SNBL	**Experiment title**: BAG proposal in Macromolecular Crystallography for the University of Oslo	**Experiment number**: 01-02-732
Beamline: BM01A	**Dates of experiments**: From: 12-NOV-05 08:00 to: 15-NOV-05 08:00	**Date of report**: 25-NOV-05
Shifts: 9	**Local contact(s)**: Dr. Philip PATTISON	*Received at UNIL:*

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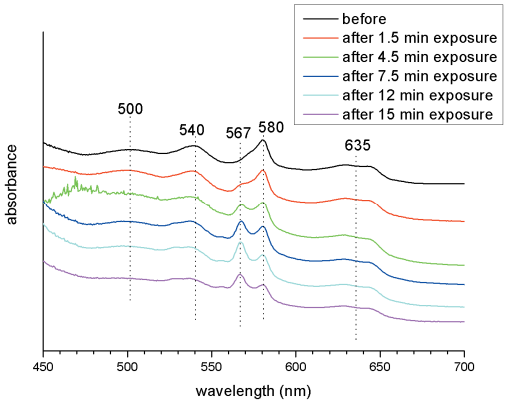
1. MYOGLOBIN

The main goal of this project has been to investigate the peroxidase reaction cycle in myoglobin by trapping intermediates in the cycle. Two of the intermediates have been determined, the compound II and the compound 0 equivalent. The introduction of a microspectrophotometer at SNBL has shown that the reaction site of compound II is not significantly reduced during datacolletion, while compound 0 is actually generated from compound III by the synchrotron radiation. The resting form also becomes reduced by the synchrotron radiation.

Several short datasets have been obtained to try to overcome the effect of radiation damage (reduction of metal centre by radicals produced by the high-energy X-ray beam). The reduction was last time followed in time (See figure to the right, which shows the reduction of the resting form with exposure time, by disappearance of 580 nm and appearance of 567 nm peak).



This time we planed to investigate the influence of lower temperature on the observed radiation damages. Due to mainly an unnoticed leak in the He-gas pumping system, we were not able to get any complete He-cooled dataset but instead 3 partial and incomplete datasets.

We also collected a dataset on myglobin crystallized from deutorated solutions and reacted with D_2O_2 to investigate the effects of deuterium on the hydrogen bondings in the reaction site.

We have tried to change the kinetic of the reaction and trap other intermediates by the use of larger organic peroxides. This time we got a dataset to 1.15 Å of a myoglobin crystal soaked in peracetic acid.

Data Set	*Mb7-deutorated*		*Mb7-peracetic acid*		*Mb7-helium (partials)*	
	Over all	Outer Shell	Over all	Outer Shell	Over all	Outer Shell
Low resolution limit (Å)	22.0	2.0	30	1.21	21.0	1.48
High resolution limit (Å)	1.9	1.9	1.15	1.15	1.40	1.40
Rmerge	0.066	0.379	0.059	0.508	0.145	0.470
Mean(I)/sd(I)	11.6	2.7	13.4	2.1	7.2	1.9
Completeness (%)	91.1	91.4	99.5	99.2	82.5	83.7
Multiplicity	2.1	2.1	3.7	3.4	2.2	2.2

2. LECTINS

A part of this project concerns the lectin domains of heat-labile enterotoxin from the bacterium *E. coli*. We have very recently obtained two data sets of a variant of this lectin domain in complex with its primary receptor, the GM1 ganglioside, at improved resolution. These datasets are currently under investigation, and should provide a more detailed view of the receptor binding.

Data Set	*GM1_1*		*GM1_2*	
	Over all	Outer Shell	Over all	Outer Shell
Low resolution limit (Å)	34.65	2.21	24.72	2.32
High resolution limit (Å)	2.10	2.10	2.20	2.20
Rmerge	0.051	0.401	0.064	0.394
Mean(I)/sd(I)	12.4	2.4	14.1	2.6
Completeness (%)	99.2	99.5	96.3	95.7
Multiplicity	2.1	2.1	3.1	2.2

3. CHORISMATE MUTASES

Chorismate mutase (EC 5.4.99.5) catalyzes the rearrangement of chorismate to prephenate, an important step in the synthesis of aromatic amino acids. To solve the heatedly debated question if it is the positive charge or the substrate geometry imposed by the active site residues, which is of ultimate importance for the catalytic competence of this enzyme, we are investigating a semisynthetic chorismate mutase variant, in which the positively charged Arg 90 is replaced by the sterically very similar, but neutral amino acid analogue citrulline.

The previously available crystal form has been determined to be unsuitable for soaking in the transition state analog (TSA) or the substrate. To address this problem we have very recently characterized a new crystal form at the SNBL. The crystals will however still need to be improved as the small crystals tested (~50µm) diffracted too weakly to make collection of a complete data set feasible. Future data collection on this crystal form might also need some special precautions since one cell axis appears to be rather long (~450Å).

Related Publications in this periode using SNBL data:

[1] Hersleth, H.-P., Uchida, T., Teschner, T., Røhr, Å. K., Shünemann, V., Nilsson, K., Hsiao, Ya-wen, Trautwein, A. X., Ryde, U., Görbitz, C. H., Kitagawa, T., & Andersson, K. K. (2005) Structure of the Intermediates in the Myoglobin-peroxide Reactions. *Acta Cryst.* A**61**, C214 (poster)

[2] Røhr, Å.K., Dalhus, B., Görbitz, C. H., & Andersson, K.K. (2005) A Tri-nuclear Metal Cluster in Reduced Mouse Ribonucleotide Reductase R2 Subunit. *Acta Cryst.* A**61**, C211 (poster)