Probing the Microbial-Mineral Interface by Neutron Reflectivity

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Outline

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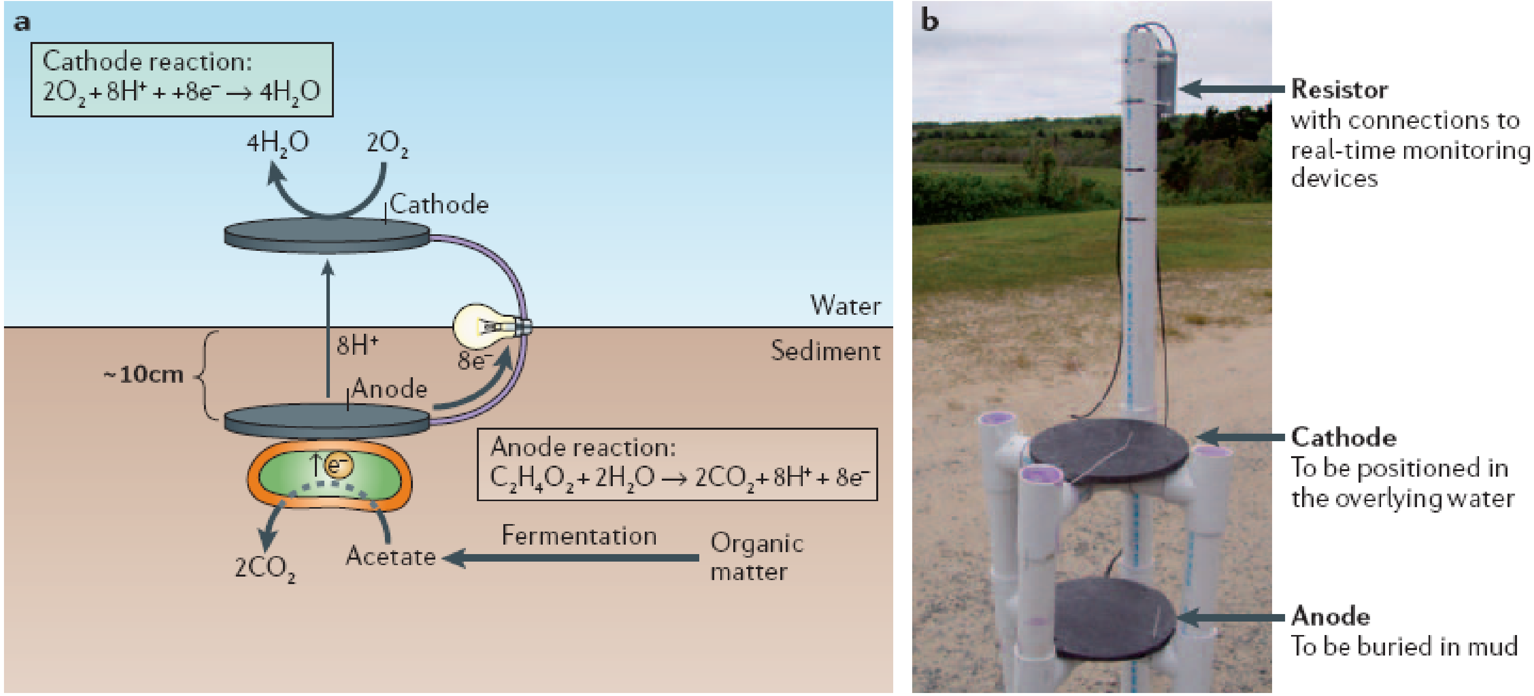
  - **Motivation, Scientific Challenges**
- **Model System**
  - **Bacterial-Mineral Interface**
- **Neutron Reflectivity**
  - **Experimental Challenges**
- **Complementary Methods**
  - **Structure, Electrochemistry**
- **Future Research Perspectives**

Mineral respiration by *Geobacter sulfurreducens*

- ***Geobacter sulfurreducens***
  - gram-negative, anaerobic
  - transfers electrons to soluble and insoluble electron acceptors
- **Reductive precipitation of contaminant metals and radionuclides**
  - Uranium: U(VI) → U(IV)
  - Technetium: Tc(VII) → Tc(IV)
  - Chromium: Cr(VI) → Cr(III)
- ***Geobacter* is predominating in many subsurface environments, also at DOE contamination sites**

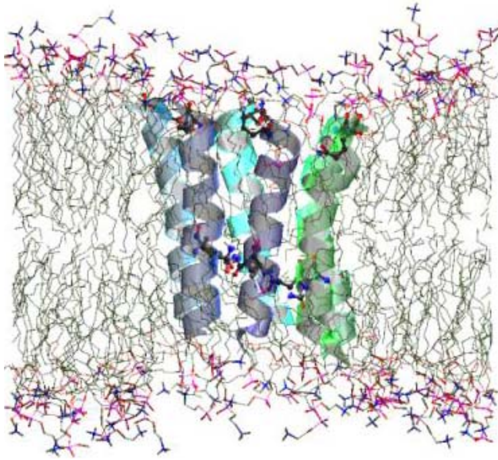
Microbial fuel cells

- ***Geobacter sulfurreducens*** **is capable of direct electron transfer to insoluble electron acceptors (Fe_2O_3, MnO_2, Graphite)**
- **Design of microbial fuel cells to generate electricity**



Lovley D.R., Nature Reviews, **4**(7), 497-508 (2006).

Scientific Challenges



- Fundamental knowledge gaps exist in the electron transfer reaction mechanisms between microorganisms and sediment mineral surfaces
- Outer membrane cell protein (e.g. cytochrome) function and transport are poorly known

Objectives

- Proof-of-principle experiments to study the molecular interaction of microbial proteins with mineral surfaces with biomimetic lipid model membranes and neutron reflectivity
- Reveal molecular arrangement to understand mechanisms of electron transport and function of bio-macromolecules (proteins and lipids) on mineral surfaces

Hypothesis

- Transport of electrons by cytochromes across the cell membrane, and direct contact with minerals, are necessary for the reduction of minerals such as iron oxide

Cytochromes identified to be involved in Fe(III) reduction

The genome of *Geobacter sulfurreducens* contains **111** c-type cytochrome coding sequences (43 unique) with up to 27 heme moieties.

