Biogeochemistry Grand Challenge

"Electron Transfer at the Microbe-Mineral Interface"

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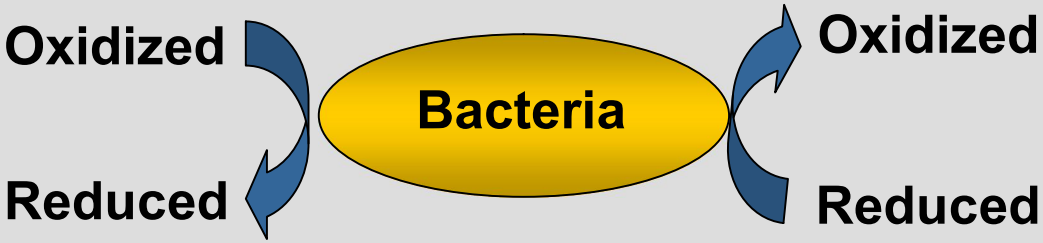
Poorly Explored Terrain: The Mineral-Microbe Interface

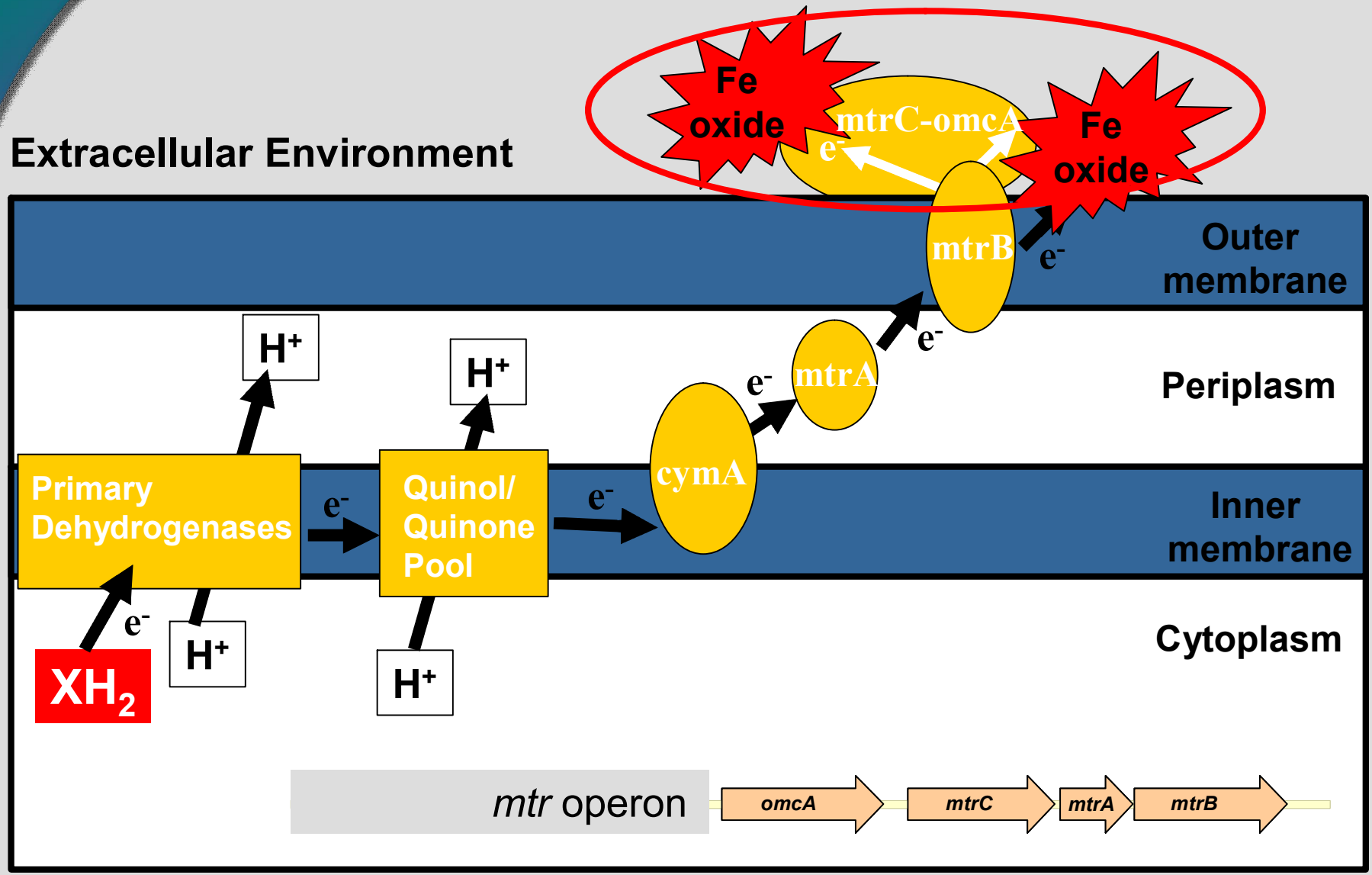
- Microorganisms influence their environment through energy and materials transfer across a complex biologic-solvent-solid interface
- The interfacial region is dynamic with chemistry and structure determined by interplay and response

