

# **The Structures of Fumarate Reductase and Succinate Dehydrogenase from *E. coli* : Crystallization of Two Complex II and their Methodology Implications.**

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In the past few years high-resolution structures for several of the membrane-bound electron transport chain respiratory complexes, including the structure of *E. coli* Complex II analogous to the mitochondrial respiratory Complex II, have become available. Nevertheless, the number of membrane proteins that have been crystallized in forms that diffract to high resolution remains relatively small in comparison to the number of soluble proteins. Membrane proteins, typically multi-subunit complexes, are not only tricky to handle biochemically, they are also notoriously difficult to overexpress, purify and seldom yield high quality 2D or 3D crystals. Several unique characteristics of membrane proteins, however, often make it difficult to apply the methods available for the purification utilized to isolate water-soluble, non-membrane associated proteins. The structure of Fumarate Reductase from *E. coli* we reported in June of 1999 was exciting (1,2,3). It became the first available for a family of membrane-bound enzymes with important function in central carbon metabolism and energy production in cells. They catalyze succinate-fumarate interconversion coupled to the reduction/oxidation of quinone (Q). Recently, we reported the structure of Succinate Dehydrogenase a member of this family, which is both a citric acid cycle enzyme and respiratory complex II of the mitochondrial respiratory chain (4,5). In this section the focus will be review of various parameters for reproducible preparations of highly pure complexes including conditions of a new purification strategy, which played a major role. The isolated and crystallized enzymes show the same sub-unit stoichiometry and contain all types of redox cofactors known to be present in these two *E. coli* enzymes. The optimized procedures may be applicable to the isolation and crystallization of other membrane proteins.

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  3. Cesar Luna-Chavez, Tina Iverson, Douglas Rees, Gary C. Cecchini. *Overexpression, purification and crystallization of the membrane bound Fumarate Reductase from E. coli*. Protein Expression and Purification (2000). 19:188-196
  4. Collaborative work between Molecular Biology, Department of Veterans Affairs Medical Center. Department of Biological Sciences, Imperial College London. Department of Biochemistry, Uppsala, University. Department of Biophysics, UCSF. Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University
  5. Victoria Yankovskaya, Rob Horsefield, Susanna Tornroth, Cesar Luna-Chavez, Hideto Miyoshi, Christophe Leger, Bernadette Byrne, Gary C. Cecchini and So Iwata. *Architecture of Succinate Dehydrogenase and Reactive Oxygen Species Generation*. Science (2003). 299:700-704