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A molecular movie of structural changes in bacteriorhodopsin

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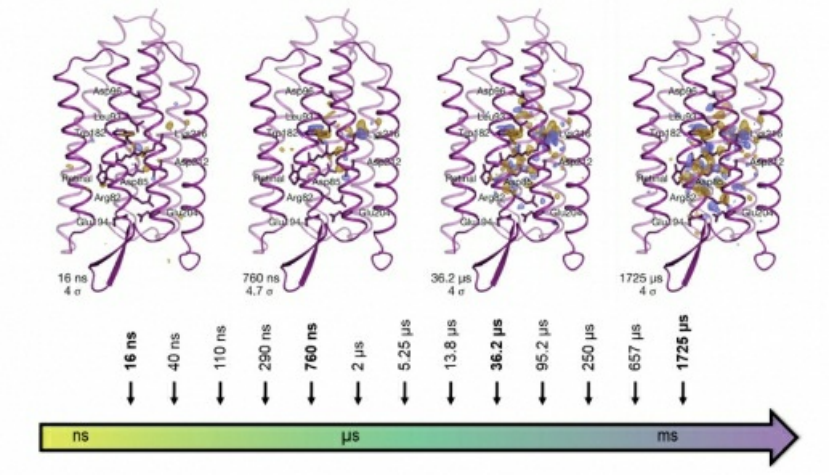
Bacteriorhodopsin (bR) is a light-driven proton pump derived from Halobacterium salinarum. The protein comprises a seven-transmembrane helix and contains a retinal molecule that is covalently bound to Lys216. The all-trans retinal undergoes isomerization to the 13-cis configuration by absorption of light, which causes a structural change in the protein via several intermediates called as K, L, M, N and O. The structural change results in proton transfer from cytoplasmic side to extracellular side, and the protein recovers the initial state (photo-cycle). The generated proton concentration is utilized for ATP production by ATP synthase.

Considerable effort has been made to understand how structural changes in bR transport a proton uphill against a transmembrane potential. More than one hundred crystallographic structures of bR have been deposited to the protein data bank to date. Cryo-trapping studies of bR from many laboratories provided structural information about structural changes during the photo-cycle. Despite these successes, a number of scientific weaknesses are apparent from that work. In the first instance, intermediate trapping studies were performed at low temperature and thus were not truly time-dependent. In the second instance, conventional crystallography is subject to radiation damage and early results have been criticized for this reason.

We circumvent these concerns by recording a three-dimensional movie of structural changes in bR at room-temperature at 2.1 Å resolution using time-resolved serial femtosecond crystallography at the SPring-8 Angstrom Compact Free Electron Laser (SACLA). Recent advent of intense, femtosecond X-ray pulses from X-ray free electron laser (XFEL) has enabled to acquire diffraction patterns from protein microcrystals before the onset of radiation damage. In serial femtosecond crystallography (SFX), intact microcrystals are continuous delivered by an injector, allowing "damage free" structure at physiological temperature. This technique is of great utility for time-resolved experiments in measuring ultra-fast reactions in proteins when combined with an excitation laser in a pump-probe setting.

Using pump-probe time-resolved SFX technique, we observed conformational changes in bR at thirteen time-points from nanoseconds to milliseconds following photo-activation (Figure). Our data revealed that an initially twisted retinal displaces Trp182 and Leu93 towards the cytoplasm and allows a water molecule (Wat452) to order between Leu93, Thr89 and the Schiff base (SB) on the retinal in the L-state. Hydrogen-bonding interactions from the protonated SB, which is a proton donor, to Wat452 or Thr89 create a pathway for proton transfer to a proton acceptor, Asp85. This observation explains how the SB makes contact with Asp85 despite been turned towards the cytoplasmic side by photo-isomerization. We found that once a proton is transferred, the hydrogen-bonding interaction between Asp85 and Thr89 is lost, which breaks the connectivity to the extracellular side of the protein. The resulting cascade of structural changes throughout the protein provided unprecedented insight into how structural changes in bR conspire to achieve unidirectional proton transport.

[1] Nango, E., Royant, A., Kubo, M., Nakane, T., ..., Neutze, R., and Iwata, S. (2016). Science 354, 1552-1557



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