

# Functional Characterization of Microbial Macromolecules

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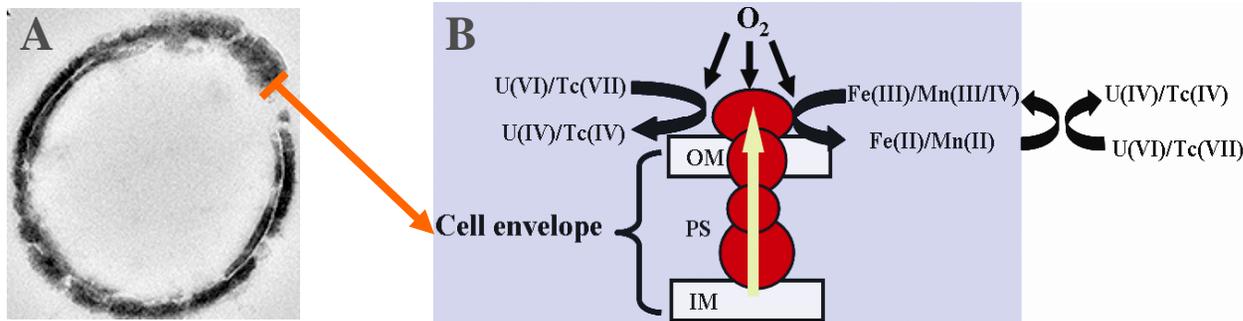
➤ **Objective:** To understand the molecular mechanisms by which microbial macromolecules (e.g. redox proteins) engage and react with U and Tc contaminants and with redox-active metals, such as Fe and Mn.

➤ **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.



# Science Problems

Valence transformations of metals occur via electron transfer (ET) processes facilitated by redox-active biomolecules in the microbial cell envelopes.



Marshall et al., 2008

- Undetermined ET mechanisms from the redox-active biomolecules to U, Tc and Fe/Mn oxides.
- Poorly characterized molecular mechanisms that regulates ET across the microbial cell envelope.
- Lack of detailed understandings of how O<sub>2</sub> impacts these ET processes.

## SFA Hypothesis

Reductive 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.

## Research Team

**PNNL:** Liang Shi (Protein Biochemistry), Zheming Wang (U and Fe reactions) and Dave Kennedy (U and Fe reactions)

**External collaborator:** David Richardson of University of East Anglia



# Relationship with Other SFA Projects

Identification of Hanford-relevant microorganisms & their biomolecules directly involved in valence transformations of U, Tc and Fe(III)/Mn(IV) oxides (Konopka & Beliaev projects)



Functional characterization of microbial macromolecules



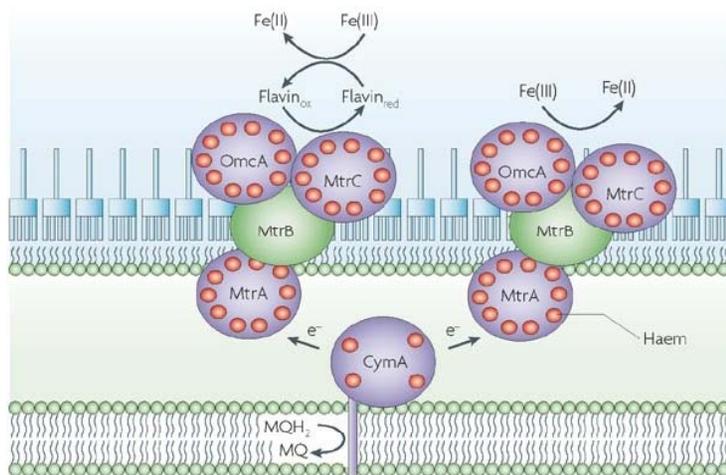
Molecular modeling the elementary biomolecular electron transfer processes (Rosso project)



