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Solute binding proteins and their cognate ligands: structure, function and their role in functional annotation

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Abstract

The uptake of exogenous solutes is mediated by transport systems embedded in the plasma membrane and drive active transport even at μM to nM solute concentrations. In many of these systems a periplasmic Solute-Binding Protein (SBP) is utilized to bind their cognate ligands with high affinity and deliver them to the membrane bound translocator subunits. Active transport systems with SBP components are traditionally divided into three main families based on their energetic coupling mechanism, primary sequence and subunit composition: tripartite ATP-independent periplasmic transporters (TRAP), ATP binding cassette transporters (ABC) and tripartite tricarboxylate transporters (TTT). Knowledge of the cognate ligand for the SBP component of the transporter can provide crucial data for functional assignment of co-located or co-regulated genes.In the present study, the structural and functional characterizations of several solute binding proteins have been carried out. Proteins were cloned from genomic DNA, expressed by autoinduction and purified by a combination of Ni-NTA and size exclusion chromatography. The purified SBPs were screened using differential scanning fluorometry (DSF) and a >400 compounds ligand library. Two of the SBPs exhibited DSF hits that were novel for their respective transport family. Crystallization trials of proteins have been conducted with their respective DSF ligand hits. Those SBPs that have structures determined and their respective interactions with co-crystallized ligands will be presented. Cocrystallization with DSF determined ligands resulted in structures of Avi_5305 in complex with D-glucosamine and D-galactosamine, the first structure of an ABC SBP with an amino sugar.

The rate at which genome sequencing data is accruing demands enhanced methods for functional annotation and metabolism discovery. Solute binding proteins (SBPs) facilitate the transport of the first reactant in a metabolic pathway, thereby constraining the regions of chemical space and the chemistries that must be considered for pathway reconstruction. We describe high-throughput protein production and differential scanning fluorimetry platforms, which enabled the screening of 158 SBPs against a 189 component library specifically tailored for this class of proteins. Like all screening efforts, this approach is limited by the practical constraints imposed by construction of the library, i.e., we can study only those metabolites that are known to exist and which can be made in sufficient quantities for experimentation. To move beyond these inherent limitations, we illustrate the promise of crystallographic- and mass spectrometric-based approaches for the unbiased use of entire metabolomes as screening libraries. Together, our approaches identified 40 new SBP ligands, generated experiment-based annotations for 2084 SBPs in 71 isofunctional clusters, and defined numerous metabolic pathways, including novel catabolic pathways for the utilization of ethanolamine as sole nitrogen source and the use of d-Ala-d-Ala as sole carbon source. These efforts begin to define an integrated strategy for realizing the full value of amassing genome sequence data.

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