

# Functional Characterization of Microbial Proteins Involved in Biogeochemical Electron Transfer Reactions

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## Introduction and Objectives

### Scope

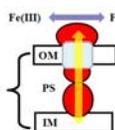
As part of the PNNL SESP SFA's Biogeochemical Electron Transfer Science Theme, this research project focuses on developing a molecular-level understanding of key microbial protein-mediated Fe(III) redox reactions in the subsurface and their constitutive relationships to redox transformation of U(VI), Tc(VII), Pu(IV), and Pu(VI).



### Motivation

U, Tc and Pu are primary risk-drivers at Hanford site and the valence state of U, Tc and Pu impacts their geochemical behavior. Many Fe(II)- and Fe(III)-containing minerals exist in Hanford sediment where they can serve as energy sources for Fe(II)-oxidizing microorganisms and as terminal electron acceptors for Fe(III)-reducing microorganisms, respectively. The microbial proteins involved in Fe(III) redox reactions affect the valence state of U, Tc and Pu directly and indirectly by affecting the supply and availability of reactive Fe(II).

### Important Science Issues

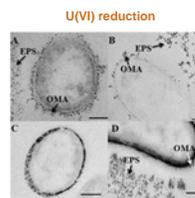
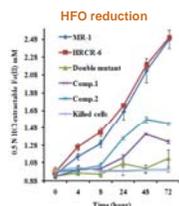


Microbial proteins involved in extracellular electron transfer (ET) reactions play critical roles in Fe(III) redox transformations. These microbial proteins, which are strategically located in the microbial cell envelope, form pathways to facilitate ET across the cell envelope. c-Type cytochromes are the key components of these ET pathways. The molecular mechanisms used by these microbial proteins for extracellular ET are poorly characterized. The results of these proposed studies will contribute to resolution of PNNL SESP SFA hypotheses pertaining to the role of subsurface microenvironments and transition zones as dominant regions of contaminant oxidation-reduction reactions at Hanford.

### Approach

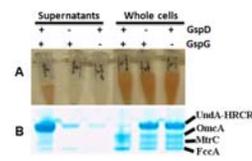
A coupled approach with experimental characterization and molecular modeling are used to gain the mechanistic insights at the molecular level for the ET reactions mediated by the redox proteins of Hanford-relevant and model microorganism

## Characterization of UndA-HRCR-6



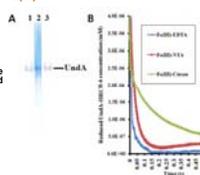
- In collaboration with the Bellaev/Marshall SFA task, seven U(VI)- and Tc(VII)-reducing *Shewanella* isolates from the Hanford Reach of the Columbia River (HRCR) were selected. These strains were PCR-screened for the presence of *mtrC*/*omcA*/*undA*-like genes in their genomes.
- All *Shewanella* strains screened were found to contain *mtrC*-like genes. In addition, they have either *omcA*- or *undA*-like genes.
- The entire coding region of an *undA*-like gene was cloned from HRCR-6 and sequenced. HRCR-6 may be relevant to the IFRC project since it was isolated at the U-seeps near the 300 area.
- UndA-HRCR-6 has 11 putative heme-binding sites and is 73% and 93% identical to the UndA1 of *S. putrefaciens* CN-32 and the UndA of *S. salicis* OS23, respectively.
- In vivo, UndA-HRCR-6 complemented the impaired phenotype of a *S. oneidensis* MR-1 mutant lacking MtrC and OmcA in both Fe(II) oxide- and U(VI)-reduction assays.

### Translocation by T2S



In *S. oneidensis* MR-1, the recombinant UndA-HRCR-6 was translocated across the bacterial outer membrane by the type II secretion system (T2S).

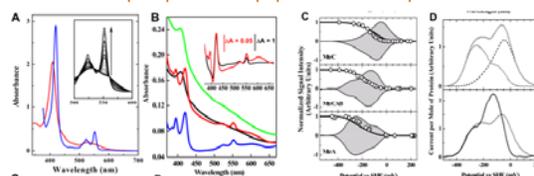
### Reduction of chelated Fe(III)



- UndA-HRCR-6 is purified as a functional c-type cytochrome from the membrane fraction and the purified UndA-HRCR-6 reduces chelated Fe(III).
- UndA-HRCR-6 has functional roles in extracellular metal reduction.

