SNBL	**Experiment title**: BAG proposal in Macromolecular Crystallography for the University of Oslo & Oslo University Hospital	**Experiment number**: 01-02-1040
Beamline: BM01A	**Dates of experiments**: From: 29-OCT-14 08:00 to: 04-NOV-14 08:00	**Date of report**: 20-JAN-15
Shifts: 18	**Local contact(s)**: Vadim DIADKIN	*Received at UNIL:*

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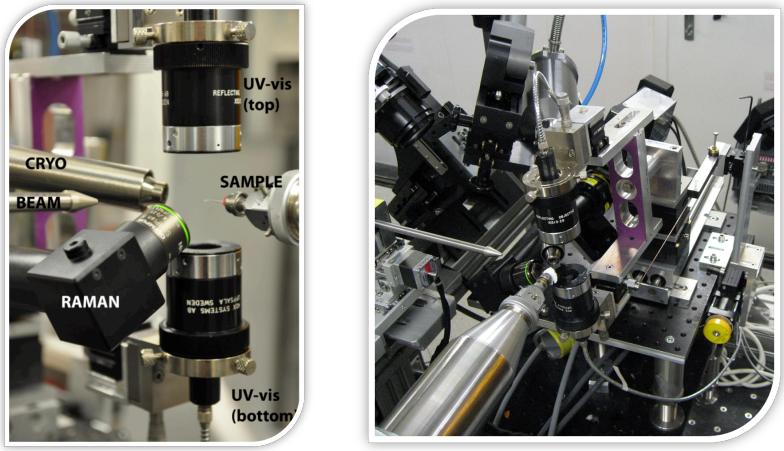
This report is for the 18 first shifts of proposal 01-02-1040.

In the spring 2014 Åsmund K. Røhr was at SNBL to update, build and automate a combined setup for X-ray diffraction, UV-vis and Raman spectroscopy at BM01A for *in situ* measurement. During the curretn beamtime the setup was tested on some model systems.



The new combined X-ray diffraction, UV-Vis and Raman spectroscopy setup at BM01A

VALIDATION OF NEW SINGLE CRYSTAL SPECTROSCOPY SETUP

The equipment was mounted according to the manual without any problems. It can be noted that the Raman laser intensity always should be controlled before starting the experiments. One limitation of the current setup is the UV-vis lamp that has low or no intensity below 350-400 nm. Since many biological cofactors absorb light in the near UV region important spectral information becomes unavailable.

The mounted equipment was stable throughout the experiment, and sample exchange did not disturb the position of the lenses. Also, the software interface and scripting language worked perfectly allowing a wide range of different experiments to run automatically.

SPECTROSCOPIC STUDIES OF LPMOs

The redox state of copper in crystals of lytic polysaccharide monooxygenases was investigated with Raman and UV-vis. We obtained excellent Raman spectra using the red laser, however, relevant Raman modes involving copper could not be assigned with certainty. For these experiments it would be an

advantage to use the green laser to enhance the relevant copper bands. This will be done during the next experiment. The LPMOs have weak UV-vis absorption bands in the spectral region around 400 nm, thus the current UV-vis lamp limits the sensitivity of these measurements.

MYOGLOBIN AND RADIATION DAMAGE

To be able to study the structure of redox proteins with X-ray diffraction, complementary spectroscopic methods are needed for two reasons: i) to prove the redox state in the crystals and ii) to monitor potential X-ray induced reduction of the redox site.

We have for many years studied the peroxidase reaction cycle in myoglobin (Mb) by trapping intermediates in the cycle, and these intermediates are very easily reduced by the X-rays. With the new combined setup we continued to study this model system with respect to spectroscopic monitoring of the radiation damage. With the new UV-vis/Raman/X-ray setup, we were able to automatically collect real-time UV-vis spectroscopy during X-ray exposure for determining the lifedoses for the different intermediates. By comparing this results with previous measurement we can see the influence of using a shorter wavelength (0.7 Å) and lower flux on the different intermidates. The data was also supplemented with Raman spectra taken before and after X-ray exposure.

COMBINED *IN SITU* STUDIES OF FLAVODOXIN-LIKE PROTEIN NRDI

Another of the model systems we used to test out the combined setup was the flavodoxin-like protein NrdI. For the oxidised state we were able to program a real-time monitoring of the X-ray radiation by using Raman spectroscopy. We were also able to generate the blue semiquinone state, and follow the radiation induceed reduction of this state with a combined UV-vis/Raman/X-ray setup. First the best orientation of the crystal for obtaining optimal UV-vis and Raman spectra was determined, and then a cycling with automatic taking consequitive UV-vis, Raman, X-Ray diffraction data was repeated five. This resulted in an advanced monitoring and characterisation of the radiation-induced reduction of this intermediat state, resulting in linking the spectroscopic changes with structural changes.

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**, 1828-1838.
- 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.
- Røhr, Å.K., Hersleth, H.-P., van Beek, W., Diadkin, V., Wiker, G., Chernyshov, D. & Anderson, K.K. Combining Protein X-Ray Crystallography and Single-Crystal Spectroscopy - A New *in situ* Setup at BM01A at ESRF. *Norwegian Synchrotron and Neutron User Meeting*, 19th–20th January 2015, Sola, Norway.
- Lofstad, M., Gudim, I., Skråmo, S., Hammerstad, M., Røhr, Å.K., Anderson, K.K. & Hersleth, H.-P. Crystallisation of ferredoxin/flavodoxin-NADP(H) oxidoreductases, flavodoxins, ferredoxins and redox partners in *Bacillus cereus*. *15th International Conference on the Crystallization of Biological Macromolecules (ICCBM15)*, 17th–20th September 2014, Hamburg, Germany.
- Hersleth, H.-P., Zhao, X., Magliozzo, R.S. & Andersson, K. K., Structural insight into the function and anti-TB pro-drug activation by KatG. *XXIII Congress and General Assembly of the International Union of Crystallography,* 5th–12th August 2014, Montreal, Canada. *Acta Cryst*. A**70**, C707 (2014).
- Skråmo, S., Lofstad, M., Monka, S., Hammerstad, M., Røhr, Å.K., Anderson, K.K. & Hersleth, H.-P. Structural studies of ferredoxin/flavodoxin-NADPH reductases, flavodoxins, ferredoxins and redox partners in *Bacillus cereus*. *18th Annual Conference of the Swedish Structural Biology Network*, 13th–16th June 2014, Tällberg, Sweden.
- Lofstad, M., Hersleth, H.-P., Røhr, Å.K., Hammerstad, M. & Andersson, K. K., Nitric oxide synthase and possible redox partners in *Bacillus cereus*. *XXIII Congress and General Assembly of the International Union of Crystallography,* 5th–12th August 2014, Montreal, Canada. *Acta Cryst*. A**70**, C1657 (2014).
- Hammerstad, M., Hersleth, H.-P., Tomter, A.B., Røhr, Å.K. & Andersson, K. K., Structural insight into the function and anti-TB pro-drug activation by KatG. *XXIII Congress and General Assembly of the International Union of Crystallography,* 5th–12th August 2014, Montreal, Canada. *Acta Cryst*. A**70**, C434 (2014).