Ligand dissociation and recombination of Nitrosyl-myoglobin in physiological media studied by ultrafast X-ray spectroscopy and X-ray Diffuse Scattering

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Myoglobin is a small protein consisting of a single polypeptide chain of 153 amino acid residues and a heme group as its active center. It plays a central role in several biological functions based on detection, transport, release and binding of molecular ligands such as O_2, CO, NO, CN, etc. The unligated high-spin (HS) form (deoxyMb) binds the ligands at the Fe center of the porphyrin, leading to a change to the planar low-spin (LS) ligated form. Ligation causes differences in spin, electronic configuration and geometric structure of the heme which determine the biological function of the protein.

Nitrosyl-Myoglobin (MbNO), in particular, is important due to its role in various neurophysiological responses. The ultrafast photodissociation of low spin, planar MbNO leads to a state similar to the HS deoxyMb. However, the return to the initial state occurs on multiple timescales (from sub-ps to 100s ps), which are much shorter than in the case of the CO ligand. In particular, the formation of an unusual HS domed ligated MbNO species is considered one of the intermediates on the way back to the planar form. Previous X-ray absorption studies with 70 ps resolution supported the latter hypothesis,[1,2] however the weakness of the signal called for further studies, while the poor temporal resolution hindered a description of the earlier time kinetics. In particular, is the relaxation back to planar a cascade via spin states or is it due to steric hindrances? In order to elucidate these aspects, we combined femtosecond Fe K-edge X-ray absorption spectroscopy (XAS) with X-ray emission spectroscopy (XES) and X-ray diffuse scattering (XDS) at the FXE instrument of the European-XFEL (Hamburg) and at SACLA (Japan). XAS probes the unoccupied density of states (DOS) and the local structure around the Fe atom, while XES the occupied DOS and the spin state of the intermediates. XDS probes the structural changes of the protein scaffold. This is the first time these three methods are combined together to investigate a biological system in solution. We will present our results from these measurements and cast them in the context of on-going studies on biosystems at XFELs.

1. Silatani, M. *et al.* NO binding kinetics in myoglobin investigated by picosecond Fe K-edge absorption spectroscopy. *Proc. Natl. Acad. Sci.* **112,** 12922–12927 (2015).
2. Lima, F. A. *et al.* Probing the electronic and geometric structure of ferric and ferrous myoglobins in physiological solutions by Fe K-edge absorption spectroscopy. *Phys Chem Chem Phys* **16,** 1617–1631 (2014).