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Publication Date

2007-01-10

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Protein Complex Analysis Project (PCAP): Project Overview

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The Protein Complex Analysis Project (PCAP) has two major goals: **1**. to develop an integrated set of high throughput pipelines to identify and characterize multi-protein complexes in a microbe more swiftly and comprehensively than currently possible and **2.** to use these pipelines to elucidate and model the protein interaction networks regulating stress responses in *Desulfovibrio vulgaris* with the aim of understanding how this and similar microbes can be used in bioremediation of metal and radionuclides found in U.S. Department of Energy (DOE) contaminated sites.

PCAP builds on the established research and infrastructure of another Genomics:GTL initiative conducted by the Environmental Stress Pathways Project (ESPP). ESPP has developed *D. vulgaris* as a model for stress responses and has used gene expression profiling to define specific sets of proteins whose expression changes after application of a stressor. Proteins, however, do not act in isolation. They participate in intricate networks of protein / protein interactions that regulate cellular metabolism. To understand and model how these identified genes affect the organism, therefore, it is essential to establish not only the other proteins that they directly contact, but the full repertoire of protein / protein interactions within the cell. In addition, there may well be genes whose activity is changed in response to stress not by regulating their expression level but by altering the protein partners that they bind, by modifying their structures, or by changing their subcellular locations. There may also be differences in the way proteins within individual cells respond to stress that are not apparent in assays that examine the average change in a population of cells. Therefore, we are extending ESPP's analysis to characterize the polypeptide composition of as many multi-protein complexes in the cell as possible and determine their stoichiometries, their quaternary structures, and their locations in planktonic cells and in individual cells within biofilms. PCAP will characterize complexes in wild type cells grown under normal conditions and also examine how these complexes are affected in cells perturbed by stress or by mutation of key stress regulatory genes. These data will all be combined with those of the ongoing work of the ESPP to understand, from a physical-chemical, control-theoretical, and evolutionary point of view, the role of multi-protein complexes in stress pathways involved in the biogeochemistry of soil microbes under a wide variety of conditions.

Essential to this endeavor is the development of automated high throughput methods that are robust and allow for the comprehensive analysis of many protein complexes. Biochemical purification of endogenous complexes and identification by mass spectrometry is being coupled with *in vitro* and *in vivo* EM molecular imaging methods. Because no single method can isolate all complexes, we are developing two protein purification pipelines, one the current standard Tandem Affinity Purification approach, the other a novel tagless strategy. Specific variants of

each of these are being developed for water soluble and membrane proteins. Our Bioinstrumentation group is developing highly parallel micro-scale protein purification and protein sample preparation platforms, and mass spectrometry data analysis is being automated to allow the throughput required. The stoichiometries of the purified complexes are being determined and the quaternary structures of complexes larger than 250 kDa are being solved by single particle EM. We are developing EM tomography approaches to examine whole cells and sectioned, stained material to detect complexes in cells and determine their localization and structures. New image analysis methods will be applied to speed determination of quaternary structures from EM data. Once key components in the interaction network are defined, to test and validate our pathway models, mutant strains not expressing these genes will be assayed for their ability to survive and respond to stress and for their capacity for bioreduction of DOE important metals and radionuclides.

Our progress during the first year of the project includes constructing genetically altered *D. vulgaris* strains and using them to test a range of affinity tags for the purification of protein complexes and EM localization of complexes in cells, developing automated primer design algorithms for high throughput recombinant DNA and strain engineering, establishing cost effective strategies to produce up to 200 L cultures of *D. vulgaris* per week, establishing an optimized four-step tagless fractionation series for the purification of water soluble protein complexes, adopting an efficient PVDF membrane micro titer plate-based methods for mass spectrometry sample preparation, determining the structure of the 1 MDa *D. vulgaris* Pyruvate Ferredoxin Oxidoreductase complex to 17Å resolution by single particle EM, and constructing relational databases for biomass production, genetically manipulated *D. vulgaris* strains, single particle EM, and tagless complex purification. Further details on these and other results are provided in Subproject specific posters.