Biogeochemistry Grand Challenge

"Electron Transfer at the Microbe-Mineral Interface"

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Pacific Northwest National Laboratory

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Environmental Molecular Sciences Laboratory

OBER/EMSL Grand Challenge Concept

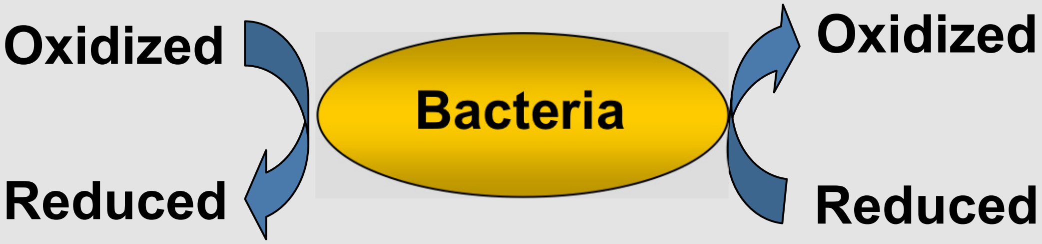
"A coordinated, multi-investigator research effort to resolve a challenging scientific issue not accessible to the single investigator"

- Align with OBER research portfolio in environmental science and environmental biology
- Address an important/impactful science topic
- Complement multiple programs (NABIR, EMSP, GtL)
- Involve a multidisciplinary, collaborative team
- Synergistic use of computational and instrumental capabilities in EMSL

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

BGC Science Topic

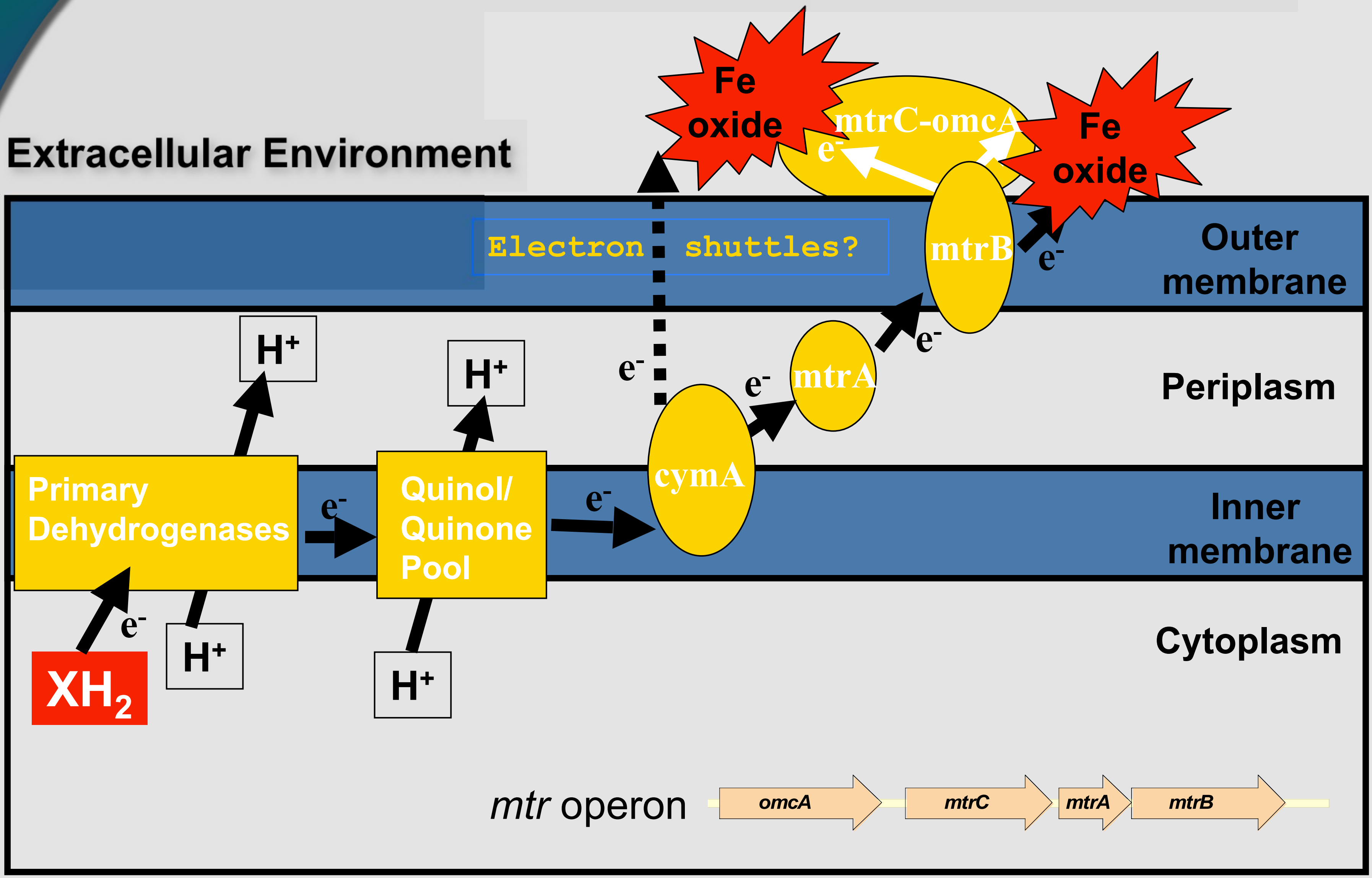
Molecular basis for electron transfer at the microbe mineral interface