# FY 09 Work Scope

## Scopes

### EMSL Biogeochemistry Grand Challenge (BGC): electron transfer mechanism at the microbe-mineral interface.



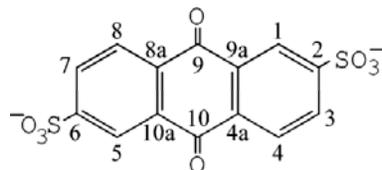
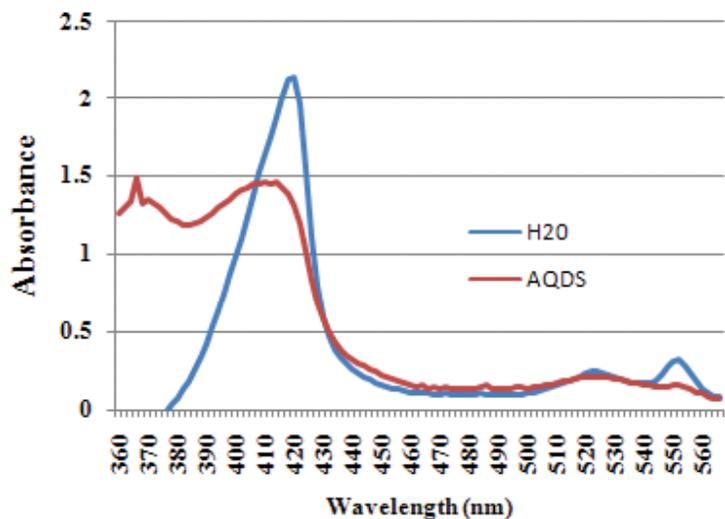
Extracellular electron transfer pathways of *Shewanella oneidensis* MR-1 (Fredrickson et. al., 2008)

- Determine interfacial electron transfer between STC and hematite electrode (protein film voltammetry).
- Investigate electron transfer from MtrC/OmcA to FMN, AQDS and other electron shuttles (stopped-flow technique/theoretical computation).
- Clone and sequencing the *mtrC/omcA/undA1*-like genes from the Columbia river isolates and purify and characterize the MtrC/OmcA/UndA1-like proteins.
- Characterize MtrABC complex by cross-linking (in collaboration with Josh Adkins).
- Characterize the outer membrane proteins that physically interact with MtrC/OmcA, such as SOA0110 and SO0404.

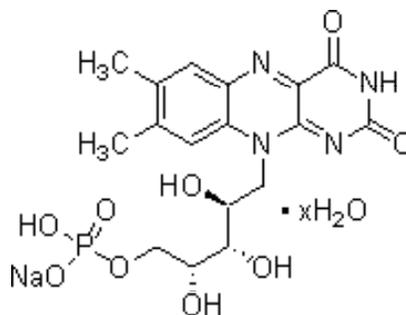
# Investigation of electron transfer from MtrC/OmcA to different electron shuttles

B. The electron shuttles that are planned to be tested.

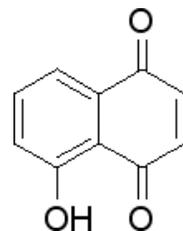
A. Re-oxidation of reduced MtrC by AQDS.



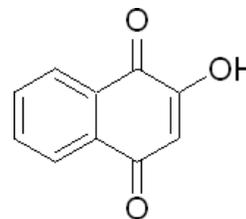
AQDS ( $E_m = -184$  mV)



FMN ( $E_m = -200$  mV)



