Important Findings in Last Year

1. Demonstration of metallic-like conductivity along protein filaments: a paradigm shift in biological electron transport.


3. Demonstration that anaerobic reduced sediments can be conductive, but by mechanisms other than electron conduction through pili.

4. Demonstration of the utility of genome-scale modeling of community dynamics in the subsurface.

5. Demonstration of the potential importance of protozoan grazing in uranium bioremediation.


Important Findings in Last Year

1. Demonstration of metallic-like conductivity along protein filaments: a paradigm shift in biological electron transport.


Metallic-Like Conductivity is Significantly Different than Previously Considered Modes of Biological Electron Transfer

Hopping or Tunneling Electron Transfer is the Known Mechanism for Biological Electron Transfer

In Metallic-Like Conductivity Electrons are Delocalized
Metallic-Like Conductivity is a Paradigm Shift

Within the context of this present analysis, it is apparent that proteins are large band gap materials, approaching 3 eV and there is no possibility of “metallic-like” conductivity even at the highest doping levels, that is, on D/A transfer within the molecule. Delocalized coherent transport is dismissed in biomolecules due to their lack of periodicity, random fluctuations, and limited conductance values from experiments.


Pili and Flagella are Specifically Produced When *Geobacter* are Grown on Insoluble Electron Acceptors

Flagella Aid in the Search for Fe(III) Oxide

Geobacter Specifically Expresses Flagella when only Fe(III) Oxide is Available as an Electron Acceptor

Geobacter follows Fe(II) Gradient Associated with Fe(III) Oxides Under Anaerobic Conditions to Locate Fe(III) Oxides


Transcriptional Regulation of PilA Expression with Specific Upregulation During Growth on Fe(III) Oxide Suggested Special Role for Pili in Fe(III) Oxide Reduction

Pili are Required for Fe(III) Oxide Reduction
(T. Metha ca. 2003)

*Geobacter sulfurreducens*

![Graph showing the reduction of Fe(III) oxide by different strains of *Geobacter sulfurreducens*. The graph compares the growth of the wild type, PilA complement, and PilA-deficient mutant strains over time.](image-url)
Pili are Also Required for Fe(III) Oxide Reduction in *Geobacter metallireducens*

Data Supporting Initial Suggestion that \textit{Geobacter} Pili are Conduits for Long-Range Electron Transport

- PilA specifically expressed during growth on Fe(III) oxide
- Knocking out PilA eliminates Fe(III) oxide reduction
- Fe(III) oxide associated with pili
- Pili are electrically conductive across their width

Conclusion: long-range electron transport Not involving cytochromes

OmcS is Specifically Associated with Pili in Fe(III) Oxide-Grown *G. sulfurreducens*

It was Concluded that OmcS Could **NOT** Account for Conductivity Along the Pili Because the Spacing Between the Cytochromes was Too Great

Alignment of the $c$-Type Cytochrome OmcS along Pili of *Geobacter sulfurreducens* $\n$\nChing Leang,* Xinlei Qian,§ Tünde Mester,‡ and Derek R. Lovley

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Immunogold localization revealed that OmcS, a cytochrome that is required for Fe(III) oxide reduction by *Geobacter sulfurreducens*, was localized along the pili. The apparent spacing between OmcS molecules suggests that OmcS facilitates electron transfer from pili to Fe(III) oxides rather than promoting electron conduction along the length of the pili.