- Perplexing science question (unresolved after more than 20 years of research)
- Requires a multidisciplinary science team
  - Biochemistry, molecular biology, microbiology
  - Interfacial geochemistry and mineral physics
  - Molecular spectroscopies
  - Computational science
- Broad science applications
  - Environmental remediation, energy
  - Biogeochemical cycles and a sustainable planet
  - Life origin
  - Engineered devices

The Science Problem is Now Tractable

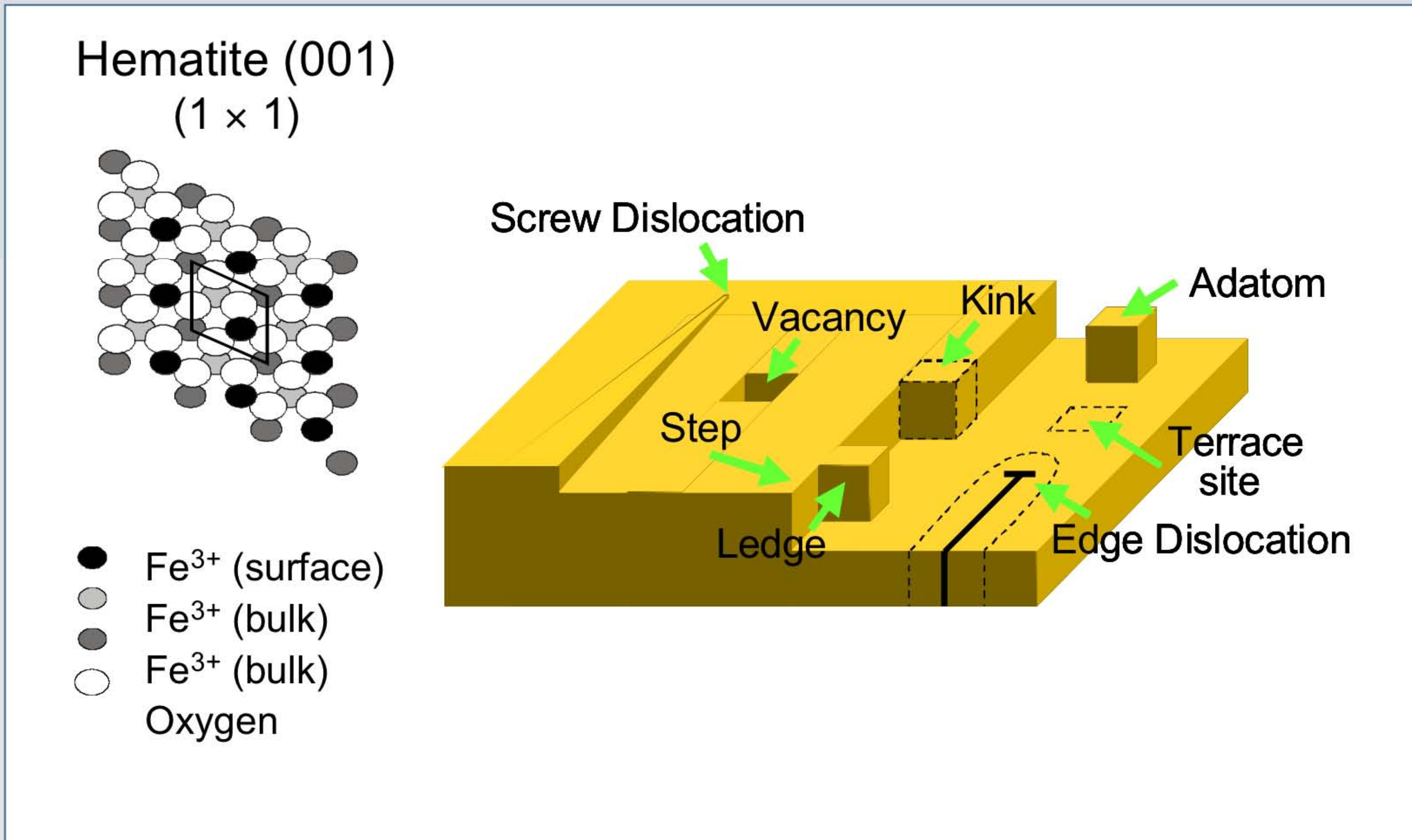
- Genome sequence & genetic systems for relevant microorganisms (*Shewanella oneidensis* MR-1)
- Advances in controlled cultivation techniques and understanding of organism physiology
- New spectroscopic and microscopic capabilities at EMSL and other DOE user facilities
- Recent progress in molecular modeling of large and complex systems

Current Conceptual Model for Electron Transfer to Extracellular Substrates



(D. Richardson)

