2. Functional Attributes of ABC Transporter Complexes from *P. fluorescens* That Mediate the Cellular Response to Changes in Environmental Nutrients

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Project Goals: The Argonne Environment Sensing and Response (ESR-SFA) program addresses the hypothesis that cellular behavior can be modeled through an understanding of the biological interface with the environment and the cellular responses that originate from the cell/environment interaction. The overall objective is to identify the molecular basis of cellular transport and sensory pathways that mediate the response to environmental nutrients. This study provides an overview of molecular approaches used to assign functional attributes to sparsely annotated transporter and sensor proteins with sufficient detail to predict the cellular response to specific environmental ligands. Experimental characterization of microbial transport and sensor proteins will facilitate the development of system-level predictive models for cellular response to nutrient availability. The ultimate goal of this work is to enable synthetic biology applications that will allow the manipulation of a system's response in a predictable way in order to maximize bioenergy production while minimizing negative environmental impact.

The Functional Characterization subgroup of the ESR-SFA applies a suite of biochemical and biophysical methods for protein production and functional annotation of transport machinery and regulatory networks. The experiments focused initially on characterization of ABC import complexes for sulfur compounds in four Plant Growth Promoting Bacterial (PGPB) strains of *Pseudomonas*. This pilot project focuses on methionine as the availability of this compound impacts cell metabolism via three major routes: as a sulfur source, its use in protein translation initiation and synthesis, and synthesis of the methyl group donor S-adenosylmethionine. Multiple ABC transporter complexes implicated in the import of methionine are encoded in the genomes of the four target strains: PF-5, Pfl01, SBW25, and WH6 strains.

Primary objectives of this subgroup are the profiling the ligand binding capabilities, mapping the interaction domains/peptides of the component proteins, and characterization of structure function relationships using biophysical methods. To achieve these objectives we have devised soluble and membrane protein expression strategies for the solute binding protein (SBP), permease, and ATPase component proteins of the ABC transporter complex. The majority of these components, comprising ~40 individual genes, have been successfully expressed and purified either individually or as complexes and functional characterization studies are ongoing. Profiling of the ligand binding specificity of the individual SBPs demonstrate these organisms can import a variety of sulfur-containing compounds with some organisms having multiple bacterial transporters with predicted specificity for the same ligand. This set of methionine importers also contains ATPase components with regulatory domains identified using the Pfam database and many of these ATPase proteins have been expressed in soluble form. An important question we will undertake is the examination the ligand binding specificity of these ATPase regulatory domains as it is not known if the ligand-binding capacity changes with specificity identical to that of the solute-binding protein.

The set of sulfur compound transporters from *P. fluorescens* PGPB strains was additionally used as a model ABC transport system for the production of intact, transmembrane transporter assemblies for functional characterization experiments. Here, success requires co-expression of permease and ATPase components. Multiple approaches were successful in producing purifiable quantities of the membrane-localized complex that contained affinity tag fusions for use in chromatography, cellular localization, and quantitation. Conditions that fostered efficient purification of permease-ATPase complexes utilizing affinity-tag fusions were discovered and optimized using the automated Maxwell purification system (Promega). Standardized protocols have been developed to produce samples of intact transporters with > 90% purity from whole cells in less than 4 hours. Expression yields approach 0.5 mg/L of cell culture and have been shown to be scalable. The most unexpected finding discovered in the course of methods development for transmembrane systems is a potential for cross-talk between components derived from different operons within the same organism. We used co-purification to demonstrate the feasibility for generation of hybrid methionine transporter assemblies from multiple ATPase components and an affinity-tagged permease. Because they are regulated differently, it is unclear if these heterologously- expressed *in vivo* findings from altered regulatory scenarios have any relevance to structures that are formed and operating in *P. fluorescens* cells, but we intend to examine fully the extent of these promiscuous interactions and to characterize how prominent their presence and utility is in Nature.

As many of the proteins involved are membrane-bound, or have membrane-bound components in multi-subunit complexes we evaluated the utility of SAXS/WAXS which can be performed on membrane proteins in their native lipidic environment, such as micelles, bilayers, and nanolipoprotein particles (nanodiscs). This model independent low resolution structural analysis by SAXS demonstrates the first successful step for determining membrane protein structures in nanodiscs. Current studies are focused on development of the data analysis methodology. Most *ab initio* SAXS analysis algorithms are based on proteins in buffer solution and do not consider the different electron density distribution in the phospholipid bilayer. This presents a major challenge in that existing programs cannot be extended to model nanodiscs. The problem is further complicated by the presence of embedded guest proteins in the nanodisc. As a consequence, we are developing custom software for modeling and fitting routines.

Specifically, we are utilizing a course-grained modeling approach to model the different scattering bodies within the nanodiscs, i.e. proteins, lipid head groups, and lipid tails which all have different electron densities and scatter differently. To do so, we plan to assemble course-grained scattering bodies as two protein belts wrapped around a cylindrical phospholipid bilayer. Next, a number of lipid bilayers are removed from the center of the nanodiscs and the void is filled with protein electron density scattering bodies. Using molecular constrained ab initio shape determination methods, the scattering curves are calculated and the model is refined iteratively to fit the experimental data. The goal of this work is to calculate the low resolution structures of membrane proteins incorporated into nanodiscs, and to determine conformational changes upon binding ligands or other soluble proteins.

The submitted manuscript has been created by UChicago Argonne, LLC, Operator of Argonne National Laboratory ("Argonne"). Argonne, a U.S. Department of Energy Office of Science laboratory, is operated under Contract No. DE-AC02-06CH11357.Brookhaven National Laboratory is operated under Contract No. DE-AC02-98CH10886.This contribution originates from the "Environment Sensing and Response" Scientific Focus Area (SFA) program at Argonne National Laboratory. This research was supported by the U.S. Department of Energy (DOE), Office of Biological and Environmental Research (BER), as part of BER's Genomic Science Program (GSP).