SNBL	**Experiment title**: BAG proposal in Macromolecular Crystallography for the University of Oslo	**Experiment number**: 01-02-784
Beamline: BM01A	**Dates of experiments**: From: 13-JUN-08 08:00 to: 17-JUN-08 08:00	**Date of report**: 29-AUG-08
Shifts: 12	**Local contact(s)**: Dr. Dmitry CHERNYSHOV	*Received at UNIL:*

Names and affiliations of applicants (* indicates experimentalists):

Prof. K. Kristoffer Andersson, Department of Molecular Biosciences, Univ. of Oslo, Norway
Prof. Carl Henrik Gørbitz, Department of Chemistry, Univ. of Oslo, Norway
Prof. Ute Krengel, Department of Chemistry, Univ. of Oslo, Norway
Dr.scient. Hans-Petter Hersleth, Department of Molecular Biosciences, Univ. of Oslo, Norway*
Cand.scient Åsmund K. Røhr, Department of Molecular Biosciences, Univ. of Oslo, Norway*
Mast. Sci.. Inger Kirstine Oslbu, Department of Molecular Biosciences, Univ. of Oslo, Norway*

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 microspectrophotometry at SNBL previously. This time the different states, as well as the radiation induced changes were studied by single crystal online Raman spectroscopy. The Raman spectroscopy complement the X-ray diffraction and microspectrophotometry studies, and gives further insight into the specific and subtle changes in the haem enviroment. Assigment of the Raman peaks, and interpretation of the observed changes in the Raman spectra are ongoing. In Figure 1, the single crystal Raman spectra (at 785 nm exication) for the different states: resting ferric, compound II and compound III are shown before and after X-ray diffraction data collection as well as after annealing of the X-ray exposed crystal. Several changes are observed, which need to be further interpreted. Microspectrophotometry was used to confirm the states, and controll X-ray diffraction data sets were collected for the different states. A reduced laser power had to be used not to destroy the haem protein crystals, especially the compound III state was very easily destroyed. For the next beamtime oxygen-18 and deuterium exchange experiment will be performed to futher characterise the changes.

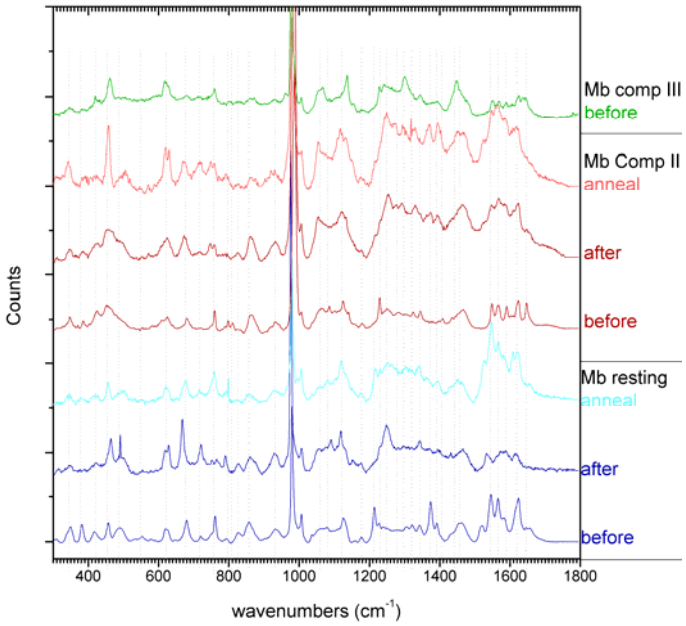

Figure 1: *Single-crystal Raman spectroscopy of the different state sof myoglobin before and after X-ray exposure*

2. Ribonucleotide Reductase proteins

The enzyme Ribonucleotide Reductase (RNR) converts the four ribonucleotides to their corresponding deoxyribonucleotides that are necessary for DNA synthesis.Currently there are 3 major classes of RNR that have been characterized, all of them utilizing different cofactors to initiate enzymatic catalysis. The class I RNR has been further divided into two subclasses, class Ia and class Ib. Eukaryotes encode the class Ia type only, whereas prokaryotes can depend on both. Interestingly, it seems that class Ib RNR can provide deoxyribonucleotides under both aerobic and anaerobic growth conditions. The Ribonucleotide Reductase system in *Bacillus cereus* consists of at least 4 proteins; NrdE, NrdF, NrdH, and NrdI. We have performed studies of the flavine co-factor in NrdI at SNBL. Until recently the function of this protein was unknown, however, a novel paper propose that it can reduce the damaged di-iron centre in NrdF and help reactivating the di-iron-tyrosine radical site in NrdF (I. Roca, *et al.*, 2008).When the oxidized flavin mononucleotide (FMN) cofactor in NrdI is reduced to the semiquinone form the protein undergoes a structural change. In addition to determine the ~1.1 Å 3D- structure of NrdI we have conducted a spectroscopic investigation of the flavin cofactor in the protein crystal. Both the oxidized and semiquinone forms of FMN in NrdI single crystals have been characterized with light absorption and Raman spectroscopy prior to and after data collection.

