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Advances in Membrane Protein Structural Biology:

Lipidic Sponge Phase Crystallization, Time-Resolved Laue Diffraction and Serial Femtosecond Crystallography

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Abstract

Membrane proteins carry out many essential tasks in cells such as signaling and transport, or function as electron carriers in photosynthesis and cellular respiration. The aim of this thesis has been to develop new and improve existing techniques for elucidating the structure and function of membrane proteins.

Membrane proteins are difficult to crystallize due to their combination of hydrophilic and hydrophobic domains. Part of this thesis was therefore dedicated to the development of a membrane protein crystallization screen based on mimicking the protein's native environment. The screen, consisting of 48 different lipidic sponge phase (LSP) conditions, was tested on eleven different membrane proteins and gave crystal leads for eight of these. One of these leads was the photosynthetic reaction center of the purple bacterium *Blastochloris viridis* (RC_{vir}). Two high-resolution structures to 1.86 Å and 1.95 Å were obtained from data collected using different radiation doses and revealed a new space group and novel crystal packing along with a number of lipid-protein interactions.

Using this new crystal form the electron-transfer reaction of RC_{vir} was studied by time-resolved Laue diffraction where data were collected on crystals illuminated with light at room temperature. This revealed a reproducible movement of the highly conserved TyrL162 residue towards the special pair upon photoactivation. These results were combined with molecular dynamics studies to propose a coupling between the conformational orientation and protonation states within a bacterial reaction center.

Finally, the LSP method was extended to a batch type of crystallization approach. This provided a large volume of micron-sized crystals suitable for structure determination at the Linac Coherent Light Source, a recently commissioned X-ray free electron laser (XFEL) facility. Data from hundreds of microcrystals were collected to low resolution and revealed yet another space group and crystal packing. After the commissioning of a high-resolution beamline, the structure of RC_{vir} was solved to 3.5 Å resolution. This represents the highest resolution membrane protein structure determined using XFEL radiation to date.