ENIGMA Biotechnology: Membrane Protein Complexes – Their Roles in *Desulfovibrio vulgaris* Stress Response, and in the Establishment and Maintenance of Communities

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Project Goals: Key aims of this project are – to develop a system for the high-throughput isolation and identification of membrane protein complexes, optimizing this process for effectiveness across a range of sample types including planktonic cultures and biofilms; to apply this system in the study of DOE relevant microbes such as *Desulfovibrio vulgaris* in order to detect and characterize changes in their membrane protein complexes brought about by environmental stressors, and through the role of these proteins in the establishment and maintenance of communities.

A central goal of the ENIGMA consortium is to develop robust molecular-level models capable of predicting how target microbes respond to a range of environmental conditions. In support of this goal, our interests within ENIGMA have centered on the dynamic role of membrane protein complexes in this process. Cell membranes represent the “front-line” of cellular defense and the interface between a cell and its environment. Significant changes in response to environmental conditions are expected to take place through the proteins situated within these membranes. Membrane protein-associated changes may occur in the form of abundance level, protein-protein interactions, post-translational modifications and even mutations. To understand some of the earliest and perhaps most critical responses to stress, characterization of these changes on a molecular level is needed.

The study of membrane proteins presents a major challenge in protein biochemistry; to address this we have developed a unique high-throughput process for the isolation and identification of untagged membrane protein complexes that features mild, but effective, detergent solubilization, liquid chromatography and native electrophoresis methods. We have been applying this system in two main areas of investigation, one of which has been our work on developing a *D. vulgaris* membrane protein complex database covering standard and stressed growth conditions, and the second to characterize the roles of selected membrane proteins in the establishment and maintenance of communities.

Our study of membrane protein complexes in the outer-membrane of *D. vulgaris* grown under standard conditions is complete and we are at an advanced stage with the inner-membrane component. An interactome of proteins identified in *D. vulgaris* outer-membrane preparations is in the final stages of refinement. These preparations have yielded 69 outer-membrane protein identifications (which is over 80% of the number expected); 90% of these proteins were found to be in complexes. The most prevalent categories of proteins detected were the lipoproteins, and proteins with non-specific annotations (hypothetical and conserved hypothetical). This compendium of *D. vulgaris* outer-membrane protein complexes will serve as an essential reference for the detection and characterization of environment-driven changes in these proteins. Processing of outer-membrane proteins from stressed *D. vulgaris* cultures (including growth to stationary phase, and growth under

elevated levels of nitrate or NaCl) has recently been completed. Initial analysis of stress-associated changes in outer-membrane protein abundance suggests that for many proteins there are significant differences between these changes and the changes in expression level inferred from mRNA experiments. Efforts on the preparation of the inner-membrane protein interactome and completion of the analysis of stress-induced changes occurring in the outer-membrane proteins of *D. vulgaris* are on-going.

In addition to our studies on large-volume planktonic monocultures, we have refined the methods employed in the pipeline so that they may be used to process samples derived from more native-like sources. Recent improvements made in pipeline sensitivity and resolution are now allowing us to work successfully with relatively small cell samples such as biofilms. We anticipate that through additional optimization of the system, we will be able to process yet smaller samples, not only cultured in the lab but obtained directly from field sites. To assess the potential for discovery from such sources, we have begun pilot studies on biofilm samples. Early results contain evidence of protein changes occurring during the transition from stationary phase to biofilms, suggesting that this will be a productive direction for future studies of microbial communities. Recent work by the Stahl group on adaptive evolution experiments with co-cultures of *Methanococcus maripaludis* and *D. vulgaris,* has identified mutations of soluble and membrane protein genes likely to be important in establishing the syntrophic mutualism between these species. These results suggest a large influence of acquired membrane protein mutations leading to improved growth rates within this co-culture community. We have begun to process membranes from clonal isolates to characterize changes in the *D. vulgaris* membrane protein population. Interestingly, the most abundant protein of the *D. vulgaris* outer-membrane (DVU_0799) is also the most consistently mutated protein in these experiments. Therefore, a key goal of ours will be to purify and functionally characterize those mutated membrane proteins found to play a role in facilitating improved rates of growth.

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