ESRF	Experiment title: Structural determination of the L-intermediate in the photocycle of bacteriorhodopsin.	Experiment number: LS-1467
Beamline: ID14, EH1	**Date of Experiment:** from: **October 27 1999** to: **October 30 1999**	**Date of Report:** **24 February 2000**
Shifts: 9	**Local contact(s):** Dr. Hassan Belrahli	*Received at ESRF:* 28 FEV. 2000

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Report:

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 electro-chemical 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 Å 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 structure, also derived from data collected at the ESRF last February, of the low-temperature K-intermediate of the bacteriorhodopsin photocycle [2]. That work showed the early structural rearrangements immediately following retinal isomerisation, and provided clues as to the nature of later movements which facilitate efficient vectorial proton transport.

In collaboration with Dr. Thomas Ursby, of the ESRF, a trapping protocol for the L-state was developed. In this trapping protocol the temperature of each crystal; the intensity with which each crystal was illuminated with green light; the duration of the period when each crystal was illuminated; and the length of a delay following illumination prior to the crystal being frozen, we all variables which were examined. Once the trapping protocol was established, crystals containing a high population of the trapped L-state were mounted on the X-ray camera of ID14-EH1 and diffraction data were collected.

Although this was the first official experiment of EH1 of ID14, there were only a small number of minor problems associated with the performance of the beamline components. When we occasionally suffered minor technical problems Dr. Hassan Belrhali was able to sort out these promptly. Overall we were very happy with the performance of this new station.

A large number of crystals of bacteriorhodopsin with the trapped intermediate were screened on the basis of their diffracting power, and a number of X-ray diffraction data sets were recorded. Of particular note, two data sets to 2.1 Å resolution (one with 24 % twinning and the other with very low twinning) were recorded and both showed a high population of the trapped L-intermediate. X-ray diffraction data sets were examined initially using difference electron density maps, and even at the time of this experiment it was clear that the structural changes associated with the L-intermediate were entirely consistent with our published structure of the K-intermediate [2]. Indeed, all the observations previously reported for the K-state were observed to have become extended and have propagated outwards from the protein's core. A novel mechanism of vectorial proton transport emerged from this diffraction data, and manuscript describing these findings is currently under review [3].

[1] Belrhali, H., Nollert, P., Royant, A., Menzel, C., Rosenbusch, J. P., Landau, E. M., & Pebay-Peyroula E., *Protein, lipid and water organisation in bacteriorhodopsin crystals: a molecular view of the purple membrane at 1.9 Å resolution*, Structure **7**, 909 (1999).

[2] Edman, K., Royant, A., Nollert, P., Belrhali, H., Pebay-Peyroula, E., Hajdu, J., Neutze, R & Landau, E. M., *High resolution X-ray structure of an early intermediate in the bacteriorhodopsin photocycle,* Nature **401**, 822-826 (1999).

[3] Royant, A., Edman, K., Ursby, T., Peyba-Peyroula, E., Landau, E. M. & Neutze, R., *Helix deformation coupled to vectorial proton transport during the bacteriorhodopsin photocycle*, manuscript under review.