	Experiment title: Femtosecond Crystallography: Characterizing ligand migration pathways in proteins	Experiment number: MX-381
Beamline: ID09B	Date of experiment: from: 16/05/2005 to: 21/05/2005	Date of report: 06/09/2005
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Report:

We studied flash photolysis of CO in native myoglobin crystal in the presence of pressurized Xenon.

The crystals were mounted in 1-mm quartz capillaries closed at one end and filled with a mixture of 25% CO and 75% Xenon. We injected UV curing epoxy into the other end of the capillary and compressed with a syringe the gas column from 32 mm to 10 mm length, and cured the plug of epoxy inside the capillary by UV light. The crystal was thus in an atmosphere of 2.4 atm Xe and 0.8 atm CO.

To photolyze the crystal, we used laser pulses with a wavelength of 520-560 nm of about 100 ps duration and a power density of 1.2 mJ/mm². These were generated by the TOPAS OPA set to 520 nm, by injection into a 200-μm glass fiber. This stretched the pulse duration from 100 fs to ca 100 ps wavelength and caused the spectrum to shift and broaden from 12 to 40 nm FWHM. The fiber output was imaged by a 2:1 reducing lens assembly onto the sample to an elliptical spot of 125x400 μm (FWHM), where a pulse energy 47 μJ was measured.

We collected a time series of six time points from 100 ps to 10 μs in one step per decade on a single crystal. All time points were collected simultaneously in a single run. Each Laue image was obtained using 16 single pulses at 3.3 Hz repetition rate, using the undulator U17 at 6 mm gap in 4-bunch mode with 8.6-7.6 mA per bunch. This gave us a wavelength band of 0.824 to 0.855 Å (FWHM). To obtain a complete data set we used 30 angular settings of the crystal with a spacing of 2°, covering a range of 60°.

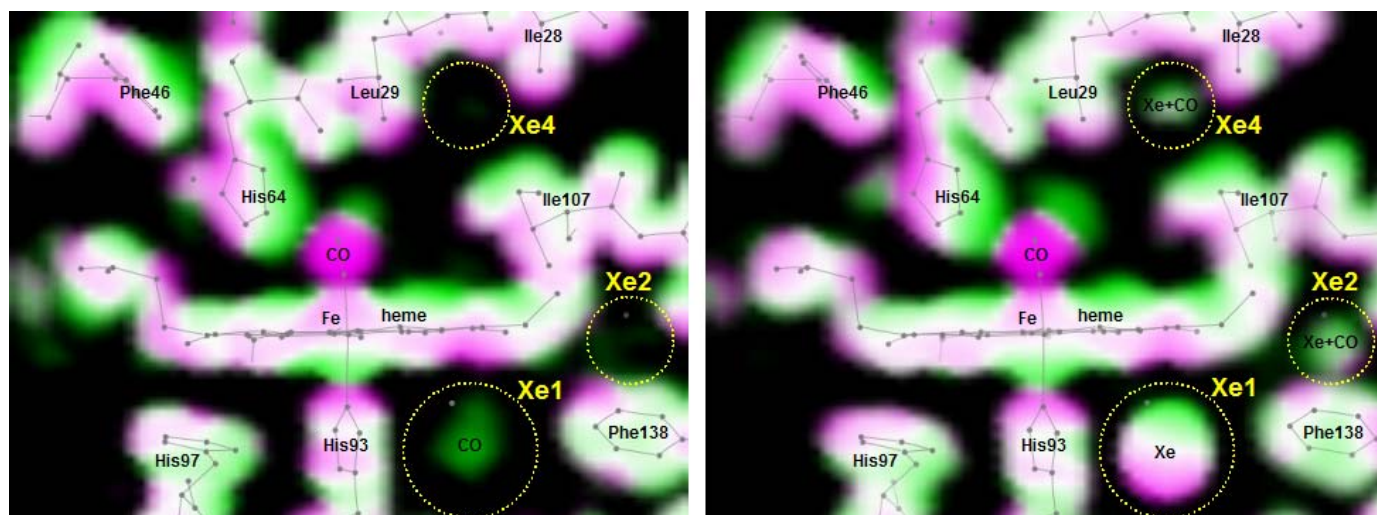


Figure 1. Superposition of the electron density maps of unphotolyzed (magenta) and photolyzed (green) MbCO; the photolyzed state was determined 1 μ s after photolysis. Where the electron densities overlap, the two colors combine to white. The magenta to green color gradient depicts the direction of atomic motion. In the absence of Xe (left), CO accumulates in the Xe1 site. In the presence of Xe (right), the Xe1 site is plugged, and CO sequesters in both Xe4 and Xe2.