Molecular Mechanisms Underlying the Metallic-Like Conductivity of *Geobacter* Pili

Madeline Vargas[1,3], Nikhil S. Malvankar[1,2], Pier-Luc Tremblay[1], Kelly Flanagan, Pranav Patel[1], Manju L. Sharma[1] and Derek R. Lovley[1] (PI; dlovley@microbio.umass.edu)

[1]Department of Microbiology, [2]Department of Physics, University of Massachusetts, Amherst, Mass. [3]Dept. of Biology, College of the Holy Cross, Worcester, Mass.

The discovery of long-range electron transport along the pili of *Geobacter sulfurreducens* via metallic-like conductivity is a paradigm shift in biological electron transfer and materials science with important implications in bioremediation and bioenergy. The metallic-like conductivity of the *Geobacter* pili differs significantly from previously described biological electron transport in which electrons hop or tunnel between discrete redox-active molecules, such as cytochromes. This finding also contradicts the long-standing belief that biological proteins act as insulators and can not be conductive. In the last year we have made significant progress in understanding the mechanisms for the metallic-like conductivity of *Geobacter* pili and the role of pili in extracellular electron transfer.

Additional evidence for metallic-like conductivity along pili was the finding that decreasing the pH of the pili preparations enhanced conductivity by as much as 100 fold. This response is characteristic of materials with metallic-like conductivity, but inconsistent with the alternative model of electron hopping/tunneling via cytochromes. In fact, the highest conductivity was observed at pH 2, a pH at which cytochromes are denatured.

Structural studies of *Geobacter sulfurreducens* pili using X-ray diffraction revealed overlapping pi orbitals. This is significant because pi orbital stacking is the basis for the metallic-like conductivity observed in synthetic conducting polymers, such as polyaniline. This finding led to the hypothesis that aromatic amino acids exposed on the outer surface of the pili play a key role in establishing the metallic-like conductivity of the pili.

In order to investigate this possibility, we genetically constructed mutant strains in which alanine was substituted for individual aromatic amino acids located at the carboxyl terminus of PilA, the structural pilin protein. Through these studies we identified an aromatic amino acid moiety that is required for the conductivity of pili sheared from the cells and for long-range electron transfer via pili attached to viable cells. The strain with this single amino acid substitution continued to produce pili that were functional in other aspects, such as promoting attachment to surfaces. These results are consistent with the hypothesis of metallic-like conductivity, but inconsistent with the alternative model of cytochromes mediating electron transport along pili.

Although *c*-type cytochromes can not account for conduction along pili, the cytochromes OmcS and OmcE are positioned outside the cell and are involved in electron transfer to Fe(III) oxide. Previous studies have demonstrated that OmcS is localized on the pili and is thought to facilitate electron transfer from pili to Fe(III) oxide. In order to localize OmcE, whole cells were treated with antibodies raised to OmcE, followed by secondary gold-labeled antibodies. Examination with transmission electron microscopy revealed that, unlike OmcS, OmcE is associated with the outer cell surface rather than the pili. These results suggest that a potential role of OmcE is to facilitate electron transfer to the pili. However, the ability to cells to adapt to deletion of the gene for OmcE indicates that there may be multiple proteins capable of this electron transfer step, a possibility that will be evaluated with adaptive evolution studies.