



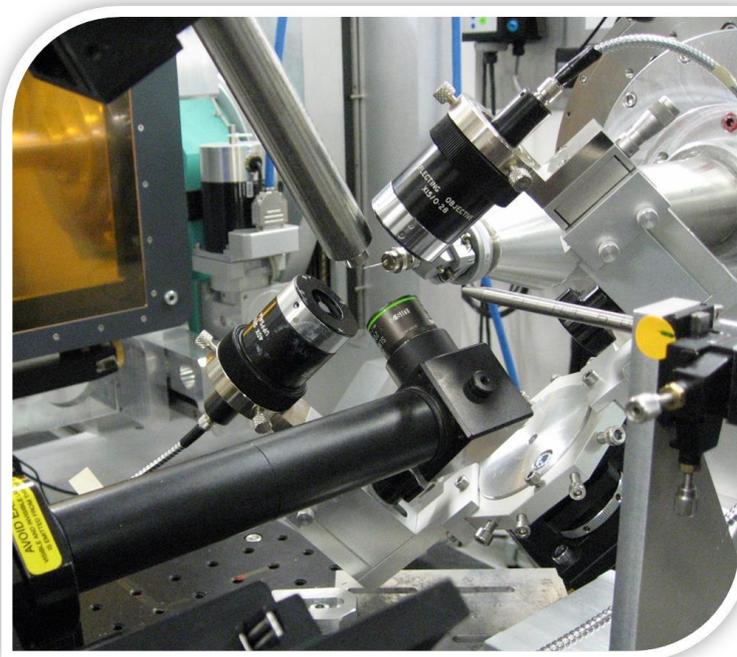
Experiment title: BAG proposal in Macromolecular Crystallography for the University of Oslo & Oslo University Hospital	Experiment number: 01-02-955	
Beamline: BM01A	Dates of experiments: From: 01-MAR-13 08:00 to: 05-MAR-13 08:00	Date of report: 30-JUL-13
Shifts: 12	Local contact(s): Vadim DIADKIN	<i>Received at UNIL:</i>
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This report is for the 12 first shifts of proposal 01-02-955, the remaining 9 shifts will be scheduled during the autumn 2013.

1. IMPLEMENTING OF THE UV-VIS MICROSPECTROPHOTOMETER ONLINE AT THE PILATUS SETUP AT BM01A

The UV-vis microspectrophotometer was previously made online on the MAR345 setup at BM01A, but with the upgrading to the PILATUS setup the system had to be rebuilt. Part of the beamtime was used together with the SNBL team to make a first prototype of a combined UV-vis, Raman and X-ray setup with the new diffractometer at BM01A. This resulted in a initial setup that was usable, and worked quite nice, but need to be further developed for optimal use.

Figure show the initial setup with the UV-vis probe, Raman probe and cryo mounted simultaneously on the Pilatus X-ray setup.



2. MYOGLOBIN AND RADIATION DAMAGE

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, by using the new initial UV-vis/Raman/X-ray setup, we performed experiments with X-ray exposure together with real-time UV-vis spectroscopy monitoring of the radiation-damage to estimate the lifedoses with a shorter wavelength (0.7 Å) and lower flux than at standard protein beamlines. The effect of annealing above the glass transition temperature was also tested out.

3.FLAVODOXIN REDUCTASES FLD1 AND NRDI

There are three flavodoxins in *B. Cereus* that can represent the electron delivering system for both ribonucleotide reductase and nitric oxide synthase. We have previously solved the structure of FLD1 and NrdI, but several aspect remains to be assessed. When FLD1 and NrdI is chemically reduced a peptide flip of a glycine residue is observed above the flavin ring making a hydrogen bond to the flavin in the reduced state. If this flip can occur through X-ray induced reduction at low temperature, or if the crystal is heated above the glass transition temperature, was tried answered. Through the new setup a combined UV-vis monitoring, programmed temperature jumps, and several X-ray data sets collected at different points was performed. Additionally, chemically reduced data was collected as comparison.