Bacterial mediation of geochemical reaction



- Microorganisms mediate kinetically inhibited, but thermodynamically favorable reactions
- ΔE for metabolism and growth

Current Conceptual Model for Electron Transfer to Extracellular Substrates: *Shewanella*



Scientific Challenges

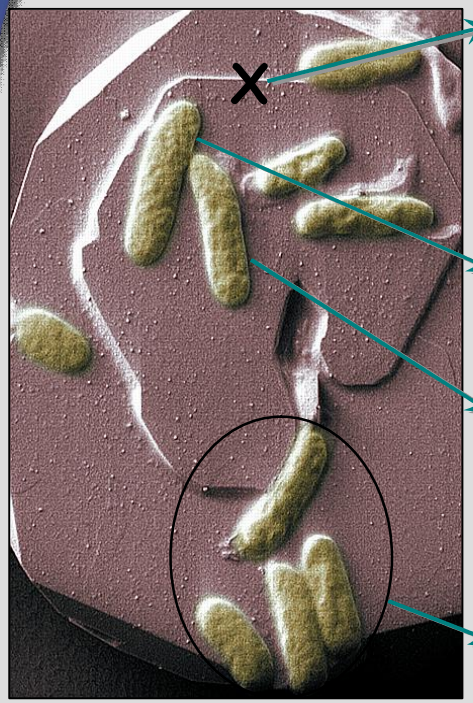
- Microbe-mineral interface is a nano-scale domain
  - Unique & dynamic architecture of biologic and inorganic components
  - Instrumentation needed with high spatial and molecular sensitivity
- Membrane proteins involved
  - Difficult to purify, crystallize, characterize etc.
  - Methods for *de novo* structure determination lacking
  - *In vitro* model systems require design to identify & evaluate mechanisms
- Interfacial effects may predominate
  - Complex orientation, electrostatic, and solvation issues
  - Coupled experiment & molecular modeling key to understanding

Outer Membrane *c*-Type Cytochromes

- Large (>50 kDa) metalloproteins common to multiple metal-reducing organisms
- Common electron transport chain components, plausible terminal reductase(s)
- Unique, multiple (10x in MR-1) redox centers (N-Fe-S heme sites) store and deliver electron density
- Orientation, solvation, and structure control individual heme properties
- Intermolecular heme coupling drives reactivity

Science Approach for Two Year Study (FY05 – FY06)

<u>Hypothesis:</u> Outer membrane cytochromes MtrC/OmcA responsible for direct e^--transfer to mineral substrates [Fe, Mn oxides]



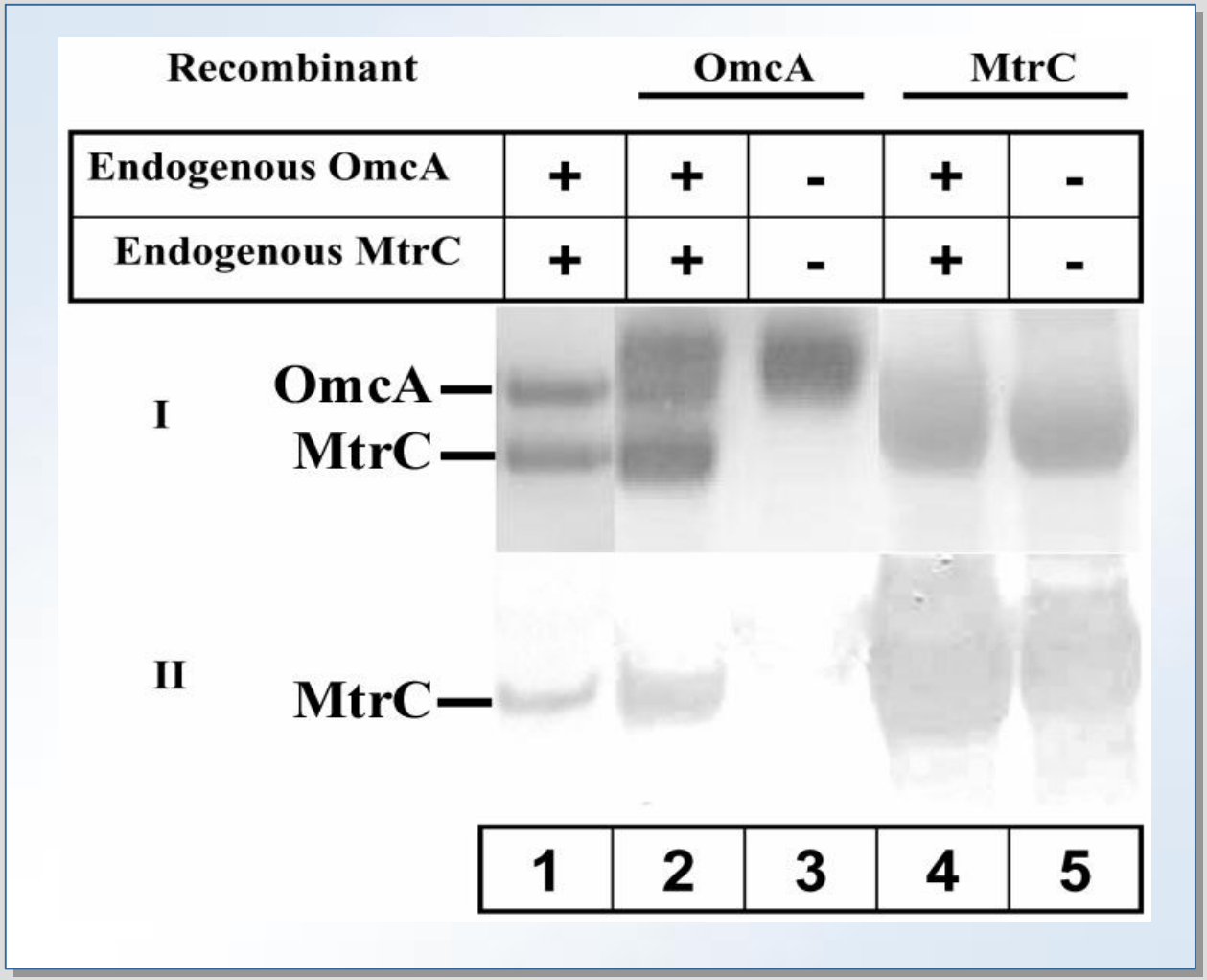
- Physical/mineralogic controls on electron transfer
  - defects
  - free energy
  → Mechanistic numeric experiments
    - heme-heme
    - heme-surface
  → Mechanisms and physical constraints

