ESRF	**The redox structure of haem- and flavoproteins by combining X-ray diffraction and UV-vis and Raman spectroscopy**	**Experiment number**: A01-2 1276
Beamline: BM01	**Date of experiment**: from: 01FEB2023 to: 04FEB2023	**Date of report**: 12MAR2023
Shifts: 9	**Local contact(s)**: Charles McMonagle	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Hans-Petter Hersleth, University of Oslo, Department of Biosciences, P.O. Box 1066 Blindern, 0316 Oslo, Norway*

Marta Hammerstad, University of Oslo, Department of Biosciences, P.O. Box 1066 Blindern, 0316 Oslo, Norway*

Åsmund Røhr Kjendseth, Norwegian University of Life Sciences, P.O. Box 5003, 1432 Ås, Norway*

Report:

The overall focus of the project is deciphering reaction mechanisms of selected cofactor proteins. These systems are very labile for radiation damage of the redox sites during diffraction data collection, which makes *in situ* studies using single-crystal UV-vis and Raman spectroscopy inevitable to both prove and trap different redox states and to monitor the potential radiation damage.

We have previously taken part in developing an *in situ* spectroscopy setup at BM01. After the refubishing and update of the ESRF, we had to update and adjust this setup. In the previous beamtime we focused on re-establishing the UV-vis *in situ* single-crystal setup in combination with X-ray diffraction at BM01, and in this beamtime to re-establish the whole *in situ* spectroscopy setup with both UV-vis and Raman spectroscopy. The updated setup is shown in Figure 1. Both UV-vis and Raman light is aligned to hit a crystal centered in the X-ray beam. The crystal is manually mounted and centered and orientation optimised for best UV-vis and Raman

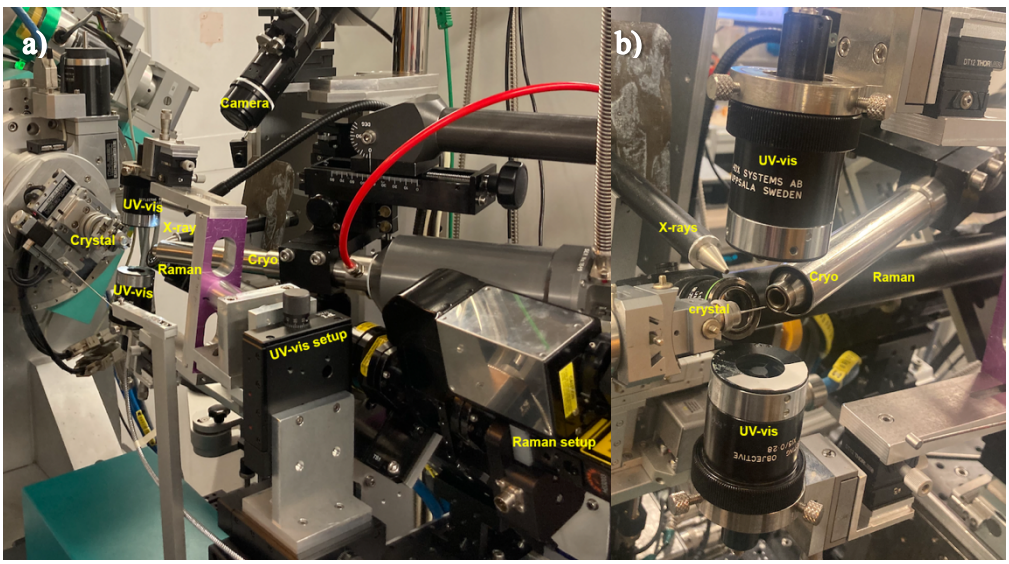

Figure 1 – a) Re-established UV-vis and Raman in situ setup at BM01, b) zoomed in.

spectra, while the data colletion can be script based and performed from the control cabin. There the running of both X-ray, Raman and UV-vis can be executed as shown in Figure 2.

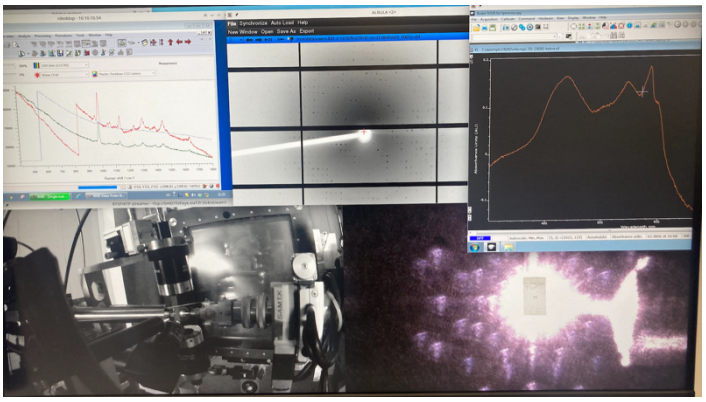

Figure 2 – The running of X-ray, UV-vis and Raman from BM01 control cabin

The re-established setup was used to investigate different effects on both haem-, flavo- and copper proteins. The setup worked well, and the data are still processed, therefore two examples of data collected is shown below. For myoglobin compound II H_2O_2 and D_2O_2 generated crystals were compared with both single-crystal UV-vis and Raman spectroscopy (Figure 3). It has been debated if the iron-oxo compoud II state is protonated or not.The initial results show similar UV-vis spectra, while there are difference in the Raman spectra compared to previously colleted Raman spectra on H_2O_2 generated compound II. These data will be futher analysed. For NrdI protons are involved in the redox reaction, thefore the effect of NrdI in H_2O and D_2O was compared, and the changes induced by X-ray data collection. Raman data was collected at different timepoints during the X-ray data collection (Figure 4). Some changes was observed and the data will be further processed and analysed. For the copper proteins the Raman spectra was successfully obtained by the use of 532 nm laser.

