Exploiting Genetic Variation for Insights into Structure and Biology of Membrane Proteins

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Genetic variation within families of proteins having common functional properties has been exploited to expedite structural biology since Kendrew identified sperm whale myoglobin as being especially propitious for crystallographic analysis. The subsequent expansion of sequence information and recombinant DNA technology empowered a related structural genomics approach to protein structure, and the New York Consortium on Membrane Protein Structure (NYCOMPS) has applied this approach successfully to membrane proteins. We target families defined in a given genome, currently the membrane proteins of *Homo sapiens*, and seek homologs in any available genome. Structural results from a homolog are used to generate structure-inspired hypotheses that may be tested in experiments both on the structural prototype and on the genuine target.

Here, we exemplify the NYCOMPS approach with three recent examples: (1) Bax inhibitor-1 (BI-1) is an anti-apoptotic protein that mediates a calcium leak and is representative of a ubiquitous family of transmembrane-Bax-inhibitor-motif (TMBIM) proteins. We solved crystal structures of a *Bacillus* TMBIM protein, and we characterized the pH dependence of calcium uptake into cells and proteoliposomes (Chang *et al.*, *Science* **344**, 1131, 2014). (2) TSPO proteins have been implicated in steroid and porphyrin transport; thus they are also called translocator proteins. Our biochemical studies, inspired by the structure of a bacterial homolog, showed that TSPOs have a previously uncharacterized activity to degrade protoporphyrin IX into bilindigen, which we implicate in the control of reactive oxygen species (ROS). We find this same activity in eukaryotic TSPOs, including polymorphic variants (Guo *et al.*, *Science* **347**, 551, 2015). (3) Human bestrophin-1 (Best1) is a calcium-activated chloride channel from the retinal pigment epithelium, where mutations are associated with macular degeneration disease. We solved the crystal structure is of a *Klebsiella* homolog, and we performed mutational analyses of channel activity in this bacterial bestrophin and in human Best1 (Yang *et al.*, *Science* **346**, 355, 2014). Future work will study human Best1 disease mutations.