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Membrane Protein Crystallization in Bicontinuous Lipid Systems. Pia Wadsten, Annemarie Wöhri, Arjan Snijder, Richard Neutze, Sven Engström, Dept. of Chemical and Biological Engineering, Chalmers Univ. of Technology, Gothenburg 412 96, Sweden.

In 1996, a new concept for membrane protein crystallization in the cubic phase was introduced. The method has been successful for proteins with small hydrophilic domains where a phase change, from a curved bilayer to a flat bilayer plays an important role in the crystallization process. The cubic phase is stiff and time consuming to handle. The phase also puts restrictions on the hydrophilic parts of the protein due to limited aqueous domain size. The method however yielded crystals from two photosynthetic reaction centers (RC) with larger hydrophilic domains as a result from the transformation cubic phase-to-liquid phase. Simulating the crystallization conditions in a small angle X-ray setup we conclude that the cubic phase in the RC case forms a sponge phase, which can be visualized as a 'melted' cubic phase. The presence of this phase should play the same role as the lamellar phase for smaller proteins, but with larger aqueous pores for the accommodation of RCs hydrophilic domain. Here we introduce a new method using the sponge phase directly in a hanging drop setup. Crystals of RC from *Rhodobacter sphaeroides*, diffracting to 2.1 Å, were obtained and the practical advantages of the sponge phase make it a potent tool for protein crystallization.