EXPERIMENTAL REPORT

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High resolution structure of the M-intermediate of bacteriorhodopsin.

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Bacteriorhodopsin is the simplest known light driven proton pump and, as such, provides an ideal system for studying a basic function in bioenergetics. By harvesting light so as transport protons across a cell membrane, this integral membrane protein is able to create a trans-membrane proton-motive potential. This electrochemical potential is then used by ATP-synthase to synthesize ATP (from ADP+P), which acts as the basic energy currency of the cell. For reasons of its simplicity, stability and availability, bacteriorhodopsin has become one of the most important model systems within the field of bioenergetics.

Bacteriorhodopsin's ground state structure at 1.9 A resolution [1] shows the location of a number of key water molecules associated with the proposed proton pumping mechanism. A full understanding of the vectorial proton translocation mechanism, however, requires a detailed structural characterization of the photointermediates. This experiment was aimed to build upon our previous two structures, also derived from data collected at the ESRF, of the low-temperature K [2] and L [3] intermediates of the bacteriorhodopsin photocycle. That work showed the early structural rearrangements immediately following retinal isomerisation, and their evolution towards the extracellular medium, presenting an explicit mechanism for how the initial proton transfer event achieves vectoriality within the photocycle.

This experiment aimed to determine the next intermediate of the photocycle, the M-state. In this state bacteriorhodospin is known to undergo large scale movements on its cytoplasmic side [4,5], although the X-ray structures to date [6,7,8] have not revealed this unequivocably. To overcome ambiguities as to the temperature of the crystal arising from the M-state trapping protocols to date, we did not pre-freeze and then thaw crystals while being illuminated [6,7.8]. Instead we used a freeze-trap methodology. Spectra taken on

crystals trapped in this manner showed almost complete conversion into the M-state. Data collected from these crystals diffracted in the range from 2.5 to 2.0 Å. Difference Fourier maps have been calculated from these data sets, and show a continuous evolution of electron density changes from the K-state to the L-state to the M-state. Refinement of the M-state is now underway. A high degree of mosaic spread induced by the light-triggering, however, indicates that large scale movements took place within the crystals.

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