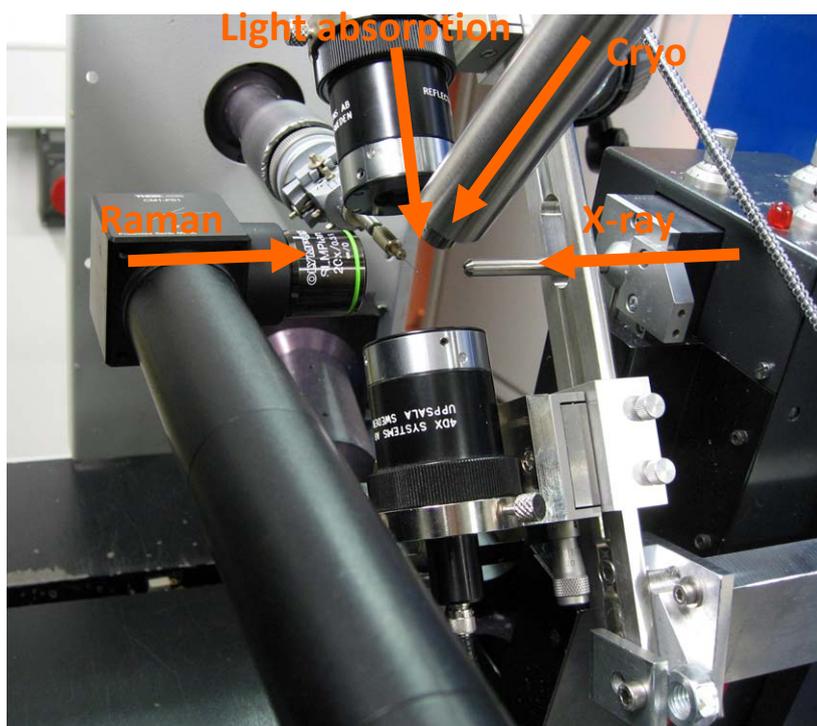




	<b>Experiment title:</b> Application for a combined online microspectrophotometry and Raman spectroscopy setup at BM01A SNBL for studies combining crystallography and spectroscopy, e.g. studies of haem- and flavoproteins.	<b>Experiment number:</b> MX-909
<b>Beamline:</b> BM01A	<b>Dates of experiments:</b> From: 05-NOV-09 08:00 to: 09-NOV-09 08:00	<b>Date of report:</b> 28-FEB-10
<b>Shifts:</b> 12	<b>Local contact(s):</b> Yaroslav FILINCHUK	<i>Received at UNIL:</i>
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### A combined setup for X-ray diffraction, online Raman spectroscopy and online microscpetrophotometry.

SNBL (BM01) has already a setup for online Raman spectroscopy, but have until now only had the microspectrophotometer for single-crystal light absorption studies offline. This application set out to put it online, and to test out the setup with haem- and flavoproteins. The setup can be seen below. Some futher improvement will be performed at a later stage. The setup was built by Wouter van Beek.



The combined setup was tested out on the haem protein myoglobin and the flavodoxin-like protein nrdI, and it worked nicely. Some small adjustments need to be made, but we were able to use the setup for investigating a few new aspects of our myoglobin research.

The main focus of the **MYOGLOBIN** project has been to investigate the peroxidase reaction cycle in myoglobin (Mb) by trapping intermediates in the reaction cycle. Several of these intermediates have been determined, the so-called compound II equivalent and the compound 0 equivalent as well as the resting state (metMb). Several of these states experience some radiation damage of the metal site as shown by light absorption microspectrophotometry and online Raman spectroscopy at SNBL previously. This time we tested out the new combined online microspectrophotometry and online Raman spectroscopy setup. We have previously followed the reaction:



in crystals with X-ray diffraction and off-line microspectrophotometry. This time we followed it with the combined online Raman and microspectrophotometry. The Raman will help to better characterise the different states by the observed changes of the different haem vibrations and specially the important Fe-O bond stretch during the reaction.