The Surfaces of Fe(III) Oxides are Complex

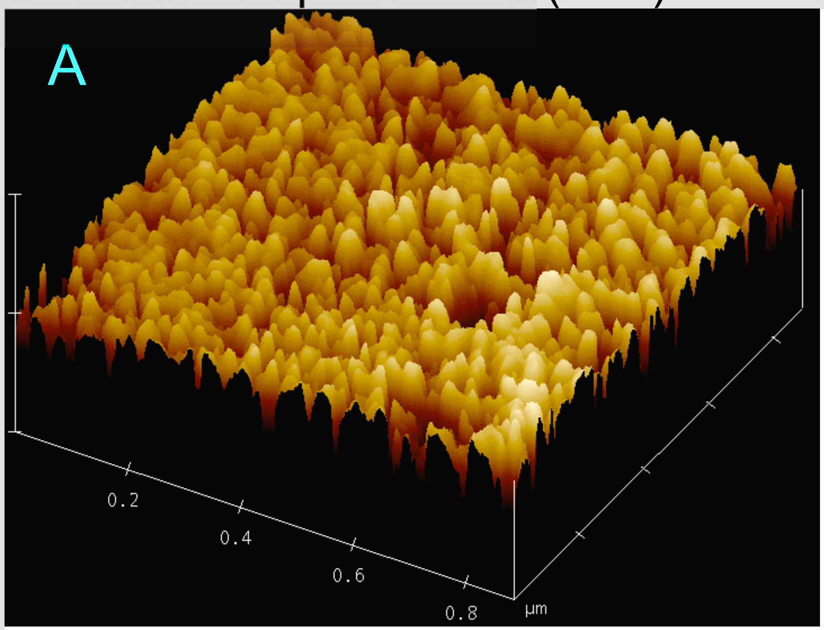


(Kevin Rosso)

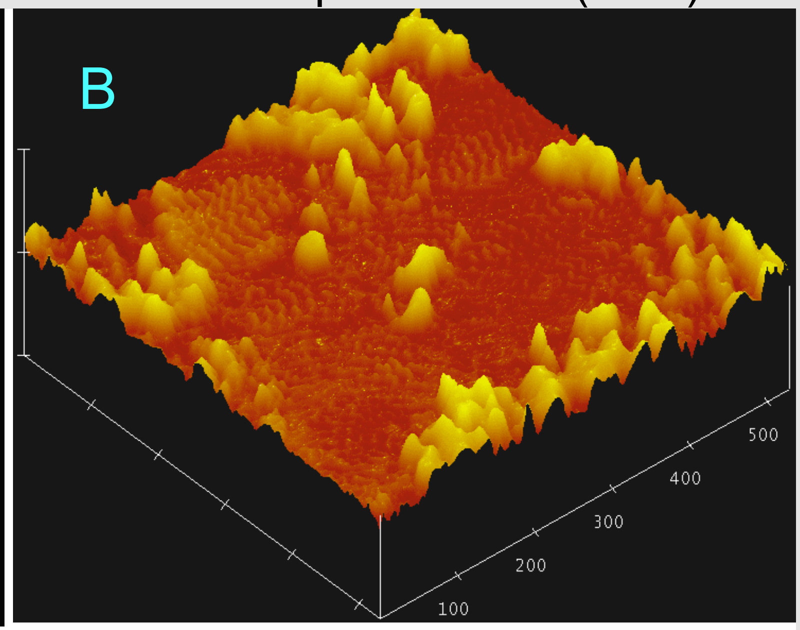
The Surfaces of Bacterial Cells are Equally Complex & *Dynamic*

Atomic Force Microscopy Images of *S. oneidensis* Cell Surface

Electron acceptor limited (EAL)



Electron acceptor excess (EAX)



(Y.A. Gorby, P. Lu)

Scientific Challenges

- Microbe-mineral interface is a nano-scale domain
  - Unique & dynamic architecture of biologic and physical entities
  - Instrumentation needed with high spatial and molecular sensitivity
- Membrane proteins involved
  - Difficult to purify, analyze and crystallize
  - *In vitro* model systems requires design to identify & evaluate mechanisms
- Interfacial effects may predominate
  - Complex orientation, electrostatic, and solvation issues
  - Coupled experiment & molecular modeling key to understanding

Hypothesized Electron Transfer Model to Solids for *Shewanella oneidensis* MR-1

- Outer membrane cytochrome(s) MtrC responsible for direct e^--transfer to Fe(III) oxide
  - Previous investigations support role of mtr proteins in Fe(III) reduction & localization of MtrC/OmcA to OM
- Hypothesis allows detailed scientific planning
  - Isolation/purification/molecular characterization of MtrC and associated proteins
  - Design of *in vitro* models and *in vivo* studies with Fe(III) oxides
  - Formulation of appropriate molecular models
- Contingency plans and flexibility to address alternative models, organisms, pending results

BGC Team

Coordinators: John Zachara and Jim Fredrickson

Internal Science Team Leaders
A. Beliaev, M. Bowman, S. Chambers, T. Droubay, M. Dupuis, D. Gaspar, Y. Gorby, M. Kennedy, B. Lower, P. Lu, S. Ni, M. Romine, K. Rosso, L. Shi, and T. Straatsma

External Science Team Leaders
T. Beveridge, (U. of Guelph); G. Brown, (Stanford); T. DiChristina, (Georgia Tech.); C. Eggleston, (U. of Wyoming); S. Fendorf, (Stanford); G. Geesey, (Montana State U.); M. Hochella, (V.P.I.); K. Nealson, (USC); D. Saffarini, (U. of Wis.-Milwaukee); and D. Richardson, (U. of East Anglia)