- Protein isolation and purification
  → Characterization
    - structure
    - redox properties
    - complexes
  → *In vitro* studies
    - metal complexes
    - metal solids
  → Fundamental rates and characteristics

- Mutagenesis and complementation
  ΔMtr, ΔOmC, ΔGsp, etc
  → Phenotype characterization

- Protein localization
  - periplasm, OM, and other features
  → Interfacial architecture
    - distances
    - functional groups
    - forces
  → *In vivo* studies
    - attachment
    - biofilm
    - products
  → **An Integrated Model**

OmcA and MtrC Co-Purify

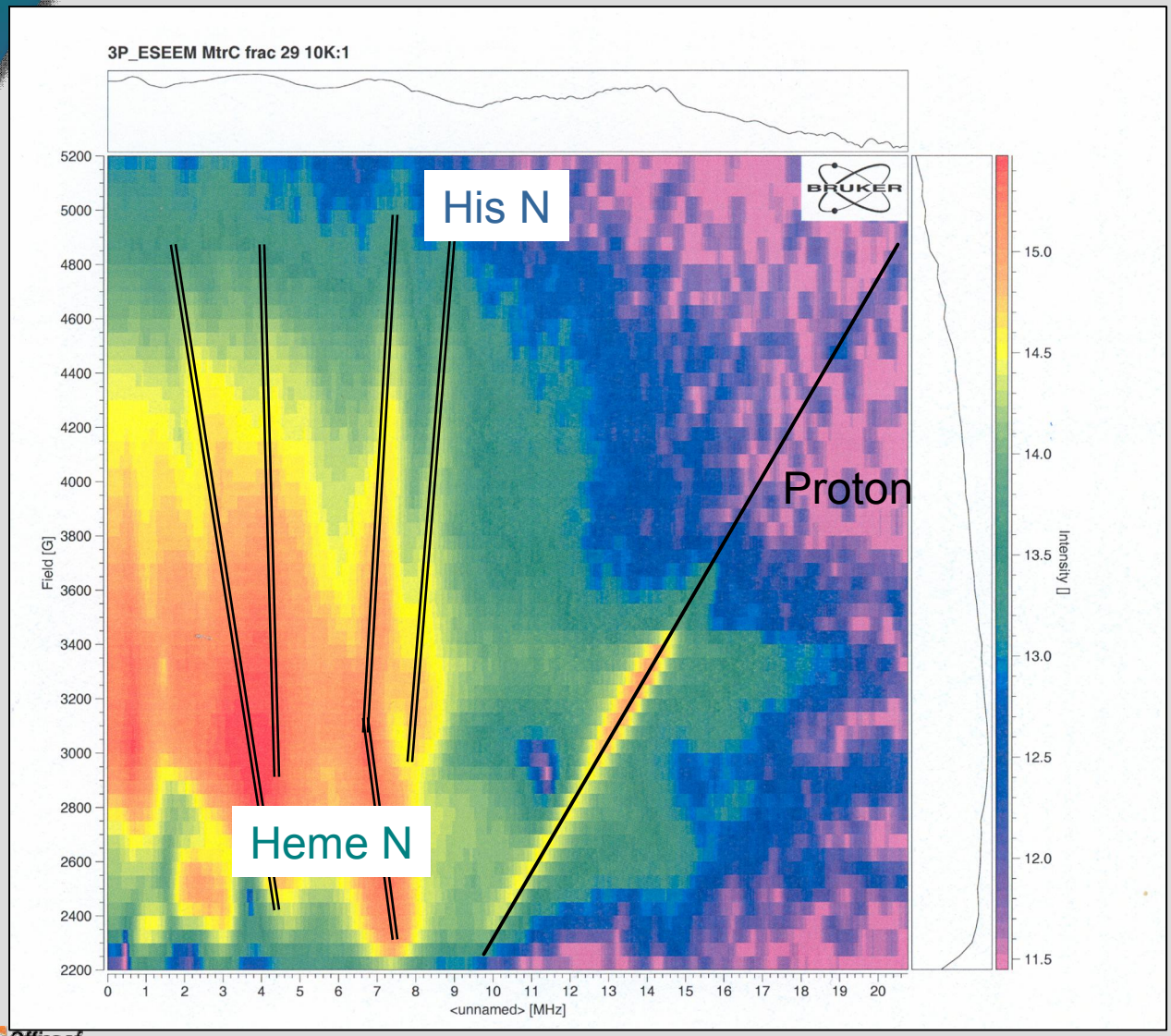


Heme stain

Western blot

L. Shi, PNNL

Electron Spin Echo Envelope Modulation (ESEEM) of MtrC (EMSL)