There are multiple competing/complementary models for extracellular electron transfer in Fe(III)- and electrode-reducing microorganisms (8, 18, 20, 44). Which mechanisms prevail in different microorganisms or environmental conditions may greatly influence which microorganisms compete most successfully in sedimentary environments or on the surfaces of elec-
The Separation Between OmcS Appeared to be Too Far for Electron Transfer Along Pili Via Cytochromes

Typical Spacing Between OmcS clusters of 100-200 nm

This conclusion has subsequently been confirmed with atomic force microscopy.
Therefore, it is physically impossible for cytochromes to account for conduction along the length of the pili.
First Demonstration of Conduction Along the Length of *Geobacter* Pili

Physiologically Relevant Conditions:
No chemical fixative
Pili still hydrated

The Conductivity of Filament Preparations can be Attributed to PiliA-Pili

The Initial Exponential Increase in Conductivity as Temperature is Lowered is Similar to that of Synthetic Organic Polymers that Exhibit Metallic-Like Conductivity. Traditional electron hopping/tunneling will NOT exhibit this temperature response.
Treatment with a Cytochrome Denaturing Agent (BME) Did Not Affect Pili Conductance

Protein Gel Stained for Heme

Conductivity (µS/cm)

After denaturing  Before denaturing

Control (Buffer without ME)  Control (Buffer with ME)  Wild Type without ME  Wild Type with ME
Proton Doping Increases the Conductivity of Synthetic Organics with Metallic-Like Conductivity such as Polyaniline

(Alan Macdiarmid, Nobel lecture, 2001)

Protonation of imine and amine Nitrogen sites gives rise to an Unpaired electron on each of the imine nitrogen sites.

(Heeger, Metallic and semiconducting Polymers 2010)
The Conductivity of Pili Increased Over Two Orders of Magnitude with Decreasing pH and Was Highest at a pH that Denatures c-Type Cytochromes

Furthermore, an increase in conductivity with decreasing pH is consistent with an increase that would be expected with metallic-like conductivity as the result of proton doping, there is no model in which proton doping would enhance electron hoping.
In metallic polymer polyaniline, the peak at 25° corresponds to face-to-face interchain stacking distance between phenyl rings.

In synthetic conducting proteins with delocalized electronic states, an intermolecular distance ~ 3.5 Å has been reported to facilitate efficient charge delocalization and has been observed in many conductive materials based on aromatic ring stacking.

Thus, aromatic ring stacking may confer metallic conductivity to pili.
X-ray Diffraction Patterns of Purified Pilin Preparations
Indicate the Presence of Overlapping π Orbitals

A peak at 25° (d-spacing ~ 3.5 Å) is indicative of overlapping π orbitals.
The Hypothesis that Aromatic Moieties in the Non-Conserved Carboxyl Portion of the Molecule are Responsible for Conductivity is Being Tested via Amino Acid Substitution
A Strain of *Geobacter sulfurreducens* in which an Alanine was Substituted for an Aromatic Amino Acid in the PilA Sequence Produced Pili and Properly Localized Cytochromes, But was Deficient in Fe(III) Oxide Reduction

For more details see poster by Madeline Vargas et al.
Although Cytochromes can NOT Account for Conductivity Along the Pili the Cytochrome OmcS is Essential for Fe(III) Oxide Reduction

The gene for OmcS is the most highly upregulated gene during growth on Fe(III) oxide…

… and deleting the gene for OmcS Specifically Inhibits Fe(III) Oxide reduction, not reduction of soluble Fe(III) 
(Mehta et al. 2005)
Model for Electron Flow to Fe(III) Oxide: Electrons Flow Along Pili and the Outer Surface C-Type Cytochrome OmcS Facilitates Electron Transfer onto Fe(III) Oxides

Additional Support for Model: Charge injected into untreated individual pili still attached to cells moves through pili and into OmcS (Nikhil Malvankar unpublished data).

Adaptive Evolution to Select for Superior Fe(III) Oxide Reduction Leads to Mutations in Regulatory Systems that Lead to Increased Expression of Pili

Metallic-Like Conductivity of Pili is Involved in Electron Transfer in Current-Producing Biofilms and in Direct Interspecies Electron Transfer


Important Findings in Last Year

Subsurface Microbial Fuel Cell for Monitoring Microbial Activity

Cathode and Anode Separated by 8-10 Meters

Direct Correlation Between Current Production and Rates of Methanogenesis in Methanogenic Sediments

![Graph showing the correlation between current production and CH₄ production rate.](image)

- Current (uA) on the y-axis.
- CH₄ production rate (ppm h⁻¹) on the x-axis.

