ESRF	**The redox structure of haem- and flavoproteins by combining X-ray diffraction and UV-vis and Raman spectroscopy**	**Experiment number**: 01-02-1249
Beamline: BM01	**Date of experiment**: from: 15FEB2022 to: 17FEB2022	**Date of report**: 26FEB2022
Shifts: 6	**Local contact(s)**: Vadim Diadkin	*Received at ESRF:*

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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. The beamtime in this round focused on re-establishing the UV-vis *in situ* single-crystal setup in combination with X-ray diffraction at BM01. The updated setup is shown in Figure 1. The UV-vis light is aligned to hit a crystal centered in the X-ray beam. The crystal is mounted and centered manually, while the optimalisation of the crystal orientation to obtain the best UV-vis spectrum and collection of UV-vis spectra are script based and performed from the control cabin.

The re-established setup was used to investigate the X-ray radiation-induced reduction of different redox states of horse heart myoglobin as a model system.

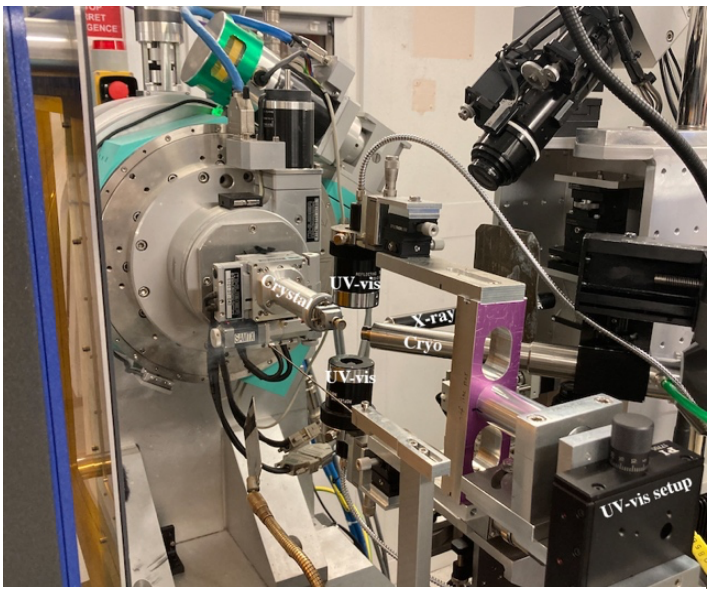

Figure 1 - The re-established UV-vis in situ setup at BM01

Both the radiation-indcued reduction of the ferric and ferryl horse heart myoglobin crystals could easily be monitored with the *in situ* UV-vis spectroscopy setup. In Figure 2 the gradual radiation-induced reduction of the ferryl myoglobin compund II with increased absorbed dose is observed from the reduction of the peaks at 541 and 580 nm, and by the increase of the peak at 568 nm. The radiation-damage occurs from the first photon that hits the crystals, and the reduction rate is faster in the beginning as seen from Figure 2D. We have previously pragmatically suggested that a lifedose of such redox cofactor proteins is when 90% of the original state remains. It can be observed here that this would be after a few 1/100 parts of a MGy of dose. Similar measurements have been done for the ferric myoglobin form, and show a faster reduction of the ferric than the ferryl state. It has been suggested that reduction of these states can be the influence by X-ray wavelength. Compard to previous studies on the myoglobin system, a shorter wavelength of radiation have been used in these experiment, 0.68 versus 0.93 Å. We observe a sligtly longer lifedose, however, since the previous results are from another beamline, further studies are needed. From these observation we initiated a teadious composite data collection study to be able to obtain complete merged datasets of these states within the lifedoses. Several datasets were collected, but additional datasets need to be collected during the next beamtime to obtain complete lifedose datasets and structures. For each dataset a UV-vis spectrum was obtain before and after the data collection.

