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Cytochrome *ba*₃ Oxidase in Nanoscale Lipid Bilayers: A Roadmap to Crystallization. V. Mitch Luna, James A. Fee, C. David Stout, Dept. of Molecular Biology, The Scripps Research Institute, La Jolla, CA, 92037.

Cytochrome *ba*₃ oxidase is a respiratory enzyme that catalyzes the reduction of molecular oxygen to water in aerobic organisms. Here we present a novel assembly procedure of *Thermus thermophilus* cytochrome *ba*₃ oxidase in nanoscale lipid bilayers (nanodiscs) as a model for integral membrane protein incorporation in nanodiscs. This procedure increases homogeneity for the purpose of high-resolution structure determination through crystallization and avoids the use of detergents. Utilizing the His-tag engineered at the N-terminus of subunit I, we are able to bind cytochrome *ba*₃ oxidase to Nickel resin and conduct the nanodisc assembly on the resin, which allows us to separate empty nanodiscs, and nanodiscs that incorporated the cytochrome *ba*₃ oxidase. Through this procedure, we are able to obtain cytochrome *ba*₃ oxidase in a native, detergent free, lipid bilayer environment. Here the enzyme is fully active as a cytochrome c oxidase and retains its unique spectroscopic properties. Cytochrome *ba*₃ oxidase in nanodiscs offers a unique avenue for structure determination and presents a membrane protein in a more native-like lipid environment.