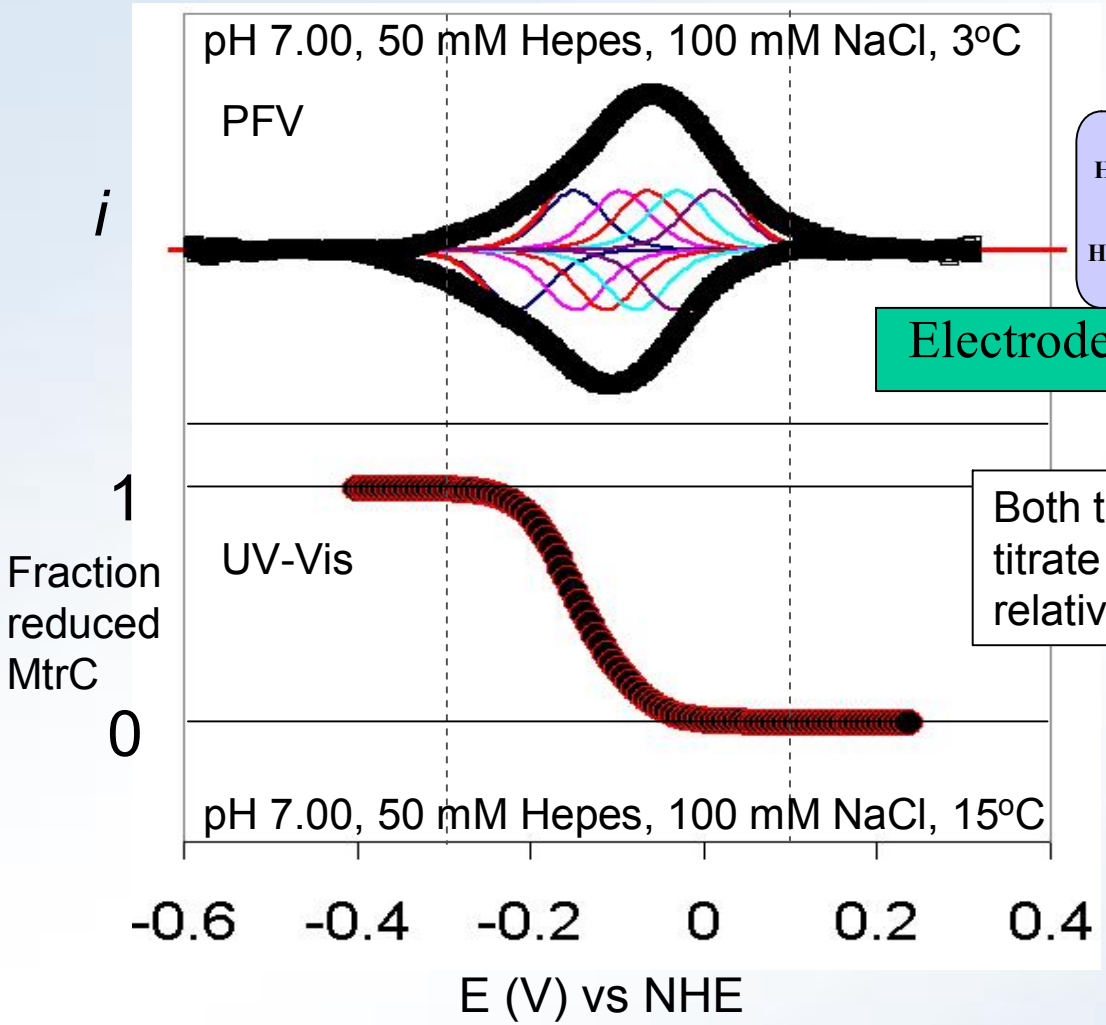
Nitrogen Hyperfine Couplings/MHz

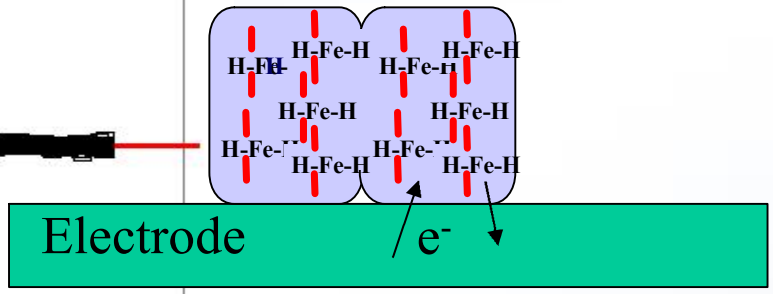
His	4.6	5.1	5.8
Heme	6.0	5.9	4.0

ESEEM frequencies along horizontal axis as a function of magnetic field along the vertical axis. There is a ridge from protons on the right-hand side of the spectrum. The other features are from nitrogens of heme and histidine coordinated to the heme iron. These ESEEM spectra indicate that the two his rings that serve as axial ligands are parallel to each other.

M. Bowman, PNNL

Adsorbed (PFV) vs. Solution (UV-Vis) Redox Behavior MtrC