EMSL Team
Dave Wunschel, Ravi Kukkadapu, Alice Dohnalkova, and Marat Valiev

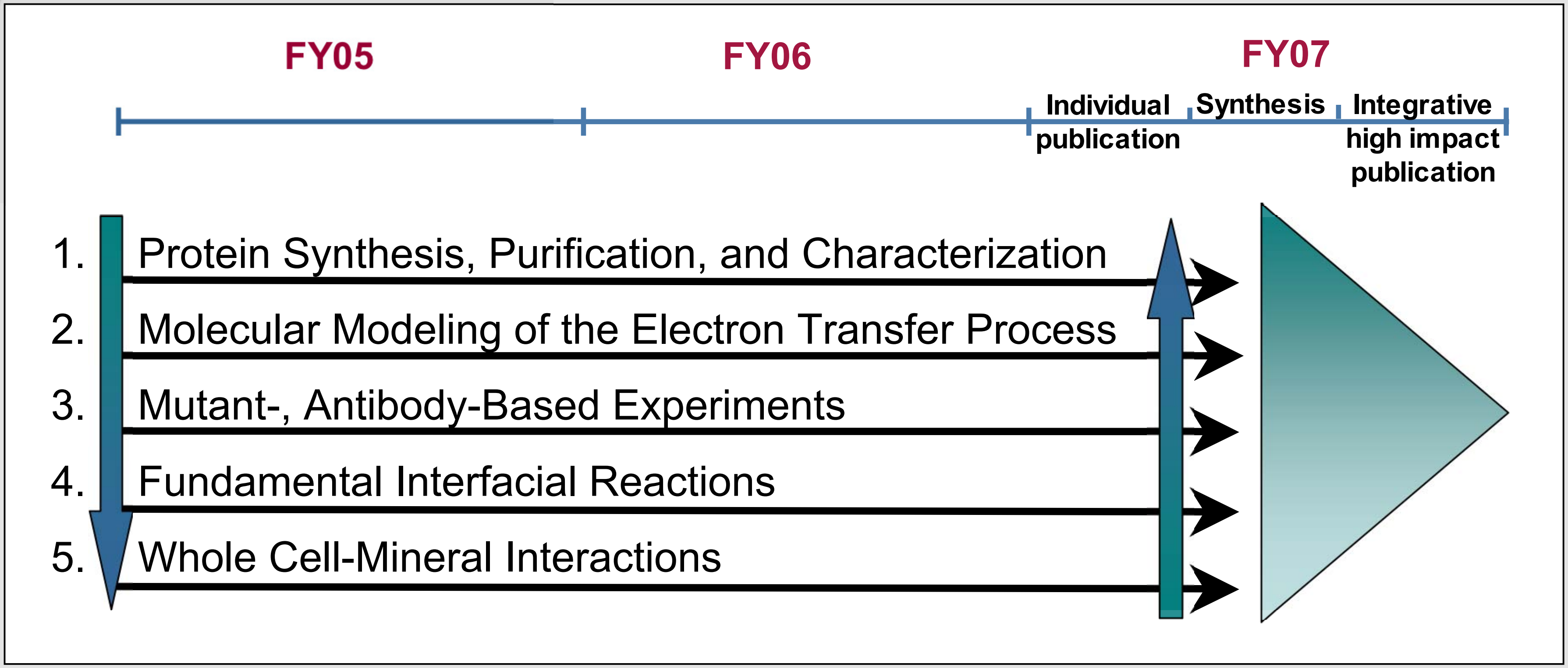
Select EMSL Capabilities

- Electron Paramagnetic Resonance (heme center electrochemistry)
- Mass Spectrometry (protein and protein complex characterization)
- Scanning Probe Microscopies (nanometer scale surface characterization)
- Scanning and Transmission Electron Microscopies (imaging of all forms)
- Surface Enhanced and Resonance Raman Spectroscopy (heme identification and localization)
- Molecular Beam Epitaxy (synthesis of thin films)
- Photoelectron Diffraction (surface structure)
- Mössbauer Spectroscopy (Fe coordination environment)
- Molecular Science Computing Facility (numeric experiments of electron transfer)

