

# Functional Characterization of Key Proteins Involved in Metal Redox Transformations

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**Objective:** To understand the molecular mechanisms by which microbial macromolecules (e.g. redox proteins) engage and react with U and Tc contaminants and with redox-active metals, such as Fe and Mn.

**Scope:** Molecular characterization of the redox-active biomolecule-facilitated electron transfer processes that are used for valence transformation of U, Tc, Fe and Mn in the cell envelope of Hanford-relevant microorganisms.

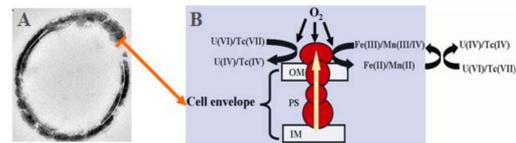
## Hanford/DOE Site Motivation

- U and Tc are primary risk-drivers at Hanford & other DOE/IFRC sites & the valence state of U and Tc impacts their geochemical behavior.
- A variety of microbial redox-active macromolecules affect the U and Tc valence states directly & indirectly by reactive products, such as Fe(II).

## PNNL SFA Hypothesis

Redox valence transformations of U and Tc occur in microenvironments where: i) O<sub>2</sub> is consumed by microbial respiration; ii) microbes populate their cell envelope with redox-active biomolecules; and iii) ubiquitous ferrous containing mineral solids are exposed for dissolution and reaction.

## Important Science Issues



- Valence transformations of metals occur via electron transfer (ET) processes facilitated by redox-active biomolecules in the microbial cell envelopes.
- The biomolecule-mediated ET mechanisms in microbial cell envelopes are poorly characterized.

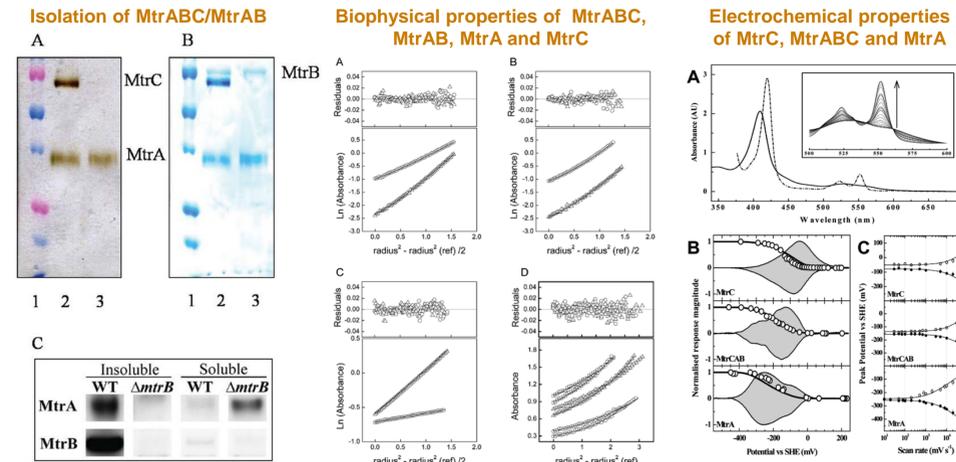
## Relationship with Other PNNL SFA Projects

Identification of Hanford-relevant microorganisms & their biomolecules directly involved in valence transformation of U, Tc and Fe/Mn (Konopka & Breliaev projects)

Functional characterization of microbial macromolecules

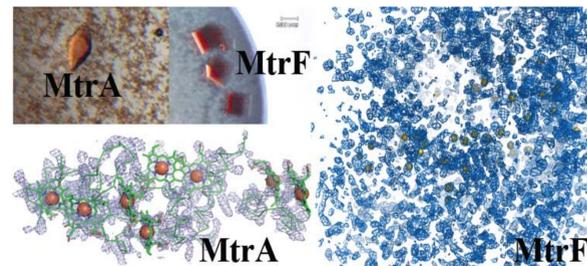
Molecular modeling the elementary biomolecular electron transfer processes (Rosso project)