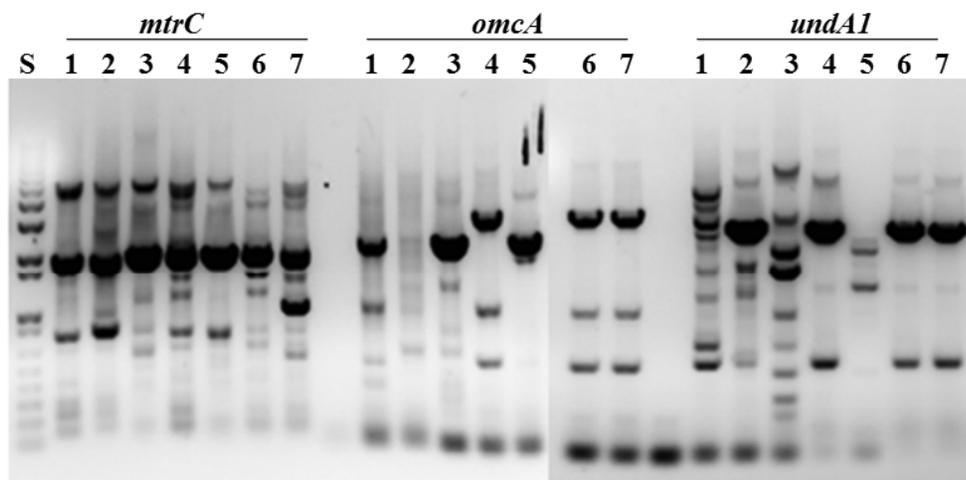
NQJ ( $E_m = -3$  mV)



NQL ( $E_m = -137$  mV)

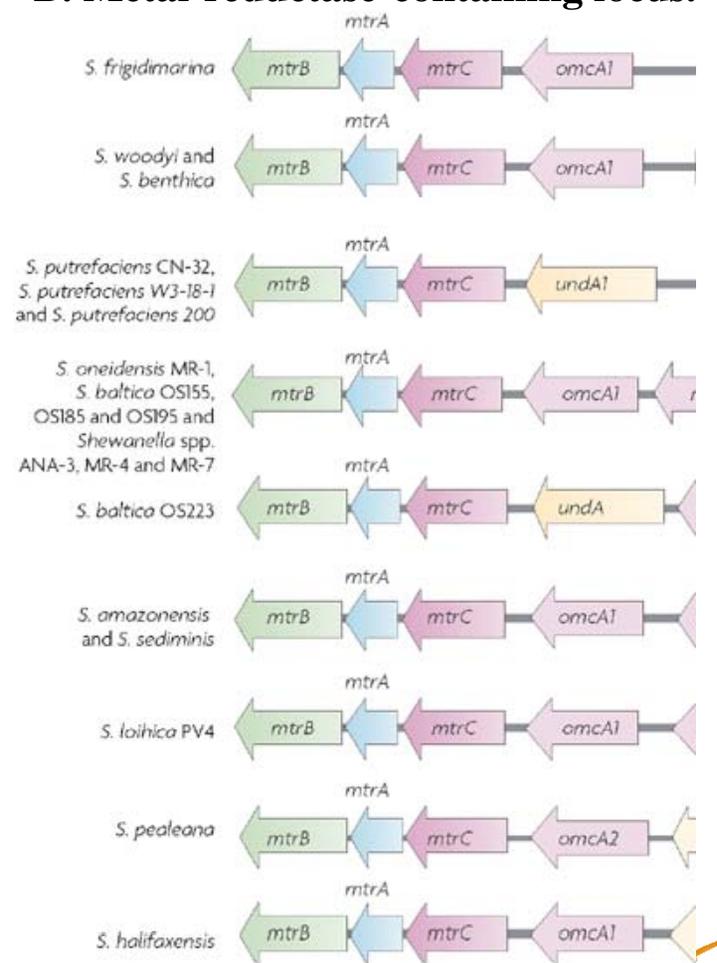
# Cloning and sequencing of the *mtrC/omcA/undA1*-like genes from the strains isolated from the Hanford Reach of the Columbia River (HRCR)

## A. PCR-amplification of *mtrC/omcA/undA1*-like genes.



**S: standard**  
**1: HRCR-2**  
**2: HRCR-3**  
**3: HRCR-4**  
**4: HRCR-6**  
**5: HRCR-7**  
**6: HRCR-9**  
**7: HRCR-14**

## B. Metal-reductase-containing locus.



(Fredrickson et. al., 2008)

