| SNBL | **Experiment title**:<br>BAG proposal in Macromolecular Crystallography for the University of Oslo | **Experiment number**:<br>01-02-732 |
|---|---|---|
| **Beamline**:<br>BM01A | **Dates of experiments**:<br>From: 18-FEB-05 08:00 to: 21-FEB-05 08:00 | **Date of report**:<br>28-FEB-06 |
| **Shifts:**<br>9 | **Local contact(s)**:<br>Dr. Philip PATTISON | *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
Ass. Prof. Ute Krengel, Department of Chemistry, Univ. of Oslo, Norway
Dr. Mats Ökvist, Department Chemistry, Univ. of Oslo, Norway*
Cand.scient Hans-Petter Hersleth, Department of Chemistry, Univ. of Oslo, Norway*
Cand.scient Åsmund K. Røhr, Department of Molecular Biosciences, Univ. of Oslo, Norway*
Siv.ing. Glareh Askarieh, Department of Chemistry, Univ. of Oslo, Norway*


## 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. This time we continued to try to trap new intermediates as well as studying further the radiation influenced reduction of some of our states. Two datasets were collected at 165 K which is just above the glass-transition for our cryo-solution. This would lead to more movements, and possible more radiation damage. We could for the intermediate that does not normally get reduced observe reduction. We also collected data on a crystal that had been irradiated with intense white radiation at ID09B. In addition some partial datasets were collected for radiation damage purposes

| *Data Set* | *Mb-cmp-III-303* | | *Mb-cmp-II-210* | | *Mb-rest-111* | |
|---|---|---|---|---|---|---|
| | Over all | Outer Shell | Over all | Outer Shell | Over all | Outer Shell |
| Low resolution limit (Å) | 34.0 | 1.9 | 28.5 | 1.53 | 34.0 | 1.95 |
| High resolution limit (Å) | 1.8 | 1.8 | 1.45 | 1.45 | 1.85 | 1.85 |
| Rmerge | 0.123 | 0.437 | 0.072 | 0.415 | 0.135 | 0.438 |
| Mean(I)/sd(I) | 11.1 | 3.1 | 9.7 | 2.1 | 6.9 | 2.8 |
| Completeness (%) | 99.9 | 99.9 | 98.5 | 99.5 | 89.6 | 90.9 |
| Multiplicity | 3.5 | 3.5 | 2.4 | 2.4 | 2.3 | 2.2 |

## 2. MOUSE RIBONUCLEOTIDE REDUCTASE

A datasett of the apo mouse R2 protein was collected. Earlier studies have indicated some strange rearagnments of Phe side chains upon iron binding in the protein. We want to confirm that this observation is linked to the activation process of the protein. The second dataset (not fully processed yet) was collected at a crystal soaked in ferrous iron an methanol. The goal was to reproduce the trinuclear metal cluster previously observed in this protein. These datasets are currently under investigation.

| *Data Set* | *mR2_1* | |
|---|---|---|
| | Over all | Outer Shell |
| Low resolution limit (Å) | 37.01 | 2.21 |
| High resolution limit (Å) | 2.10 | 2.10 |
| Rmerge | 0.075 | 0.277 |
| Mean(I)/sd(I) | 14.5 | 4.1 |
| Completeness (%) | 97.8 | 97.6 |
| Multiplicity | 3.9 | 3.7 |

## 3. LECTINS

Several lectins are under current investigation, in order to reveal the structural determinants of protein-carbohydrate interactions. In particular, our research targets two highly homologous legume lectins from *E. cristagalli* (ECL) and *E. corallodendron* (ECorL) as well as the lectin from the *Marasmius oreades* mushroom (MOA), which all have related but distinct binding properties. For MOA, a 2.5 Å (native) data set is available. Several heavy atom derivatives has been tested with negative results in search of one which would yield high enough resolution (possibly at a higher brilliance beamline) to give useful phase information. A related project concerns the lectin domains of heat-labile enterotoxin from the bacterium *E. coli*, cholera toxin and interesting toxin hybrids and their interactions with carbohydrate ligands. Extensive screening of crystals with new and previously tried ligands has been performed with limited success due mainly to small crystal size.

## 4. 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 (BsCMCit). We have very recently obtained crystals of this protein in complex with the transition state analog (TSA) and a first data set has been collected at the SNBL (see below). We expect to collect higher quality data at a later date as the crystal showed some problems with ice-rings, and possibly a satellite crystal, which has yet to be resolved. The structure of the exported chorismate mutase from Mycobacterium tuberculosis has recently been published. To further examine the question if there is any binding site for feed-back regulation by aromatic amino acids soaking experiments has been performed with phenyl alanine and tryptophan. Two data sets were recently collected and are currently being evaluated (see below). Preliminary results indicate the need to collect higher resolution data.

*Preliminary* processing results for datasets:

| Data Set | MtCM +Trp | | MtCM +Phe | | BsCMCit +TSA | |
|---|---|---|---|---|---|---|
| | Over all | Outer Shell | Over all | Outer Shell | Over all | Outer Shell |
| Low resolution limit (Å) | 14.79 | 2.42 | 42.95 | 2.37 | 20.37 | 1.95 |
| High resolution limit (Å) | 2.30 | 2.30 | 2.25 | 2.25 | 1.85 | 1.85 |
| Rmerge | 0.111 | 0.55 | 0.073 | 0.30 | 0.087 | 0.43 |
| Mean(I)/sd(I) | 10.23 | 2.10 | 10.18 | 2.54 | 9.56 | 3.67 |
| Completeness (%) | 99.54 | 99.55 | 96.90 | 97.71 | 89.44 | 74.62 |
| Multiplicity | 3.53 | 3.44 | 2.05 | 2.04 | 1.86 | 1.82 |

**Related Publications in this periode using SNBL data**:


[1] Hersleth, H.-P., Ryde, U., Rydberg, P., Görbitz, C. H. & Andersson, K. K. (2006) Structures of the high-valent metal-ion haem-oxygen intermediates in peroxidases, oxygenases and catalases. *J. Inorg. Biochem.* (Review in press)

[2] Kolberg M., Logan D.T., Bleifuss G., Potsch S., Sjoberg B.M., Graslund A., Lubitz W., Lassmann G.N., Lendzian F. (2005) A new tyrosyl radical on Phe(208) as ligand to the diiron center in Escherichia coli ribonucleotide reductase, mutant R2-Y122H - Combined X-ray diffraction and EPR/ENDOR studies. *J. Biol. Chem.* **280** 11233-11246