	Experiment title: Structural studies of the archaeal rhodopsins	**Experiment number**: MX-27 & MX-78
Beamline: ID14/2 ID09	**Date of experiment**: 28/08/2002 & 06/12/2002 30/04/2003 to 06/05/2003	**Date of report**: 03/09/2003
Shifts: 2 + 1 + 9	**Local contact(s)**: Edward Mitchell & Michael Wulff	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Gergely Katona*[1], Pontus Gourdon*[1], Karl Edman*[1], Richard Neutze*[1], Antoine Royant*[2], Eva Pebay-Peyroula*[3], Javier Navarro*[4], Ehud Landau[4]

[1]*Department of Molecular Biotechnology, Chalmers University of Technology.*

[2]*ESRF*

[3]*IBS, Universite Joseph Fourier*

[4]*Membrane Protein Institute, Universit of Texas Medical Brach,*

Report:

These experiments aimed to characterise structural rearrangements in the photocycles of bacteriorhodopsin and sensory rhodopsin II at ID14 EH2 and ID09. A number of other studies were performed in parallel during these beamtime allocations, in particular studies on lipidic cubic pase crystals of the bacterial reaction centre of *Rhodobacter spheroids*. These results have been described in other reports also submitted in this reporting period.

In previous work at ID14 EH2, our studies on the light driven structural rearrangements in bacteriorhodopsin aimed to respond to some controversy. Our X-ray structure of predominantly the L-intermediate contained some contamination from a later intermediate [1,2]. As such we repeated the trapping experiment using red light illumination at 150 K. Spectral analysis shows pure L-intermediate results under these conditions. The structural changes observed in this experiment were in full agreement (as viewed by the difference Fourier map) with the work previously published and fully justified the mechanistic interpretation given then. The

experimental results from these studies have now been presented in both a review and a research article [3,4].

Nevertheless, bacteriorhodopsin as a field is no stranger to controversy. With the L-state controversy now effectively dealt with, there have been two recent publications [5,6] which challenged our K-state result [7]. Although the trapping protocols used in these studies were very similar to our earlier work, both claim that the correct structure for K is closer to the bR ground state than what was clearly visible in our published difference Fourier map. As such these experiments on bacteriorhodopsin set out to repeat our earlier work using a modified trapping protocol for the K-intermediate which could not be regarded as controversial. Working at 100 K we illuminated crystals first with green light, to as to build up a high population of the K-intermediate, then with lower-intensity red light so as to depopulate the K-intermediate. The use of ID09 was an advantage with this protocol since we could program how to illuminate the crystals on-line, and thereby collect each frame twice – first after green light illumination and followed by red light illumination. With this protocol, a modification of that of reference [6], difference density maps could be calculated from data collected from the same crystal. During these experiments we collected a number of data sets up to 2.3 Å resolution. Because of the influence of mirohedral twinning (about 35 % in this case – a bit borderline in our experience) and the slightly lower resolution than our earlier study [7], we have not yet made a firm decision as to whether or not the data is worthy of publication, or if the crystals should first be improved and the experiment repeated.

Our work on sensory rhodopsin II attempted to go beyond our recently published results from the K-intermediate of pSRII [8] and to characterize the later intermediates. This work involved first jumping the crystals to higher pH (from 4.6 to about pH 8) prior to illuminating and collecting X-ray diffraction data. Spectral studies show that the M-state is easily trapped in pSRII crystals at high pH with a good population. However, in this experiment our attempts to jump the pH of crystals were not successful (*ie.* resulted in disordering of the crystal lattice) and appear to not tolerate a pH jump of more than one or so pH units. All in all no new structural results for pSRII emerged from these experiments, and X-ray diffraction data were not collected to a resolution better than about 2.7 Å. In order to go beyond this point in studies of pSRII we therefore need to have a lot of crystals available so as to pinpoint the region where it is still possible to get reasonable diffraction from the crystals after a pH jump and illumination.

References:

[1] Royant, A., Edman, K., Ursby, T., Pebay-Peyroula, E., Landau, E.M. and Neutze, R. (2000) Nature 406, 645-8.
[2] Royant, A., Edman, K., Ursby, T., Pebay-Peyroula, E., Landau, E.M. and Neutze, R. (2001) Photochem Photobiol 74, 794-804.
[3] Neutze, R., Pebay-Peyroula, E., Edman, K., Royant, A., Navarro, J. and Landau, E.M. (2002) Biochim Biophys Acta 1565, 144-67.
[4] Edman, K. et al. (2003) Manuscript under review.
[5] Schobert, B., Cupp-Vickery, J., Hornak, V., Smith, S. and Lanyi, J. (2002) J Mol Biol 321, 715-26.
[6] Matsui, Y., Sakai, K., Murakami, M., Shiro, Y., Adachi, S., Okumura, H. and Kouyama, T. (2002) J Mol Biol 324, 469-81.
[7] Edman, K., Nollert, P., Royant, A., Belrhali, H., Pebay-Peyroula, E., Hajdu, J., Neutze, R. and Landau, E.M. (1999) Nature 401, 822-6.
[8] Edman, K., Royant, A., Nollert, P., Maxwell, C.A., Pebay-Peyroula, E., Navarro, J., Neutze, R. and Landau, E.M. (2002) Structure 10, 473-82.