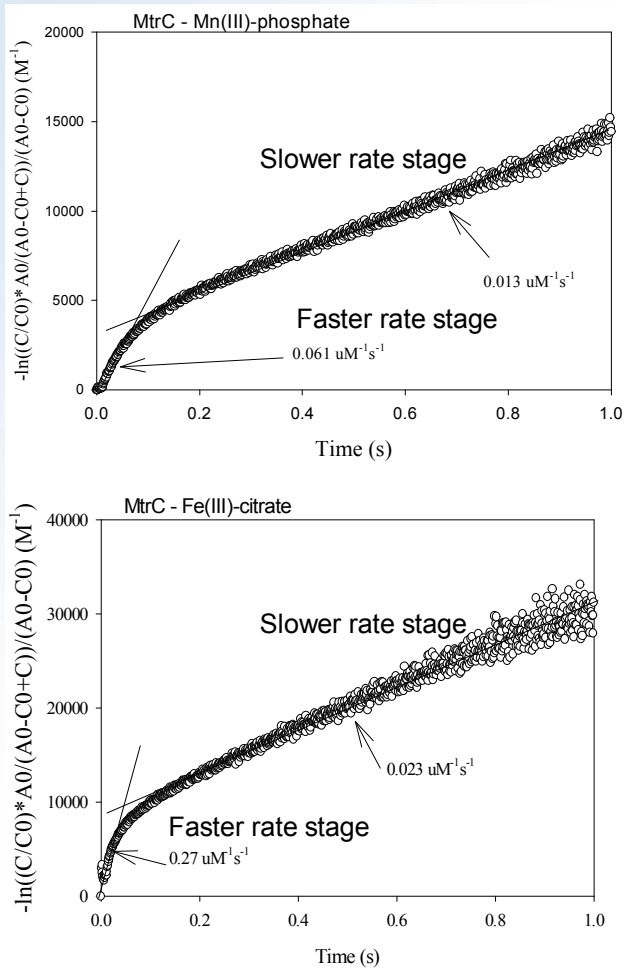




Both techniques show the hemes titrate in the region +100 to -350 mV relative to NHE

Purified OmcA and MtrC are Functional Metal Reductases

(stop-flow spectrophotometry-EMSL)



Rate constants of OmcA and MtrC

	MtrC ($\mu M^{-1}s^{-1}$)	OmcA ($\mu M^{-1}s^{-1}$)
Fe(III)-Citrate	0.023; 0.27	0.014; 0.035
Mn(III)-pyrophosphate	0.015; 0.066	0.024; 0.039
Uranium citrate	0.0039 (s^{-1})	NO
MnO_2	0.0105 (s^{-1})	0.0068
HFO	<3.8 x 10^{-5} (s^{-1})	<3.8 x 10^{-5} (s^{-1})
Hematite	<3.8 x 10^{-5} (s^{-1})	<3.8 x 10^{-5} (s^{-1})

