36. Development of Quantitative Protein Biomarker Assays for Enzymes Involved in Bacterial Iron and Uranium Reduction

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Project Goals: The overall goal of this project is to develop quantitative MS/MS based assays for peptides specific to iron and uranium reducing proteins. The suite of peptides to be monitored includes representatives from proteins across a wide range of iron and uranium reducers. Synthetic, isotopically labeled peptide standards will be used for assay validation and to enable absolute quantification of target proteins in laboratory cultures as well as field samples from uranium contaminated groundwater.

Molecular biological tools hold promise for monitoring *in situ* remediation. While DNA/RNA based MBTs are becoming widespread, proteins MBTs are not as common. However, protein biomarker assays directly detect (and ideally quantify) the catalysts and therefore have a more direct relationship to instantaneous *in situ* capabilities. Our objectives in this study are to develop quantitative, mass spectrometry-based assays that will allow absolute and specific detection of a suite of iron and uranium reductases in pure culture proteomes and in metaproteomes from environmental samples.

In this work, we combine comparative genomics and high-throughput proteomic data to identify a collection of proteotypic peptides (PTPs) for proteins involved in iron and uranium reduction in six phylogenetically diverse bacteria: *Geobacter sulfurreducens, Geobacter bemidjiensis, Shewanella oneidensis, Anaeromyxobacter dehalogenans, Desulfovibrio desulfuricans,* and *Desulfotomaculum reducens*. Proteins include those previously identified in the literature as well as those discovered by our project. The list of proteins includes several cytochromes, pili proteins, membrane-bound oxidoreductases, and cytoplasmic iron reductases.

The selected method for quantification of proteins of interest is by multiple reaction monitoring (MRM) of PTPs via tandem mass spectrometry. Two to three PTPs per protein of interest are being selected for MRM based upon several criteria: previous detection in shotgun proteomic surveys of trypsin-digested proteomes, conservation across orthologs of the protein in other species, uniqueness to the protein of interest or its orthologs, and absence of methionine residues. Additionally, preference is given to PTPs that are located at the active site (if known) of the protein and have relatively large parent ion intensities during shotgun proteome characterizations relative to other PTPs in the proteins. Isotopically labeled synthetic peptides will be used to validate the MRM assays and to enable development of quantitative standard curves for multiple transitions (parent ion/fragment ion pairs) for each peptide. Assays will be used on proteomes from laboratory cultures as well as metaproteomes recovered from aquifers at uranium contaminated field sites.

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