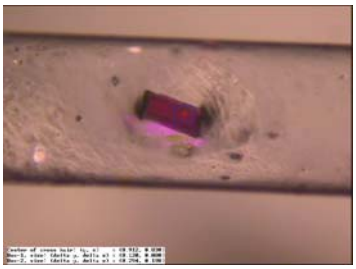
Proposal Code: **MX-213**
Proposal Title: Structural dynamics: real time resolved diffraction of photolytic intermediates of hemoglobin
Beam line: ID09B from 18 May 2004 to 25 May 2004

Experimental report.
During this experiment we have carried out two experiments, the main one was on a mutant Hb (called MISA-Hb), where two residues in the pocket on the distal side of the heme are mutated in both the alpha and beta chains: Leu(B10)Tyr and His(E7)Gln that was crystallized in the T state. The crystals were subsequently soaked with CO that binds only to the beta chains. This procedure was tested for the first time at ESRF on crystals to be exposed at room temperature (20°C) after mounting in quartz capillaries.



These samples were subsequently analyzed spectrophotometrically to ascertain their ligation state, and subsequently underwent time resolved data collection after laser photolysis.

Figure 1. Crystal of Hb MISA, prior to data collection.

Most of the beam time was used to test the samples, in order to define a soaking protocol that would not lead to loss of diffraction due to the quaternary transition to the high affinity R state, but at the same time yielded crystals with fully carboxylated heme group on the beta chains.
We have collected a total of 13 data sets from 7 crystals (out of the 50 which underwent soaking, mounting and spectroscopic characterization).
Several time points were collected, from the earliest possible delay (100 psec) to 300 microsec.
Subsequent data analysis showed that only two data sets (100 psec and 1 μsec) resulting from one crystal mounted in an atmosphere of CO:N2=1:20 displayed at the same time high resolution (1.9 Å) and CO photolysis, clearly showing in the electron density maps CO photodissociation, heme iron out of the plane displacement, and displacement of Cysteine β93.

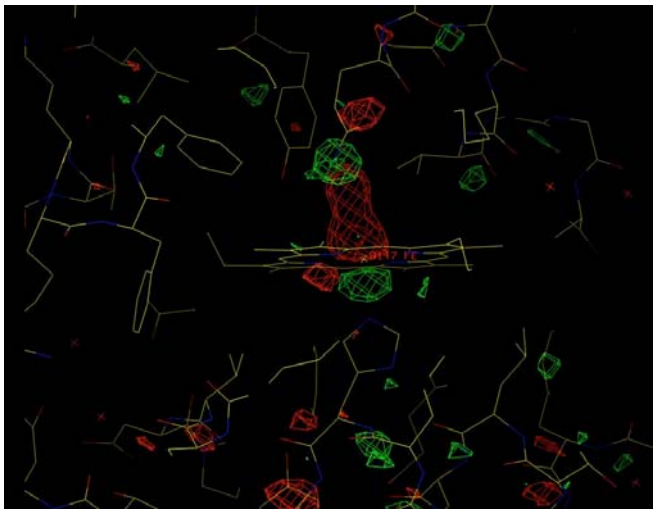
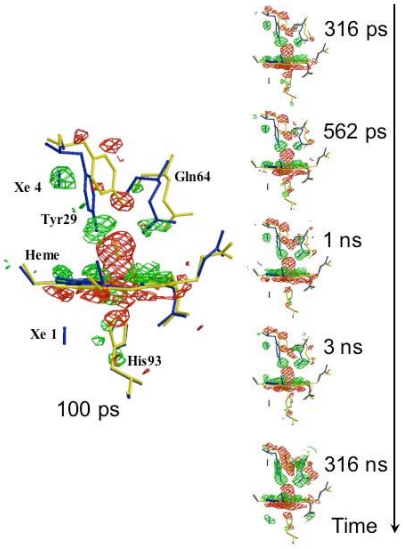


Figure 2. Dark-light electron density map of Hb-MISA.
Red=negative density
Green=positive density

We used the remaining beam time, after having exposed all the crystals of Hb-MISA available, to collect real time resolved data on Mb-YQR, for which we lacked early dealy time points (from 100 psec to 3.6 nsec).



This experiment resulted in data of excellent quality, shown in figure 3, that are the subject of a publication in preparation, in which we confirm the observation previously reported (1) and also a delay in heme relaxation, complete only after 1 nsec, which seems to be due to a network of interactions that couple full heme doming to the transition of amino acids in the distal site to the deoxy conformation, via two phenilalanines on the CD corner (Phe46 and Phe43).

Figure 3. Early time evolution of Mb-YQR after CO photolysis.

References.

1. Bourgeois D, Vallone B, Schotte F, Arcovito A, Miele AE, Sciara G, Wulff M, Anfinrud P, Brunori M. (2003) Complex landscape of protein structural dynamics unveiled by nanosecond Laue crystallography. Proc Natl Acad Sci U S A.100:8704-9.