	Experiment title: Structural rearrangements in the photocycles of bacteriorhodopsin and sensory rhodopsin II	**Experiment number**: LS-2158
Beamline: ID13 ID14/2	**Date of experiment**: from: Jan 30 2002 to: Jan 31 2002 from: March 6 2002 to: March 9 2002	**Date of report**: 02/09/2002
Shifts: 3 + 9	**Local contact(s)**: Dr. Manfred Burghammer & Dr. Sigrid Kozielski	*Received at ESRF:*

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Report:

This experiment aimed to characterise structural rearrangements in the photocycles of bacteriorhodopsin and sensory rhodopsin II at ID14 EH2. In addition, one day of beamtime was allocated on ID13 as part of the ESRF policy to BAG several experiments together into one experiment. In addition to a number of unsuccessful studes on new membrane proteins, the beamtime on ID13 yeilded our first diffraction results from the C-terminus of Mucin (described in more detail in report for LS-2168) and also diffraction results on the lipidic cubic pase crystals of the bacterial reaction centre of *Rhodobacter spheroides* (described in more detail in report for LS-2158). A paper describing this lipidic cubic phase structure is now in preparation.

In the work at ID14 EH2, our studies on the light driven structural rearrangements in bacteriorhodopsin were first 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 [1] and fully justifies the mechanistic interpretation given at that point, despite the small levels of contamination by other species in the earlier work. A manuscript describing these results will soon be submitted for publication, and a brief presentation of this structural result is also now in press in a longer review in Biochimica Biophysica Acta [3].

Our work on sensory rhodopsin II attempted to go beyond our recently published results from the K-intermediate [4] and to characterize the later intermediates. This work involved two procedures: first to flash the crystals at room temperature using a millisecond flash followed by rapid freezing; and also to try to first jump the crystals to higher pH prior to illuminating and collecting X-ray diffraction data. Due to support from Dominique Bourgeois, we could use the cryobench room of the ESRF for spectral characterization of a number of trapping conditions. These showed mixed results, but with very promising spectra at higher pH. While in this experiment our attempts to jump the pH were not successful (*ie.* resulted in disordering of the crystal lattice) later experiments at the Swiss Light Source found conditions where the crystals could be raised to slightly basic pH. We also screened X-ray diffraction from crystals grown at slightly basic pH, and they diffracted but, since they were small, to lower resolution than when the crystals are grown at pH 4.6 [5]. All in all no new structural results for pSRII emerged from this experiment, despite a significant number of X-ray diffraction data sets being collected from pSRII after reaction initiation by bright light. In a recently submitted experimental application our ideas on how to go beyond these difficulties, and proceed to structurally characterize the later photo-intermediates of pSRII, have been described. We believe that, with a willingness to modify the trapping protocol and change the pH, we will have success on the later intermediates of the photocycle of pSRII despite the relatively disappointing results from this experiment.

References:

[1] Royant *et al. Nature* **406**, 645 - 648 (2000).
[2] Royant *et al, Photochemistry & Photobiology* **74**, 794 - 804 (2001).
[3] Neutze *et al. Biochim. Biophys. Acta.* in press (2002).
[4] Edman *et al. Structure* **10**, 473 - 482 (2002).
[5] Royant *et al. Proc. Natl. Acad. Sci. USA* **98**, 10131-10136 (2001).