SNBL	**Experiment title**: BAG proposal in Macromolecular Crystallography for the University of Oslo	**Experiment number**: 01-02-752
Beamline: BM01A	**Dates of experiments**: From: 24-AUG-06 08:00 to: 27-AUG-06 08:00	**Date of report**: 01-SEP-06
Shifts: 9	**Local contact(s)**: Dr. Philip PATTISON	*Received at UNIL:*

Names and affiliations of applicants (* indicates experimentalists):

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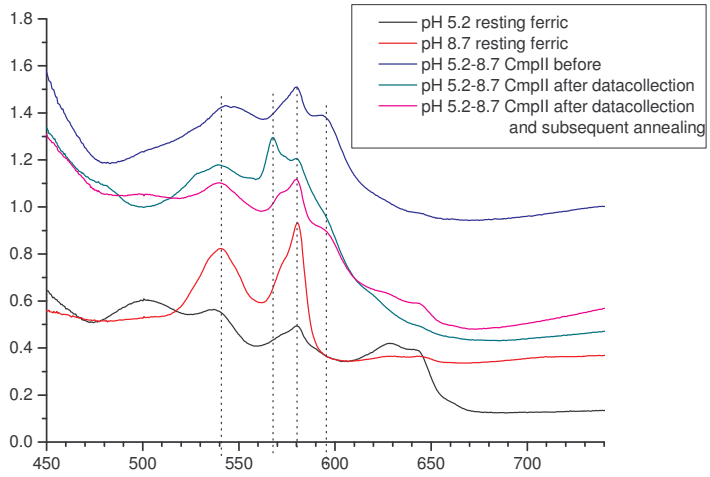
A GENERAL COMMENT

During this 9 shifts we experienced some heavy difficulties with the CCD detector and it's software which froze during data collection and also made some other faults. Due to this we were only able to collect a few datasets, and not as much as we had planned.

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 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 four short 1 hour datasets were collected to investigate further the potential radiation damage of the compound II state at different pH's. As seen from the single crystal light absorption spectra some damage is observed, but interesting not a reduction back to the resting state, but to an unstable intermediate state.



Data Set	*CmpII pH 5.2*		*CmpII pH 6.8*		*CmpII pH 8.7*	
	Over all	Outer Shell	Over all	Outer Shell	Over all	Outer Shell
Low resolution limit (Å)	34.0	1.5	34.0	1.5	34.0	1.5
High resolution limit (Å)	1.5	1.6	1.5	1.6	1.5	1.6
Rmerge	0.064	0.209	0.158	0.466	0.082	0.404
Mean(I)/sd(I)	10.2	2.9	7.6	2.3	10.2	2.6
Completeness (%)	73.3	67.6	81.0	81.0	95.8	89.4
Multiplicity	1.6	1.5	2.2	1.9	1.8	1.7

2. MOUSE RIBONUCLEOTIDE REDUCTASE

The enzyme ribonucleotide reductase (RNR) catalyses the reduction of all four ribonucleotides to their corresponding deoxyribonucleotides, an essential step in the synthesis of DNA in all living cells. This time two datasets were collected. One with an attempt to co-crystallize mouse ribonucleotide reductase with the inhibitor resveratrol. The second with a peptide inhibitor c-130 that prohibits the protein-protein interaction of mouse ribonucleotide Reductase R1 and R2 subunit. Preliminary analysis indicates a successful binding of the inhibitor to the R2 protein.

Data Set	R2 with resveratrol		R2 with c-130	
	Over all	Outer Shell	Over all	Outer Shell
Low resolution limit (Å)	34.0		34.0	
High resolution limit (Å)	2.50		2.20	
Rmerge	0.116	0.412	0.118	0.56
Mean(I)/sd(I)	11.2	1.9	10.6	2.2
Completeness (%)	96	73	99	100
Multiplicity	3.4	2.5	3.5	3.5

3. FAB MURINE 7A7

Ligand-induced signaling from receptor tyrosine kinases (RTKs) of the epidermal growth factor receptor (EGFR) family regulates many cellular processes, including proliferation, cell motility, and differentiation. Perturbations in these cellular signals can lead to malignant transformation, and the correlation between EGFR and cancer has been firmly established. Antibodies have being developed against the extracellular domain of the EGFR aiming for the inhibition of the binding to the EGF molecule. We are interested in the structural studies of three of those antibodies, isolated or in complex with the receptor. The name of these antibodies are: the murine antibody mR3, its humanized hR3 and another murine, the called 7A7. The structure of Fab hR3 has already been determined and crystals of Fab 7A7 has been obtained. We have collected a complete data set of the Fab fragment of the antibody 7A7 to about 2.0 Å resolution at SNBL. We have not yet been able to resolve the space group, since we have a problem with multiple diffraction patterns.

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.* **100**, 460-476 (Review)

[2] Hersleth, H.-P., Uchida, T., Røhr, Å. K., Teschner, T., Schünemann, V., Nilsson, K., Hsiao, Ya-wen, Trautwein, A. X., Ryde, U., Görbitz, C. H., Kitagawa, T., & Andersson, K. K. Structural Studies of intermediates in the reaction between myoglobin and peroxides. *38th International School of Crystallography: The Structure and Function of Large Molecular Assemblies,* 9th –18th June 2006, Erice (Sicilia), Italy

[3] Hersleth, H.-P., Uchida, T., Røhr, Å. K., Teschner, T., Schünemann, V., Nilsson, K., Hsiao, Ya-wen, Trautwein, A. X., Ryde, U., Görbitz, C. H., Kitagawa, T., & Andersson, K. K. Structural studies of intermediates in the myoglobin-peroxide reaction. *8th European Conference on Biological Inorganic Chemistry,* 2nd – 6th July 2006, Aveiro, Portugal.

[4] Hersleth, H.-P., Uchida, T., Røhr, Å. K., Teschner, T., Schünemann, V., Nilsson, K., Hsiao, Ya-wen, Trautwein, A. X., Ryde, U., Görbitz, C. H., Kitagawa, T., & Andersson, K. K. Structures of intermediates in the myoglobin-peroxide reaction. *Peroxidase 2006 meeting* 6th–9th July 2006, Aveiro, Portugal..

[5] Andersson, K. K., Hersleth, H.-P., Uchida, T., Røhr, Å. K., Teschner, T., Schünemann, V., Nilsson, K., Hsiao, Ya-wen, Trautwein, A. X., Ryde, U., Görbitz, C. H., Kitagawa, T. Crystallographic and spectroscopic studies of two of the intermediates in the myoglobin-peroxide reaction. *The 20th IUBMB International Congress of Biochemistry and Molecular Biology and 11th FAOBMB Congress, Japan.*

[6] Andersson, K. K., Spectroscopic studies of the murine and human p53 induced ribonucleotide reductase R2 protein. *8th European Conference on Biological Inorganic Chemistry,* 2nd – 6th July 2006, Aveiro, Portugal, S7.6