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Experiments Toward Crystallization of Transhydrogenase. M. Yamaguchi¹, H.A. Heaslet¹, M. Yeager², C.D. Stout¹; ¹Depts. of Molecular Biology¹, and Cell Biology², The Scripps Research Institute, La Jolla, CA.

Transhydrogenase (TH) is essential enzyme in mitochondria that couples hydride transfer between NAD(H) and NADP(H), bound to extramembranous domains, to proton translocation through a membrane-intercalated domain. It is the only respiratory membrane component lacking structural data for an intact complex. The mechanism for transducing binding energy into conformational change and proton translocation is unknown, but TH resembles ATP synthase in that the proton gradient is utilized for chemical bond formation in the absence of net redox. The *E. coli* enzyme is a 200 kD homodimer of gene products, the *alpha* subunit containing the NAD(H) binding domain and four trans-membrane helices, and the *beta* subunit, containing the NADP(H) binding domain and nine TM helices. Together, the 13 TM helices comprise the proton channel of each monomer. The approach to crystallization of TH is combining expression methods, mutagenesis, His-tag design, and detergent solubilization, with biochemical methods, activity assays, and electron microscopy. The results are integrated to assess TH monodispersity and conformational homogeneity for 2D and 3D crystallization screening. Observations and progress with these experiments will be reported.