GÖTEBORGS UNIVERSITET

INSTITUTIONEN FÖR KEMI OCH MOLEKYLÄRBIOLOGI

Production and Structural Dynamics of Microbial Rhodopsins

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Abstract

Rhodopsins are a family of membrane proteins that are found in a wide range of organisms and provide them with the ability to sense and react to light. When light is absorbed by these proteins, structural changes occur that initiate the pumping of protons, opening of a channel or signal transfer. The rhodopsins have different functions but they are structurally similar. This makes the protein family interesting from the perspective of structural biology, a field that aims to link function of proteins to structure and dynamics.

The structure of a protein may be obtained with X-ray crystallography. It requires a pure protein sample that can form protein crystals, data collection at an X-ray source and analysis of data for structural modelling and interpretation. In the last ten years, the development of X-ray free electron lasers (XFELs) generating very intense and short X-ray pulses has made it possible to capture structural changes in real-time with a time resolution of femtoseconds. This technique, called time-resolved serial femtosecond crystallography (TR-SFX), is particularly suited for studying structural dynamics of light-activated proteins.

The first part of this thesis is about production of channelrhodopsins. Channelrhodopsins are light-gated ion channels that we aim to produce for a future TR-SFX experiment. We describe protein production in the yeast *Pichia pastoris* and strategies to handle glycosylations of the proteins. We also establish protocols for purification and screen for microcrystals in the lipidic cubic phase.

The second and major part of this thesis is about structural dynamics of bacteriorhodopsin, a light-driven proton pump and by far the most studied microbial rhodopsin. We perform a TR-SFX study on bacteriorhodopsin that reveal structural changes in the time window from nanoseconds to milliseconds. In addition, we develop tools for comparison and analysis of structures and difference Fourier electron density maps from intermediate trapping studies and TR-SFX. These analyses give new insights in the mechanism of proton pumping.