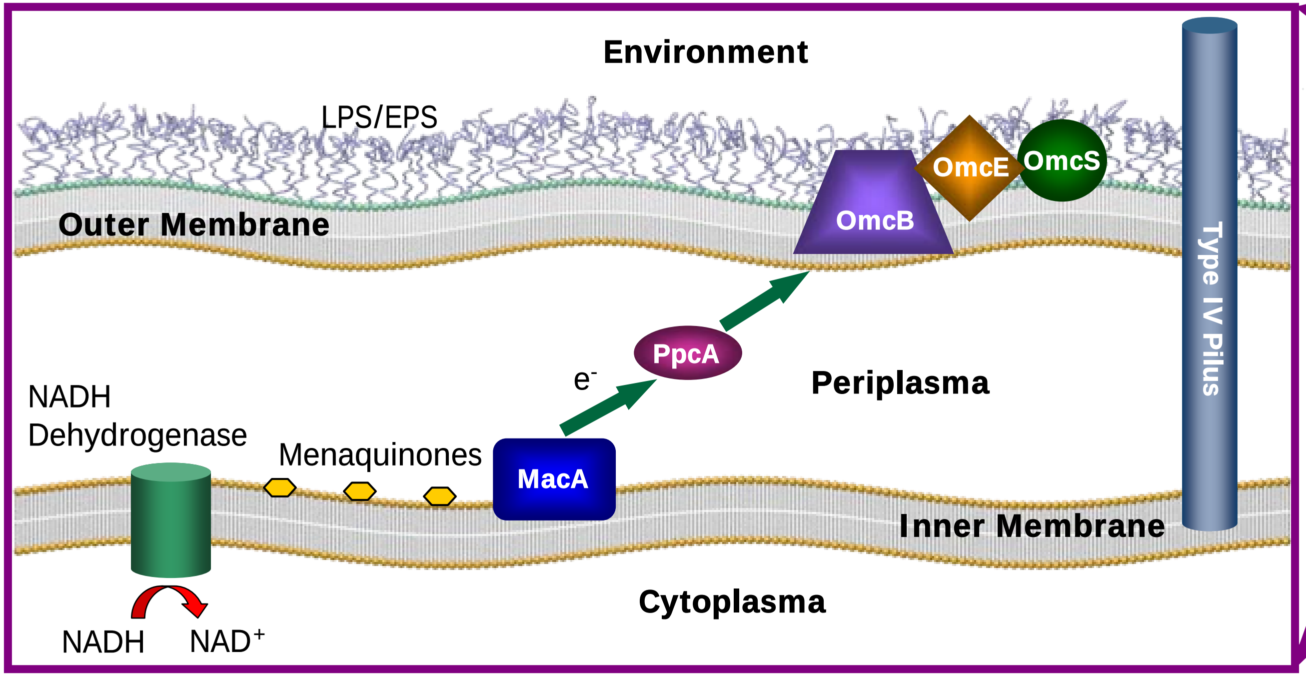
MacA	36 kDa	2 hemes	associated to inner membrane
PpcA	9.6 kDa	3 hemes	shuttles electrons between inner and outer membrane
OmcB	86 kDa	12 hemes	most abundant cytochrome at outer membrane
OmcC	89 kDa	12 hemes	highly homologous to OmcB
OmcE	30 kDa	4 hemes	probably involved in transmembrane electron transfer
OmcS	50 kDa	6 hemes	extracellular surface (LPS/EPS layer) possibly terminal electron donor

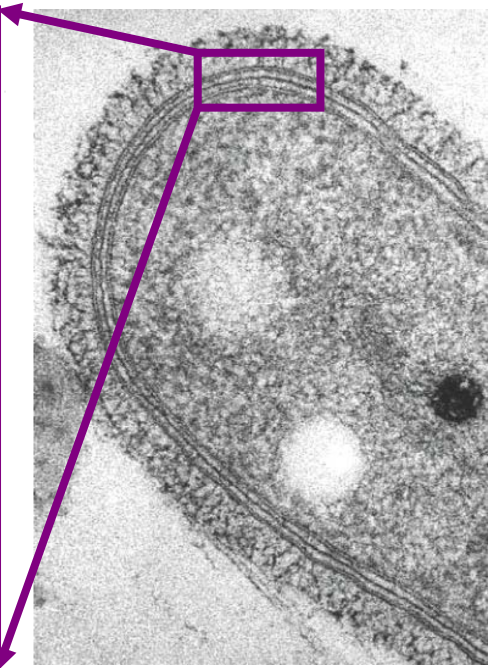
Plasma membrane **Periplasmatic space** **Plasma membrane**

Methé, B.A. et al., Science 302, 1967 (2003)

Proposed mechanism for extracellular electron transfer to insoluble electron acceptors

Simplified model of the electron transfer system in *Geobacter sulfurreducens*



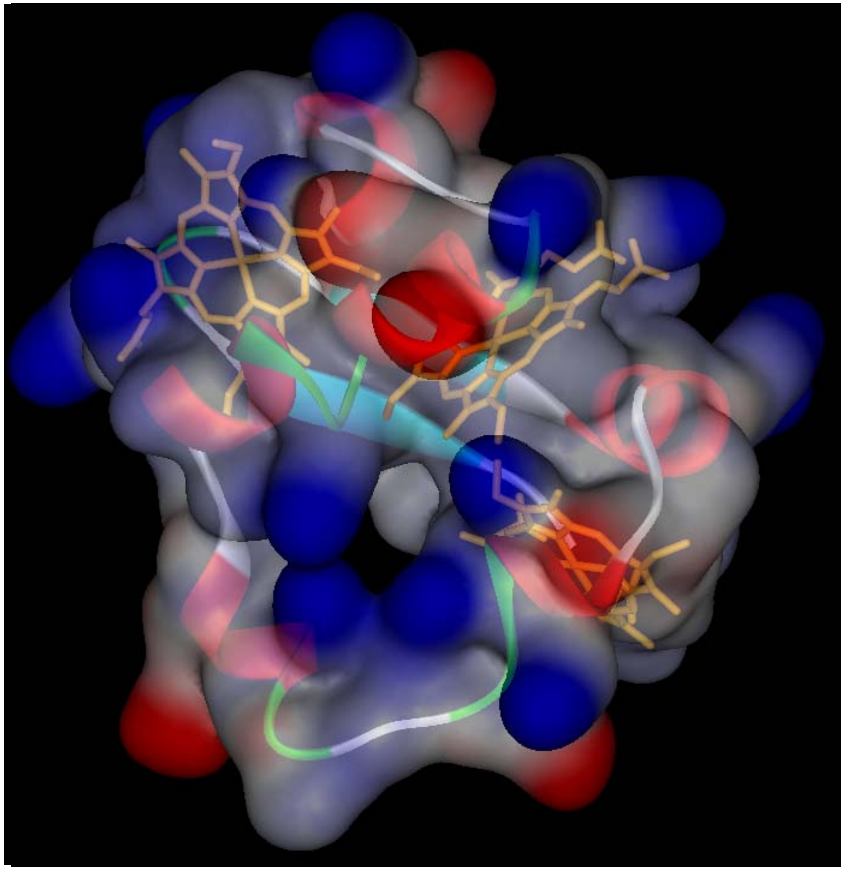


Gram-negative bacterial cell

Lovley, Nature Reviews Microbiology, 4(7),497-508, 2006
Weber *et al.*, Nature Reviews Microbiology, 4(10), 752-764, 2006

