Spatioelectrochemistry : The Molecular Basis for Electron Flow Within Metal-Reducing Biofilms.

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Project Goals: Electrochemical, spectral, genetic, and biochemical techniques have provided evidence that multiple redox proteins and structural macromolecules outside the cell work together to move electrons long distances between *Geobacter* cells and to metals. This extracellular matrix contains many stable proteins not easily solubilized by standard methods, in addition to complex polysaccharides. Thus, much of these components were likely lost or ignored in previous proteomic and biochemical surveys. We aim to define this matrix and develop new tools to visualize it in action. The goals of this project are to 1) identify protein and polysaccharide elements crucial to both the assembly and function of the extracellular conductive matrix, 2) expand spectroelectrochemistry techniques to define the mechanism and route of electron transfer through the matrix, and 3) we will combine this knowledge of matrix proteins and their role in multicellular electron transfer to visualize redox and gene expression gradients in space over time.

When bacteria change the state of metals in the environment, they transport electrons unprecedented distances from intracellular metabolic reactions to distant mineral surfaces. This electron movement drives subsurface bioremediation, controls aquifer chemistry, and powers new microbial energy generation applications. Yet a molecular understanding of how this electron transfer is accomplished by *Geobacteraceae,* who are among of the most predominant bacteria in such systems, remains one of the grand challenges in microbial environmental processes.

Direct measurements of living biofilms using electrochemistry has revealed electron hopping between *Geobacter* redox proteins to be a rate-controlling step at all stages of growth. Direct spectral analysis of living biofilms has confirmed that *c*-type cytochromes are a major reservoir of charge in these films, and that these cytochromes experience a bottleneck to oxidation when electrons must be transferred longer distances. Fine-scale immunogold labeling has discovered gradients in cytochrome abundance throughout these films, further suggesting the presence of gradients within this biofilms. Genetics has discovered new polysaccharide biosynthesis operons, and secretion systems essential for the attachment of *Geobacter* to metals and other cells in the biofilm. Biochemistry has shown that the extracellular space acts anchors a diverse assemblage of essential *c*-type cytochromes and adhesion proteins outside the cell. We hypothesize that this data converges on a model of electron transfer mediated by multiple cooperating proteins,

ultimately limited by hopping between a highly adaptable network of cytochromes, which are attached to polysaccharides and pili.

Our project hypothesizes that it is the components of this matrix, and its high adaptability, that explains why *Geobacter* can so easily interface with a wide range of mineral surfaces, grow as multicellular networks, and quickly adapt to disruptions in single cytochromes.

To accomplish these goals, we are screening mutant libraries for strains defective in different developmental stages of this matrix using a combination of traditional and high-throughput approaches, and developing biochemical extraction methods for separating and analyzing this matrix. Key to this phase is to identify proteins required for specific developmental stages such as surface recognition, vs. self-recognition, or cell-surface electron transfer vs. cell-cell electron exchange. In parallel, we are inventing spectroelectrochemistry cells able to monitor cytochrome redox states in living biofilms, and engineering proteins for spatiotemporal localization of activity. This multidisciplinary approach aims to link quantitative data for specific reactions that occur at the biotic-abiotic interface with genes and expression patterns that can be used in predictive modeling, environmental monitoring, and design of bacteria with altered conductive properties.