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Structural Studies of *E. coli* Transhydrogenase A Multi-technique Approach for Membrane Proteins. Holly Heaslet, Mutso Yamaguchi, Vidyasankar Sundaresan, Mark Yeager, C. David Stout, Dept. of Molecular Biology, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037 USA.

Although there has been impressive progress in high-resolution structure analysis of membrane proteins, the generation of 3D crystals is still very difficult. Cryo-EM and image analysis is an alternative approach for obtaining structural information. While obtaining 2D crystals can also be a time consuming and difficult process, there is the important advantage of being able to observe functionally important states of the membrane protein in a lipid bilayer environment. There is also the option of examining detergent-protein complexes as single particles. *E. coli* transhydrogenase (TH) is an integral membrane proton pump that catalyzes the formation of NADPH to by hydride transfer between two large soluble domains. The high-resolution crystal structures of the two extra-membranous, nucleotide-binding domains, domains I and III, and the domainI/domainIII complex have been previously determined. The goal of this project is to determine the three-dimensional structure of intact *E. coli* transhydrogenase (TH). The structure of intact TH would give valuable insight into the proton translocation mechanism, as well as the mode of coupling of proton pumping to hydride transfer. We are using a combination of novel crystallization methods and electron microscopy techniques to obtain structural information about full-length TH.