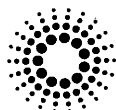

ESRF

	Experiment title:	Experiment number:
	Crystal structure of bacteriorhodopsin from Halobacterium salinarium	LS-701
Beamline: BLl	**Date of Experiment:** from: 30-4-97 to: 2-5-97	**Date of Report:** 12-6-97
Shifts: 6	**Local contact(s):** Dr. Christian Riekel	*Received at ESRF :* 2 3 JUIN 1997

Names and affiliations of applicants (*indicates experimentalists):

Prof. Dr. Oesterhelt — MPI for Biochemistry, Germany
* Dr. Lars-Oliver Essen — Am Klopferspitz 18a
* Ralph Siegert — D-82 15 2 Martinsried
* Martin Klumpp — Germany

Report:

Bacteriorhodopsin (BR) is a paradigm for membrane proteins mediating vectorial ion transport through lipid membranes. Unlike eukaryotic and eubacterial photosynthetic reaction centers, this archaebacterial protein utilizes a covalently bound retinal as chromophore which undergoes a sequence of conformational changes during the photocycle. Therefore, a major goal for the understanding of the BR photo cycle is a structural correlation between vectorial proton transport and photoisomerisation.

Electron-crystallographic studies of the natural 2D-crystalline state of BR (purple membrane) yielded a 3.5 Å structure of this protein (Grigorieff et al., 1996). Several attempts were made in the past to generate three-dimensional crystals suitable for X-ray analysis (Michel & Oesterhelt, 1980; Michel, 1982), but did not prove to be useful for an X-ray crystallographic structure determination. A monoclinic crystal form of BR that diffracted to 3.6 Å maximal resolution was generated by epitactic growth on organic crystals (Schertler et al., 1993). Several efforts were done to improve crystal quality and to establish a suitable cryogenic protocol that enables collection of complete data from single crystals. However, so far it was not possible to process these data by

conventional methods, because monoclinic BR crystals exhibited strong internal disorder not only by showing an enormous anisotropy in regard to the diffraction limit (2.8-3.5 Å along a and b, 5-7 Å along c), but also by very high mosaicities exceeding 5-10° and strongly elongated spot shapes. As working hypothesis, we assumed that monoclinic BR crystals might be well ordered on a microscopic level, but that growth defects lead to the apparent disorder as shown so far by whole crystals. Therefore, we pursued to minimize the crystal volume interacting with the X-ray beam by applying the microfocus beam line BLMD13.

We examined 45 BR crystals ranging in size from 0.05 mm to 0.5 mm longest dimension using the microfocus beam line BLMD 13 (focus size 30 um, λ=0.7838 Å). With larger crystals, we found a strongly improved maximal resolution ranging to 2.2 Å in the best direction. Spot overlap was strongly reduced compared to studies with focus sizes larger 100 urn, albeit still not sufficient for data processing. No variation in diffraction quality was observed at different regions of large crystals thus ruling out the possibility of local well ordered regions surrounded by less ordered regions. Smaller crystals showed reduced maximal resolution (2.5-3.0 Å along a and b), but further reduction in spot overlap and anisotropic spot shape. The smallest crystal (50 urn longest dimension) was used for collection of a partial dataset. This crystal exhibited 3.5 Å resolution along the c-axis and 2.7 Å along a and b. All data could now be processed with DENZO leading to a completeness of 26 % at 3.5 Å (mosaicity 2.5°, R_{merge} 6.6 %, spg. C2, a=120 Å, b=106 Å, c=78 Å, β=96°). The crystal showed no apparent decay during total exposure time (6 hours).

In conclusion, the application of the microfocus beam line BLl/ID13 proved to be instrumental for resolving persistent problems with the internal disorder of these membrane protein crystals.

Grigorieff, N., T. A. Ceska, K. H. Downing, J. M. Baldwin, and R. Henderson. 1996. Electron-crystallographic refinement of the structure of bacteriorhodopsin. J, Mol. Biol. 259 : 393-421.
Michel, H. 1982. Characterization and crystal packing of three-dimensional bacteriorhodopsin crystals. EMBO 1.10 : 1267-1271.
Michel, H., and D. Oesterhelt. 1980. Three-dimensional crystals of membrane proteins: Bacteriorhodopsin. Proc. Natl. Acad. Sci. U. S. A. 77 : 338-342.
Schertler, G. F. X., H. D. Bartunik, H. Michel and D. Oesterhelt. 1993. Orthorhombic crystal form of bacteriorhodopsin' nucleated on benzamidine diffracting to 3.6 Å resolution. J. Mol. Biol. 234 : 156-164.