Others

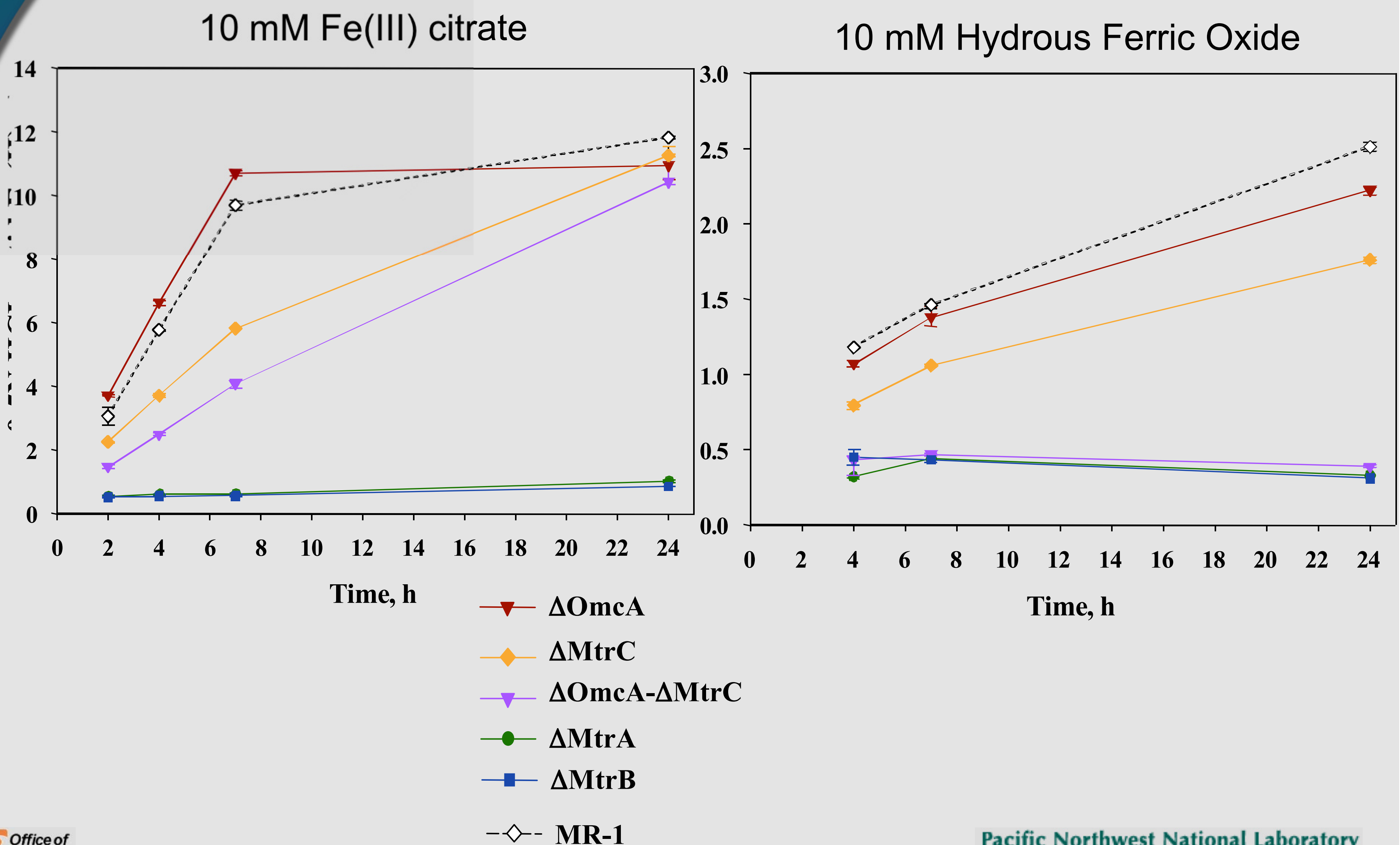
- Hard and soft x-ray spectroscopy and microscopy of different sorts at SSRL and ALS