The graph displays a trend line with an R² value of 0.8373, indicating a strong correlation between the current production and CH₄ production rate.
Microbial Community Composition in Sediments and on Anode After 64 Days of Monitoring Microbial Activity

- **Anode**
  - 69% Geobacter sp.
  - 12% β-Proteobacterium (Azospira sp.)
  - 12% Others
  - 3% Geobacter sp.

- **Sediment**
  - 1% Acidobacteria
  - 2% Clostridiales
  - 3% α-Proteobacterium (Rhizobiales)
  - 1% Bacteroidia
  - 2% Bacteroidia
  - 1% Others
3. Demonstration that reduced sediments can be conductive, but by mechanisms other than electron conduction through pili.
Graphite Electrodes Visually Oxidize the Sediment Around Them Suggesting that Electrons at Distance are Conducted to the Electrode
It has been Speculated that Pili Might Account for the Conductivity that was Inferred in Anaerobic Marine Sediments

Electric currents in sediment.

Direct Measurements of Conductivity Confirm Previous Inference of Conductivity in Reduced Sediments

Conductivity is via a mechanism other than pili—see poster by Nevin et al.
Electrobiogeochemistry: Biological and mineralogical electrical connections that influence the biogeochemical cycling of minerals and carbon in soils and sediments

Important Findings in Last Year

4. Demonstration of the utility of genome-scale modeling of community dynamics in the subsurface.


Modeling the Interaction Between Sulfate Reducers and Geobacter and In Silico Design of Bioremediation Strategies

See poster by J. Zhao et al.
Bottom-Up Genome-Scale (BUGS) Modeling: Iterative Development of *In Silico* Models of Microbial Communities

Capacity to Predictively Model Response of Microbial Community to Natural or Anthropogenic Perturbations

Modification of Model Based on Environmental Gene Expression and Proteomics Data

Pure culture studies

Pure culture sequences

Molecular Analysis of Growth and Metabolic State *In Situ*

*In Silico* Models

Environmental Analysis

Isolation

Environmental Genome Sequencing and Assembly

Predictions of Community Response
5. Demonstration of the potential importance of protozoan grazing in uranium bioremediation.
Growth Rate of Estimates of Subsurface Geobacter Community from Molecular Analysis was an Order of Magnitude Higher than the Growth Rate Observed from Increases in Cell Numbers During Acetate-Amendments at the Rifle Site

The Bloom of Geobacter Species Following the Addition of Acetate at the Rifle, CO Study Site was Accompanied by a Bloom of Bacteriovorous Protozoa in the Genus Breviata
The Bloom of *Geobacter* Species Following the Addition of Acetate at the Rifle, CO Study Site was Accompanied by a Bloom of Bacteriovorous Protozoa in the Genus *Breviata*.

![Graph showing the percentage of Breviata and Geobacter species over time.](#)
The Bloom of Sulfate-Reducing Species that Followed the *Geobacter* Bloom was Accompanied by a Bloom of Bacteriovorous Protozoa in the Genus *Hexamita*
Conclusions

• The metallic-like conductivity responsible for long-range electron transport along the length of *Geobacter* pili is a paradigm shift in biological electron transfer and has important implications for growth of *Geobacter* species on Fe(III) oxides in the subsurface.

• Subsurface microbial activity sensors are an inexpensive and simple strategy for real-time monitoring of rates of microbial metabolism in a diversity of anaerobic soils and sediments.

• Reduced sediments are conductive, but probably not due to the presence of pilin networks.

• Genome-scale modeling of subsurface community dynamics is possible and can aid in the design of improved bioremediation strategies.

• Protozan grazing may play a previously unrecognized role controlling microbial growth during anaerobic groundwater bioremediation.
Thank You