Ultrafast Electronic and Structural Dynamics of Heme Proteins Unveiled by Time-resolved X-ray Spectroscopy at XFELs

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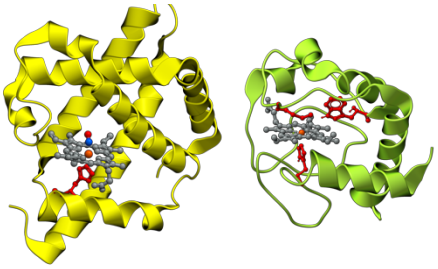

The structures of Myoglobin (left) and Cytochrome C (right).

Metalloproteins correspond to almost half of all known proteins. In these proteins, the metal-binding center is paramount to protein function and iron containing heme proteins are amongst the most important ones. Myoglobin was the first three-dimensional structure ever resolved by crystallography, and since then much effort has been dedicated to the study of the structure, function and dynamics of these subgroup of proteins. Time-resolved X-ray spectroscopy is a particularly well-suited tool to investigate these systems. The element specificity provides a direct and sensitive probe of the protein dynamics from the metal-binding center point of view. This work focuses in the investigation of light induced ultrafast electronic and structural dynamics of two important heme proteins, Nitrosyl Myoglobin (MbNO) and Cytochrome C (CytC), by femtosecond time-resolved x-ray absorption (fs-XAS) and x-ray emission (fs-XES) spectroscopies.

Upon visible photoexcitation of the heme group (530 nm) MbNO undergoes dissociation of the ligand (NO) which is accompanied by a spin change and a structural reconfiguration of the porphyrin ring. Part of the excited population undergoes recombination in multiple timescales through an intermediate state that is presumed to be a high spin domed ligated form of MbNO. We carried out a combined time-resolved non-resonant XES and Fe K-edge XAS experiment in physiological media. The results offer new insight on the dissociation-recombination dynamics and capture the signature of the proposed hexacoordinated intermediate state.

Meanwhile, in CytC we focused on the investigation of the nature of energy transfer between the tryptophan (Trp) residue and the heme, by exciting the protein in the UV region (285 nm). This process can proceed in one of two ways: via dipole-dipole coupling or double electron transfer. Calculations strongly suggest the latter is overwhelmingly more likely in CytC due to the close proximity between the Trp and the heme and the poor dipole moment overlap between them, but optical measurements are ambiguous in this respect. We recently performed fs-XAS and fs-XES experiments that contradict the theory expectations and the results, which indicates that the Trp and heme might be better described as an excitonic pair.