MS38-P11 Combining X-ray crystallography and *in situ* single-crystal UV-Vis and Raman spectroscopy to study haem- and flavoproteins

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X-ray crystallography is the central methods for studying the structure-function relation of redox proteins, however the crystal structures are missing key information. This includes oxidation state, protonation and spin state, which are essential for understanding the reaction mechanisms of these redox protein systems. Therefore, a combination of crystallographic and spectroscopic methods is vital for a more fully characterisation. Additionally, the redox sites are very labile for X-ray induced radiation damage and reduction during crystallographic data collection at synchrotrons. Therefore combining X-ray crystallography with *in situ* UV-Vis and Raman spectroscopy is important for both characterisation and radiation damage monitoring. We have used *in situ* spectroscopy setups at both X10SA at SLS, the Cryobench at ESRF and at the Swiss-Norwegian Beam Lines at ESRF. We have studied several haem- and flavonproteins such as myoglobin, cytochrome *c*, catalase-peroxidase, flavodoxin like protein NrdI, and several flavodoxins and flavodoxin reductases. These studies have resulted in complementary structural information, and shown the redox state of the solved crystal structures. With respect to radiation induced changes, we have observed for haem proteins a lengthening of the iron-oxygen bond, and for flavoproteins a bending of the flavin ring during X-ray induced radiation damage. We have been able to estimate the lifedose of different states, and these results have lead to the use of composite data collection to obtain the unreduced crystal structures of some of these redox proteins.

References: H.-P. Hersleth & K.K. Andersson. How different oxidation states of crystalline myoglobin are influenced by X-rays. *Biochim. Biophys. Acta, Proteins Proteomics* (2011), **1814** , 785-796. Å.K. Røhr, H.-P. Hersleth & K.K. Andersson. Tracking Flavin Conformations in Protein Crystal Structures with Raman Spectroscopy and QM/MM Calculations. *Angew. Chem. Int. Ed.* (2010), **49**, 2324-2327.

Acknowledgements. Financial support by the Norwegian Research Council through projects 214239/F20 / 218412/F50 / 231669/F20.

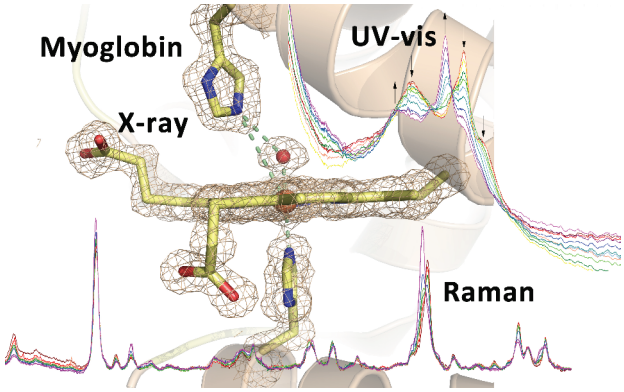

Figure 1.

Keywords: protein crystallography, single-crystal spectroscopy, haemproteins, flavoproteins, radiation damage