## Characterization of MtrABC Complex of *Shewanella oneidensis* MR-1



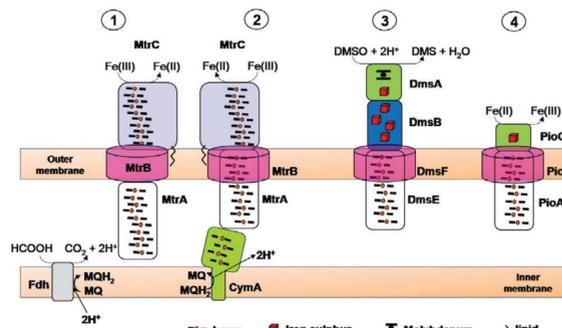
- A stable MtrAB sub-complex can assemble in the absence of MtrC, but an MtrBC sub-complex is not assembled in the absence of MtrA.
- MtrC and MtrA can form a complex *in vitro* with a *K<sub>d</sub>* of 11 ± 4 μM.
- MtrABC complex has an electron transfer capacity similar to that of MtrC, which is about 4 times higher than MtrA alone.
- When bound to MtrAB complex, the operating potential domain of MtrC is shifted by about -100 mV.
- A manuscript is in revision.

## Structural determinations of MtrA and MtrF of *S. oneidensis* MR-1

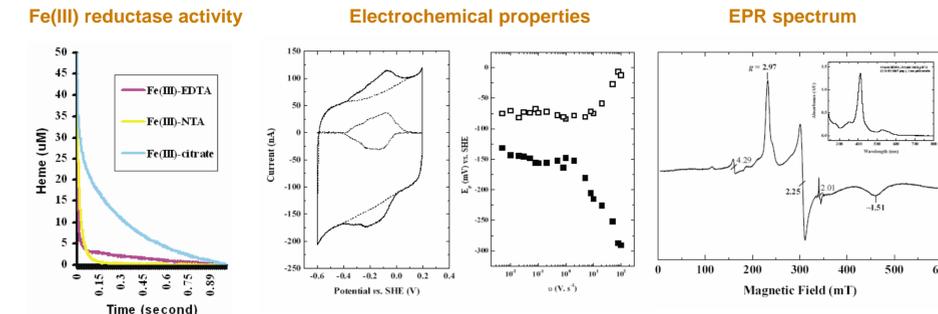


- MtrA and MtrF (an MtrC homolog) are crystallized. The efforts for determining their molecular structures are underway.
- A full dataset at 3 Å for MtrA is obtained. Relative locations of eight of ten heme groups are resolved for MtrA.
- A full dataset at 5 Å for MtrF is also obtained. All ten heme groups can be detected in MtrF.

## Conceptual models



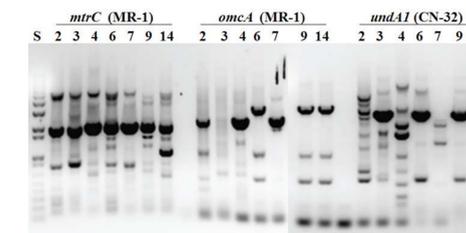
## Characterization of MtrF of *S. oneidensis* MR-1



- Purified MtrF is a functional Fe(III) reductase and can transfer electrons to the electrode surface.
- MtrF exhibits an X-band electron paramagnetic resonance (EPR) spectrum that differs from that of MtrC.

