	Experiment title:	Experiment number:
	HIGH RESOLUTION STRUCTURE OF THE M-INTERMEDIATE OF BACTERIORHODOPSIN	LS 1316
Beamline: ID13	**Date of experiment:** from: 14 Apr. 99 to: 17 Apr. 99	**Date of report:** 30 Aug. 99
Shifts: 9	**Local contact(s):** Manfred Burghammer	*Received at ESRF:* 02 SEP. 1999

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Report:

Aim of the experiment:
Resolution of the ground state structure of bacteriorhodopsin (bR) enabled to explain part of the mechanism of the bR photocycle. Characterisation of a key intermediate in the photocycle, the M-state, should provide more hints of how the protein works. As the photocycle is slowed down in a single-point bR mutant (D96N), we first focus on crystals of this mutant.

Experimental conditions:
The experiments (14 to 17 Apr.) were carried out using a MarCCD (Mar Research) detector, at a wavelength of 0.782 Å. The synchrotron was operating in the 2*1/3 filling mode. During the entire allocated beamtime, 42 crystals were tested.

Experimental method:
All crystals were grown in a lipidic cubic phase. They were extracted from the cubic phase, fished with a cryoloop, flash-frozen in liquid nitrogen, then stored in a portable dewar.
For ground state characterisation of the mutant, each crystal was transfered into the 100 K nitrogen gas stream of an Oxford CryoSystem cooler using a goniometer arc.
For M-state characterisation of the mutant, each crystal was treated off-line using various combinations of temperature control and light excitation.

Results:
5 crystals of the ground state protein were tested, and 2 of them gave high-resolution diffraction (up to 2.0 Å resolution) to yield useable datasets. Unfortunately, the high beam intensity significantly damaged the crystals, which resulted in a decrease of diffraction power and a degradation of spot shape quality.

All the remaining crystals were excited in different ways to provide optimal population of the the M-state, together with sufficient diffraction power. The crystalline quality of the samples turned out to be very sensitive to temperature variation below 273 K. Hence, the majority of tested crystals yielded non-useable diffraction, even if the characteristic yellow colour was obtained. Given that, we managed to get 4 datasets of bR mutant in the M-state. Moreover the radiation damage problems were also faced. Consequently, these datasets are only of medium to low resolution (3.5 to 4.0 Å).

Finally, all datasets collected were from significantly-twinned crystals (twinning ratio ranging from 30 to 50 %), which also lowered their quality.

Conclusions:
(a) Now that the conditions of M-state generation for the bR mutant D96N have been screened, it is possible to focus on excited crystals diffracting to high-resolution. This also would require to reduce the radiation damage by using attenuators.
(b) This mutant study provides information on how steering the experiment towards characterisation of the M-state with crystals of the wild-type protein.