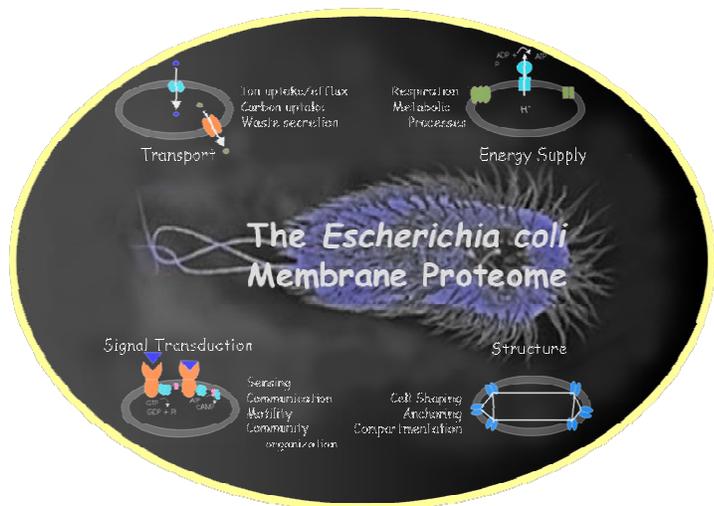


## The Membrane Proteome of *E. coli* - Cloning and Heterologous Expression in Rhodobacter Membranes

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We have targeted 444 unique membrane protein families, representing the complete membrane proteome of *E. coli*, for cloning and functional expression in the inducible intracytoplasmic membranes of *Rhodobacter sphaeroides*. These targets encompass a wide range of molecular weights, transmembrane spans, and pls. This set of genes has been cloned into a broad-host-range vector where expression is directed by the oxygen-sensitive *puf* promoter. Expression of the foreign gene and proliferation of the host membranes are autoinduced coordinately when the oxygen tension decreases as a function of increasing cell density. Analysis of the first half of the expression strains has shown that ~ 60% of the *E. coli* membrane proteins are expressed in *Rhodobacter* at levels that exceed 1 mg/L. Many can be expressed and purified at levels of 10-20 mg/L of culture.

Subcellular fractionation reveals that these *E. coli* target proteins are localized within *Rhodobacter* membranes. Ligation-independent cloning and semi-automated purification protocols have been implemented for the last half of this project, enabling a higher-throughput approach to the heterologous expression and analysis of the representative *E. coli* membrane proteome.



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