Isolation and Purification of Geobacter Cytochromes

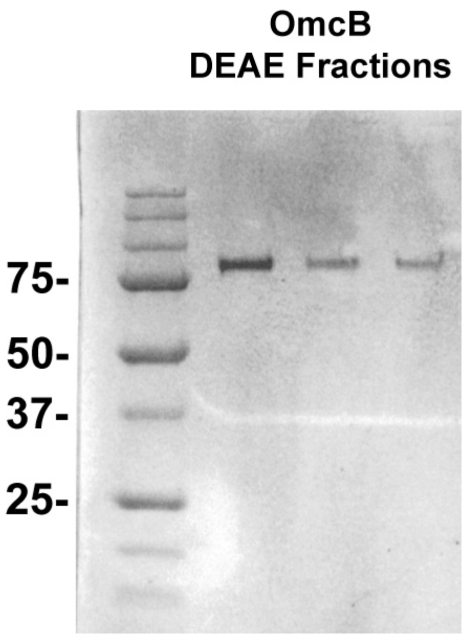
- **PpcA from wild-type *Geobacter sulfurreducens***
- **9.6 kDa periplasmatic cytochrome**
- **3 hemes with His-His coordination**
- **Hydrophobic loop**
- **Positively charged, pI 9.5**



Crystal structure of recombinant PpcA

Isolation and Purification of Geobacter Cytochromes

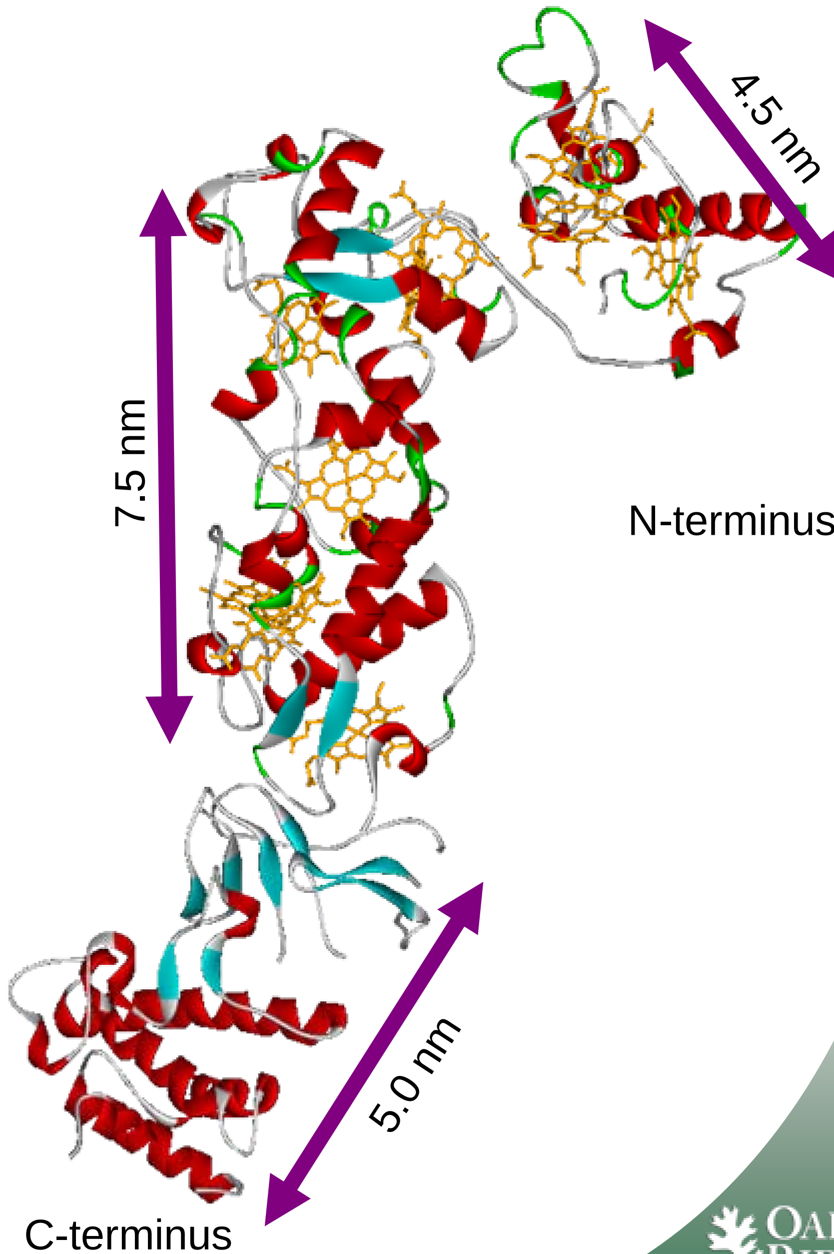
- **OmcB from a ΔOmcC mutant of Geobacter sulfurreducens**
- **86 kDa outer membrane cytochrome**
- **12 hemes with His-His coordination**
- **Flexible modules**



Purified OmcB from *G. sulfurreducens*. Molecular masses at left. Each lane is a fraction from the final purification step (ion exchange on DEAE-Sepharose).

