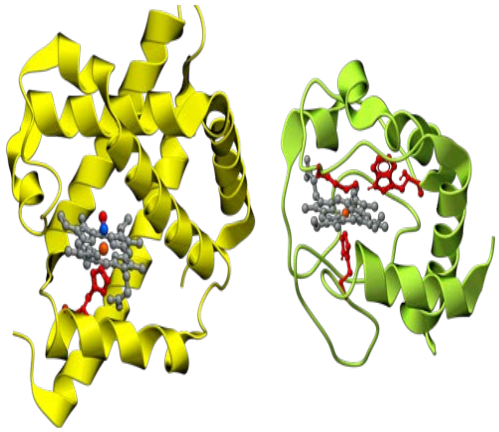
Ultrafast electronic and structural dynamics of heme proteins unveiled by time-resolved X-ray spectroscopy at XFELs

C. Bacellar[1,2], D. Kinschel[1,2], R. Ingle[2], G.F. Mancini[1,2], J. Rouxel[1,2], O. Canelli[1], B. Sorokin[1], J. Budarz[1], C. Cirelli[2], G. Knopp[2], P. Johnson[2], S. Menzi[2], G. Pamfilidis[2], J. Szlachetko[3], F.A. Lima[4], W. Gawelda[4], D.Khakhulin[4], C. Bressler[4], T. Katayama[5], Y. Obara[6], J. Nishitani[7], H. Ito[6], T. Ito[6], N. Kurahashi[8], C. Higashimura[7], S. Kudo[7], T. Suzuki[7], K. Misawa[5], C. Milne[2] and M. Chergui[1]

[1]*LSU, Ecole Polytechnique Fédérale de Lausanne, Switzerland,* [2]*SwissFEL, Paul Scherrer Institute, Switzerland,* [3]*Institute of Physics, Jan Kochanowski University in Kielce, Poland,* [4]*European XFEL, Germany,* [5]*XFEL Division, Japan Synchrotron Radiation Research Institute, Japan,* [6]*Tokyo University of Agriculture and Technology, Japan,* [7]*Kyoto University, Japan,* [8] *Sophia University, Japan,* **camila.bacellar@epfl.ch**

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 x-rat 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, and particularly X-ray Emission (XES), 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.



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

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 the relaxation process fowling excitation of the heme at 350-400 nm. Ferrous heme proteins such as the MbNO example above are known for undergoing dissociation of the axial ligand, however this is not observed in their ferric counterparts. This has led to a long discussion of the relaxation pathway involved in these types of systems, which for the most part was believed to be entirely via vibrational cooling back to the ground state. We recently performed fs-XAS and fs-XES experiments that challenge this interpretation evidencing the presence of heme doming and de-excitation via high spin states.