



	Experiment title: Bacteriorhodopsin Active Site and C-terminal Deletion Mutants	Experiment number: TC-150
Beamline: ID13	Date of experiment: from: 9 of May to: 10 of May 2003	Date of report: 27.6.2003
Shifts: One	Local contact(s): Dr. Manfred Roessle	<i>Received at ESRF:</i>

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Report:

High resolution x-ray structures of the bacteriorhodopsin carboxyl terminal deletion mutants would validate that the transmembrane domain folding is not perturbed by the truncation. The double-site bacteriorhodopsin mutants, potential photocycle intermediate structures, are likely to reveal re-arranged hydrogen bonding. High resolution is needed to correctly determine hydrogen bonds and locate waters.

Results: The test experiments (9 to 10 May 2003/one shift) were carried out on beamline ID13/ESRF. During the entire allocated test time several wild-type and mutant crystals were tested, and most of them gave high-to-medium diffraction (up to 2.0 Å resolution) to yield useable datasets.

Two full datasets collected were a hexagonal double mutant (D85N/T121V) bR crystals grown in bicelles, the diffraction limit of the crystals were at a medium resolution of 2.3 Å (from three crystals; twinning fraction 44 %), and a rhombic C-terminal deletion mutant formed also in bicelles, the diffraction limit of this crystal was at 3.5 Å (single crystal; no twinning detected).

In conclusions: The results demonstrate the use of bicelle matrice for obtaining hexagonal, P6(3), crystals of membrane protein bacteriorhodopsin, and its active site mutants, to high resolution x-ray analysis.

Now the low resolution dataset of the bR C-terminal deletion mutant have been collected. To obtain high resolution structure, further testing must be done.