Homology model of *Geobacter* omcB

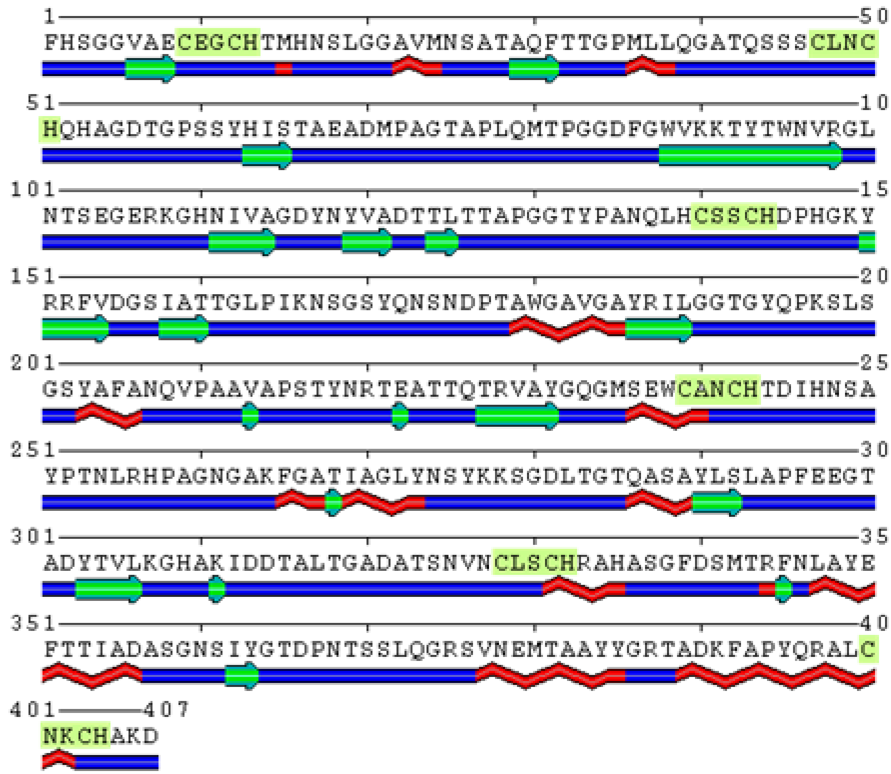
- **12 heme moieties**
- **Hydrophobic membrane anchor**
- **Template structure: hexadecaheme cytochrome Hmc from *Desulfovibrio vulgaris***
- **E-value of alignment: $6.5 \cdot 10^{-12}$**
- **3 domains**
- **Flexible linker**



Model created using *Phyre and LOOPP*
http://www.sbg.bio.ic.ac.uk/~3dpssm/
http://cbsuapps.tc.cornell.edu/loopp.aspx

Outer membrane cytochrome – OmcS

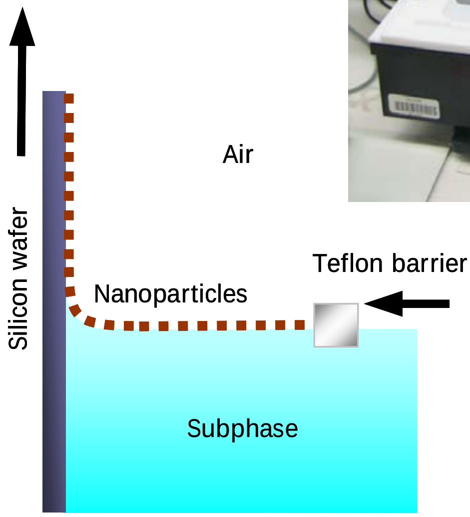
- **50 kDa**
- **6 hemes**
- **OmcS may be the terminal electron donor transferring electrons to mineral surfaces**
- **OmcS is associated with lipopolysaccharides in the outer membrane and may also play an essential role in the conductivity of pili**



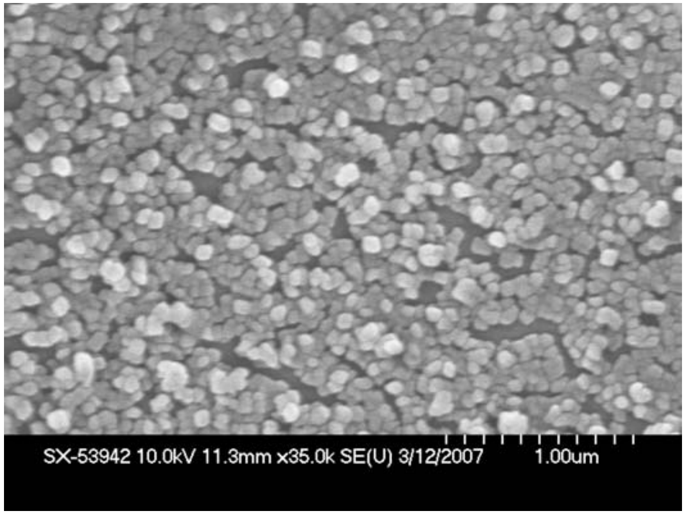
Secondary structure prediction for OmcS

Model system

- **Langmuir-Blodgett deposition of Fe_2O_3 nanoparticles**



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

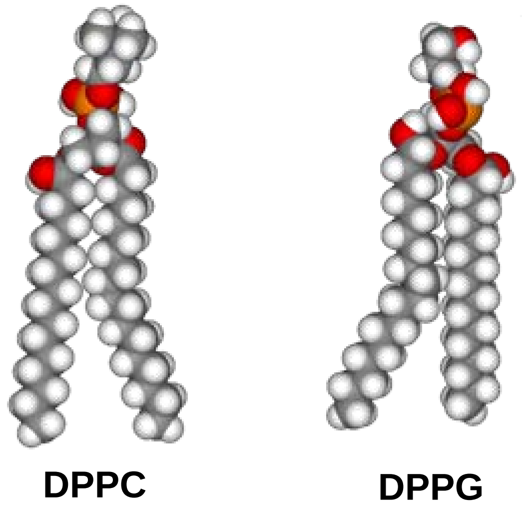


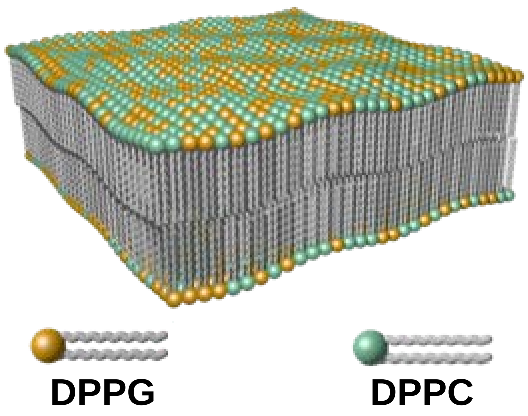
SEM image of an iron oxide nanoparticle monolayer on silicon.

