



ESRF

Beamline:

ID2-BL4

Shifts:

2

Experiment title:

High resolution crystal structure of the photosynthetic reaction centre from *Rhodobacter sphaeroides*

Date of Experiment:

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Local contact(s):

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Report:

Applying a new crystallization procedure the resolution of crystals from the membrane-bound reaction centre from the purple bacterium *Rhodobacter sphaeroides* has been improved from 3 Å to 2.6 Å (1). Recently, the structures of three mutants of this reaction centre have been solved (not yet published). It was supposed that the crystals from the wild type reaction centre grown under improved conditions diffract better than 2.5 Å at station ID2-BL4 (high brilliant beam). Since no cryogenic buffer has been developed so far, the temperature at the crystal was adjusted to 0° to 5° C during X-ray exposure. Crystals of several sizes were mounted. A few relatively big crystals (approx. 2.0 x 1.5 mm) diffracted beyond 2.5 Å. After 4 to 5 exposures the resolution got worse. A shift of the crystal by 0.2 mm along the long crystal axis improved the resolution again. Longer, but thinner crystals showed the same behaviour. With these crystals it was possible to perform more than 10 shifts along the *long* crystal axis during the X-ray exposure. For this reason, two 3-4 mm long crystals with a "thickness" of about 0.8 mm and one shorter crystal with

the same “thickness” were chosen for a high resolution data set. The crystal shifts were performed after 4-8 exposures. At an X-ray wavelength of 0.892 Å the crystal-to-image-plate distance was chosen between 400 and 310 mm corresponding to resolutions down to 2.0 Å at the outer edge of the image plate. Preliminary processing of the images with denzo and scalepack yielded completenesses of at least 98.5% above 2.5 Å and at least 20% between 2.4 and 2.5 Å. The fitted mosaicity was approx. 0.07. Furthermore, one crystal of a mutated reaction centre was exposed. It is supposed that within this mutant a hydrogen bond is formed between the protein scaffold and a bacteriochlorophyll molecule. The desired resolution of 2.8 Å could be obtained (about 2.7 Å). Although smaller in size, this crystal was not less stable against radiation damage compared to the wild type reaction centre. Finally, several crystals of reaction centres with exchanged quinone Q_A were tested. Although some of these crystals were of a huge size (2.5 x 1.5 x 1.0 mm), they diffracted to lower resolution compared to the crystals from the wild type reaction centre (2.7 Å vs. 2.4 Å). Nevertheless, with one of these crystals a data set was collected. The obtained resolution is sufficient to determine the position of the exchanged quinone within the quinone binding site.

Literature:

1. U. Ermler, G. Fritsch, and H. Michel
“Crystal structure of the reaction centre from *Rhodobacter sphaeroides* to 2.65 Å: Cofactors and Protein-Cofactor Interactions”. *Structure* 2,925-936 (1994).