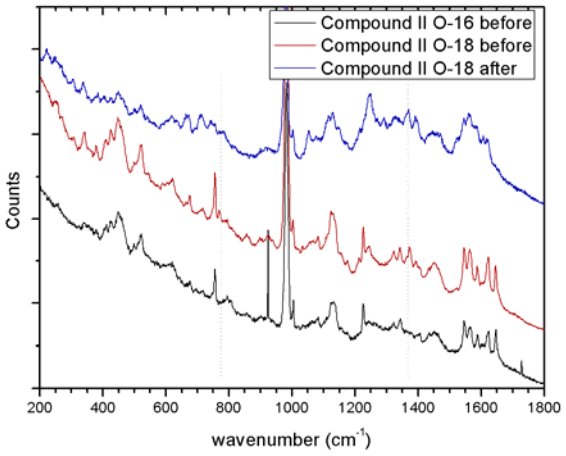
SNBL	**Experiment title**: BAG proposal in Macromolecular Crystallography for the University of Oslo	**Experiment number**: 01-02-826
Beamline: BM01A	**Dates of experiments**: From: 06-FEB-09 08:00 to: 10-FEB-09 08:00	**Date of report**: 28-FEB-09
Shifts: 12	**Local contact(s)**: Yaroslav FILINCHUK	*Received at UNIL:*

Names and affiliations of applicants and users (* 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. Hans-Petter Hersleth*, M.Sc. Åsmund K. Røhr*, Department of Molecular Biosciences, Uni. of Oslo, Norway
M.Sc.Gabriele Cordara*, M.Sc.Steffi Munack*, Siri Trygsland*, Espen Haugen* at Dept. of Chemistry, Uni. 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 light absorption microspectrophotometry and online Raman spectroscopy at SNBL previously. This time we supplemented the Raman spectrosopy data collected last time. To be able to assign the oxygen generated Raman modes in Mb after the reaction with hydrogen peroxide we used O-18 enriched hydrogen peroxide to assign them since they would shift about 25 cm^{-1} compared to when O-16 hydrogen peroxide was used. A dataset of this intermediate genereated with O-18 was collected to 1.3 Å. In addiation a dataset of the compound III to 1.5 Å was collected with both Raman spectra and light absorption spectra before and after data collection. 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 the figure to the right the single crystal Raman spectra (at 785 nm exication) for the compound II generated with O-16 and O-18 hydrogenperoxide are shown before and after X-ray diffraction data collection. Several changes are observed, which need to be further interpreted.



2. THE NrdI FLAVOPROTEIN

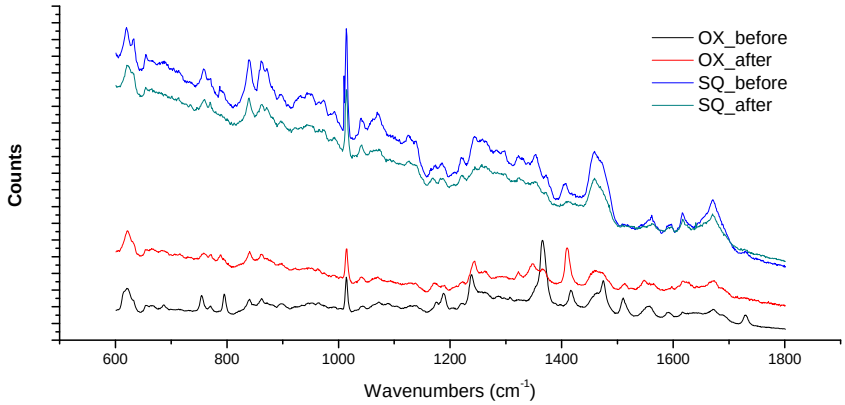
The enzyme Ribonucleotide Reductase (RNR) converts the four ribonucleotides to their corresponding deoxyribonucleotides that are necessary for DNA synthesis. The Class Ib Ribonucleotide Reductase system in *Bacillus cereus* consists of at least 4 proteins; NrdE, NrdF, NrdH, and NrdI. We have performed 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 oxidised flavin mononucleotide (FMN) cofactor in NrdI is reduced to the semiquinone form the protein undergoes a structural change. We have determined structures of the NrdI protein with the oxidised and semiquinone cofactor to 1.10 and 1.15 Å, respectivly. Raman spectra were recorded before and after data collection for both states (see figure below) of the crystal with the new Raman probe equipped with a camera assisting focusing the laser beam. The new setup with a camera mounted at the laser probe was indeed helpful, saving us many hours searching for a suitable orientation of the crystal for recording Raman spectra.

To examine the radiation induced reduction of the oxidized FMN cofactor a crystal was exposed to X-rays for a limitited amount of time prior recording of Raman spectra before and after annealing. Doing this enabeld us to obsere which peaks in the Raman spectrum that change upon X-ray radiation.

Work is in progress to assign the Raman modes that we observe prior and after data collection for both the oxidized and semiquinine form of the NrdI protein. In addition we plan to conduct ONIOM optimisations on the NrdI protein and DFT frequency calculations on the cofactor in both redox states. Comparing experimental spectra and structures with those calculated will possibly able us to relate spesiffic changes in spectra for the oxidised and semiquinone form of FMN with the structures and also give us insight in the mechansim of radiation damage to the FMN cofactor.



3. MARASMIUS OREADES AGGLUTININ

Marasmius Oreades Agglutinin is a lectin showing high affinity for the disaccharide unit Gal-alpha-1,3-Gal when displayed on carbohydrate chains in terminal position. The presence of this disaccharide on the human blood groub type B branched trisaccharide and on the Galili/xenotransplantation epitope makes this lectin a useful tool both for blood typing and as a molecular marker.

