| ESRF | **Experiment title:** HIGH RESOLUTION STRUCTURES OF **MEMBRANE PROTEIN CRYSTALS GROWN IN LIPIDIC CUBIC PHASES** | **Experiment number:** **LS 934** |
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| **Beamline:** ID13 | **Date of experiment:** from: 18 Apr. 98 to: 19 Apr. 98 from: 24 Apr. 98 to: 26 Apr. 98 | **Date of report:** 28 Aug. 98 |
| **Shifts:** 9 | **Local contact(s):** Hassan Belrhali and Manfred Burghammer | *Received at ESRF:* 01 SEP. 1998 |

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**The aim** of the experiments was (a) to enhance the quality of the high resolution data sets already recorded on ID13 (see previous reports and Eva Pebay-Peyroula *et al.*, Science, 1997) and (b) to explore the possibilities to trap the bR in one of the transition states, the M-state, that would give us insights on the proton translocation mechanism.

**Experimental conditions:** 9 shifts were allocated to the bR project on the ESRF micro-focus beamline ID13. The experiments have been carried out in 2 runs: 3 and 6 shifts. The synchrotron was operating in the 2/3 filling mode. The electron beam value was 200 to 160 mA. The new ID13 Kozu monochromator was set to a 0.689 Å wavelength during all the data collections. The EMBL 30 cm diameter online image plate was set in front of the ID1 3 high precision kappa diffractometer at a fixed distance of 450 mm, hence allowing diffraction patterns to be recorded up to a 2.15 Å resolution limit. During the entire allocated beamtime, 26 micro-crystals were diffracted (crystal sizes ranging from 20 to 50 microns in the larger dimension and 5 to 10 microns in the thickness).

**Experimental method:** All crystals were grown in a lipidic cubic phase (Landau *et al.*, 1996). Crystals extracted from the cubic phase are fished in a loop and flash-frozen in the 100 K $N_2$ gas stream of an Oxford CryoSystem® cooler.

The micro-crystals are then centered in the beam with the help of two optical devices mounted 90 degrees apart from one another. As the microcrystals are embedded in the lipid matrix, it is difficult to observe and align them. An improvement was achieved, due to the installation of a color CCD camera mounted vertically (Navitar® Zoom). A first shot is taken to control the diffraction quality of non-illuminated crystals. For the M state experiment, when the crystal was suitable, a combination of illumination and temperature modification was applied and a data collection procedure initiated.

**Remark:**

Luecke *et al.* (Science, 1998) have shown that bR crystals obtained by the lipidic cubic phase method are hemihedrally twinned. Careful inspection of our previous data sets revealed that our crystals also present some twinning and that the twinning ratio varies from 20 % to 50 %. The crystal used for the determination of the ground state structure of BR showed a twinning of 18%. The data could be corrected from the twinning leading to a better refinement (coordinates deposited in the PDB). At the present stage the crystallisation conditions controlling the twinning are still under investigation.

**Results:** Most of the crystals did not diffract to high resolution and were not used for data collections. The best result we had is a data set from 72 images (rotations of 1.0 degree, exposure of 60s) up to 3.75 Å resolution. The overall data set has a mean I/Sigma of 5 with a Rsym of 10.1% (from 8.5 % at 17 Å to 18.8 % at 3.75 Å). The overall completeness is 71% with a redundancy of 4. The estimated twinning ratio is 37%. A first refinement attempt was done using the program Shellx-97 (Sheldrick *et al.*, 1997) which is the only program that refines twinned crystals but its refinement algorithm requires a high resolution limit i.e. a suitable number of data over parameter ratio. Sigmaa-weighted electron density maps revealed modifications around the retinal moiety that we could unfortunately neither refine nor model because (a) of poor data quality (low resolution limit and weak redundancy) and (b) the twinning ratio.

**Conclusion:** (a) the M state trapping protocol has to be improved for the bR crystals in order to maintain their diffraction properties. These experiments allowed already the exploration of various possibilities. (b) We need to select or produce untwinned crystals if possible. (c) A detector with a lower readout time would be appropriate in order to reduce the radiation damage.

**References:**


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