Overall BGC Research Structure

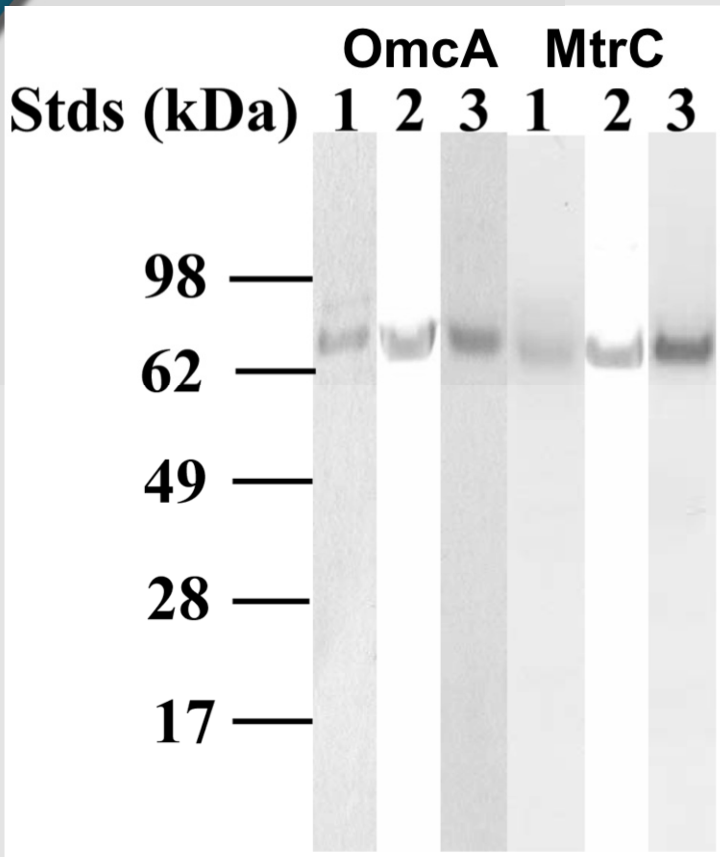


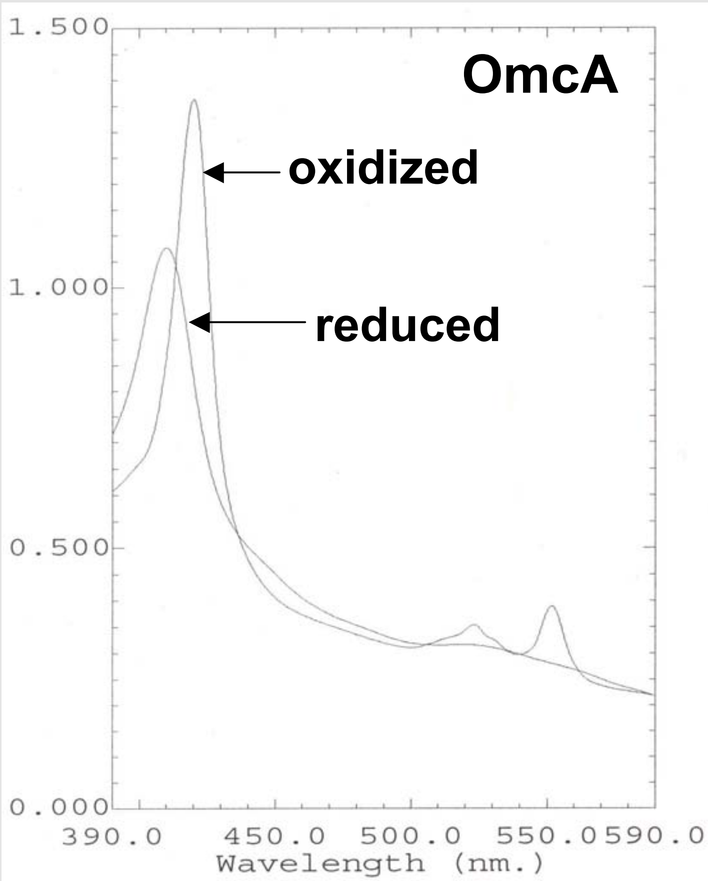
hypothesis verification ⇒ mechanistic function ⇒ in vivo behavior

Fe(III)-Reducing Phenotypes of *mtr* Mutants



Purification and Characterization of OmcA and MtrC





(1) Gel-code blue

(2) Western blot w/ anti-V5 antibody

(3) Heme stain

(L. Shi, PNNL)

Fe(III)-Reductase Activities of *Shewanella* Outer Membrane Cytochromes

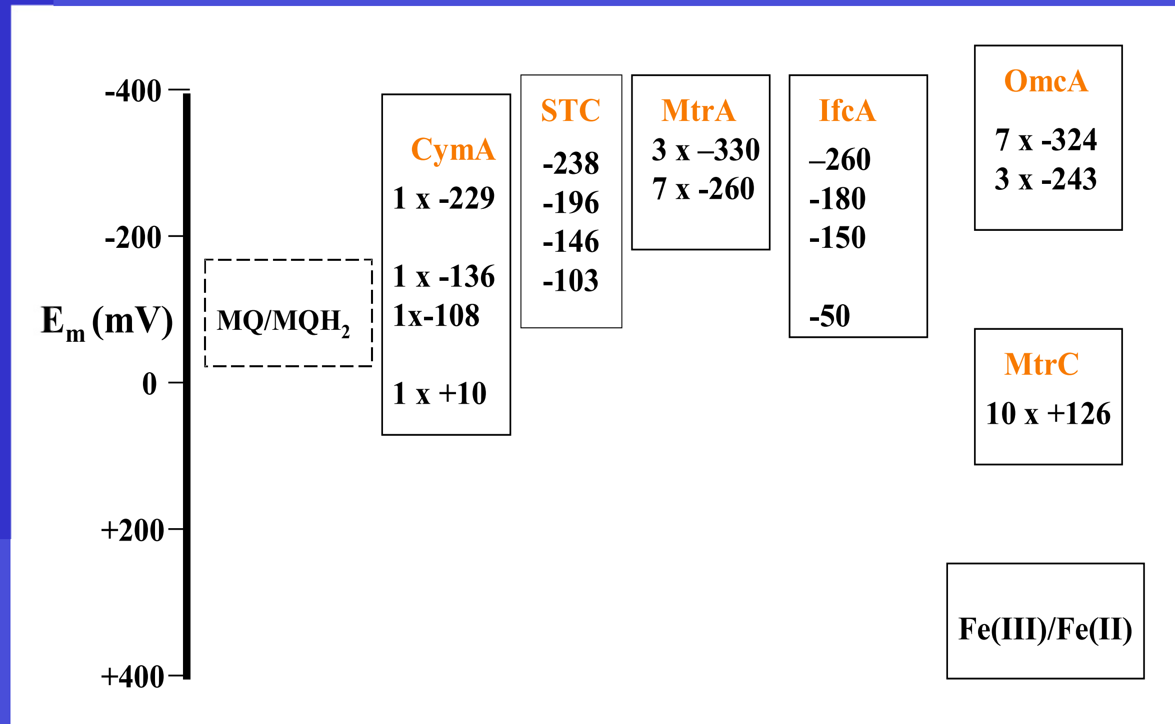
Protein	Specific activity (nmole Fe reduced min^{-1} mg^{-1} [n=3])*
MtrC	1569 ± 125
OmcA	1496 ± 229
MtrC+OmcA	2115 ± 144

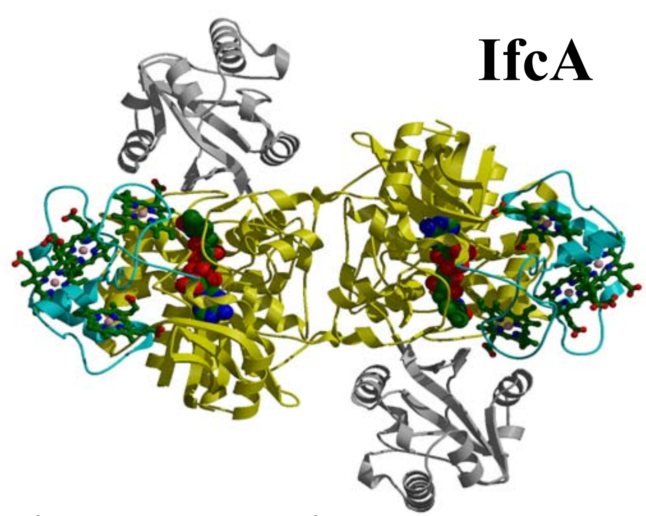
(*NADH + Fe(III)-NTA)