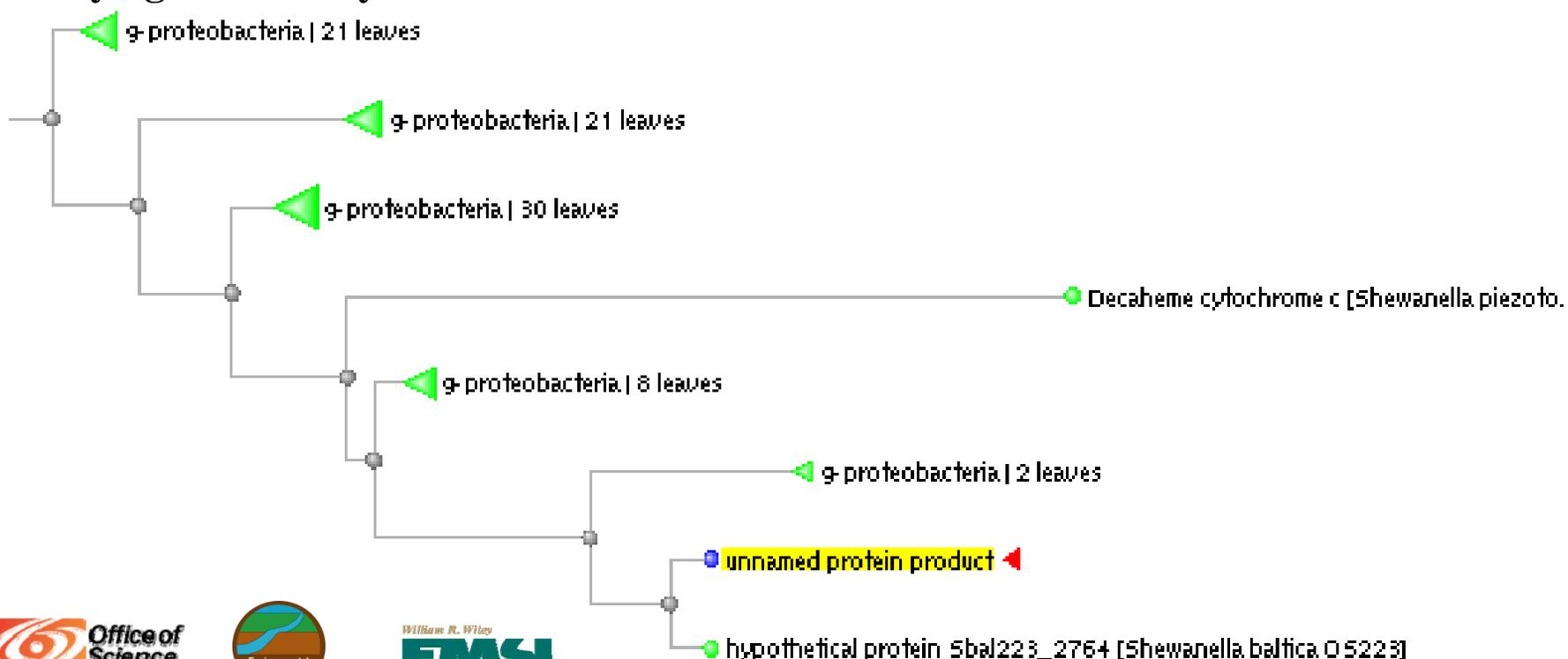
**Pacific Northwest**  
 NATIONAL LABORATORY