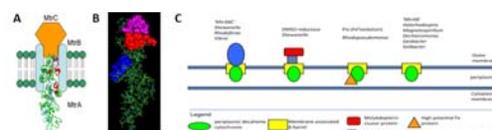
## Characterization of Mtr proteins of *Shewanella oneidensis* MR-1

### Spectropotentiometric properties of the MtrABC proteins



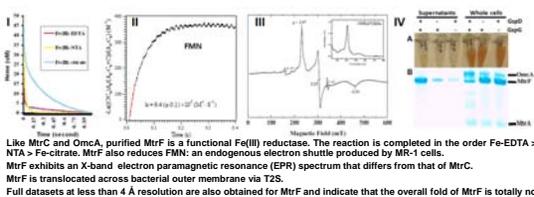
- Characterization of Mtr proteins was carried out mainly by the Richardson's group at University of East Anglia.
- Purified MtrABC complex exhibits a UV-vis spectrum that is typical of a c-type cytochrome and can transfer electrons across a lipid bilayer.
- The redox properties of MtrC and MtrA are modulated on formation of the MtrABC complex.

### Conceptual model of MtrABC complex



- A model is proposed for the modular organization of MtrABC complex.
- Consistent with this model, protein cross-linking results and a structural model of MtrA show that i) MtrA physically interacts with MtrB and MtrC and ii) ~50% of the MtrA heme chain is embedded within MtrB.
- This model could also apply more widely to mechanisms of electron exchange between related proteins present in a number of other Gram-negative bacterial genera and extracellular electron sources and sinks.

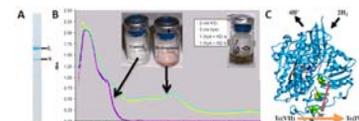
### Characterization of MtrF



- Like MtrC and OmcA, purified MtrF is a functional Fe(III) reductase. The reaction is completed in the order Fe-EDTA > Fe-NTA > Fe-citrate. MtrF also reduces FMN, an endogenous electron shuttle produced by MR-1 cells.
- MtrF exhibits an X-band electron paramagnetic resonance (EPR) spectrum that differs from that of MtrC.
- MtrF is translocated across bacterial outer membrane via T2S.
- Full datasets at less than 4 Å resolution are also obtained for MtrF and indicate that the overall fold of MtrF is totally novel.

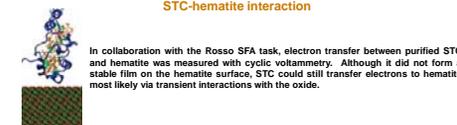
## Characterization of other redox proteins

### Purification and characterization the *S. oneidensis* MR-1 [NiFe]-H<sub>2</sub>ase



[NiFe]-hydrogenase of *S. oneidensis* MR-1 (SO-[NiFe]-H<sub>2</sub>ase) was successfully purified. The purified SO-[NiFe]-H<sub>2</sub>ase reduces Tc(VII) *in vitro* assays.

### STC-hematite interaction



In collaboration with the Rosso SFA task, electron transfer between purified STC and hematite was measured with cyclic voltammetry. Although it did not form a stable film on the hematite surface, STC could still transfer electrons to hematite most likely via transient interactions with the oxide.

### Microbial proteins involved in extracellular oxidation of Fe(II)



- MtrAB/PioAB homologues are found in the neutrophilic Fe(II)-oxidizing bacteria *Gallionella ferruginea* ES-2 and *Sideroxydans lithotrophicus* ES-1.
- Investigations of MtrABs of *G. ferruginea* ES-2 and *S. lithotrophicus* ES-1 are being initiated.

## Summary and Conclusions

- UndA-HRCR-6 is an outer membrane c-type cytochrome that has functional roles in extracellular metal reduction.
- MtrABC complex has two functional modules: MtrAB and MtrC. MtrAB module translocates electrons across the outer membrane to MtrC that serves as an extracellular module to mediate electron transfer to solid and extracellular soluble substrates.
- MtrAB homologues are found in a number of other Gram-negative bacterial genera, including neutrophilic Fe(II)-oxidizing bacteria, and other outer membrane ET systems, such PioAB of the Fe(II)-oxidizing bacterium *R. palustris*.
- MtrF is translocated across the bacterial outer membrane by T2S and reduces chelated Fe(III) *in vitro* and *in vivo*.

## Future Research

- Investigate other key proteins found in HRCR-6.
- Investigate the roles of MtrDEF in Fe(III) oxide and Cr(VI) reduction.
- Determine MtrDEF interactions by cross-linking (in collaboration with Joshua Adkins/Mary Lipton).
- Molecular modeling of interfacial ET between MtrF and hematite (in collaboration with the Rosso task).
- Investigate MtrABs found in *G. ferruginea* ES-2 and *S. lithotrophicus* ES-1 (in collaboration with the Roden task).

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