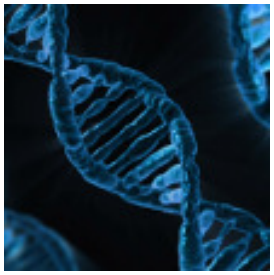

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Neutron crystallography of membrane proteins

Hydrogens play a crucial role for protein function and involved in almost every mechanism. They are critical in understanding the function of various proton pumps such as bacteriorhodopsin (BR) and cytochrome oxidase C. Their light or redox driven action and unidirectional proton pumping mechanism motivates the structural study of these membrane proteins. With the advancement in technology, neutron crystallography is used to locate hydrogen as it is not visible by X-ray crystallography. Since it requires larger crystals, no neutron structures of these membrane proteins have been determined yet. In order to maximize crystal size, we need large amount of protein to feed the crystals. Thus, we focused on optimizing the largescale production of membrane protein. In the initial stage, we used OmpF as a model system due to its high stability, yield and solubility in aqueous solutions to determine the neutron structure at a later stage. A comparative study for the production of OmpF was done considering various parameters such as temperature, media, optical density at wavelength of 600 nm and inducing conditions by Isopropyl ⍁-D-1-thiogalactopyranoside (IPTG). The most challenging part was to avoid the improper folding of protein and extraction of outer membranes to the maximum. Thus, after systematic trials with different conditions, we were able to optimize the protocol for large scale purification of OmpF. We also reproduced the published crystallization conditions for OmpF. The next step will be to grow crystals large enough for neutron studies.

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