Membrane Protein Crystallization Using Cubic Lipid Phases, Bicelles and Vapor Diffusion

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The Cubic Lipid Phase (CLP) method for membrane protein crystallization has been refined to allow large-scale screening of membrane proteins. Various parameters (CLP lipid, water content, bilayer lipid additive, pH, ionic strength, precipitating agent etc.) can be varied. Several distinct seven-transmembrane proteins were crystallized and their high-resolution structures determined. In cases where the CLP method fails, the bicelle method or detergent-based methods were employed to crystallize other membrane proteins.

Bacteriorhodopsin (BR): High-resolution maps from X-ray diffraction of bacteriorhodopsin crystal obtained in CLP and some of its photointermediates have yielded insights to how the isomerization of the bound retinal drives ion transport. Although some important mechanistic details are still undecided, the events of the photochemical cycle are now understood to reflect changes in specific hydrogen bonds of protein groups and bound water molecules in response to motions of the retinal chain. A nearly complete lipid bilayer is also part of the x-ray-derived atomic model. Surprisingly, we were unable to use the CLP method to obtain crystals of the A215T mutant of BR, only the bicelle method provided results.

Anabaena SR (ASR): The structure of a sensory rhodopsin from the cyanobacterium Anabaena has been determined to 1.9 Å resolution using the CLP method. This represents the first eubacterial rhodopsin structure. In comparison to the archael rhodopsins BR and SR there are many striking rearrangements and shifts in hydrogen bonding patterns on both the extracellular and the cytoplasmic half of the receptor. Also, the cytoplasmic face, which is thought to interact with its soluble transducer (ASRT), is structurally well defined and very different from that of the archael rhodopsins. More recently, we determined the structure of a single-site mutant (D217E) that pumps proton in the opposite direction in a different spacegroup using the CLP approach. The structure of ASRT has also been determined: it forms a C₄ tetramer with a new all-beta fold.

Xanthorhodopsin (XR): a light-driven ion pump from the halophilic eubacterium Salinibacter ruber found in saltern crystallizer ponds of Spain, contains a blue-absorbing carotenoid that functions as a light-harvesting antenna for its retinal chromophore. This protein only crystallized from bicelles. In addition to the adaptations to bind and accurately position the carotenoid antenna for efficient excited-state energy transfer to the retinal, XR exhibits major structural differences to the previously studied microbial pumps and photoreceptors. We also determined the ring structures of a pentameric (C₅) and a hexameric (C₆) proteorhodopsin, from bicelles and CLP, respectively.

Lastly, I will present the results of structural & functional studies on a system responsible for acid tolerance in certain pathogenic bacteria. The system is able to maintain a periplasmic pH of ~6 even when the medium has a pH of 2. The buffering system involves the enzyme urease that readily hydrolyzes urea into NH₃ and CO₂, which in turn act as a proton sink to reduce [H⁺] by four orders of magnitude. This membrane protein crystallizes as a C₆ hexameric using vapor diffusion after many rounds of optimization, including a detergent mix and E. coli polar lipids.