EXPERIMENTAL REPORT

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Time resolved X-ray diffraction studies of the structural intermediates of the bacteriorhodopsin photocycle.

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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 transmembrane proton-motive potential. This electrochemical potential is then converted by ATP-synthase into ATP, which acts as the basic energy currency of the cell. For reasons of its simplicity, 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 photo-intermediates. This experiment 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.

In these two experiments we attempted to use monochromatic X-ray diffraction, combined with a stroboscopic pump-probe approach, so as to recover the structural rearrangements of the bacteriorhodopsin photocycle on a millisecond time-scale. On this time-scale bacteriorhodospin is known to undergo large scale movements on its

cytoplasmic side [4]. Spectroscopic studies were performed in advance using the microphotospectrometry facilities built up at the ESRF. These showed that a 10 ms flash was sufficient to gain almost complete conversion into the M-state from the ground state. These studies also showed that, at 6 C the rate of decay of the M-state in the crystalline phase was considerably slower than what is expected at the same temperature in the purple membrane. This arises from the influence of the crystal packing on the protein's dynamics. Unfortunately, the very large-scale movements which we wished to characterize, led rapidly to disordering of the crystals, especially along the c-axis, and it was not possible to collect complete data sets. Instead, we collected a number of different time points shooting parallel to the c-axis (two dimensional, not three dimensional, data). In addition, we developed a method of flashing and freezing crystals on the millisecond time-scale, and this cumulated in a successful experiment (LS-1616 on ID14-EH2, see report) which led to high-quality data of the M-intermediate. The time at ID09 was invaluable because a number of technical problems relating to our trapping protocol were identified and could be modified.

[1] Belrhali, H. et al. *Structure* **7**, 909-917 (1999)

[2] Edman, K. et al. *Nature* **401**, 822-826 (1999)

[3] Royant, A. et al. *Nature* **406**, 645-648 (2000)

[4] Vonk, J. *EMBO J.* **19**, 2152-2160 (2000)