ESRF	**Experiment title:** **Structural intermediates of sensory rhodopsin II & bacteriorhodopsin**	**Experiment number:** **LS-2039**
Beamline: ID14, EH2	**Date of Experiment:** from: **Sept. 21st 2001** to: **Sept. 24th 2001**	**Date of Report:** **Feb. 28th 2002**
Shifts: **9**	**Local contact(s):** Dr. Antoine Royant	*Received at ESRF:*

Names and affiliations of applicants (*indicates experimentalists):

Prof. Ehud Landau* (Dept. Molecular Microbiology, University of Basel)
Prof. Javier Navarro (Dept. Physiology & Biophysics, University of Texas)
Prof. Eva Pebay-Peyroula* (IBS, Université Joseph Fourier)
Mr. Antoine Royant* (ESRF)
Dr. Richard Neutze* (Dept. Biochemistry, Uppsala University)
Mr. Karl Edman* (Dept. Biochemistry, Uppsala University)

Report:

Sensory rhodopsins belongs to a sub-family of heptahelical proteins containing a retinal chromophore. These photo-receptors mediate the cascade of vision in animal eyes and photo-taxis in archaebacteria and unicellular flagellated algae. Signal transduction by these photoreceptors occurs by means of a transducer protein. The two archaebacteria sensory rhodopsins (sensory rhodopsin I and II) mediate positive and negative phototaxis in the host organism. Crystals of sensory rhodopsin II were grown in a lipidic cubic phase [1] in the laboratory of Profs. Landau and Navarro. The aim of this experiment was to trap an early intermediate in the photocycle of sensory rhodopsin II in 3D crystals and then to collect X-ray diffraction data to high resolution from this structural intermediate. Preliminary studies in advance of this experiment had shown reproducible structural rearrangements, and hence the primary issue was the quality of the diffraction data for publication purposes.

Crystals of pSRII were extracted from the lipidic cubic phase using lipase [2] and were mounted upon cryo-loops and frozen in liquid nitrogen. A blue laser was coupled to a fibre optic and this transported blue light up to within a few hundred microns of the crystal. The crystal was cooled to 100 K and X-ray diffraction data were collected while the crystal was continuously illuminated. Single crystal microspectrophotometry experiments in advance had confirmed that the low temperature K-intermediate of sensory rhodopsin II builds up under these conditions. The performance of beamline ID14 EH2 was excellent, and we were able to collect a number of diffraction data sets from these crystals. In one case, diffraction data to 2.3 Å resolution showed an extremely low noise difference Fourier map due to the crystallographic occupancy being relatively high (50 %). This was judged to be suitable for publication purposes, since all observed structural rearrangements were consistent with trial trapping and diffraction experiments a few months earlier.

Since it was anticipated that publishable diffraction date would be recorded, progress on a manuscript was rapid, and the work entitled *"Early structural rearrangements in the photocycle of an integral membrane sensory receptor"* has recently been accepted for publication by Structure [3]. Of particular interest is that, while ground states of bacteriorhodopsin and sensory rhodopsin II are very similar,

we observed subtle differences (relative to bacteriorhodopsin) in the early relaxation of the retinal chromophore after photoisomerisation at low temperature. This work builds upon our publication of the ground state of sensory rhodopsin II in *Proc. Natl. Acad. Sci. USA* a few months earlier [4].

In addition, some further experiments on the structural intermediates of the photocycle of bacteriorhodopsin were pursued. An earlier publication of ours [5] on the L photointermediate of bacteriorhodopsin had drawn some criticism on the basis of spectral concerns. Although, following a more detailed spectral analysis [6], this criticism turned out to be unfounded, additional X-ray diffraction experiments were warranted. In particular, for technical reasons it was argued that a trapping protocol using red light at 150 K rather than green light at 170 K [5] would provide a more satisfactory trapping protocol. While it is true that the spectral results are somewhat improved using the red-light protocol, X-ray diffraction experiments following this trapping protocol revealed exactly the same structural rearrangements as we have previously published [5]. So as to quell any possibility for further controversy, we will seek to publish our diffraction data from the red-light experiment at 150 K in the near future.

[1] Landau, E. M. & Rosenbuch, J. P. Lipidic cubic phases: a novel concept for the crystallisation of membrane proteins. *Proc. Natl. Acad. Sci. USA* **93**, 14532-14535 (1996).

[2] Nollert, P., & Landau, E. M. Enzymic release of crystals from lipidic cubic phases. *Biochem. Soc. Trans.* **26**, 709-713 (1998).

[3] Edman, K., Royant, A., Nollert, P., Maxwell, C. A., Pebay-Peyroula, E., Navarro, J., Neutze, R., Landau, E. M., Early structural rearrangements in the photocycle of an integral-membrane sensory receptor, *Structure* in press (2002).

[4] Royant, A., Nollert, P., Edman, K., Neutze, R. Landau, E. M., Peyba-Peyroula, E., Navarro, J. X-ray structure of sensory rhodopsin II at 2.1 Ångstrom resolution. *Proc. Natl. Acad. Sci. USA* **98**, 10131-10136 (2001).

[5] Royant, A., Edman, K., Ursby, T., Pebay-Peyroula, E., Landau, E. M., & Neutze, R. Helix deformation is coupled to vectorial proton transport in the photocycle of bacteriorhodopsin, *Nature* 406, 645-648 (2000).

[6] Royant, A., Edman, K., Ursby, T., Pebay-Peyroula, T., Landau E. M., & Neutze, R. Spectroscopic characterisation of bacteriorhodopsin's L-intermediate in 3D crystals at 170K, *Photochemistry & Photobiology* 74, 194-804 (2001).