The protein has an N-terminal ricin B chain-like sugar binding domain, featuring three sugar binding sites with the same specificity, but different affinity, and a C-terminal domain of unknown function characterized by an alpha/beta fold. Although showing no significative sequence homology with any other protein (put aside a lectin from the mushroom *Polyporus Squamosus*, which is structurally and functionally not yet characterized), the recently published three-dimensional structure of MOA points to a structural similarity of the C-terminal domain to many different enzymes, among those N-glycanases, N-acetyltransferases, transglutaminases and cysteine proteases. Particularly striking is the conservation of the geometry of the catalytic triad (His218/Cys191/Asp235) in yeast peptide *N*-glycanase (yPNGase) and equivalent residues in MOA (His257/Cys215/Gln274) – residues, which are accessible and reactive (as confirmed by the modification of Cys215 by MalNEt, which was used as an additive for crystallization).

Among the various strategies adopted to asses the possible catalytic activity of MOA, we are trying to obtain evidence of binding by a PNGase peptide innhibitor: a preliminary dataset from a MOA crystal soaked with the inhibitor, collected on our in-house diffractomer, showed binding of the inhibitor to MOA, but the data set is not optimal and we therefore attempted to get better data (and higher resolution) at the SNBL. While we did obtain a data set of good quality, the resolution was even slightly worse (3.0 Å compared to 2.7 Å) and we do not observe evidence for inhibitor binding. This could be

due to the use of different crystal forms of MOA, which, although relatively faster growing (few weeks versus three months) are more tightly packed and might therefore be unsuitable for soaking. This will have to be further investigated.

Data Set	*MOA_Inh2*	
	Over all	Outer Shell
Low resolution limit (Å)	34.38	3.16
High resolution limit (Å)	3.00	3.00
Rmerge	0.090	0.304
Mean(I)/sd(I)	13.3	3.5
Completeness (%)	99.3	99.9
Multiplicity	4.9	4.8

4. CHORISMATE MUTASE

Chorismate mutase formally catalyses the Claisen rearrangement of chorismate to prephenate within the shikimate pathway. This pathway and hence chorismate mutase only exists in bacteria and fungi but not in plants and animals, so that inhibitors of the enzyme may be potential drugs to treat microbial infections. Based on the solved three-dimensional structure of the secreted chorismate mutase from *M. tuberculosis*, a structure-driven drug design approach is currently being undertaken, and it has already led to the synthesis of potential inhibitor molecules.

Several crystals of the secreted chorismate mutase *MtCM were soaked with synthesized inhibitors and have been screened, but no dataset could be collected due to high mosaicity and ice-formation on crystals.

5. BACTERIAL TOXINS

The lectin domains of two bacterial toxins are currently under investigation to shed further light on host-pathogen recognition mechanisms: Cholera Toxin B subunit (CTB), from *Vibrio cholerae* (ElTor biotype) and Heat Labile Enterotoxin B subunit, (LTB) from *E. coli*.

It is suspected that binding of CT to blood group determinants plays a major role in the infection process, and understanding the binding geometry and the molecular determinants involved in the interaction is a key factor in formulating hypotheses about the mechanism of action of the toxin itself.

Crystals of CTB from the ElTor biotype of *Vibrio cholerae* co-crystallized with the GM1 receptor were screened, but no dataset could be collected due to very weakly or badly diffracting crystals.

A peculiar feature of LTB is that it displays ligand promiscuity, whereas CTB is specific for GM1 in spite of almost identical binding sites: further analysis of LTB-GM1 interaction by means of X-ray diffraction is a good tool to determine the structural determinants at the heart of the change in specificity.

Two crystals of the LTB-sugar complex were screened at SNBL, but none of them gave any data that could be used for structure determination. Further refinement of crystallization conditions is underway to produce bigger or better diffracting crystal forms of the complex.

Related Publications in this periode using SNBL data:

Hersleth, H.-P., Hsiao, Y.-W., Ryde, U., Görbitz, C.H. & Andersson, K.K. (2008) The Influence of X-Rays on the Structural Studies of Peroxide-Derived Myoglobin Intermediates. *Chem. Biodiv.* **5**, 2067-2089.

Hersleth, H.-P., Hsiao, Y.-W., Ryde, U., Görbitz, C.H. & Andersson, K.K. (2008) Structural Studies of the Intermediates in the Reaction Between Myoglobin and Peroxides. Poster P78 Abstract Book. *9th European Biological Inorganic Chemistry Conference*, 2nd-6th September 2008, Wroclaw, Poland.

Hersleth, H.-P., Hsiao, Y.-W., Ryde, U., Görbitz, C.H. & Andersson, K.K. (2008) Structural studies of the intermediates in the reaction between myoglobin and peroxides. Lecture PX-S05-4. Abstract Book. *The 8th International Peroxidase Symposium*, 20th-24th August 2008, Tampere, Finland.

Røhr, Å.K., Tomter, A.B, Sandvik, G.K., Bergan, J., Barra, A.L., Nilsson, G.E, Strand, K.R, & Andersson. K.K. (2008) Spectroscopic and Structural Studies of Iron Center and Tyrosyl Radical in Mammalian, Fish and Bacterial Ribonucleotide Reductase. Session Lecture SL32 Abstract Book. *9th European Biological Inorganic Chemistry Conference*, 2nd-6th September 2008, Wroclaw, Poland.