ESRF	**Experiment title:** Picosecond time-resolved crystallography: ligand dynamics in myoglobin	**Experiment number**: MX-7
Beamline: ID09B	**Date of experiment**: from: 07/10/2002 to: 15/10/2002	**Date of report**: 1/03/2003
Shifts: 8	**Local contact(s)**: Dr. Michael Wulff	*Received at ESRF:*

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Report:

During the allocated beam time for MX-7 significant advances were made both methodologically and scientifically.

The improved focusing of the X-ray beam resulting from an upgrade of the toroidal mirror led to a new approach for photolyzing the samples. Previously, with a 300x200 μm X-ray beam, it was necessary to tune the laser to a wavelength of weak absorption, in order to obtain sufficiently deep penetration. With the new 100x100 μm focus, is it sufficient to excite the surface of the MbCO crystal and direct the X-ray along the edge of the crystal exposed to the laser beam. This can be done at a wavelength of stronger absorption where photolysis is more efficient. Since only a small fraction of the sample is exposed at a time it was possible to use several spots by translating the sample during the data collection. We were able to collect multiple time points simultaneously on the same crystal while maintaining high-resolution diffraction throughout the data set.

The upgrade of the U20 to the in-vacuum undulator U17 led to a fivefold increase in photon flux from $2 \cdot 10^9$ to $1.0 \cdot 10^{10}$ photons per pulse. This allowed us to reduce the number of pulses from 64 per image (1984 per data set) to 16 per image (496 per data set) while still saturating the detector.

From the mutant L29F, we collected a series of data sets at 3.16 ns, 31.6 ns, 316 ns and 3.16μs after photolysis, revealing the diffusion of the CO in a web of hydrophobic cavities in the interior of the protein (Figure 1). This data is complementary to a time series site from

100 ps to 3.16 ns, obtained in April 2002 during commissioning beam time, showing the fast ejection of CO from its docking site, that is characteristic for this mutant.

We obtained data from the mutant H64A, where the proximal histidine is replaced by an alanin, opening the heme pocket to the exterior. It shows a docking site very different from wild-type myoglobin, with the CO at a position where the N atom of the histidine would be in wildtype. Surprisingly, not all CO seems to escape via this pathway, since some CO is found in a hydrophobic pocket in the interior of the protein after 316 ns (Figure 2).

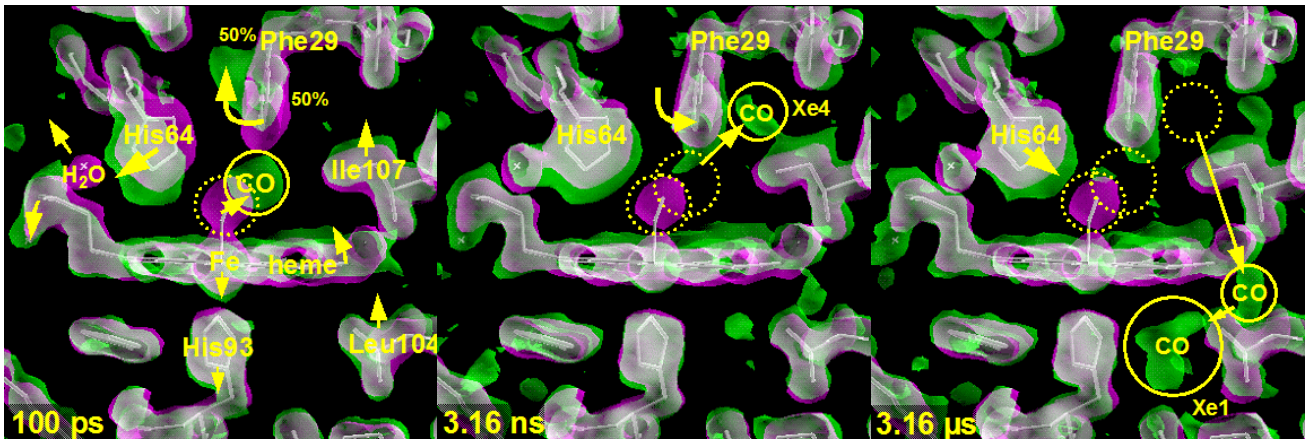

Figure 1: Ligand migration after the photolysis of L29F MbCO. The density after photolysis is rendered in green, and superimposed to the density before photolysis in magenta. Where the density remains unchanged both blend to white. The gradient magenta to green indicates the direction of motion. The solid yellow circles indicate density site we interpret as CO. The green density has been calculated to represent 100% photolysis. The actual photolysis degree was 17 %. The first time point was collected in April 2002.

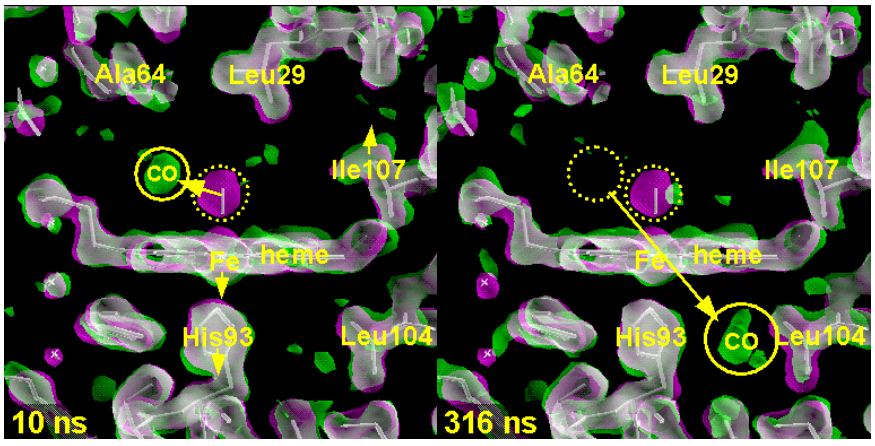

Figure 2: Ligand migration after the photolysis of H64A MbCO. The green density also represents an extrapolation from 17% to 100% photolysis.