All these studies were more test cases with the new setup, and these studies need to be complemented during the next beamtime.

Some related publications in this periode using SNBL data:

- Can M, Krucinska J, Zoppellaro G., Andersen NH, Wedekind J, Hersleth H-P, Andersson KK, Bren KL. Structural Characterization of Nitrosomonas europaea Cytochrome c-552 Variants with Marked Differences in Electronic Structure. (2013) *Chembiochem*, **14**, doi/10.1002/cbic.201300118.
- Røhr ÅK, Hammerstad M, Andersson KK. Tuning of Thioredoxin Redox Properties by Intramolecular Hydrogen Bonds. (2013) *PLoS ONE* **8**: e69411. doi:10.1371/journal.pone.0069411.
- Hersleth, H.-P., Røhr, Å.K., Lofstad, M., Hammerstad, M., Skråmo, S., Andersen, N.H., van Beek, W., Can, M., Zhao, X., Pompidor, G., Magliozzo, R.S., Bren, K.L. & Andersson, K.K. (2013). Poster. Using in situ single-crystal UV-vis and Raman spectroscopy to study the effect of X-ray radiation damage on the crystal structures of haem and flavoproteins. Poster. *16th International Conference on BioInorganic Chemistry*, 22nd–26th July 2013, Grenoble, France
- Hammerstad, M, Hersleth, H.-P., Tomter A.B., Røhr, Å.K. & Andersson, K.K. (2013). The Bacillus cereus class Ib ribonucleotide reductase NrdI-Fe2-NrdF protein complex structure favors a metal-dependent conformational basis for cofactor activation. Poster. *16th International Conference on BioInorganic Chemistry*, 22nd–26th July 2013, Grenoble, France.
- Hersleth, H.-P., Røhr, Å.K., Lofstad, M., Skråmo, S., van Beek, W., Pompidor, G. & Andersson, K.K. (2013) Using *in situ* single-crystal UV-vis and Raman spectroscopy to study the effect of X-ray radiation damage on the crystal structures of haem proteins. Lecture. *49th Norwegian Biochemical Society Contact Meeting*, 31th January – 3rd Jan 2013, Lillehammer,
- Hersleth, H.-P., Røhr, Å.K., van Beek, W., Pompidor, G. & Andersson, K.K. Radiation damage in haem and flavoproteins studied by *in-situ* single-crystal spectroscopy. Lecture. *The 27th European Crystallography Meeting (ECM27)*, 6th–11th August 2011, Bergen, Norway. *Acta Cryst. A* **68**, s135 (2012) Suppl.
- Hersleth, H.-P., Lofstad, M., van Beek, W., Pompidor, G. & Andersson, K.K. (2012) Using *in situ* single-crystal UV-vis and Raman spectroscopy to study the effect of X-ray radiation damage on the crystal structures of haem proteins. Lecture. *3rd Workshop on the Simultaneous Combination of Spectroscopies with X-ray Absorption, Scattering and Diffraction Techniques*, 4th–6th July 2012, Zürich, Switzerland.
- Røhr, Å.K., Hersleth, H.-P., W., Pompidor, G. & Andersson, K.K. (2012) Monitoring flavin X-ray radiation damage using single crystal spectroscopy. Poster. *3rd Workshop on the Simultaneous Combination of Spectroscopies with X-ray Absorption, Scattering and Diffraction Techniques*, 4th–6th July 2012, Zürich, Switzerland.
- Hersleth, H.-P., Lofstad, M., Røhr, Å.K., van Beek, W., Can, M., Zhao, X., Pompidor, G., Magliozzo, R.S., Bren, K.L. & Andersson, K.K. (2012). Poster. Using in situ single-crystal UV-vis and Raman spectroscopy to study the effect of X-ray radiation damage on the crystal structures of haem and flavoproteins. *11th European Conference on Biological Inorganic Chemistry*, 12th–16th September 2012, Granda, Spain.