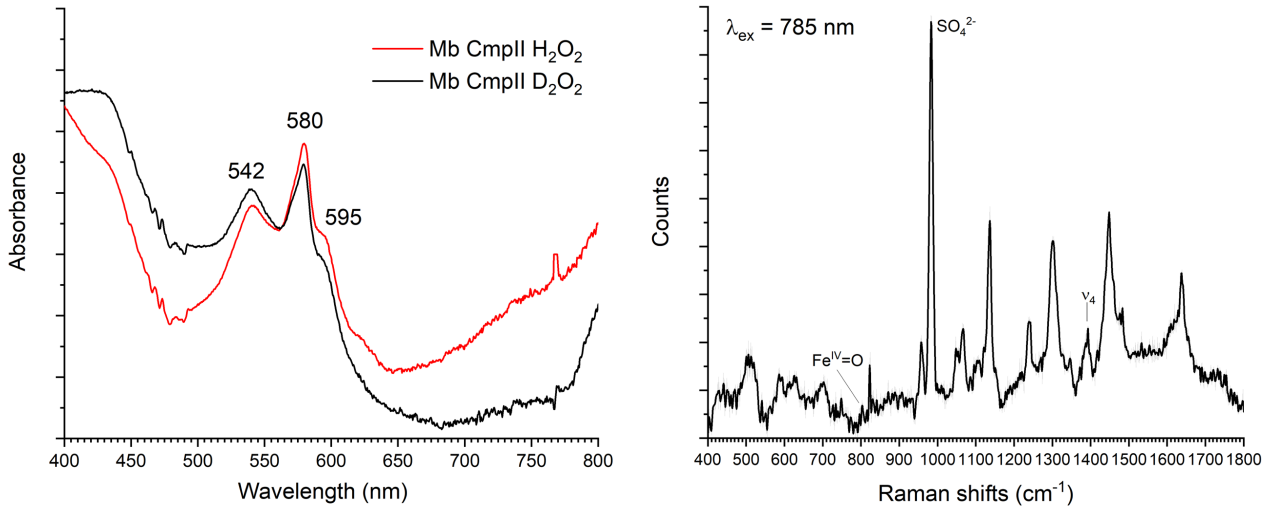

Figure 3 – Myoglobin compound II generated by H_2O_2 and D_2O_2. To the left the single-crystal UV-vis and to the right the Raman spectra.

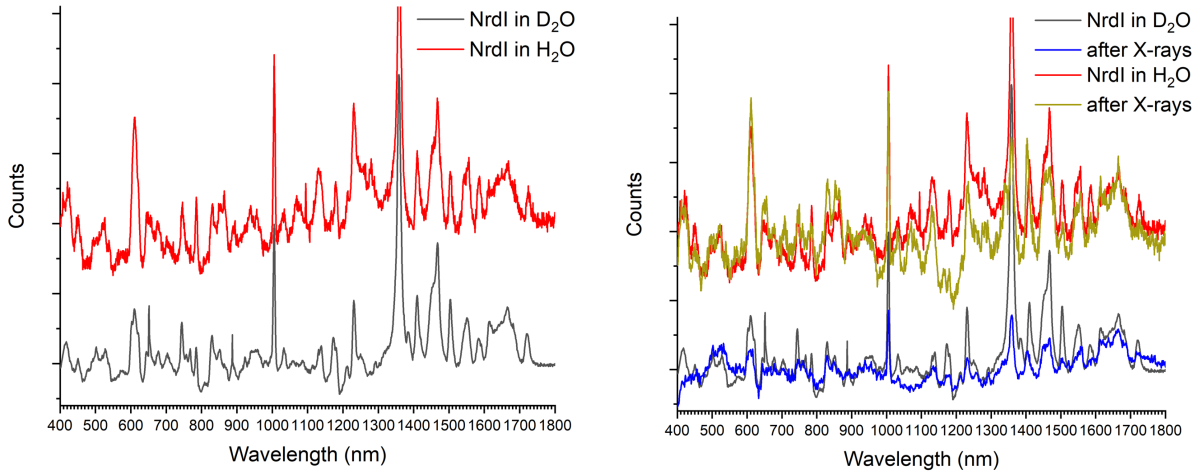

Figure 4 – NrdI single crystal-spectra Raman spectra using a 785 nm laser collected in both H_2O and D_2O, and to the right also after X-ray data collection.