



**Experiment title:**  
HIGH RESOLUTION STRUCTURES OF  
MEMBRANE PROTEIN CRYSTALS GROWN IN  
LIPIDIC CUBIC PHASES

**Experiment  
number:**  
LS 655

<b>Beamline:</b> ID13	<b>Date of experiment:</b> from: 16 Apr.97 to: 25 Apr.97	<b>Date of report:</b> 25 Aug.97
<b>Shifts:</b> 9	<b>Local contact(s):</b> Christian Riekkel and Hassan Belrhali	<i>Received at ESRF:</i> <b>29 AOUT 1997</b>

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### **Report:**

Experimental conditions:

6 shifts (15 to 17 apr.) were operated using a large MARResearch imaging plate,

Crystal to detector distance: 359 mm,  $\lambda=0.784 \text{ \AA}$

about 20 crystals tested.

3 shifts (24 to 25 apr.) using a MAR CCD detector

Crystal to detector distance: 180 mm,  $\lambda=0.784 \text{ \AA}$

about 20 crystal tested.

Results:

Among all the crystals which were tested, no improvement in the diffraction data could be achieved. The majority of the crystals exhibited either a large mosaicity along the c axis limiting the diffraction to 6 Å, or weak diffraction. The best crystal diffracted to 2.4 Å. Therefore the previous data (collected in Aug. 96 on ID13, see experimental reports 17-9-96 and 15-11-96) could not be improved.

The main reason for this is the relatively small size of the crystals available for this experiment: 10 to 30 microns in the larger dimensions compared to 20 to 50 microns in August 96. Despite the improvements implemented on the beam line (two microscopes), the crystal handling and alignment remains still difficult for such small crystals. The recent modification in the crystallization protocol leads systematically to larger crystals (comparable to that used in Aug.96) and should therefore be critical in overcoming these difficulties during the next experiments.

Despite these, the structure could be solved to 2.5 Å resolution from a data set collected in August 96. The results are in press (E. Pebay-Peyroula, G. Rummel, J.P. Rosenbusch and E.M. Landau, Science 1997). The statistics of the data collection and the model refinement are shown in the following table:

#### Data collection

resolution range: 10 - 2.4 Å

Rsym: 10%

unit cell and space group:  $a=b=61.76$  Å  $c=104.76$  Å,  $\alpha=\beta=90^\circ$   $\gamma=120^\circ$ ,  $P6_3$

completeness: 91%, redundancy: 2

#### Refinement

resolution range: 5 - 2.5 Å

number of unique reflexions ( $F > 3\sigma$ ): 5893

Rfactor= 21.2%

Rfree= 32.7%

stereochemistry:

rmsd on bond lengths: 0.016 Å, on bond angles: 2.29°

The abstract of the publication is added below.

« Lipidic cubic phases provide a continuous three-dimensional bilayer matrix which facilitates nucleation and growth of bacteriorhodopsin microcrystals. The crystals diffract X-rays isotropically to 2.0 Å. The structure of this light-driven proton pump was solved at a resolution of 2.5 Å by molecular replacement, using previous results from electron crystallographic studies as a model. The earlier structure is confirmed overall, but several significant differences are found, including loop conformations and side chain residues. Eight water molecules are now identified experimentally in the proton pathway. These findings reveal the constituents of the proton translocation pathway in the ground state. »