Structure of bacterial multiheme cytochromes at the microbial-mineral interface

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Introduction

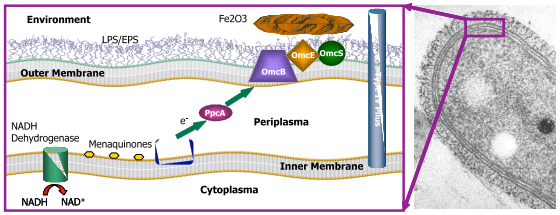
Dissimilatory metal reducing bacteria (DMRB) like *Shewanella sp. and Geobacter sp.* have attracted considerable attention because of their potential use for bioremediation by reductive precipitation and biological fuel cell applications [1,2].

DMRB are capable of anaerobic respiration by direct electron transfer to soluble metal ions and insoluble mineral surfaces as shown for the reduction of Fe(III) and Mn(IV) oxides [3]. Other relevant redox acceptors include U(VI)/U(IV), Tc(VII)/Tc(IV), Cr(VI)/Cr(III) and Hg(II)/Hg(0).

Despite of significant progress in the characterization of the proteins involved, their structures and the fundamental molecular mechanisms facilitating the electron transfer process are still unknown.

Molecular Basis for Dissimilatory Metal Reduction

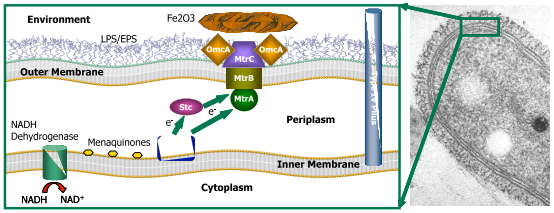
Geobacter sulfurreducens PCA



Gram-negative bacterial cell

This illustration shows c-type cytochromes identified to be involved in electron transfer to minerals. The genome of *Geobacter sulfurreducens* PCA contains a total of 111 c-type cytochrome coding sequences (43 unique) with up to 27 heme moieties. [4]

Shewanella oneidensis MR-1



Gram-negative bacterial cell

Shewanella omeidensis MR-1 employs a similar electron transport system as *Geobacter.* However, its genome contains only 42 possible *c*-type cytochrome coding sequences. [5]

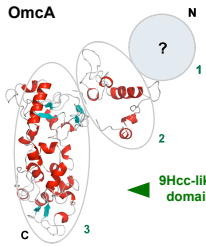
It was shown in *Shewanella oneidensis* MR-1 that outer membrane cytochromes required for electron transfer to minerals assemble into large functional complexes [6,7]. The structure and function of these reductase complexes and their interaction with insoluble minerals is not well understood and is the goal of ongoing investigations.

Objective

Develop a model system of the bacterial-mineral interface to study structure-function relationships of biological membranes and electron transport proteins with insoluble metal oxides. Neutron reflectometry is a valuable tool to obtain structural information from buried interfaces. Detailed insights into the structure of the complex biological-mineral interface can help in the development of new strategies for bioremediation processes and the design of biological fuel cells.

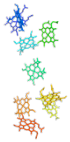
Model System

Multiheme cytochromes



Partial homology model (residues 197-687) of decaheme outer membrane cytochrome OmcA from *Shewanella oneidensis* MR-1 indicates 3 domains and cyclic heme architecture (tetraheme c_3).

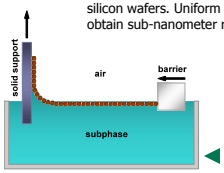
9Hcc-like domain



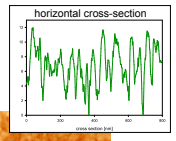
Template structure from *Desulfovibrio desulfuricans* 9HcA (PDB: 1HHC) is homologous to C-terminal domain of OmcA.

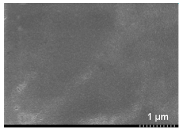
Deposition of iron oxide thin films

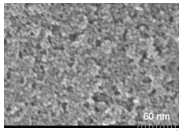
The mineral interface is represented by an iron oxide thin film deposited on 2" silicon wafers. Uniform layers with low surface roughness are a prerequisite to obtain sub-nanometer resolution of interfacial structure by neutron reflectometry.

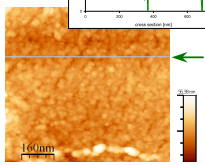


Deposition of hydrophobic iron oxide nanoparticles on silicon from solution by the Langmuir-Blodgett technique on a silicon wafer from the air/water interface.

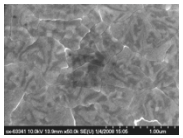


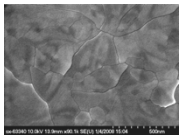


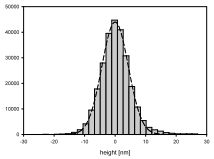




SEM and AFM images of iron oxide nanoparticle (6 nm) thin films on silicon substrates by Langmuir-Blodgett depostion.







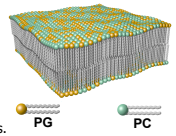
SEM images of iron oxide thin film on Si(001) by pulsed laser Deposition (PLD). 10 Hz, 10 mTorr O_2, 400 ºC, 12 min

Biomimetic model membranes

Effects of mineral surfaces on organization and phase-behavior of n[illegible] model membranes. Controlled depostion of lipid layers by fusion of Langmuir-Blodgett and Langmuir-Schäfer methods.

Bacterial lipid bilayer model membranes

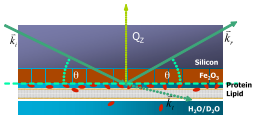
- PE – Phosphatidyl ethanolamine
- PC – Phosphatidyl choline
- PG – Phosphatidyl glycerol



These phospholipids are commonly found in bacterial cell membranes.

Phospholipids can diffuse freely within the membrane bilayer plane. Interaction with oxide surfaces can lead to demixing and formation of microdomains.

Neutron Reflectivity

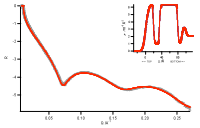


$$Q = \frac{4\pi \sin\theta}{\lambda}$$

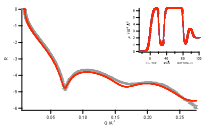
Purified outer membrane cytochromes or phospholipid bilayers interact with the mineral model interface. Using neutron reflectivity in combination with H_2O/D_2O contrast variation, the molecular self-assembly of biomolecules at the biological-mineral interface can be observed.

Experimental reflectivity curves (gray) and calculated reflectivity (red) derived from modeling of scattering length density profiles (inset).

Addition of negatively charged phospholipid PC results in changes in lipid layer structure.

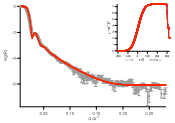


d-DPPE on SiO_2/Si
bilayer thickness: 44±3Å

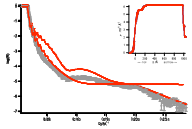


d-DPPE/PG 7:3on SiO_2/Si
bilayer thickness: 46±3Å

Surface properties of the Fe_2O_3 interface (uniform layer thickness, interfacial roughness) determine the achievable accuracy of models.



303 Å Fe_2O_3 (PLD) at Si/Air
roughness: 50 Å



920 Å Fe_2O_3 (PLD) at Si/Air
roughness: 50 Å
3 d-DPPC/PG monolayers (LB)
roughness: 5 Å

References

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