Our goal is to relate the Raman spectra to the structural changes of the flavin during data collection, and also get an improved understanding of the electron transfer mechanism in NrdI. Our results indicate quite dramatic changes in the Raman spectrum of the oxidized FMN cofactor upon data collection. We also observe quite different Raman spectra for the oxidized and Semiquinone form. The FMN redox states in the crystals have been verified from the 3D structure. Work is on the way to assign the peaks to the corresponding FMN vibration frequencies.

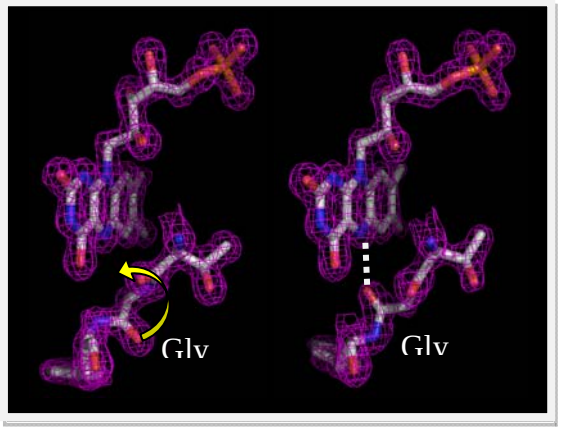

***Figure 2**: A peptide binding swaps its conformation upon reduction of the FMN_{ox} to the FMN_{sq} and a cofactor-protein H-bond is formed.*

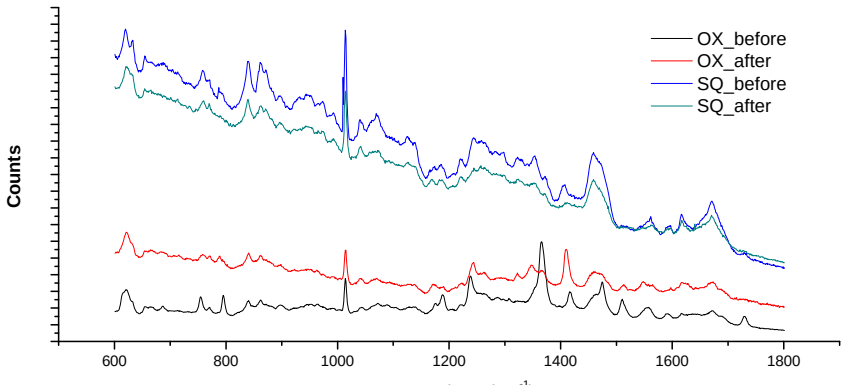

***Figure 3**: Raman spectra of NrdI single crystals with the FMN cofactor in the oxidized (OX) for semiquinone (SQ) from before and after data collection. The laser excitation frequency was 785 nm.*

Related Publications in this periode using SNBL data:

Hersleth, H.-P., Hsiao, Y.-W., Ryde, U., Görbitz, C. H. & Andersson, K. K. (2008) The crystal structure of peroxymyoglobin generated through cryoradiolytic reduction of myoglobin compound III during data collection. *Biochem. J.* **412**, 257-264.

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. (2008) Reactive Complexes in Myoglobin and Nitric Oxide Synthase. *Inorg. Chim. Acta* **361**, 831-846.

Hersleth, H.-P., Hsiao, Y.-W., Ryde, U., Görbitz, C. H. & Andersson, K. K. (2008) On the Quest for Metalloprotein Reaction Intermediates in the Radiation Damage Landscape of Protein Crystallography. Lecture. 44th Norwegian Biochemical Society Contact Meeting, 17th–19th Jan 2008, Lillehammer, Norway

Røhr, Å. K., Hersleth, H.-P. & Andersson. K. K. (2008) Electron transfer proteins in the class Ib ribonucleotide reductase system from Bacillus cereus. Poster. 44th Norwegian Biochemical Society Contact Meeting, 17th–19th Jan 2008, Lillehammer, Norway