- Nanoparticle size: 5-60 nm
- Nanocrystalline hematite (Fe_2O_3) as determined by X-ray diffraction
- Surface coverage: ~ 60%

Biomimetic model membranes

- **Biomimetic phospholipid model membranes**
- **Phosphatidylcholine (PC): zwitterionic, net charge: 0**
- **Phosphatidylglycerol (PG): anionic, net charge: -1**



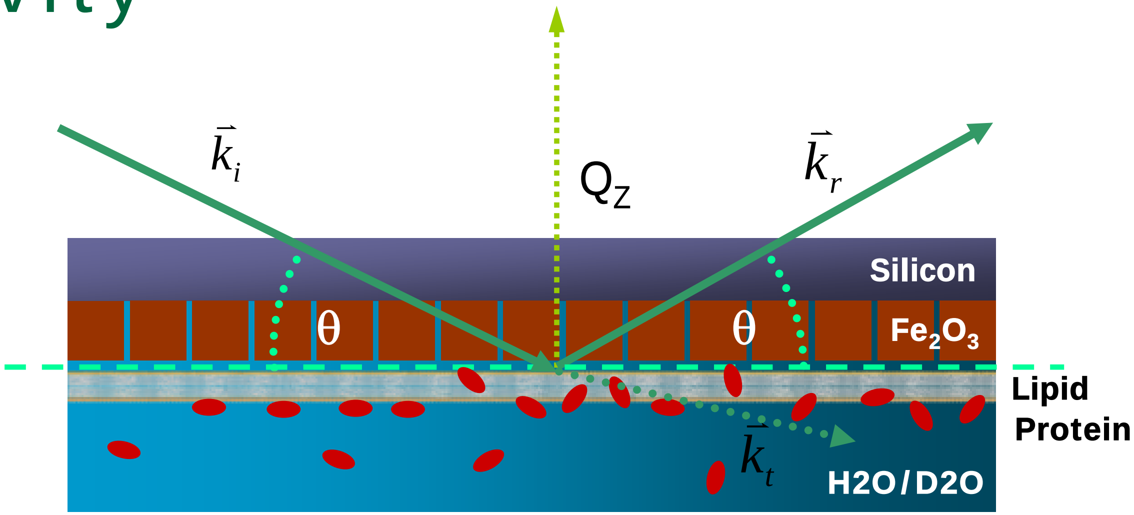


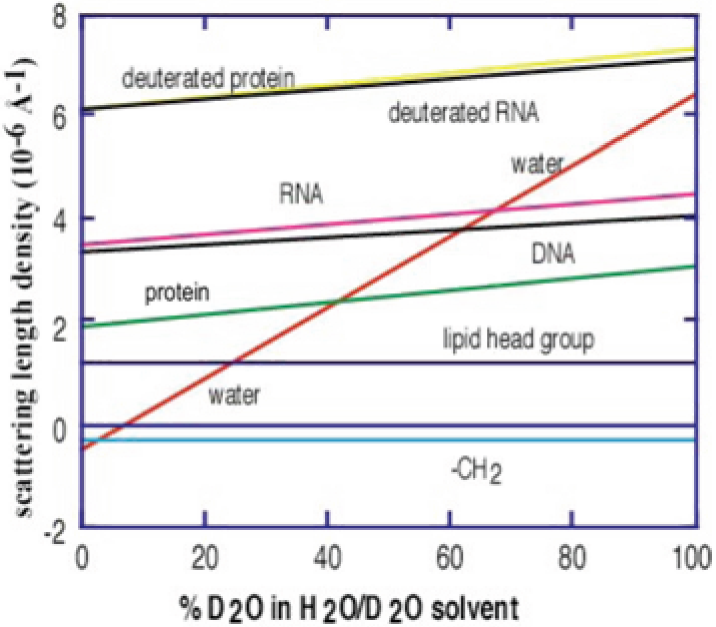
- **Uncharged and negatively charged phospholipids to mimic the electrostatic environment of the bacterial membrane**
- **Interactions with lipid membranes may induce conformational changes in the proteins**

Neutron Reflectivity

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

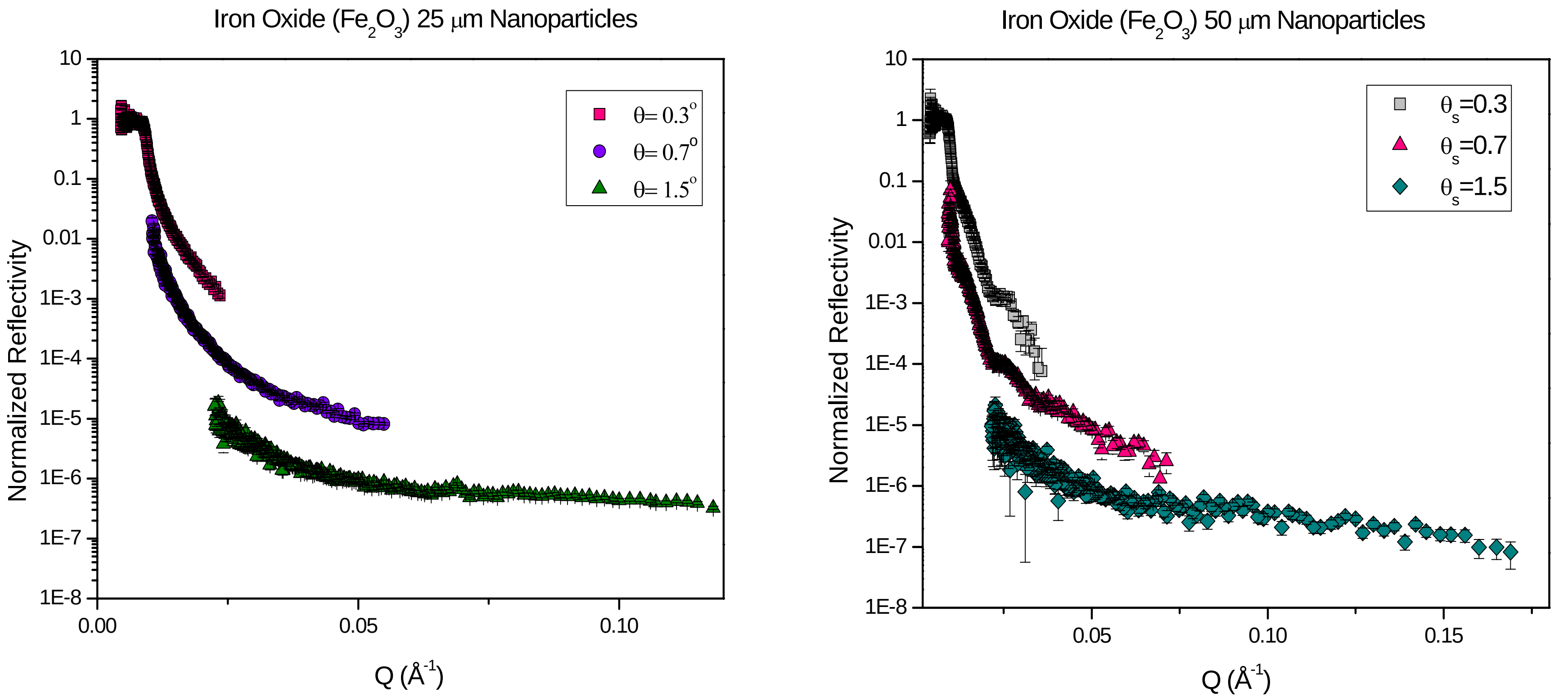
$$R(Q) \approx \frac{16\pi^2}{Q^4} N_b^2$$