## Gene cloning and protein purification of UndA-HRCR-6

PCR amplifications of *mtrC/omcA/unda1*-like genes from the strains isolated from the Hanford Reach of the Columbia River (HRCR)

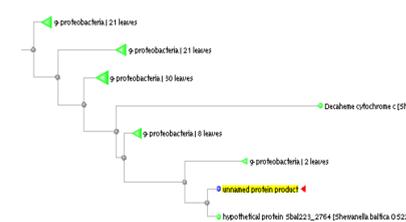


## Deduced protein sequence of UndA-HRCR-6

MMKRFNFNTATKAMLGAGLLSLLLAGCGGSDGKDGEDGKPGVGLDISQAT  
TLKATLEDVKIDNGTNSVDIVLNTANGVPVTLGLEQYQAINAGLGIKLTPESGKG  
YKTPQWVSYSINSVKAADPARSLANSYSYTDGDSAGNPITKEVKFTPGDAIQANIE  
SSCKTCLLVDSGVYRYTFQTLNLSLPAEGLLTYDPTLHRLTLELQTPGSKDA  
KLVNSHIDLPLSDNFRVAKETETRTVDLEANCCKCHSTNYSSTAKPLALHGG  
RRIGIANCOVCHTSYKSDPETSPLDMGAMVHAHKGTYAVMVGYSGTAYDFSGT  
MAKAAESGYPYREGKDVSRVTLPSVIGNCQCHSTDDKGPVDAASFHKGK  
LACASCHMSGPNVDSEWLTPEGGKDRGVGNHYHYATPEIDGPGVNLVH  
VFQNGGCASCHAEQEGESAKYHLAKANATKLLRTEYAYKLENGTDFVAKGEL  
TFTVNVHSDVAPHQDPKVKEFVVSLSLTFANGTEYTMGPRPSNGTLGRSENRSVN  
LAKVETNANLTAVPNGSKVTYTLTGKAVIGTSSVYKQVSIKGFMDGKLLICA  
NSAELDPTMDAADSNTFAPYEVIVGSKAKSASADASNVTARISVISEAKCANC  
HGEKADPSASHALTAADKPDNSCGTCHSAVPNTAVALADESCVACIINGAPAH  
SKKPFERGFVKMHIQHADRSTRVRLTTDAATFPENPANCAACHDKGQLSLAT  
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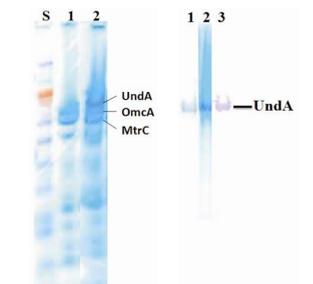
- Seven *Shewanella* strains isolated from the HRCR were tested for the presence of *mtrC/omcA/unda1*-like genes in their genomes because they all reduced U(VI) & Tc(VII).
- All tested *Shewanella* strains contain *mtrC*-like genes. In addition, they have either *omcA* or *unda1*-like genes

## Phylogenetic analysis of UndA-HRCR-6



- The entire coding region of an *unda1*-like gene cloned from the HRCR-6 is sequenced, as HRCR-6 is most likely an outer membrane c-type cytochromes.
- The deduced protein sequence of 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. baltica* OS223, respectively.

## Overexpression and purification of UndA-HRCR-6



- UndA-HRCR-6 can be overexpressed in *S. oneidensis* MR-1 cells.
- UndA-HRCR-6 is purified as a functional c-type cytochrome from the membrane fraction.

## Major Conclusions

- MtrABC complex of *S. oneidensis* MR-1 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.
- MtrF is an active metal reductase that can transfer electrons to the electrode surface.
- All tested *Shewanella* strains isolated from the HRCR contain *mtrC*-like and *omcA* or *UndA1*-like genes, consistent with their observed metal reductase activities.
- UndA-HRCR-6 is most likely an outer membrane c-type cytochromes.

## Future research

- Determine whether UndA-HRCR-6 can to complement MtrC/OmcA for reducing U(VI) and Fe(III) oxides *in vivo*.
- Characterize UndA-HRCR-6 *in vitro*, including its molecular structure.
- Characterize the redox proteins found in the cell envelopes of the microorganisms isolated from the Hanford site.