The high-valent ferryl form of myoglobin is proposed to oxidise biological active molecules as the neurotransmitter dopamin. We got initial datasets to 1.4 Å of the product from the reaction between ferrylMb and dopamin, and as well from the thymidine analog. Raman and light absorption supplemented these studies.

It has been suggested that reduced myoglobin can generate NO from reaction with nitrite. We got initial datasets of the product from the reaction with deoxyMb and nitrite, and Raman and light absorption supplemented the structure.

### **Related Publications in this periode using ESRF and BM01 data:**

- Andersson, K.K. & Hersleth, H.-P. (2009) Structural studies of the intermediates in the reaction between myoglobin and peroxides; effects of cryoradiolytic reduction. Lecture. *International Biological Inorganic Chemistry Conference*. 25<sup>th</sup> - 30<sup>th</sup> July 2009, Nagoya, Japan. *J. Biol. Inorg. Chem.* (Suppl. 1) **14**, S40.
- Andersson, K.K. & Røhr, Å.K. (2009) Stabilization of the active site thiolates in the putative nrdH-redoxin BC3987 from *Bacillus cereus*. Lecture. *Norwegian synchrotron user meeting 2009*. 18<sup>th</sup> - 19<sup>th</sup> June 2009, Lillehammer, Norway.
- Andersson, K.K. (2009) Combining X-ray diffraction and spectroscopy to study the intermediates in the reaction between myoglobin and peroxides. Lecture. *CTCC and Department of Chemistry Minisymposium on Porphyrins and Related Macrocycles*. 10<sup>th</sup> - 11<sup>th</sup> June 2009, Tromsø, Norway.
- Andersson, K.K., Hsiao, Y.-W., Görbitz, C.H., Ryde, U. & Hersleth, H.-P. (2009) Structural studies of the intermediates in the reaction between myoglobin and peroxides; effects of cryoradiolytic reduction. Lecture. *NBS-news 2009 Suppl.* 01 p.65. *45<sup>th</sup> Biochemical Contact Meeting*. 12<sup>th</sup> - 15<sup>th</sup> June 2009, Røros, Norway.
- Hersleth, H.-P. & Andersson, K.K. (2009) Combining X-ray diffraction and spectroscopy to study the intermediates in the reaction between myoglobin and peroxides. Poster. *13<sup>th</sup> SBNet Annual Conference*. 12<sup>th</sup> - 15<sup>th</sup> June 2009, Tällberg, Sweden.
- Hersleth, H.-P. & Andersson, K.K. (2009) Combining X-ray diffraction and spectroscopy to study the intermediates in the reaction between myoglobin and peroxides. Lecture. *Norwegian synchrotron user meeting 2009*. 18<sup>th</sup> - 19<sup>th</sup> June 2009, Lillehammer, Norway.
- Hersleth, H.-P., Hsiao, Y.-W., Nilsson, K., Rydberg, P., Rod, T.H., Røhr, Å.K., Ryde, U., Görbitz, C.H. & Andersson, K.K. (2009) Structural studies of the intermediates in the reaction between myoglobin and peroxides. Poster. *Summer School 2009 Computational Chemistry and Spectroscopy*, 22<sup>nd</sup> - 25<sup>th</sup> September 2009, Essen, Germany.
- Røhr, Å.K. & Andersson, K.K. (2009) Stabilization of the active site thiolates in the putative nrdH-redoxin BC3987 from *Bacillus cereus*. Poster. *Enzymes in Deoxyribonucleotide Synthesis 2009*. 29<sup>th</sup> March - 1<sup>st</sup> April 2009, Hemavan, Sweden.
- Røhr, Å.K. & Andersson, K.K. (2009) Stabilization of the active site thiolates in the putative nrdH-redoxin BC3987 from *Bacillus cereus*. Poster. *Summer School 2009 Computational Chemistry and Spectroscopy*, 22<sup>nd</sup> - 25<sup>th</sup> September 2009, Essen, Germany.
- Røhr, Å.K., Hersleth, H.-P. & Andersson, K.K. (2010) Tracking Flavin Conformations in Protein Crystal Structures with Raman Spectroscopy and QM/MM Calculations. *Angew. Chem. Int. Ed.* **49**, In Press: DOI: 10.1002/anie.200907143.