

X-ray Crystallography of Bacteriorhodopsin

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Bacteriorhodopsin (bR) is a transmembrane protein that functions as a light driven proton pump. Utilizing light energy, bR transports protons from the extracellular side to the cytoplasmic side, and generates a proton electrochemical potential across the plasma membrane. To understand the proton pumping mechanism, we have tried to elucidate the molecular structure at an atomic resolution.

A 3D crystal of bR that is made up of stacked membranes was obtained by successive fusion of the vesicular assemblies of bR. The crystallization was achieved by the following three steps. (1) Purple membrane, a 2D crystal of bR isolated from *Halobacterium halobium* strain JW3, was partially delipidated with 0.3% of Tween20. (2) Purple membrane was vesicularized by incubating at 32 °C in the presence of octylthioglucoside (detergent), ammonium sulfate (precipitant) and trehalose (cryo-protectant). (3) The resultant spherical vesicles were cooled to 10 °C and concentrated by the vapor diffusion method. Incubation for > 2 months

yielded a hexagonal crystal ($0.15 \times 0.15 \times 0.05 \text{ mm}^3$).

A single crystal was picked up with a cryo-loop, and rapidly frozen with liquid ethane. Diffraction data were collected with R-Axis IV at the station BL41XU. During data collection, the frozen crystal was maintained at 100K using a cryostream cooler. The crystal-to-detector distance was 500mm, the wavelength of X-ray was 0.708 Å and the beam size was 0.1mm. The data were divided into 60 pictures with 0.5 degree of overlap. The crystal was rotated over 2 degree for 100sec per picture.

The reflections were indexed and integrated with AUTO. Diffraction spots were observed up to 2.3 Å resolution. Completeness of the data from 50 Å to 2.5 Å was 82% with R_{sym} of 7.2%. The crystal belonged to the space group $P622$. The unit cell parameters were $a=b=102.2 \text{ Å}$, $c=112.6 \text{ Å}$, shorter about 2 Å than those obtained at room temperature.

Molecular replacement was carried out with X-PLOR using a room-temperature model previously determined at 3.5 Å. Refinement is under way.