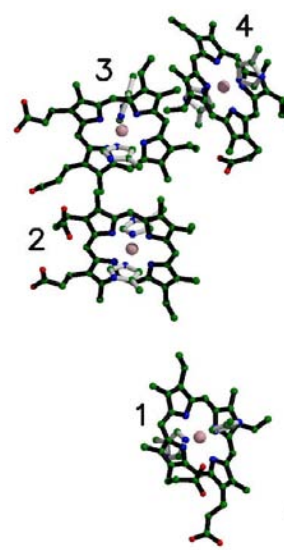
(L. Shi & D. Elias)

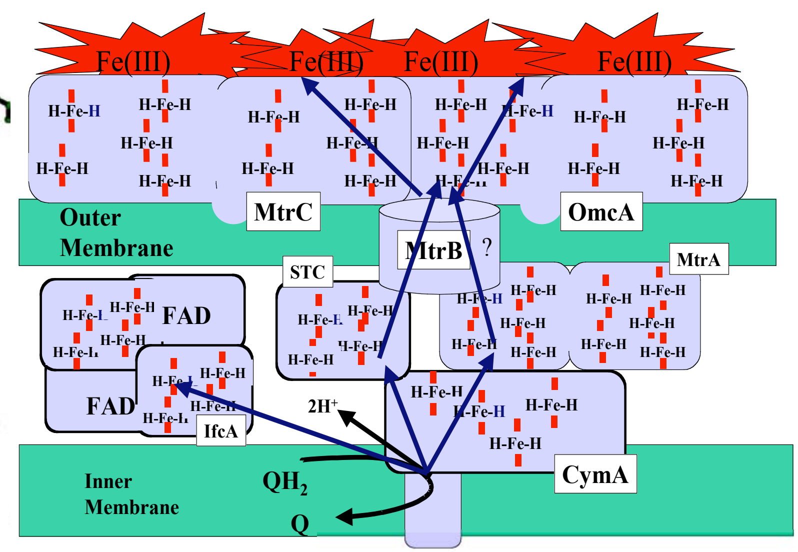
Properties of the Multi-heme Cytochromes

hemes are closely packed together (4-12Å) to facilitate rapid electron transfer ‘sucked’ through by high potential electron centres (MtrC) or acceptors (FeIII)





