ESRF

Experiment title: High resolution X-ray structure of Sensory Rhodopsin II.	Experiment number: LS-1956
Beamline: ID14, EH2	**Date of Experiment:** from: **May 5th 2001** to: **May 7th 2001**
Shifts: 6	**Local contact(s):** Dr. Steffi Arzt
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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 collect X-ray diffraction data to high resolution so as to improve the refinement of the ground state of sensory rhodopsin II from *Natronbacterium pharaonis* (pSRII).

Crystals of pSRII were extracted from the lipidic cubic phase using lipase [2] and were mounted upon cryo-loops and frozen in liquid nitrogen. The performance of beamline ID14 EH2 was excellent, and we were able to collect a number of diffraction data sets from these crystals. Furthermore, the diffraction data extended to 2.1 Å resolution, with a low degree of mosaic spread. The model which derived from this diffraction data was of sufficiently high quality to warrant publication. In our previous report (experiment LS 1766) we described diffraction data to 2.7 Å resolution, which prompted the writing of a manuscript describing this work. A manuscript describing the X-ray structure of sensory rhodopsin II at 2.1 Å resolution was submitted to Proc. Natl. Acad. Sci. USA., and has just this month been published [3]. The work suggests a mechanism for spectral tuning of the absorption peak of pSRII (which is blue shifted relative to the other archael rhodopsins) and also suggests a putative binding site for the downstream transducer protein (pHtrII). Furthermore, at 2.1 Å resolution it was possible to unambiguously assign a chloride ion bound to the protein near the active site.

In addition to the work on the ground state of sensory rhodopsin II, an aim of this experiment was to screen conditions for the trapping of the structural intermediates of pSRII. Different illumination conditions and temperatures were trialed, and the results were reproducible and encouraging. However, more work is required so as to extend the resolution to the point where the work is publishable. This will be an aim for future experiments.

[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] 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* e-manuscript released in advance of publication (2001).