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High-throughput Identification of Multi-protein Complexes via TAP tagging in
Desulfovibrio vulgaris

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The sulfate-reducing bacterium *Desulfovibrio vulgaris* has repeatedly been shown to have the potential for *in-situ* metal and radionuclide immobilization. As such, several efforts have focused on the physiology and metabolism this bacterium and the response to stress. The primary goals of this project are to understand the nature of the proteins involved, their function as complexes, and any alterations in complex composition in response to environmental stresses. We are utilizing affinity protein tagging and are comparing the efficiency of three tags; a single and two tandem tags. The tags are “*Strep-tag*” (Qiagen) that inserts a streptavidin binding peptide; CTF (a.k.a. SPA), a calmodulin binding protein (CBP), a protease (tobacco etch virus) cleavage site, and a 3 x FLAG peptide; and a new “STF” tag that replaces CBP with the streptavidin binding peptide. To test these tags, three proteins (PorB, AtpC, and DsrC) were tagged with all three. Results thus far indicate that the STF tag will be utilized. At issue with DsrC constructs is the C-terminal tag location since the penultimate C-terminal cysteine is involved in protein activity. Mass spectrometry analysis of all three affinity purified DsrC proteins showed no association with other Dsr proteins. This may be valid since all other *dsr* genes are located in an operon whereas *dsrC* is monocistronic and interaction for electron delivery may be transient. Proteins are also being tagged with tetracysteine and SNAP (Covalys) for imaging the *in-situ* location and relative protein density within the cell and as extracellular appendages at different growth states and stresses. We are also constructing a combined STF-SNAP tag, that will allow for the transition to high-throughput. Finally, we are assessing the utility of an ordered plasmid library of *D. vulgaris* DNA to allow for construction of tagged genes in *E. coli* in a high-throughput approach. The information from complex identification is expected to lead to a greater understanding of the proteins involved in metal-reduction and their protein-protein interactions, with characterization of the complete pathway(s) for these activities.