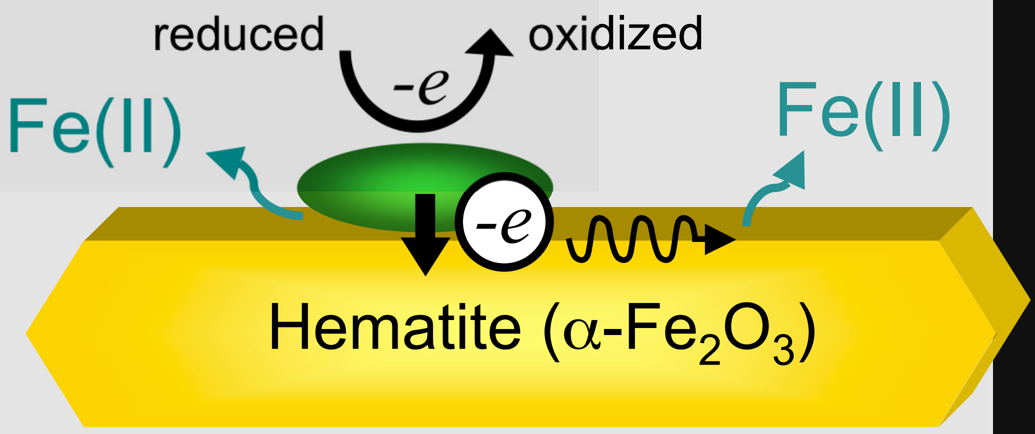


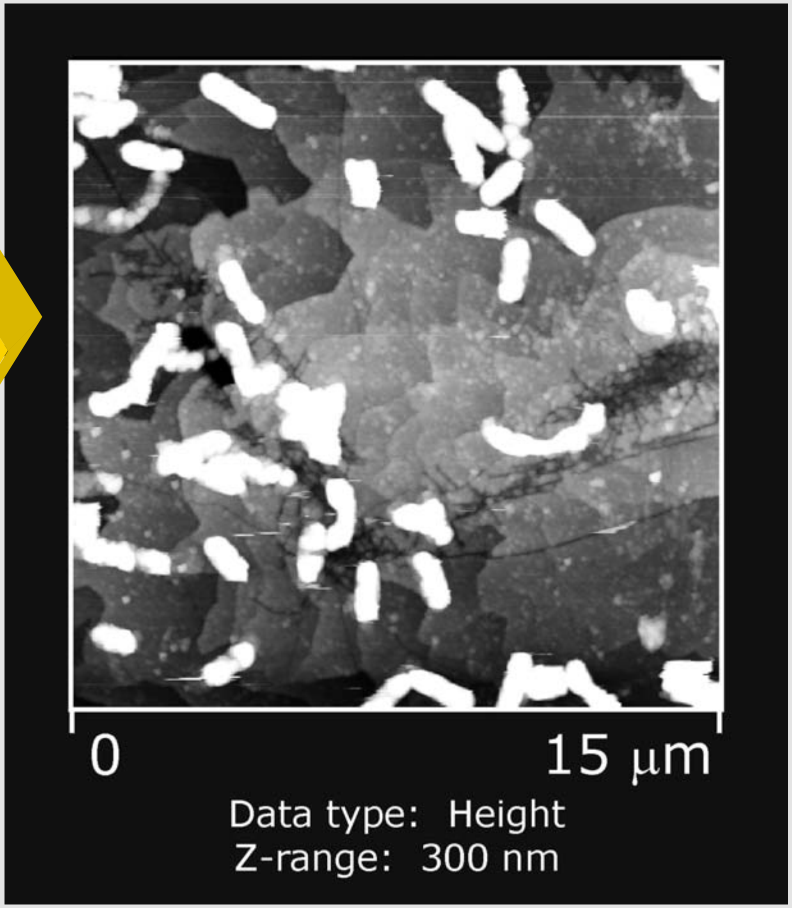


(D. Richardson)

Charge Transport Modeling

Are transferred electrons fixed or mobile? If mobile, what are the most likely release points and how easily can electrons get there?





K. Rosso

Facile electron transport through the solid implies:

- Rate limitation involving primarily the cytochrome/oxide interfacial ET steps
- A possible mechanism for acceptor site regeneration
- A possible link between metal release rates and defects

Progress To-Date

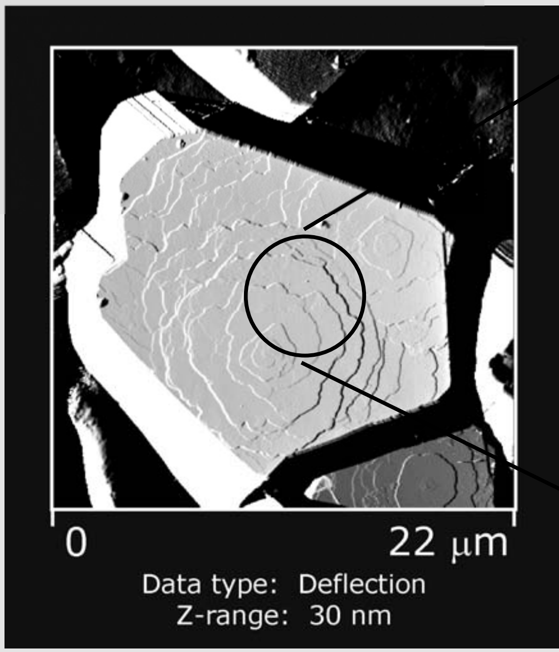
- Internal team research initiated: 12/1/04
- External team research initiated: 2/1/05
- Initial studies strive to verify the microscopic hypothesis
  - Outer membrane cytochromes MtrC and)mcA purified; characterization initiated
  - Evaluation of functionality imply the proteins function as a complex (BGC Hypothesis revised)
  - Electron transfer activities of deletion mutants (*mtr* genes) & proteins themselves are consistent with hypothesis
  - Peptide/protein antibodies generated, protein localization experiments initiated

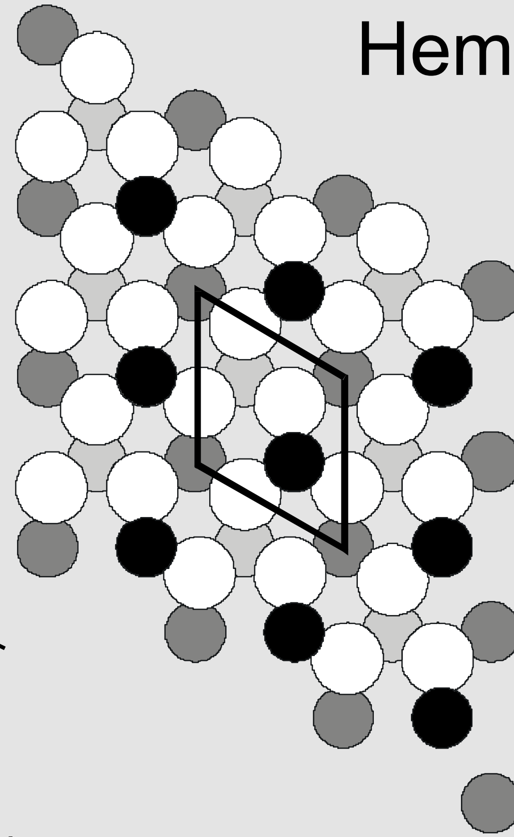
Future Accomplishments

- Demonstrate that mtrC (as a complex with omcA?) is functionally responsible for interfacial electron transfer
  - Define complexes involved and others required partners
  - Document *in vitro* and *in vivo* behavior
- Elucidate molecular mechanisms
  - Engagement reaction
  - Orientation and electronic controls
- Determine key physiologic/mineral surface controls
  - Environmental and biologic factors

Charge Transport Modeling

Are some surface sites better electron acceptors than others, and if so why?





Hematite (001)

(1 × 1)

- Fe^{3+} (surface)
- Fe^{3+} (bulk)
- Fe^{3+} (bulk)
- Oxygen

Possible implications:

- ET is more facile at certain crystal terminations
- ET depends on speciation of surface functional groups
- Surface structure is a control on ET rates from overlying cytochromes

K. Rosso