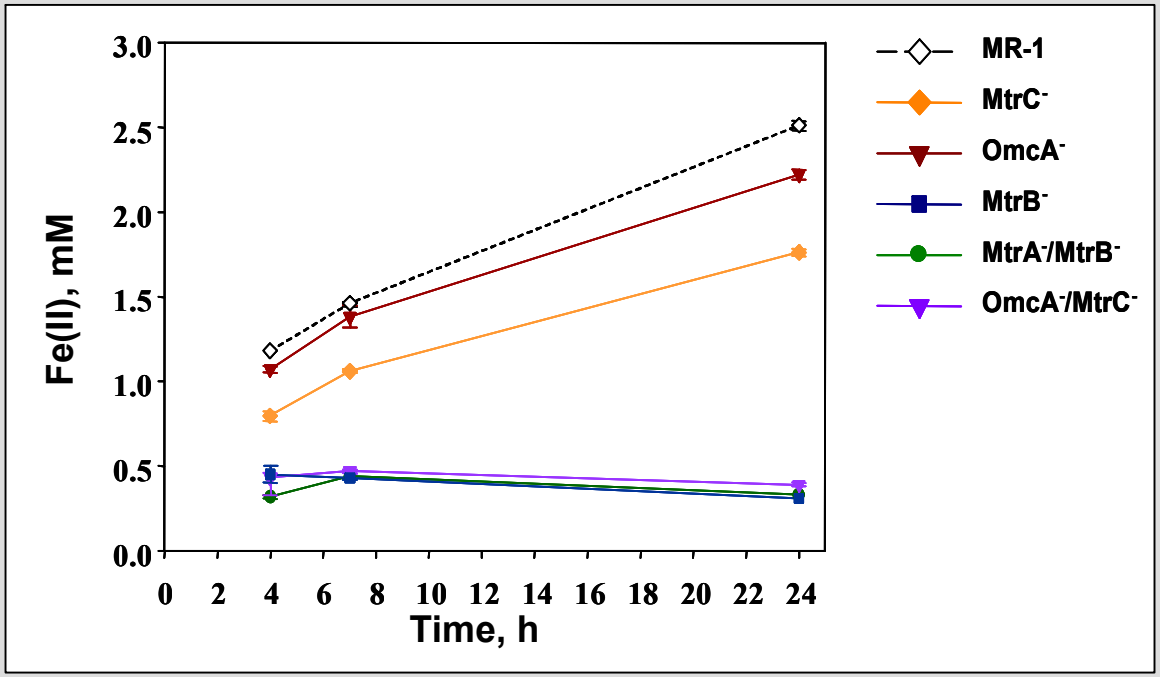
- Fe(III)/Mn(III) aqueous complex reduction by MtrC/OmcA are bi-phasic with a transition period

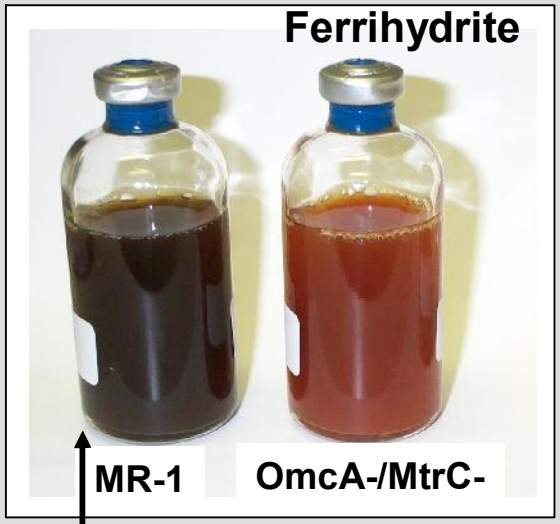
Z. Wang & L. Shi, PNNL

Mutagenesis to Identify Proteins Involved in Metal Reduction

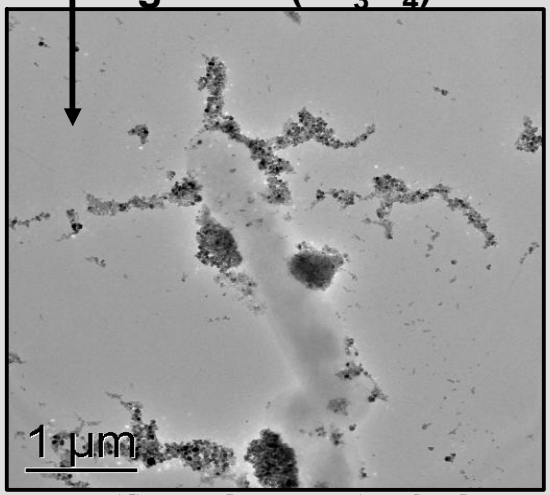
Deletion of both OMCs (MtrC & OmcA) results in extracellular electron transfer deficiency

Ferrihydrite ($5Fe_2O_3 \bullet 9H_2O$)



