Isolation and Identification of Membrane Protein Complexes in *Desulfovibrio vulgaris* Hildenborough

Peter J. Walian[1*], Ming Dong[1], Susan Fisher[2], Jil T. Geller[1], Steven Hall[2], Terry C. Hazen[1], Dominique C. Joyner[1], Mary E. Singer[1], H. Ewa Witkowska[2], Mark D. Biggin[1], Bing K. Jap[1]

[1]Lawrence Berkeley National Laboratory, Berkeley CA; [2]University of California, San Francisco CA
*Presenting Author.

An important aim of the Genomics GTL Protein Complex Analysis Project (PCAP) is the isolation and identification of membrane protein complexes from *D. vulgaris*. The cataloging of complex subunit constituents from this organism, grown under normal and stressed conditions, will support the long-range goal of modeling stress responses in *D. vulgaris* relevant to the detoxification of metal and radionuclide contaminated sites. Isolation of endogenous *D. vulgaris* membrane protein complexes in quantities sufficient for chromatographic analysis requires substantial amounts of cell membranes. Twenty liter cultures of *D. vulgaris* typically yielded about 50 milligrams of total membrane proteins. To extract inner and outer membrane protein complexes stably and with maximal yield, we have used a multi-step procedure in which the bacterial membrane is sequentially processed. Membranes were initially treated with a mild detergent to extract proteins primarily from the inner membrane. The residual membrane pellet, enriched with proteins of the outer membrane, was solubilized using a more aggressive detergent. Chromatographic procedures found effective in purifying complexes involved the use of ion exchange, hydroxyapatite and molecular sieve media. SDS-PAGE of the various chromatographic fractions was used to tentatively identify subunits of complexes based on a display of co-elution. Molecular sieve fractions were subjected to native PAGE to further isolate potential complexes. Proteins of the native gel bands, in turn, were extracted and run on SDS-PAGE. These gels revealed whether a putative complex was indeed composed of lower molecular weight subunits, and provided samples well suited for in-gel processing and mass spectrometry analysis. In this manner, a number of homo- and heteromeric complexes, ranging in weight from about 70 to 400 kDa, have been identified in this first year. Use of a free-flow electrophoresis device being developed in our group in combination with larger-scale processing of cell membranes is expected to significantly improve the isolation and identification of complexes over the next project year.