Identification of binders to the vSGLT transporter for structural studies

Jay Prakash Kumar\textsuperscript{1}, Vinod Nayak\textsuperscript{2}, Balaji Rao\textsuperscript{3}, S. Ramaswamy\textsuperscript{2}, Jeff Abramson\textsuperscript{4}

\textsuperscript{1}Institute For Stem Cell Biology And Regenerative Medicine, Bangalore, India, \textsuperscript{2}Institute For Stem Cell Biology And Regenerative Medicine, Bengaluru, India, \textsuperscript{3}Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, United States, \textsuperscript{4}Department of Physiology, UCLA, Los Angeles, United States

E-mail: kumarjp@instem.res.in

Na+/galactose transporters (SGLTs) are integral membrane proteins, which co-transport Na+ with sugars from the periplasmic space into the cytoplasm. According to the alternating access model for secondary active transporters, these proteins alternate between outward and inward-facing conformations during the transport cycle. The currently available structures from a bacterial homolog of SGLT from Vibrio parahaemolyticus, (vSGLT) are in the substrate-bound inward-occluded and the substrate-free inward-open conformations. Despite many efforts, structures of the outward conformations remain elusive.

Isolation of distinct conformations of a transporter is a major obstacle for X-ray crystallography due to their conformational heterogeneity. Crystallization chaperones based on various protein scaffolds have emerged as a promising tool to increase the crystallization probability of a selected target protein. Sso7d is a highly stable binding protein derived from the hyperthermophilic archaeon Sulfolobus solfataricus. It has a versatile scaffold for generating binding protein for a wide spectrum of targets. Sso7d-derived proteins are far easier to produce in bacteria and due to their small size, they are capable of targeting areas that are not accessible to standards antibodies.

The main goal of this project is the identification of binders to Na+/Galactose transporter from Sso7d yeast surface display library for structural studies; and the determination of the structure of the vSGLT/binder complex. As mentioned above, we aim to identify binders that would bind the vSGLT transporter in a specific conformation.

To achieve these goals, we screened the Sso7d yeast surface display library for clones that encoded possible binder. We identified a binder that bound to vSGLT and we were able to show binding using purified proteins. To obtain a higher affinity binder, the initial clone was mutagenized using error-prone PCR to obtain high-affinity binders. We are currently attempting to crystallize the complexes of the various high-affinity binders with vSGLT.


Keywords: vSGLT, Sso7d, Yeast surface display