

## Final Report

### Mechanisms for Electron Transfer Through Pili to Fe(III) Oxide in *Geobacter*

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The project was highly successful. Not only was it demonstrated for the first time that the pili of *Geobacter sulfurreducens* are conductive along their length, but also a new basic paradigm for biological electron transport was revealed. A summary of results leading to peer-reviewed publications follows.

The most stunning result of these studies was the finding that the pili of *G. sulfurreducens* possess metallic-like conductivity and that it is the metallic-like conductivity along pili that is required for long-range electron transport to Fe(III) oxides. The metallic-like conductivity was discovered in studies in which networks of pili sheared from the outer surface of *G. sulfurreducens* were placed on gold electrode arrays. Conductivity measurements demonstrated long-range electron transport through the pili network for distances of over a centimeter. This demonstrated for the first time that electrons could be transported along the length of *G. sulfurreducens* pili.

Even more surprising were studies that evaluated the impact of temperature and pH on conductivity. Lowering the temperature from room temperature initially resulted in a logarithmic increase in conductivity. This temperature response contrasts with the response of typical biological electron transfer, which relies on electron hopping or tunneling between discrete electron carriers. In typical biological electron transfer the rate of electron transfer decreases with decreasing temperature. This is the opposite of what was observed with the pili. However, the temperature response of the conductivity in the pili had a pattern very similar to that observed in synthetic organic conducting polymers, such as polyaniline, which possess metallic-like conductivity.

Another surprising response was the impact of pH. Decreasing the pH of the pili preparations from pH 10.5 to 2 increased the conductivity by 100-fold. This was despite the fact that most biological proteins are expected to be denatured at pH 2. However, the

dramatic increase in conductivity is also a response expected for metallic-like conductivity.

Metallic-like conductivity in synthetic organic conducting polymers can be attributed to overlapping pi-pi orbitals of aromatic rings. Evidence for such pi-pi stacking was apparent from X-ray diffraction analysis of pili preparations.

This publication of these results in *Nature Nanotechnology* was accompanied by a News and Views article in *Nature Nanotechnology* and significant coverage both in the scientific and popular press. Our paper has had very high citation and download rates as well as social media attention scores that rank it at the top of all journal articles of similar age.

Genetic studies were undertaken in order to further investigate the potential mechanisms for metallic-like conductivity in *G. sulfurreducens* pili. These studies were summarized in a paper published in *mBio*. In a protein overlapping pi orbitals can only result from close packing of aromatic amino acids. In order to determine if aromatic amino acids in the pili was responsible for the metallic-like conductivity, a strain of *G. sulfurreducens* was genetically constructed in which an alanine was substituted for each of the five carboxyl terminus aromatic amino acids in PilA, the protein subunit that assembles into the pili. This strain was designated strain Aro-5. Strain Aro-5 produced pili that resembled those of wild-type *G. sulfurreducens* in transmission electron micrographs. Furthermore, the *c*-type cytochrome OmcS, which associates with wild-type pili, also associated with the pili of strain Aro-5. However, the pili of strain Aro-5 had greatly reduced conductivity and lacked the capacity for long-range electron transfer to Fe(III) oxide. These results supported the concept that the metallic conductivity of *G. sulfurreducens* pili can be attributed to the close packing of aromatic amino acids, which promote metallic-like conductivity through overlapping pi-pi orbitals.

Our report of metallic-like conductivity in *G. sulfurreducens* pili was met with some skepticism in the scientific community because it was such a dramatic departure from the typical mechanism for biological electron transport. For example, it was suggested in several review articles that the conductivity of the pili was probably due to electron hopping between cytochromes associated with the pili. To further examine this, we imaged *G. sulfurreducens* with atomic force microscopy. This analysis clearly demonstrated that the cytochromes on the pili were spaced too far apart for electron hopping. A paper summarizing these results was published in *Energy and Environmental Science*, which has a very high impact factor (>15).

In addition to conducting electrons to Fe(III) oxide, it was considered that the pili might also play an important role in electron transfer to contaminant metals, such as uranium that are also reduced outside the cell. However, it was found that pili were not required for effective U(VI) reduction. Further analysis of a suite of mutants in which one or more *c*-type cytochrome genes were deleted revealed that a diversity of outer surface *c*-type cytochromes had the potential to contribute to U(VI) reduction. It was necessary to delete genes for all of the major outer surface *c*-type cytochromes in order to substantially

reduce rates of U(VI) reduction. These results are important because they demonstrate that although pili are required for growth of *Geobacter* species on Fe(III) oxides in the subsurface, the concurrent extracellular reduction of soluble contaminant metals, such as uranium, may take place at the outer cell membrane. A paper summarizing these results was published in *Applied and Environmental Microbiology*.

There is a diversity of known *Geobacter* species. In order to determine whether the model developed for long-range electron transfer in *G. sulfurreducens* applied to other *Geobacter* species, a genetic study was conducted with *Geobacter metallireducens*. Like *G. sulfurreducens*, *G. metallireducens* required pili in order to reduce Fe(III) oxide. This was not surprising because the PilA sequences of *G. sulfurreducens* and *G. metallireducens* are highly conserved. In contrast, there is poor homology between the outer surface *c*-type cytochromes of the two species. However, genetic analysis revealed several outer-surface *c*-type cytochromes that are required for Fe(III) oxide reduction by *G. metallireducens*. These results suggested that several *G. metallireducens* *c*-type cytochromes might function like *G. sulfurreducens* outer surface *c*-type cytochromes, despite their lack of sequence similarity. A paper summarizing these results was published in *Applied and Environmental Microbiology*.

