SNBL	**Experiment title**: BAG proposal in Macromolecular Crystallography for the University of Oslo	**Experiment number**: 01-02-784
Beamline: BM01A	**Dates of experiments**: From: 16-NOV-07 08:00 to: 19-NOV-07 08:00	**Date of report**: 26-NOV-07
Shifts: 9	**Local contact(s)**: Dr. Philip PATTISON	*Received at UNIL:*

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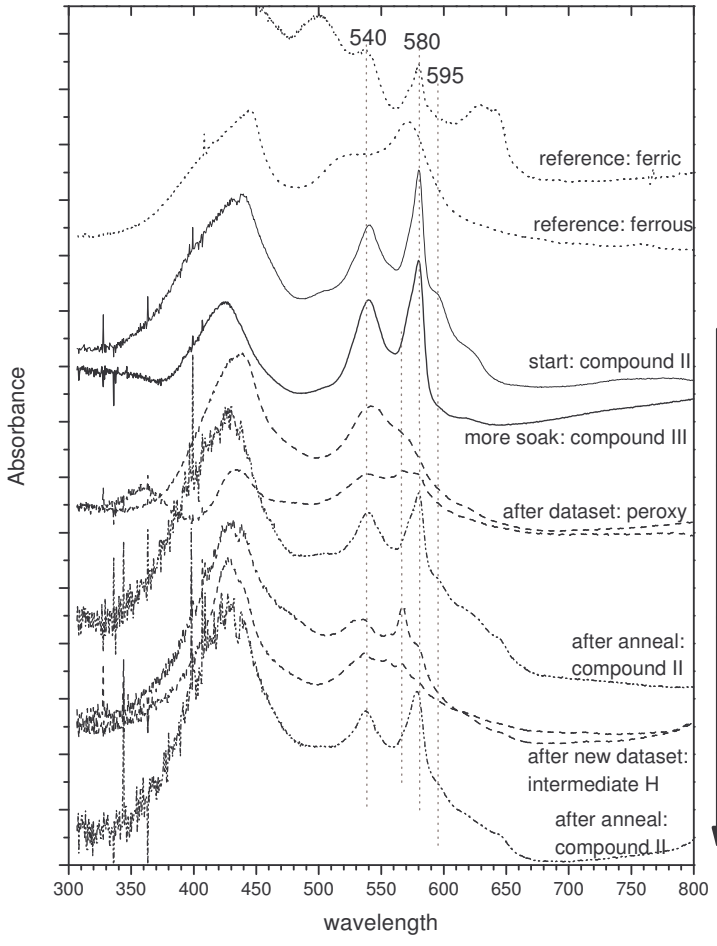
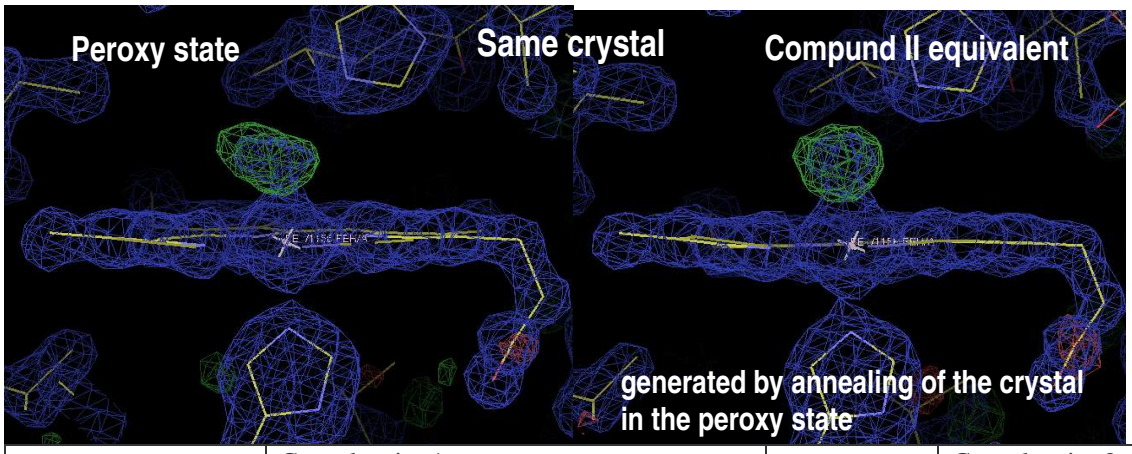
1. Myoglobin

