ESRF	**Experiment title**: Crystallographic studies of the haem protein myoglobin in combination with online microspectrophotometry	**Experiment number**: MX-885
Beamline: ID14-2	**Dates of experiments**: From: 21-FEB-09 09:30 to: 22-FEB-09 08:00	**Date of report**: 29-AUG-10
Shifts: 3	**Local contact(s)**: Dr. David Flot	*Received at UNIL:*

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Report:

The focus of our studies of combining protein crystallography and online UV-vis microspectrophotometry has been on haem protein These proteins are a good candidates to be studied with microspectrophotometry since they have characteristic absorptions in the 350-700 nm range.

Myoglobin

The main function of myoglobin is oxygen storage in heart and skeletal muscle, but myoglobin exhibit peroxidase-activity during oxidative stress. We have through previous structural studies tried to characterise and understand the reactions between myoglobin and peroxides. These reaction intermediates are relevant because myoglobin is proposed to take part as scavenger of reactive oxygen species during oxidative stress, and because these intermediates are similar amongst the haem peroxidases and oxygenases. We have in our previous studies shown that these different myoglobin states are influenced by the X-rays used. In this study, we have in detail investigated the impact that X-rays have on these different oxidation states of myoglobin. An underlying goal has been to find a way to be able to determine mostly unreduced states. We have by using single-crystal light absorption spectroscopy found that the different oxidation states of myoglobin are to a different extent influenced by the X-rays (e.g. ferric Fe^{III} myoglobin is faster reduced than ferryl Fe^{IV}=O myoglobin, with lifedoses of 0.01 MGy and 0.03 MGy, respectively) (*Figure 1*). We observe that the higher oxidation states are not reduced to normal ferrous Fe^{II} or ferric Fe^{III} states, but end up in some intermediate and possibly artificial states. For ferric myoglobin, it seems that annealing of the radiation-induced/reduced state can reversibly more or less give the starting point (ferric myoglobin) (*Figure 1A*). Both scavengers and different dose-rates might influence to which extend the different states are affected by the X-rays. Our study shows that it is essential to do a time/dose monitoring of the influence X-rays have on each specific redox-state with spectroscopic techniques like single-crystal light absorption spectroscopy. This will determine to which extend you can collect X-ray diffraction data on your crystal before it becomes too heavily influenced/reduced by X-rays.

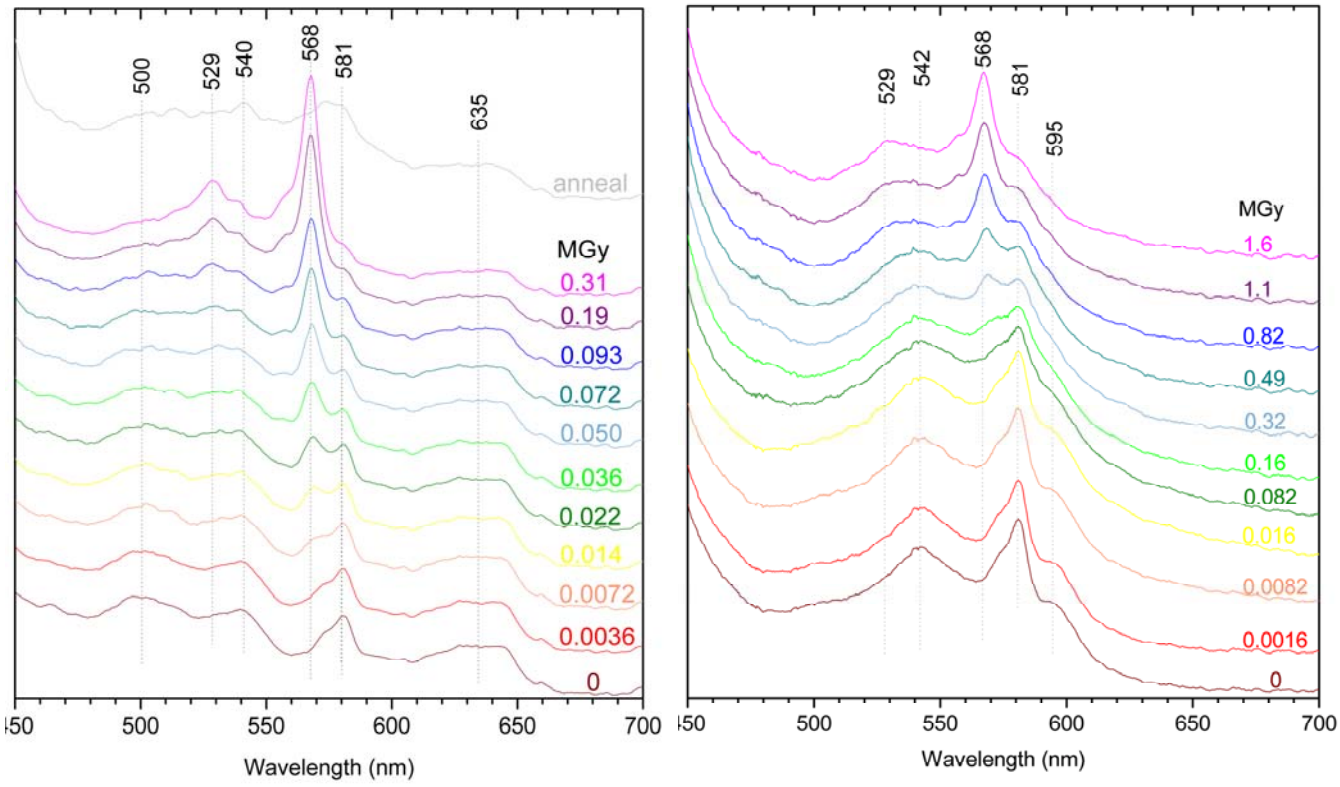

Figure 1: *UV-vis single-crystal spectra of A: ferric Fe^{III} myoglobin and B: ferryl $Fe^{IV}O$ with increasing X-ray dose*

Publication resulting from this beamtime:

Hersleth, H.-P. & Andersson, K.K. (2010). How different oxidation states of crystalline myoglobin are influenced by X-rays. *Biochim. Biophys. Acta* doi:10.1016/j.bbapap.2010.07.019