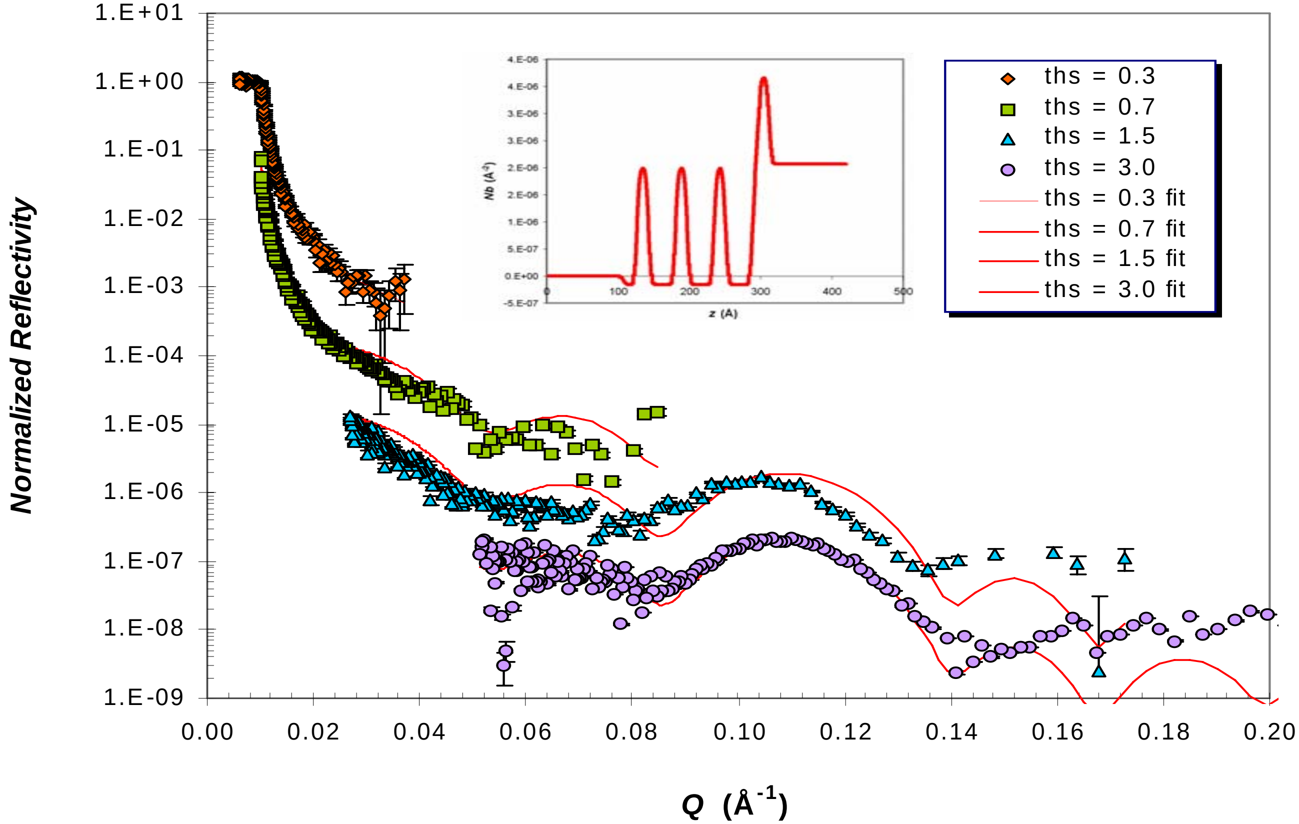
- Unique properties of neutrons to probe the complex biological-mineral interface
- Contrast variation to resolve model ambiguity
- Production and purification of deuterated proteins in the ORNL deuteration lab

Iron Oxide Nanoparticle Thin Films



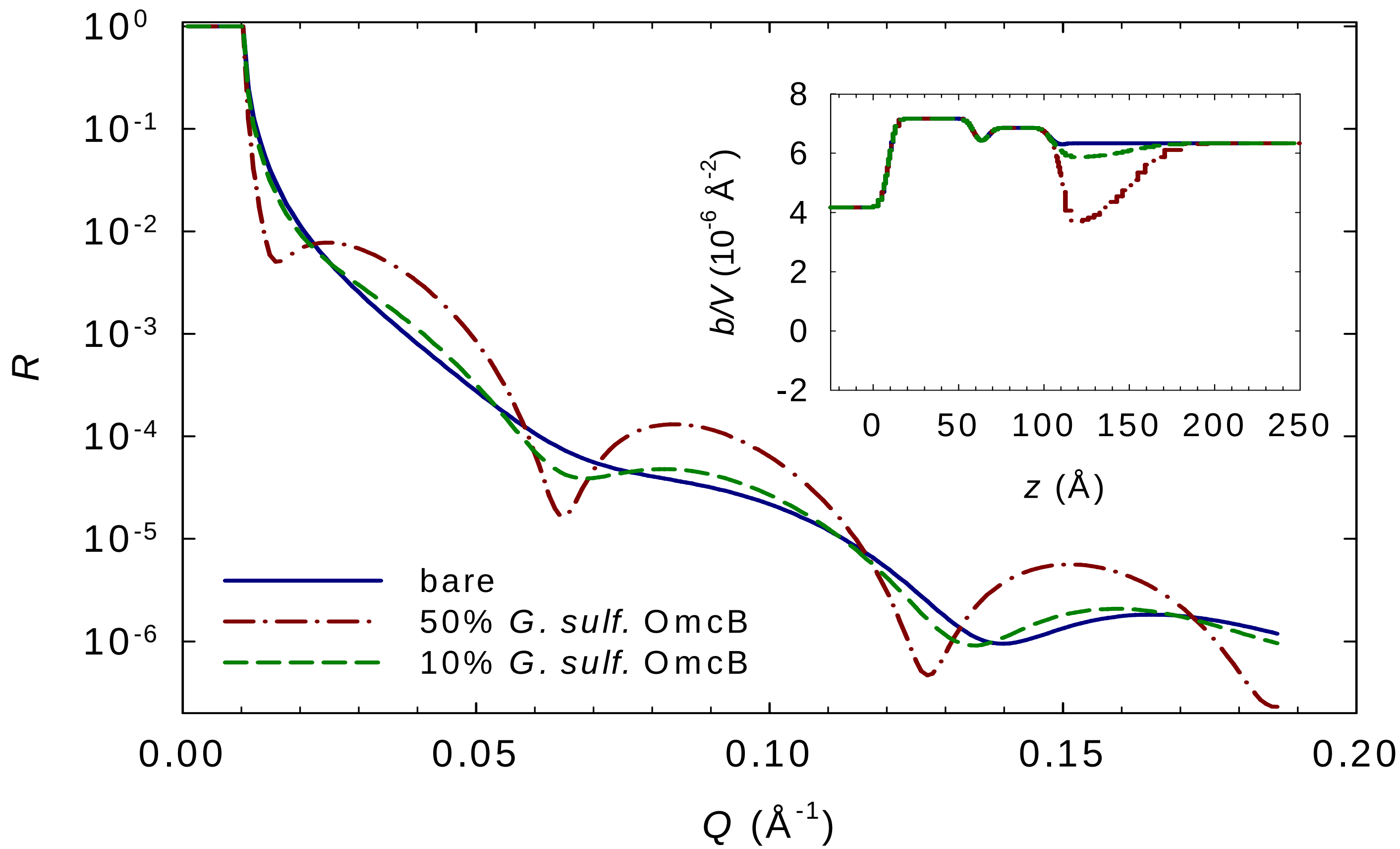
- Reflectivity profiles from 25 and 50 µm iron oxide nanoparticle thin films deposited by the Langmuir-Blodgett technique. Data acquired at SNS BL-4B.

