**s8a.m4.o1** **3D Crystallization and Structural Studies of Integral Membrane Proteins in Lipidic Cubic phases.** P. Nollert[1], M.L. Chiu[2], M.C. Loewen[3], A. Royant[4], H. Belrhali[4], K. Edman[5], J. Hajdu[5], R. Neutze[5], E. Pebay-Peyroula[6], E.M. Landau[7]. *[1]Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143-0448, USA [2]Department of Chemistry, Seton Hall University, 400 South Orange Avenue, South Orange, NJ 07079, USA [3]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02138, USA [4]ESRF, rue Jules Horowitz, B.P.220 F-38043 Grenoble Cedex, France [5]Department of Biochemistry, Uppsala University, Biomedical Center, Box 576, S-751 23, Uppsala, Sweden [6]Institut de Biologie Structurale, CEA-CNRS and Université Joseph Fourier, 41 rue Jules Horowitz, F-38027 Grenoble Cedex 1, France [7]Department of Physiology and Biophysics and The Membrane Protein Laboratory, The University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555-0641, USA.*

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A comprehensive understanding of the mechanisms of action of membrane proteins requires the elucidation of their structures to high resolution. To date, less than twenty high resolution structures of membrane proteins have been solved (http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html), reflecting the major obstacle in this endeavor - the routine production of well-ordered 3D crystals. We have developed a novel concept for the crystallization of membrane proteins using highly viscous, optically transparent and by non-birefringent lipidic cubic phases[1,2], which are materials composed of very high amounts of hydrated lipids.

The light-induced proton pump bacteriorhodopsin (bR) is an integral membrane protein found in the plasma membrane of *Halobacterium salinarum*. Hexagonal micro crystals of bR grown in a monoolein mesophase diffracted isotropically to better than 1.9 Å resolution. The crystal structure was solved to 2.5 Å resolution[3]. We have recently solved the structure of bR to 1.9 Å resolution, revealing the protein embedded within lipids and including water molecules, thus elucidating the structure of the purple membrane[4]. In parallel, we have demonstrated that bR molecules packed in the 3D crystals undergo a light-induced photocycle that is indistinguishable from that of bR in the native purple membrane[5]. This finding was followed by the determination of the high-resolution structure of the K intermediate of bR's photocycle, illustrating the early rearrangements that occur upon photoexcitation[6].

We showed previously that soluble proteins could be readily crystallized from the complementary aqueous compartments of cubic phases[7]. Recently, we succeeded in crystallizing four additional integral membrane proteins from one single mesophase system based on monoolein, thus demonstrating the general applicability of this approach[8]. Together with the application of a rapid enzymatic hydrolysis of the lipids comprising the cubic phase and release of the crystals immobilized therein[9], we now have a general methodology for generating well-ordered 3D crystals of membrane proteins and their investigation by a variety of biophysical and biochemical methods.

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**s8a.m4.o2** **Crystal structure at 2.2 Å resolution of fumarate reductase, a respiratory membrane protein complex from *Wolinella succinogenes*.** C.R.D. Lancaster, *Max Planck Institute of Biophysics, Department of Molecular Membrane Biology, Heinrich-Hoffmann-Str. 7, D-60528 Frankfurt am Main, Germany*, *lancaster@mpibp-frankfurt.mpg.de;http://www.biophys.mpg.de/~lancaster*

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Succinate dehydrogenases (succinate:quinone reductases, SQR) and fumarate reductases (quinol:fumarate reductases, QFR) catalyse the oxidation of succinate to fumarate as well as the reverse reaction. SQR (respiratory complex II) is involved in aerobic metabolism as part of the citric acid cycle and of the aerobic respiratory chain. QFR is involved in a form of anaerobic respiration with fumarate as the terminal electron acceptor, and is part of the electron transport chain catalysing the oxidation of various donor substrates (e.g. NADH, $H_2$ or formate) by fumarate. These reactions are coupled via an electrochemical proton gradient to ADP phosphorylation with inorganic phosphate by ATP synthase. QFR and SQR complexes are collectively referred to as succinate:quinone oxidoreductases (EC 1.3.5.1) and are predicted to share similar structures. The complexes consist of two hydrophilic and one or two hydrophobic, membrane-integrated subunits. The larger hydrophilic subunit A carries covalently bound flavin adenine dinucleotide (FAD) and subunit B contains three iron-sulphur centres. QFR of *Wolinella succinogenes* and SQR of *Bacillus subtilis* contain only one hydrophobic subunit (C) with two haem *b* groups. In contrast, SQR and QFR of *Escherichia coli* contain two hydrophobic subunits (C and D) which bind either one (SQR) or no haem *b* group (QFR).

We have determined at 2.2 Å resolution the structure of the two haem groups containing *W. succinogenes* QFR by X-ray crystallography[1]. Based on the structure of the three protein subunits and the arrangement of the six prosthetic groups, we propose a pathway of electron transfer from the quinol-oxidising dihaem cytochrome *b* to the site of fumarate reduction and a mechanism of fumarate reduction. Our structure is different from that of the haem-less QFR of *E. coli*, described at 3.3 Å resolution[2], mainly with respect to the structure of the membrane-embedded subunits and the relative orientations of soluble and membrane-embedded subunits.

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