SNBL	**Experiment title**: BAG proposal in Macromolecular Crystallography for the University of Oslo - Studies of proteins in the Oslo region	**Experiment number**: 01-02-696
Beamline: BM01A	**Dates of experiments**: From: 18-JUN-05 08:00 to: 21-JUN-05 08:00	**Date of report**: 01-AUG-05
Shifts: 9	**Local contact(s)**: 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

Cand.scient Hans-Petter Hersleth, Department of Chemistry, Univ. of Oslo, Norway*

Dr. Matthias Kolberg, Department of Molecular Biosciences, Univ. of Oslo, Norway*

Dr. Armelle Varnier Agasøter, Department of Molecular Biosciences, Univ. of Oslo, Norway*

1. Crystal structures of nitrogen oxides synthase

Nitrogen oxide synthase (NOS) is the protein responsible for the generation of NO through the conversion of the amino acid L-argine to L-citrulline in two reaction cycles. We collected last time native data for the bovine endothelial NOS oxygenase domain (beNOSox) expressed in *e.coli*. This time we soaked the crystal with a biopterin analog: 4-aminobiopterin. Microspectrophotometry was used to check the state of the beNOSox crystals before and after data collection.

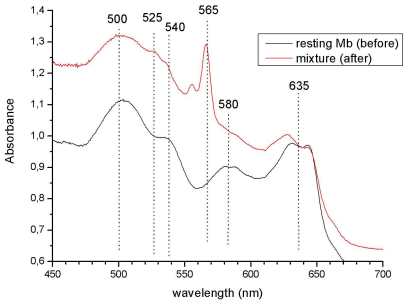
Dataset	Completeness	R(sym)	Resolution	I/sI
nos05	98.5 (90.7)	0.086 (0.39)	2.80	13.5 (3.6)

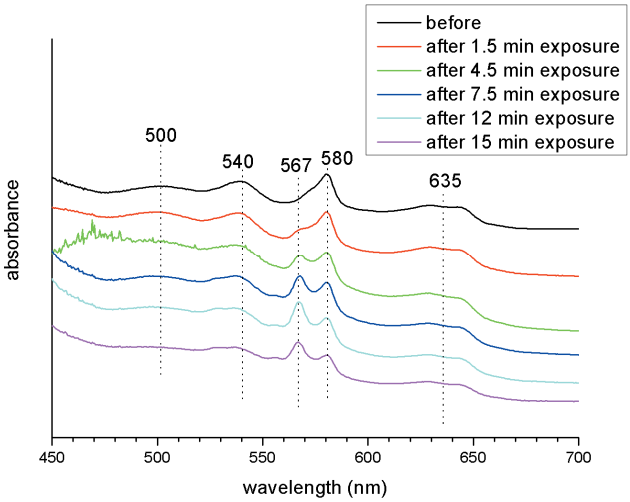
Values in parentheses are for the highest resolution bin

2. Crystal structures of 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 another compound III by the synchrotron radiation. The resting form also get reduced by the synchrotron radiation.

Several short datasets were obtained to try to overcome the effect of radiation damage (reduction of metal centre by radicals produced by the high-energy X-ray beam). We have seen that by using 1/20 of the exposure time for a normal myoglobin dataset, a mixture of the normal resting form and the radiation reduced form (peaks at 525 and 565 nm) were observed by microspectrophotometry as seen in the figure to the right. The structure of this mixture showed a longer Fe-O distance than the fully radiation reduced form. This time even shorter exposure time was investigated. A dataset of total exposure of 15 min was checked with microspetrophotometry every 2-5 min. This showed that the formation of the reduced intermediate occurred already after 1.5 min but at low percentage (see figure to the right). We then collected 6 datasets to try to recombine equally exposed parts of these datasets to be able to follow the changes shown by microspectrophotometry by structure.





In addition short datasets of compound III were obtained at both pH 6.8 and 8.7.

Related Publications in this periode using SNBL data:

[1] Hersleth, H.-P., Uchida, T., Teschner, T., Røhr, Å. K., Shünemann, V., Nilsson, K., Hsiao, Ya-wen, Trautwein, A. X., Ryde, U., Görbitz, C. H., Kitagawa, T., & Andersson, K. K. (2005) Combination of protein crystallography and microspectrophotometry. Trapping intermediates in the myoglobin-peroxide reaction cycle. *3rd Norwegian User Meeting – Application of Synchrotron Radiation,* 10th – 11th March 2005, Trondheim, Norway. (poster)

[2] Hersleth, H.-P., Uchida, T., Teschner, T., Røhr, Å. K., Shünemann, V., Nilsson, K., Hsiao, Ya-wen, Trautwein, A. X., Ryde, U., Görbitz, C. H., Kitagawa, T., & Andersson, K.K. (2005) Structural Studies of the intermediates in the myoglobin-peroxide reaction. *37th International School of Crystallography: Evolving methods in macromolecular crystallography,* 12th –22nd May 2005, Erice (Sicilia), Italia, P18, 198 (poster)

[3] Hersleth, H.-P., Uchida, T., Teschner, T., Røhr, Å. K., Shünemann, V., Nilsson, K., Hsiao, Ya-wen,Trautwein, A. X., Ryde, U., Görbitz, C. H., Kitagawa, T., & Andersson, K. K., (2005) High resolution crystal structures of two of the intermediates in the myoglobin peroxide reaction. *11th International Conference on Bioinorganic Chemistry,* 31th July – 5th August 2005, Ann Arbor, USA.(poster)

[4] Strand, K.R, H.P. Hersleth, Å.K. Røhr, B. Dalhus, S. Karlsen, M.Kolberg, K. Nilsson, Y. Hsiao, T.H. Rod, U. Ryde, C.H. Görbitz and K. K. Andersson (2005) Structures of the intermediates in the myoglobin-peroxide reaction and di and tri nuclear iron clusters in the R2 Subunit of Ribonucleotide Reductase. *The biannual 2003/2004 activity report and highlights of SNBL* (draft version) 3-5.

[5] Hersleth, H.P, T. Uchida, Å.K. Røhr, T. Teschner, V. Schnünemann, K. Nilsson, Y. Hsiao, T.H. Rod, A.X. Trautwein, U. Ryde, C.H. Görbitz, T. Kitagawa, and K. K. Andersson (2005) High resolution crystal structures of two of the intermediates in the peroxide reaction of myoglobin. *15th IUPAB & 5th EBSA International Biophysics Congress*. August 27th–1st September 2005, Montpellier, France (poster)

[6] Strand K.R., Å. K. Røhr, B. Dalhus, S. Karlsen, M. Kolberg, C.H. Gørbitz, A.-L. Barra and K.K. Andersson (2005) A novel cluster and spectroscopic studies of the radical iron site in mouse ribonucleotide reductase *15thIUPAB & 5th EBSA International Biophysics Congress,* August 27th–1st September 2005, Montpellier, France A. 107. (talk and poster)

[7] Talks K. Kristoffer Andersson

9 March 2005, Department of Chemistry, NTNU, Trondheim. Recent spectroscopical, mechanistic and structural studies of oxygen or hydrogen peroxide complexes of heme proteins myoglobin and Nitric Oxide Synthase.

11 March 2005, 3rd Norwegian User meeting -Application of Synchrotron Radiation SNBL, Trondheim A novel cluster, Crystallographic and spectroscopic studies of the radical-iron site in mouse ribonucleotide reductase