The main goal of this project has been to investigate the peroxidase reaction cycle in myoglobin (Mb) by trapping intermediates in the cycle. Two of the intermediates have been determined, the so-called compound II equivalent and the compound 0 equivalent as well as the resting state. Several of these states experience some radiation damage of the metal site as investigated by the microspectrophotometer at SNBL. This time we tried to follow the reaction from ferricMb to compound II to compound III to compound 0 and back to compound II in the crystal and by light absorption. As the spectra show we were able to make this cycle. We generated compound II by soaking the crystal in hydrogen peroxide, soaked it longer to get compound III, this state get reduced in the beam to a peroxy state (see spectrum). We collected a set 1 to ensure a complete reduction before the data set was collected as Set 2. The peroxy state can be seen in the electron density map below) Through annealing of this peroxy state the compound II was generated (density map below).
We did the same on another crystal, and also made a fluorid complex to study the radiation damage for this compound.





	Crystal series 1				Crystal series 2		
	Set 1	Set 2	Set 3	Fluorid	Set 1	Set 2	Set 3
Resolution limit (Å)	21.7-1.60	34.1-1.50	34.1-1.60	34.1-1.55	26.6-1.35	34.0-1.6	26.9-1.82
Rmerge (%)	7.9 (44.8)	6.3 (43.9)	5.4 (42.7)	7.5 (27.1)	4.3 (34.2)	5.1 (11.7)	4.1 (12.0)
Mean(I)/sd(I)	10.3 (2.3)	12.7 (2.3)	13.9 (2.4)	12.8 (3.2)	14.5 (2.4)	19.8 (8.1)	20.8 (6.9)
Completeness (%)	98.1 (99.5)	99.5 (100.0)	99.7 (99.9)	82.7 (75.2)	60.1(49.6)	95.3(82.5)	95.4(78.9)
Multiplicity	2.7 (2.7)	3.6 (3.0)	3.7 (3.1)	2.8 (2.1)	2.4 (2.0)	3.4 (3.2)	3.3 (2.1)

Highest resolution bin in paranthesis

2. L-leu:L-val

We also tried to collect data on an amino acid L-leu:L-val complex, but the crystals diffracted not to high enough resolution.

3. nrdI

We have recently started the characterization of the ribonucleotide reductase system of *Bacillus cereus.* This bacterium is readly involved in food poising, and are closley related to the leathal phatogen *Bacillus anthracis*. Our aim is to understand the basic function of all the protins known to be involved in aerobic deoxyribonucleotide synthesis in this bacterium. One of the proteins encoded by the RNR operon in *B. cereus,* nrdI, have an unknown function. A major goal is to elucidate the function of the nrdI protein applying both biochemial and structural techniques.

nrdI contains a flavine mononucleotide (FMN) cofactor, thus we suggest that it is involved in a electron transfer reaction within the RNR enzyme system. Earlier experiments conducted by our group also show a protein protein interaction between the nrdI protein and the RNR holoenzyme.

We have collected three data sets of nrdI, two of the oxidized state to 1.1 Å resolution and one of the reduced state to 1.5 Å resolution. To verify the oxidation state of the FMN cofactor singe crystal light absorption spectra was recorded before and after data collection.

Additionally, two new students were trained in using a synchrotron beamline.

Related Publications in this periode using SNBL data:

[1] Hersleth, H.-P., Uchida, T., Røhr, Å. K., Teschner, T., Shünemann, V., Kitagawa, T., Trautwein, A. X., Görbitz, C. H., Andersson, K. K. (2007) Crystallographic and Spectroscopical Studies of Peroxide-Derived Myoglobin Compound II - Presences of Protonated $Fe^{IV}O$. *J. Biol Chem.* (2007) **282**, 23372-23386.

[2] Hersleth, H.-P., Structural Studies of Hydrogen Peroxide Derived Myoglobin Complexes. (2007) Dissertation for the degree of Dr. Scient. University of Oslo, Norway. ISSN 1501-7710 Nr. 641.

[3] Hersleth, H.-P., Varnier, A., Harbitz, E, Røhr, Å. K., Schmidt, P. P., Sørlie,, M., Cederkvist, F. H., Marchal, S., Gorren, A. C. F., Mayer, B., Uchida, T., Schünemann, V., Kitagawa, T., Trautwein, A. X., Shimizu, T., Lange, R., Görbitz, C. H. & Andersson, K. K. Reactive Complexes in Myoglobin and Nitric Oxide Synthase. *Inorg. Chim. Acta* (2007). In press. Published online with DOI: 10.1016/j.ica.2007.09.045