ESRF	**Experiment title:** HIGH RESOLUTION STRUCTURES OF MEMBRANE PROTEIN CRYSTALS GROWN IN LIPIDIC CUBIC PHASES	**Experiment number:** LS553
Beamline: D2AM + ID13	**Date of Experiment:** D2AM: from: 21/8/96 to: 22/8/96; ID13: from: 24/8/96 to: 25/8/96, from: 29/8/96 to: 30/8/96	**Date of Report:** 17/9/96
Shifts: D2AM 3, ID13 5	**Local contact(s):** D2AM: ERIC FANCHON/RICHARD KAHN; ID13: ANDREAS BRAM	*Received at ESRF :* 10 MAR. 1997

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Report:

Time allocated: 8 shifts on D2AM, 9 shifts on ID13

Time used: 3 shifts on D2AM (the remaining time will be used in October)
5 shifts on ID13 (4 shifts were lost due to beamline problems)

The previous experiments performed on D2AM (experimental report, apri196) leaded to small data collections (10 to 20°) on different crystals of bacteriorhodopsin at room temperature. Although the crystals were very small (30 to 50 µm in the larger dimensions, 5 pm thickness) and the diffraction very weak, three data sets could be integrated and merged with a Rsym of 13.7% to 5Å resolution. The unit cell is $a=b=64.1Å, c=107.9Å, \alpha=\beta=90°$ and $\gamma=120°$. From symmetry considerations the space group was assigned tentatively to P63. The completeness is 60% and the redunduncy 2.5. The space group determination was compatible with packing considerations. In the a/b plane the cell dimensions are similar to the 2-dimensional crystal for bacteriorhopsin in the natural purple membrane. Assuming the conservation of the P3 symmetry in this plane which produces a trimer in the a/b plane, a second layer of trimer could be generated by the 2-fold axis along c. This packing gives 6 monomers per unit cell with a protein content of 50%. This experiment showed that the major problems come from the small size of the crystals and of the rapid decay of the diffraction after irradiation. The logical pursuit of the experiment was to use a highly focused beam and to cool the crystals to liquid nitrogen temperature.

The 3 shifts on D2AM (21/9/96) were used to test the cooling procedure. Previously, it was shown on a standard diffractometer at IBS, that the lipid cubic phase surrounding the crystal acts as a cryoprotectant. The crystals were mounted on small loops or in open capillaries and rapidly cooled to -170°. A scan on a lysozyme crystals grown in lipid cubic phases gave a data set of good quality confirming that the cubic phase itself acts as a cryoprotectant. 11 crystals of bacteriorhodopsin were tested and the best crystal diffracted to 9 Å resolution. 7 cystals at room temperature were tested with no better results. The negative results obtained in this experiment can be explained by the 16 bunch mode beam and by an unsufftcient collimation (low signal-to-noise ratio). The wavelength was set to 0.98 Å, the crystal-to-detector distance to 3 10 mm which allowed a maximum resolution of about 4.5 Å at 2θ=0°. Most of the tests were done with 1° oscillations during 60 s.

The 5 shifts on ID13 (24/8/96 and 29/8/96) were used to test several bacteriorhodopsin crystals and collect data from five of them. The detector (large MARresearch image plate) was set to 420mm, the accessible resolution was 2 Å with a wavelength of 0.69Å. The crystal sixes ranged from 20 to 50µm in the larger dimensions with a thickness of 5µm. The beamline was equiped with a microscope which allowed the vizualisation and the centering of such small crystals. Furthermore, a small camera allowed the optimisation of the crystal centering by observing directly the diffraction pattern on a monitor. Several general observations can be made:

- some crystals diffract to 2 A resolution
- the quality along the c axis varies drastically from one crystal to another
- this quality is probably related (at least partially) to the handling of the crystals
- the diffraction limits decrease rapidly, from 2 Å to 5 Å in 2 hours experiment despite the cooling.

The data collection were done with following conditions: 1° oscillation and 60s exposure.
The starting diffaction limit was 2.8Å for crystal 1 and 2Å for crystals 2,3,4 and 5.
Data processing:
Data were processed with Denzo. The unit cell for crystals 1 and 2 could not be determined probably because of a high mosaicity along the c axis.
Crystals 3,4 and 6 could be integrated reasonably to 2.4Å (below this limit I/sigma <3) with following unit cells:

- crystal 3: a=b=61.90Å, c=106.42Å, α=β=90°, γ=120°
- crystal 4: a=b=61.83Å, c=104.27Å, α=β=90°, γ=120°
- crystal 5: a=b=61.93Å, c=105.97Å, α=β=90°, γ=120°

The space group determination appears to be problematic. From packing considerations, the upper limit of monomers per unit cell is 6 (corresponding to a Laue group P6/m, P3bar1m or P3barm 1) in contradiction with the symmetry in the diffraction pattern showing a higher symmetry. The Rsym calculated in the different space groups are similar (8 to 10%, depending on the crystal and the rejection level). At this stage, the observed symmetry could be explained by a perfect twinning of the crystal. Space group investigations are still underway. Further experiments could help in understanding the problem.