ESRF	**Experiment title:** HIGH RESOLUTION STRUCTURES OF MEMBRANE PROTEIN CRYSTALS GROWN IN LIPIDIC CUBIC PHASES	**Experiment number:** LS 795
Beamline: ID13	**Date of experiment:** from: 14 Oct.97 to: 20 Oct.97	**Date of report:** 28 Feb.98
Shifts: 9	**Local contact(s):** Manfred Burghammer	*Received at ESRF:* - 2 MAR. 1998

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Report:

Experimental conditions:

3 shifts (14 to 15 Oct.) and 6 shifts (18 to 20 Oct.) were operated using a large MARResearch imaging plate,

Crystal to detetector distance: 400 mm, λ=0.784 Å (2.2 Å at the edge of the detector)

about 65 crystals tested.

Results:

We tested several crystals originating from different growing conditions and from a mutant protein. Interestingly, we found out that using the non-attenuated beam line with a synchrotron operating under a 2/3 fill mode, the intensity was so strong that the crystal died after one 60s exposure. Attenuating the beam with a 1.025 mm thick aluminium, we were able to collect data over 45 degres, similarly to what was obtained in the august 96 experiment for which the beam was in a 16bunch mode. The optimal attenuation, in term of the best compromise between signal-to-noise ration, resolution limit and crystal decay has still to be worked out and can be improved.

During these shifts, we tested various approaches for handling the crystals (from the crystallization tube to the cooling on the beam line) and improved the ratio of diffracting crystal showing low mosaicity along the c axis. The limiting factor for obtaining usable diffraction still remains the size: under 20 microns in the larger dimensions (with a thickness less than 5 microns) it was not possible to get diffraction to 2.5 A.
We were able to collect a complete data set from a native crystal of BR in the ground state with an Rsym of 11% from 20 to 2.3 Å resolution. We also collected a complete data set from a crystal which was illuminated and then cooled to 100K in which BR should be in the M-state. The crystal diffracted only to 4 Å (before and after illumination) and it was not possible to decide if the crystal contains a majority of M-state BR or a mixing of various states of the photocycle. Nevertheless, this experiment showed the possibility of illuminating crystals without destroying the crystal quality.

The 2 data sets obtained from ground-state-BR crystals are not isomorphous (aug. 96 and oct. 97) and were only partially mixed: the oct97 data from 10/3.2 Å were mixed to the aug96 data from 10/2.4 Å. The refinement of the structure could be improved with following statistics: resolution: 10/2.4 Å, 7232 independants reflections (F>50 and >2s) R=24.8% and Rfree=31.6%, the model contains 1767 atoms (1695 protein, 20 retinal and 52 water). This model was deposited in the pdb under the access number lAP9 were it is accessible (or available by email under request).