Phospholipid model membrane data



- Reflectivity profile of a phospholipid bilayer stack
- Fluid cell setup for studies in excess water

Simulated Neutron Reflectivity profiles

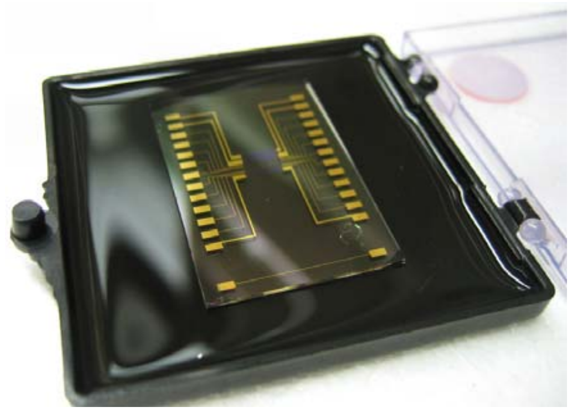


Neutron specular reflectivity from the protein *Geobacter sulfurreducens* OmcB deposited atop the fully deuterated DPPC bilayer from D_2O. A layer with 50% coverage is readily distinguishable from the bare phospholipid bilayer, while 10% coverage is more difficult to resolve and so represents a lower limit.

Complementary structural characterization

- **Atomic Force Microscopy to investigate surface properties, roughness**
- **Spectroscopic methods: Fluorescence Spectroscopy (protein-lipid interactions) and Circular Dichroism (conformational changes)**
- **Small Angle X-ray and Neutron Scattering with contrast variation to obtain solution structures of single proteins and protein complexes, influence of pH and ions**

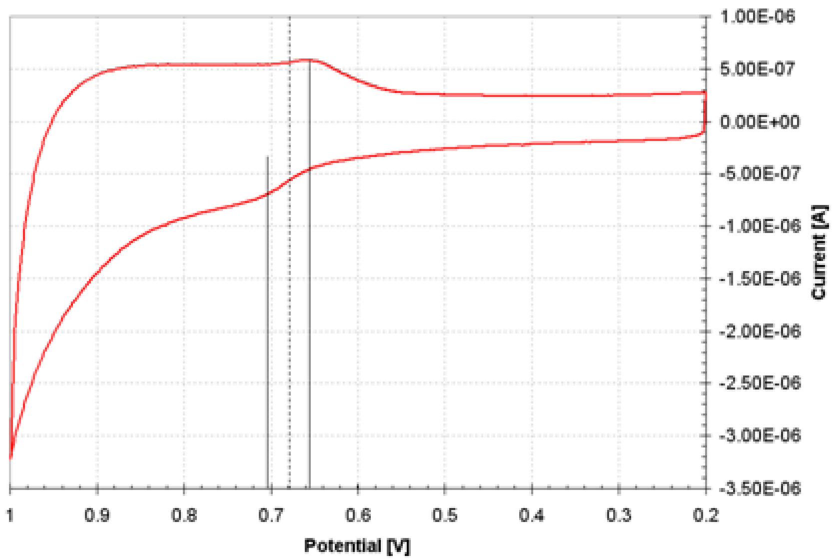
Complementary methods



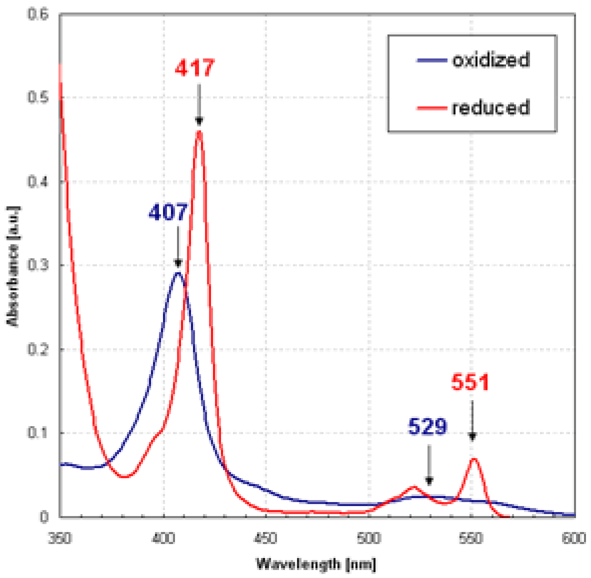
Thin layer electrode setup to study electrochemical properties of cytochrome proteins in lipid environments

Option to integrate thin layer electrochemical cell into sample holder for neutron reflectivity, simultaneous measurements

Control redox potential, electron transfer kinetics.