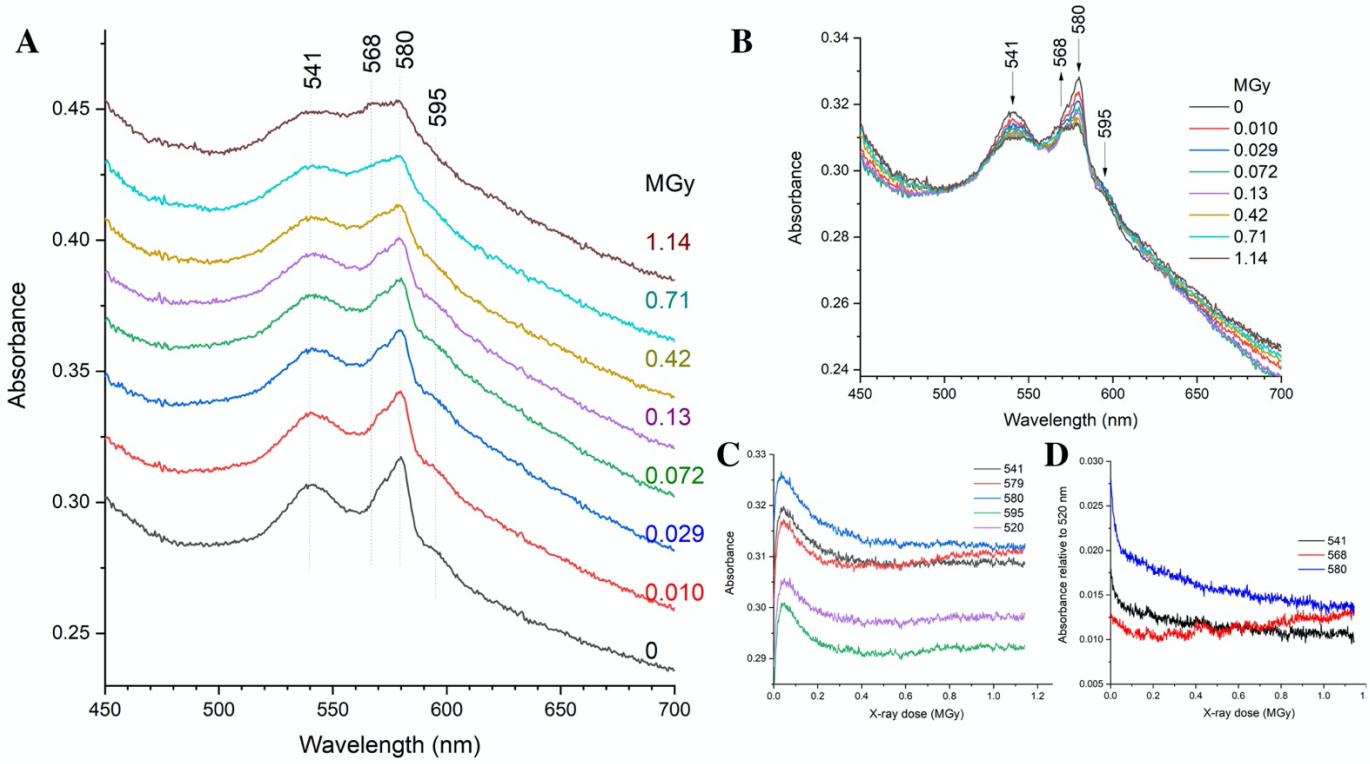

Figure 2 – Monitoring the the radiation-induced changes of the high-valent horse heart myoglobin compound II crystals with in situ single-crystals UV-vis spectroscopy. The absorbed doses were calculated with RADDOSE-3D.

As a pilot study, severel datasets were also recorded for tyrosinase proteins containing a di-copper cofaktor. The aim was to record and characterize a UV-vis signal that my be assosiated to self-hydroxylation of the enzyme. The data are still being analyzed.

The *in situ* UV-vis setup makes it relatively easy to measure UV-vis spectra of different cofactor proteins with absorption in the UV-vis range both before, during and after the X-ray diffraction data collection. For these redox systems this is essential to both know the initial state you have in your crystal, and to controll the potential radiation damage.