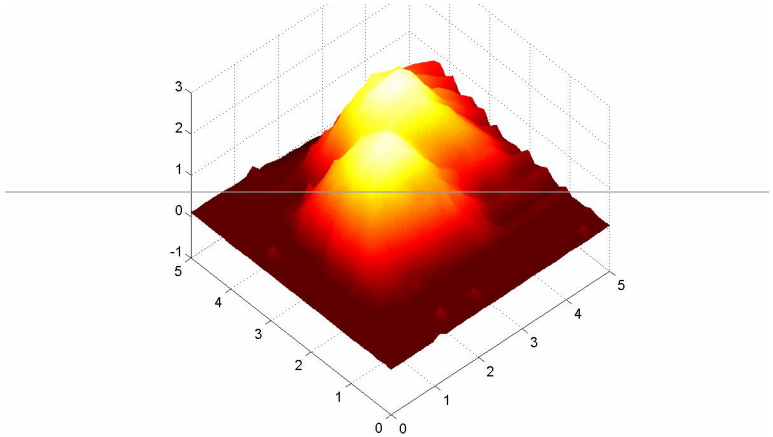


Magnetite (Fe_3O_4)

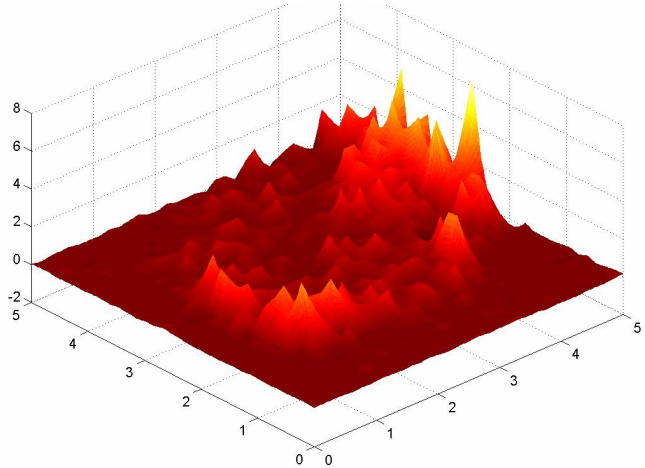


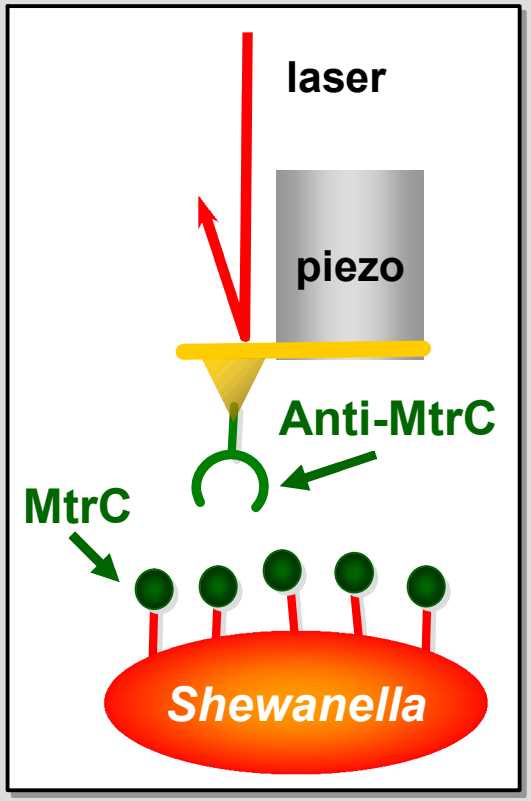
Identifying Surface Exposed MtrC on *Shewanella oneidensis* Using AFM

Topography of *Shewanella* cell obtained during force-volume mapping



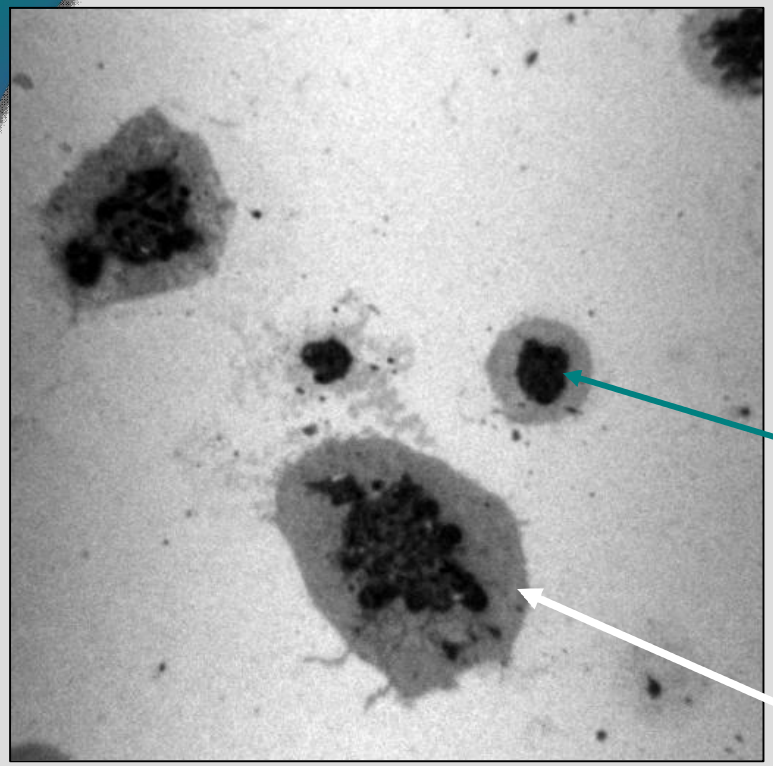
Force-Volume mapping *Shewanella* cell Using Anti-MtrC functionalized AFM tip



