High Throughput Purification and Identification of Water Soluble Multi-Protein Complex in Desulfovibrio vulgaris

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As part of the Genomics GTL Protein Complex Analysis Project (PCAP) we are developing a high throughput pipeline to purify water soluble protein complexes from *D. vulgaris*, identify their polypeptide constituents by mass spectrometry, determine their stoichiometries, and provide samples suitable for single particle EM characterization. These methods will then be used as part of PCAP's effort to model stress responses relevant to the detoxification of metal and radionuclide contaminated sites.

Our strategy uses a novel "tagless" method that fractionates the water soluble protein contents of a bacterium into a large number of fractions, and then identifies the polypeptide composition of a rational sampling of 10,000 – 20,000 of these fractions using MALDI TOF/TOF mass spectrometry. To establish this method to date: We have developed an optimized four-step fractionation scheme. We have built a prototype multi-channel, native gel electrophoresis instrument for high resolution protein separation and automated band collection that elutes a protein band into a 200 µl fraction without noticeable loss of sample. We have established an efficient, highly reproducible mass spectrometry sample preparation protocol that uses 96-well PVDF multiscreen plates. We have demonstrated that iTRAQ methodology provides quantitation of the relative abundances of polypeptides in different chromatographic fractions. We have also developed algorithms and graphical display tools for identifying protein complexes from mass spectrometry data, including a method for cluster analysis of iTRAQ data to allow detections of co migrating polypeptides and hence putative protein complexes. Finally, we have refined methods for preparing protein samples suitable for single particle EM analysis. To date, have identified and purified 15 homomeric and heteromeric water soluble protein complexes from *D. vulgaris*, of which five have been sent for EM structural determination, and one of which has been solved at a 17 Å resolution.

We are further optimizing and refining our methods to establish a fully functional high throughput pipeline.