points were taken and instantaneously frozen after 15s, 30s, and 60s of reaction.
4. Size-exclusion chromatography (SEC) purified reaction between U(VI)-carbonate and reduced MtrC baltica. MtrC baltica was separated from reaction mixture bulk by SEC, and immediately frozen. The total reaction time before freezing was measured to be 15min. [U(VI)] = 600 µM and [MtrC baltica] = 300 µM, pH 7.5, buffer [NaCl] = 50 mM and [HEPES] =100 mM.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 1. **Ion-exchange chromatography after 16.5min** | | | | | |
|  |  |  |  |  |  |
| %U(IV) | standard deviation %U(IV) | [Utot] (µM) | standard deviation [Utot] | [MtrC] (µM) | [Utot]/[MtrC] |
| U(VI)-carbonate + MtrC baltica | | | | | |
| **52.83** | 0.005 | 595.33 | 1.38 | 334 | 1.78 |
| U(VI)-NTA + MtrC baltica | | | | | |
| **97.76** | 0.002 | 654.11 | 1.61 | 297 | 2.20 |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
| 1. **Size-exclusion chromatography after 15min** | | | | | |
|  |  |  |  |  |  |
| [Ubound] (µM) | standard deviation %U(IV) | [Utot] (µM) | standard deviation [Utot] | [MtrC] (µM) | [Ubound]/[MtrC] |
| U(VI)-carbonate + MtrC baltica | | | | | |
| **54.68** | 1.12 | 595.33 | 1.38 | 334 | 0.16 |
| U(VI)-NTA + MtrC baltica | | | | | |
| **2.34** | 0.01 | 654.11 | 1.61 | 297 | 0.01 |

**Table S13**: Experimental data illustrating the HR-XANES experiment reported **Fig. 6** and **Table S12.d**. The standard deviations reported here average two technical replicates.

1. Percentage of U(IV) obtained by ion-exchange chromatography after 16.5min of reaction between reduced MtrC baltica and both U(VI)-carbonate (light blue) and U(VI)-NTA (orange). The total concentration of U, MtrC baltica and their ratio are reported in this table.
2. Concentration of U bound to MtrC baltica ([Ubound]) after 15min of reaction between reduced MtrC baltica and both U(VI)-carbonate (light blue) and U(VI)-NTA (orange). Ubound correspond to the U recovered in the first fraction eluted from the size-exclusion column, also containing the protein. It represents the U interacting with MtrC baltica.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| %U(IV) | standard deviation %U(IV) | [Utot] (µM) | standard deviation [Utot] | [MtrC] (µM) | [Utot]/[MtrC] |
| U(VI)-NTA + MtrC baltica | | | | | |
| 67.61 | 1.92 | 648.54 | 3.34 | 280.86 | 2.31 |
| U(VI)-EDTA + MtrC baltica | | | | | |
| 81.43 | 0.60 | 650.81 | 1.43 | 266.22 | 2.44 |

**Table S14**: Percentage of U(IV) obtained by ion exchange chromatography corresponding to the reaction between U(VI)-NTA or U(VI)-EDTA and reduced MtrC baltica after 2.5min, analysed by M4-edge HR-XANES (**Fig. 7**, **Fig. S12**, **Table S12.b. and S12.c.**). The total concentration of U, MtrC baltica and their ratio are reported in this table. The standard deviations reported here average two technical replicates.