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Cubic Lipid Phase & Bicelle Crystallization of Membrane Proteins Hartmut Luecke, *Center for Biomembrane System, UC Irvine, CA, USA.*

The cubic lipid phase (CLP) & bicelle methods for membrane protein crystallization have been refined to allow large-scale screening of various membrane proteins. The various parameters (CLP lipid, water content, bilayer lipid additive, pH, ionic strength, precipitating agent etc.) can be varied. Numerous distinct seven-transmembrane proteins were crystallized and their high-resolution structures determined.

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 present in the x-ray-derived atomic model.

Sensory Rhodopsin (SR): Atomic resolution structures of a phototaxis receptor in haloarchaea, the first sensory member of the widespread microbial rhodopsin family, have yielded insights into spectral tuning and the interaction face with its membrane-embedded transducer. Spectral differences between the sensory rhodopsin and light-driven proton pump bacteriorhodopsin depend largely on the repositioning of a conserved arginine residue in the chromophore-binding pocket. Information from the structures combined with biophysical and biochemical analysis have established a model for receptor activation and signal relay involving light-induced helix tilting in the receptor transmitted to the transducer by lateral transmembrane helix-helix interactions.

Anabaena SR (ASR): The structure of a sensory rhodopsin from the cyanobacterium *Anabaena* has been determined to 1.9 Å resolution. This represents the first eubacterial rhodopsin structure. In comparison to the archaeal 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 the soluble transducer, is structurally well defined and very different from that of the archaeal rhodopsins. The structure of the soluble transducer of this photoreceptor (ASRT) has also been determined - it forms a C4 tetramer with a new all-beta fold. Studies characterizing the interaction between transmembrane photoreceptor and soluble transducer are underway.

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. 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, in particular near the initial proton acceptor, Asp-96 (Asp-85 in BR), which forms a salt bridge with a histidine conserved in proteorhodopsin, as well as in the region of Arg-93 (Arg-82 in BR) and in the BC loop. The rearrangement of the latter causes the formation of a large aqueous pocket, which is accessible to the bulk solvent, in a region where the proton release group of bacteriorhodopsin (Glu-194, Glu-204, ordered waters) is located.

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Solvent-drop grinding as an alternative tool for preparation of cocrystals. Monika Oracz, Waldemar Maniukiewicz, Marek Główska, *Technical University of Lodz, Institute of General and Ecological Chemistry, Poland*

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For pharmaceutical substances existence of many forms like polymorphs and solvates is very common. Apart from these, mentioned above many active pharmaceutical ingredient compounds (API) can create cocrystals. Cocrystal is the multicomponent molecular crystal built from two or more constituencies in stoichiometric ratio. Pharmaceutical cocrystal performs when the unit cell is built of host molecules, which is the API framed with guest molecules. Nowadays, cocrystals especially pharmaceutical ones gain large concern what is connected with their patentability. Additionally, the main advantage of medical application such compounds is possibility to use them to improve commercially available drugs mostly their physicochemical properties (like solubility, stability or bioavailability) [1,2]. Cocrystals are often obtained by evaporating or cooling a saturated solution of the target compound, sublimation or crystallization from melt. Recently, large interest achieve mechanochemical methods of cocrystallization like a solid-state grinding. Cocrystals likewise grinding methods are known in literature for over 160 years but lately combination of these two issues bring science attention. The most interesting method of mechanochemical mixing is a solvent-drop grinding (SDG). Cocrystallization proceeds in the presence of several drops of a properly solvent. The mechanism of solvent influence is not exactly known yet. In SDG approach predominantly occurred solvent like: water, methanol, ethyl acetate or acetonitrile. It is assumed that at least one of the components should be soluble in applied [3].

„Solvent-drop grinding” provides an interesting alternative in contrary to traditional cocrystallization mostly because of economic and ecologic benefits. Moreover it requires small amount of the solvent, whereby provide value to the industry from both a scientific and economic perspective and it is effective approach and sometime gives possibility of create new cocrystal impossible to obtain in traditional way. SDG method can offer improvement or selectivity in the crystallization, thereby offering much wider applicability [4]. In the presentation we are going to show some examples of our recent studies on synthesis of API cocrystals by employing „solvent-drop grinding” and traditional methods.

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