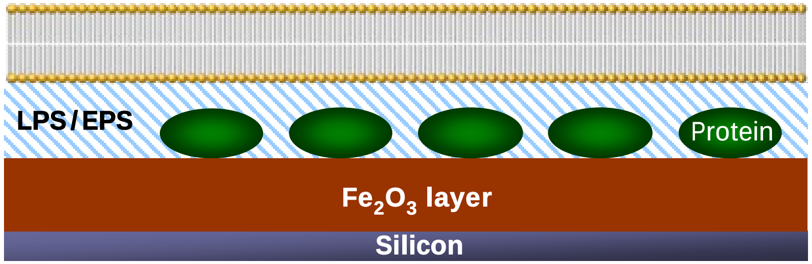
Redox potential of a small monoheme cytochrome c

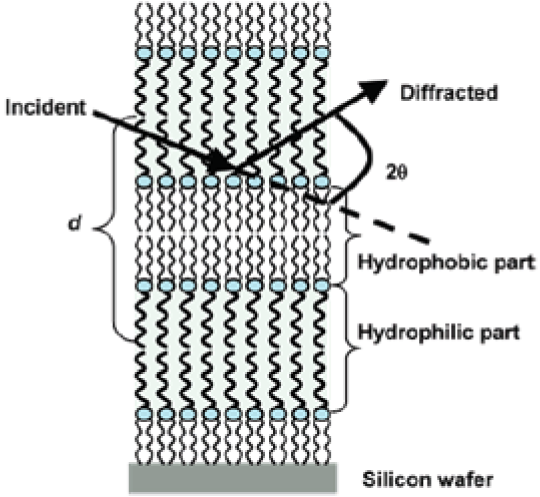


UV/VIS absorption spectra of oxidized and reduced forms of PpcA

Future research perspectives

- **Application of experimental system to study electron transport in *Shewanella oneidenis* or other microorganisms**
- **X-ray crystallographic data to obtain atomic resolution structural models**
- **Novel model systems incorporating Exopolysaccharides (EPS) and Lipopolysaccharides (LPS) from cell extracts**





- **Interaction of outer membrane cytochromes with oriented LPS layers**
  - Recent progress in characterization of LPS on solid support*

**Abraham et al., JPhysChem B, 111, 2477-2483, 2007*

Acknowledgements

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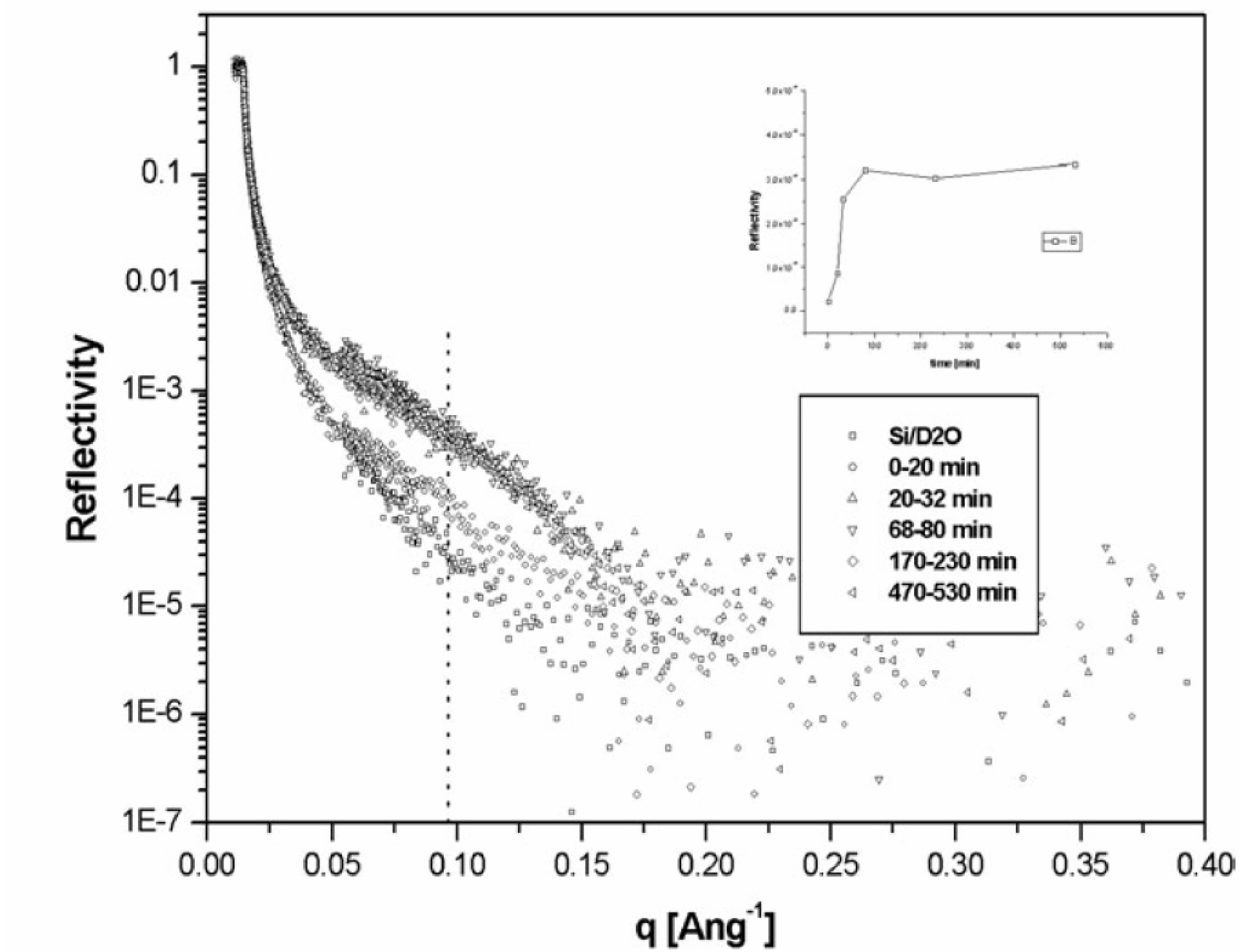
- **N. Ericson, T. McKnight, EST**
- **L. Liang, ESD**
- **ORNL LDRD program**

Single DMPC bilayer on Si



- T. Gutberlet, R. Steitz, G. Fragneto, B. Kloesgen, J. Phys.: Conden. Matter 16 (2004)

Contrast Variation

- **Calculated scattering length density (SLD) for Fe_2O_3 in the hematite crystal modification is $7.2 \cdot 10^{-6}$ A^{-2}. Surface coverage between 60% and 80%:**
- **SLD for the iron oxide layer between $4.32 \cdot 10^{-6}$ A^{-2} and $5.76 \cdot 10^{-6}$ A^{-2} corresponding to a D_2O/H_2O ratio of 70 - 92%.**