# Cloning and sequencing of the *undA1*-like gene from HRCR-6 isolate

## A. Deduced amino acid sequence of UndA-HRCR-6

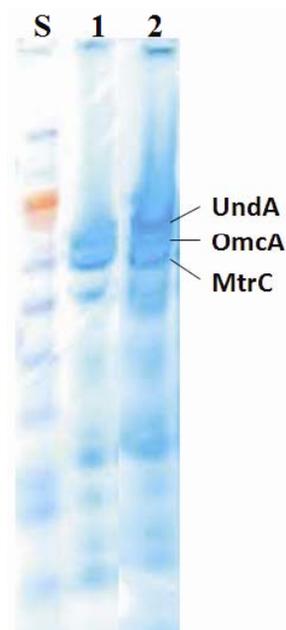
MMKRFNFNTATKAMLGAGLLSLLLACGGSDGKDGEDGKPGPVGLDISQATTLKATLEDVKIDNGTVSVDIVLTNANGVPVTGLEQYAQINAIGLGIKLTPESGK  
 GYKTPQWVSYINSVKAADPARSLANYSYTDGKDSAGNPITKEVKFTPGDAIQANIESSCKTTCLTVVDSGVYRYTFQTNLSTLPAIEGLDLTYDPTLIHRITLQLTDG  
 SKDAKLVNSHIDFLPSDNFRVAKETETRTVVDLEAN**CIKCHS**TNYSDTSSSTAKPLALHGRRIGIAN**CQVCHT**SYSKDPETGSPLDMGAMVHAIHKGTYAMVGYSGT  
 AYDFSGMTAKAAAESGYYPQYREGKDVSERVTLPVSIGN**CQSCH**STDDKGPVDAASFKHHKGLA**CASCH**MSGFNPVDNSEWLTPEEGQKDRGFVGNFYHYATPEI  
 DGIPGVNLVHVFQNGG**CASCH**AEQGEESAKYHLAKANATKLLRTEYAYKLENGTFDVAKGELTFTVNWHSADVAPHQDPKVKEFWVSLTAFNGTEYTMGPRPSN  
 GTLGRSENRI SVNLAKVETNANLTA VPNGSKVYTYLTGIIKAVIGTSSVPYKQIVSIGKGFMDGKLLICANSAELDPTMDAAIDCSNTEAPIYEVIVGSNKASFSADASN  
 VTARSIVISEAK**CANCH**GEKADFSASHALTHAADKPDNSC**GTCH**SA VPNTAVALADG**CVACH**NGAPAHSKKPFERGFDFKVMIHQIHADTRS VRRLTTDAATFPEN  
 PAN**CAACH**DKGQLSLATLGNKPAFLASTGEYSPTVAAC**CASCH**ATTATDSA VIGHFETNGGVYNAAGTYTPGSET**CATCH**GEGKSFGVDKVHPVKY

## B. Phylogenetic analysis of UndA-HRCR-6



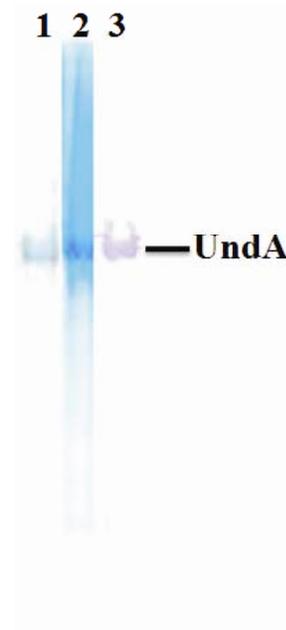
# Purification of UndA-HRCR-6

## A. Overexpression of UndA-HRCR-6 in MR-1 cells.



S: standard, 1: MR-1, 2: MR-1 + UndA-HRCR-6

## B. Purification of UndA-HRCR-6



1: Gel-code blue, 2: Heme-staining, 3: Western-blot



## Deliverables for FY09

- A. **Finish the mini-review for Environmental Microbiology Report (manuscript 1).**
- B. **Finish the measurements of electron transfer between STC and hematite electrode and provide data to the Rosso task (manuscript 2).**
- C. **Finish the measurements of electron transfer from the MtrC/OmcA to FMN, AQDS, NQJ and NQL and integrate the data with those from the Richardson task and those of theoretical computation from the Rosso task (manuscript 3).**
- D. **Clone and sequencing *cymA* (membrane & soluble forms) of MR-1 and *mtrC/omcA/undA1*-like genes of Columbia river isolates and send the constructs to the Richardson group.**
- E. **Characterize the UndA-HRCR-6.**