After long-term incubation, a strain of *G. sulfurreducens* that was deficient in the gene for PilA began to reduce Fe(III) oxide, even though it could not produce pili. After seven consecutive transfers the PilA-deficient strain adapted to reduce Fe(III) oxide as fast as the wild type. Microarray, whole genome resequencing, proteomic, and gene deletion studies indicated that this adaptation was associated with greater production of the *c*-type cytochrome PgcA, which was released into the culture medium. Further investigation revealed the PgcA was acting as an electron shuttle between the cells and Fe(III) oxide. Although this was an effective adaptation to the inability to produce pili in laboratory incubations, the pili-deficient, PgcA-overexpressing strain was outcompeted by the wild-type, pili-producing strain under conditions that simulated those found in subsurface environments. These results further demonstrated the effectiveness of long-range electron transport via pili as a strategy for Fe(III) oxide reduction. A paper summarizing these results was published in *Applied and Environmental Microbiology*.

Another major focus of the research was to elucidate the localization of proteins predicted to be on the outer surface of *Geobacter sulfurreducens*. The *c*-type cytochrome OmcE is considered to play an important role in electron transfer to Fe(III). Preliminary results from immunogold studies revealed that OmcE is associated with the outer membrane. This is in contrast to OmcS, which is also required for Fe(III) oxide reduction and is localized on the pili. However, further studies on the functional role of OmcE will be required prior to publication.

A number of invited reviews were written which further discussed the concept of long-range electron transfer through pili and its importance to the reduction of Fe(III) oxide and the bioremediation of uranium-contaminated subsurface environments. These reviews were published in high-impact journals and have been highly cited.

## **Publications**

- Malvankar NS, Vargas M, Nevin KP, Franks AE, Leang C, Kim B-C, Inoue K, Mester T, Covalla SF, Johnson JP, Rotello VM, Tuominen MT, Lovley DR. 2011. Tunable metallic-like conductivity in nanostructured biofilms comprised of microbial nanowires. *Nature Nanotechnology* 6:573-579.
- Lovley DR. 2011. Live wires: direct extracellular electron exchange for bioenergy and the bioremediation of energy-related contamination. *Energy Environ. Sci.* 4:4896-4906.
- Lovley DR, Ueki T, Zhang T, Malvankar NS, Shrestha PM, Flanagan K, Aklujkar M, Butler JE, Giloteaux L, Rotaru A-E, Holmes DE, Franks AE, Orellana R, Risso C, Nevin KP. 2011. *Geobacter*: the microbe electric's physiology, ecology, and practical applications. *Adv. Microb. Physiol.* 59:1-100.
- Malvankar NS, Mester T, Tuominen MT, Lovley DR. 2012. Supercapacitors based on c-type cytochromes using conductive nanostructured networks of living bacteria. *ChemPhysChem* 13:463-468.
- Smith JA, Lovley DR, Tremblay PL. 2012. Outer cell surface components essential for Fe(III) oxide reduction by *Geobacter metallireducens*. *Appl. Environ. Microbiol.* 79:901-907.
- Malvankar NS, Tuominen MT, Lovley DR. 2012. Biofilm conductivity is a decisive variable for high-current-density *Geobacter sulfurreducens* microbial fuel cells. *Energy. Environ. Sci.* 5:5790-5797.
- Lovley DR. 2012. Electromicrobiology. *Ann. Rev. Microbiol.* 66:391-409.
- Malvankar NS, Tuominen MT, Lovley DR. 2012. Lack of involvement of c-type cytochromes in long-range electron transport in microbial biofilms and nanowires. *Energy. Environ. Sci.* 5:8651 - 8659.
- Malvankar NS, Lovley DR. 2012. Microbial nanowires: a new paradigm for biological electron transfer and bioelectronics. *ChemSusChem* 5:1039– 1046.
- Malvankar NS, Lau J, Nevin KP, Franks AE, Tuominen MT, Lovley DR. 2012. Electrical conductivity in a mixed-species biofilm. *Appl. Environ. Microbiol.* 78:5967– 5971.
- Orellana R, Leavitt JJ, Comolli LR, Csencsits R, Janot N, Flanagan KA, Gray AS, Leang C, Izallalen M, Mester T, Lovley DR. 2013. U(VI) reduction by a diversity of c-type cytochromes of *Geobacter sulfurreducens*. *Appl. Environ. Microbiol.* 79:6369-6374.
- Vargas M, Malvankar NS, Tremblay P-L, Leang C, Smith JA, Patel P, Synoeyenbos-West O, Nevin KP, Lovley DR. 2013. Aromatic amino acids required for pili conductivity and long-range extracellular electron transport in *Geobacter sulfurreducens* *mBio* 4:e00105-00113.
- Williams KH, Bargar JR, Lloyd JR, Lovley DR. 2013. Bioremediation of uranium-contaminated groundwater: a systems approach to subsurface biogeochemistry. *Curr. Opin. Biotechnol.* 24:489-497.
- Smith JA, Tremblay P-L, Shrestha PM, Synoeyenbos-West OL, Franks AE, Nevin KP, Lovley DR. 2014. Going wireless: Fe(III) oxide reduction without pili by *Geobacter sulfurreducens* strain JS-1. *Appl. Environ. Microbiol.* 80:4331-4340.