

**Experiment title:**

BAG proposal in Macromolecular Crystallography for the University of Oslo

**Experiment number:**  
01-02-752**Beamline:**  
BM01A**Dates of experiments:**

From: 16-FEB-07 08:00 to: 20-FEB-07 08:00

**Date of report:**  
28-FEB-07**Shifts:**  
12**Local contact(s):**

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**1. nrdI**

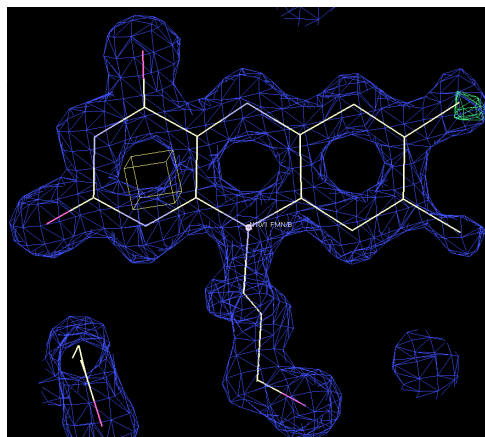
We have recently started the characterization of the ribonucleotide reductase system of *Bacillus cereus*. This bacterium is readily involved in food poisoning, and are closely related to the lethal pathogen *Bacillus anthracis*. Our aim is to understand the basic function of all the proteins known to 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 biochemical and structural techniques.

nrdI contains a flavine mononucleotide (FMN) cofactor, thus we suggest that it is involved in an 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.

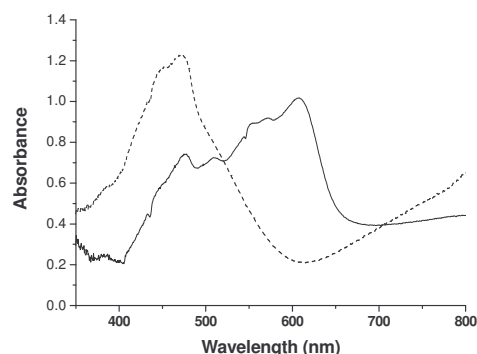
We have collected four data sets of nrdI, two of the oxidized state and two of the semiquinone state. To verify the oxidation state of the FMN cofactor single crystal light absorption spectra was recorded before and after data collection.

Data Set	nrdI 1 ox		nrdI 2 ox		nrdI 1 sq		nrdI 2 sq	
	Over all	Outer Shell	Over all	Outer Shell	Over all	Outer Shell	Over all	Outer Shell
High resolution limit (Å)	21.2	1.49	23.5	1.84	23.4	1.67	23.5	1.83
Low resolution limit (Å)	1.41	1.41	1.75	1.75	1.58	1.58	1.74	1.74
Rmerge	0.077	0.462	0.109	0.499	0.066	0.41	0.077	0.332
Mean(I)/sd(I)	10.5	3.0	9.4	3.3	13.0	3.0	12.5	3.0
Completeness (%)	99.8	100	99.3	99.6	99.3	100	98.7	98.7
Multiplicity	3.9	3.9	3.0	3.0	3.6	3.6	2.9	3.4

The single crystal light absorption spectra of oxidized (dashed) and semi-reduced (solid). nrdI. For both redox states the spectral features could be related to the solution spectra, verifying the redox state of the cofactor.



The 2Fo-Fc electron density map of the oxidized FMN cofactor countered at  $1.5 \sigma$ . A preliminary structure have been refined to  $R_{\text{work}} = 0.20$  and  $R_{\text{free}} = 0.23$  at a resolution of  $1.41 \text{ \AA}$ .

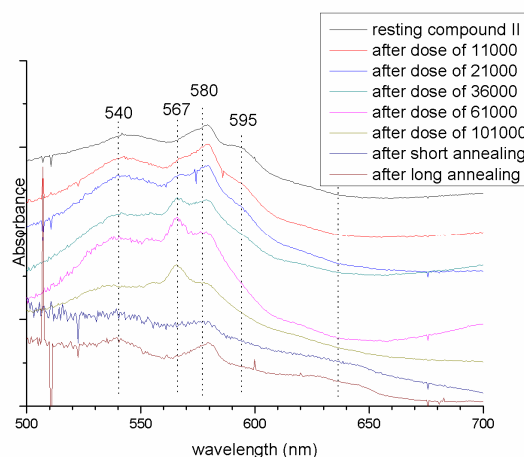


## 2. nrdH

Another protein in the RNR system of *B. cereus*, nrdH, provides the electrons necessary to reduce the ribonucleotide hydroxyl group to the catalytic subunit. Three datasets was collected, and the point group was found to be P4. The best dataset diffracted to  $\sim 1.3 \text{ \AA}$ . Unfortunately, we have not been able to process these datasets yet

## 3. 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 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 one long datasets was collected to investigate further the potential radiation damage of the compound II state. In addition several crystals were irradiated to give further insight into the radiation induced changes. The change of compound II with increasing dose can be seen from the single crystal light absorption spectra. A clear peak change from 580 to 567 nm can be observed during increased radiation of the same crystal, and a regeneration after annealing.



Data Set	CmpII pH 6.8	
	Over all	Outer Shell
Low resolution limit ( $\text{\AA}$ )	34.0	2.2
High resolution limit ( $\text{\AA}$ )	2.0	2.0
Rmerge	0.074	0.309
Mean(I)/sd(I)	10.2	3.2
Completeness (%)	95.3	94.6
Multiplicity	2.2	1.9

### 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  $\text{Fe}^{\text{IV}}\text{O}$ . *Submitted March 2007*
- [2] Hersleth, H.-P., Combining protein crystallography and single crystal light absorption spectroscopy – Structures of intermediates in the reaction between myoglobin and peroxides.. *19<sup>th</sup> Nordic Structural Chemistry Meeting*, 8<sup>th</sup> – 11<sup>th</sup> January 2007, Spåtind, Norway, Lecture