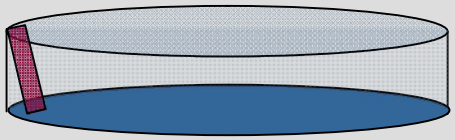


B. Lower, PNNL

Photoemission Electron Microscopy of MR-1 interactions with synthetic hematite (α-Fe_2O_3)



Fe *L*-edge



incubated 96 hours

Cell "colonies"

Extracellular material

- **X-ray Photoemission Electron Microscopy (ALS)**
- **Fe(II)/Fe(III) spatial distributions relative to cells**
- **Performed on dehydrated samples**
- **Epitaxially grown hematite (0001) thin films**

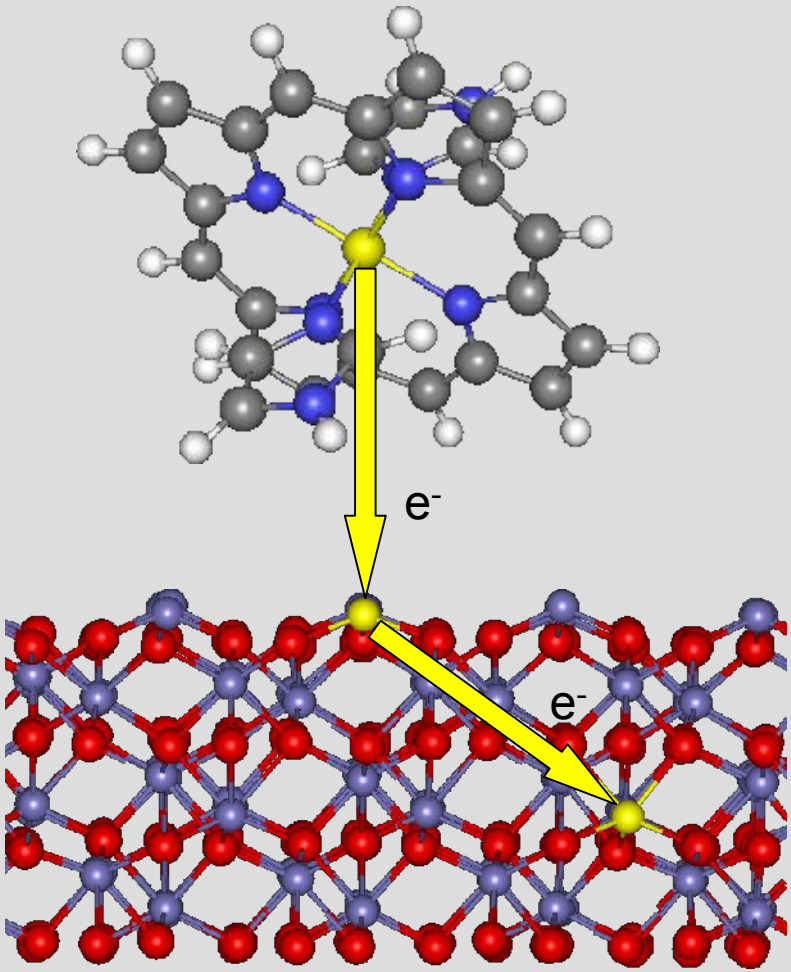
Molecular Modeling of Electron Transfer at the Cytochrome/Oxide Interface

Objectives:

- Use computational molecular modeling to provide insights on electron transfer at the cytochrome / oxide interface.
- Evaluate charge migration through the mineral substrate using molecular modeling and electron transfer theory.

Is direct ET from outer membrane cytochromes kinetically feasible?

Can ET through iron lattice explain the turnover of electron-accepting surface sites?



S. Kerisit and K. Rosso, PNNL

Interim Findings

- An extracellular protein complex consisting of two distinct cytochromes (MtrC and OmcA) appears responsible for electron transfer to solid-phase electron acceptors.
- The two cytochromes exhibit distinct redox properties that may spatially and chemically couple in ways that remain under investigation.
- Electron transfer from the complex is much slower to solid phase electron acceptors because of orientation and approach distance effects at the solid-water-organism interface. Extremely complex chemistry occurs at the scale of <10 nm.
- Absence of OmcA or MtrB individually does not eliminate extracellular electron transfer to solids; deletion of both does
- Whole cell behavior is a complex function of cytochrome localization, concentration, and dynamic cellular response to the mineral surface and its topography and energetics.