ESRF	**Experiment title:** Picosecond time-resolved crystallography: ligand dynamics in myoglobin	**Experiment number**: LS-1993
Beamline: ID09B	**Date of experiment**: from: 21/08/2001 (5/12/2001) to: 25/08/2001 (17/12/2001)	**Date of report**: 25/02/02
Shifts: 12	**Local contact(s)**: Dr. Michael Wulff	*Received at ESRF:*

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Report:

The allocation of beamtime for LS-1993 (9 shifts) was divided into two periods, one in August and the remainder in December 2001. During these periods, significant advances were made in both experimental methodology and in the quality of time-resolved x-ray diffraction data.

> *6x faster data acquisition*. Pump-probe time-resolved x-ray crystallography studies require an intense laser "pump" pulse to trigger a reaction in a protein crystal. The pump-induced heating of the protein crystal leads to distillation of water from mother liquor to cooler regions in the x-ray capillary. This loss of water can trigger precipitation of salt and can increase the mosaicity of the protein crystal. External cooling can arrest this problem. However, a very delicate balance is required: too little cooling and the problem persists; too much cooling and water distills to the protein crystal and causes it to dissolve. In the past, the only sure way to avoid distillation problems was to lower the repetition frequency of the data acquisition to 0.2-0.5 Hz. In an effort to speed up data acquisition, we isolated the crystal from the remote regions of the capillary by inserting mineral oil plugs on either side of the crystal. This innovation allowed us to increase the laser repetition frequency to 3 Hz, the maximum rate currently permitted by the heat load shutter, without suffering the ill effects of crystal heating. We can now acquire an entire time series in the length of time previously required to obtain a single time point.

Improved fs laser source. The ID09B fs laser, originally based on a BMI regenerative amplifier, had been problematic since its installation in 1997. The BMI regenerative amplifier portion was replaced by a Spectra-Physics regenerative amplifier (Hurricane) in December 2001. This installation was directed by Anfinrud and assisted by Graham Naylor and Friedrich Schotte. This process involved removing the BMI regenerative amplifier from the laser table, moving the Coherent Mira Ti:sapphire fs laser and its Verdi pump laser to new

locations on the laser table, designing and laying out an optical system to couple femtosecond pulses into and out of the Hurricane regenerative amplifier, and optimizing the focal parameters required to efficiently pump a BMI optical parametric amplifier. Once the lasers and the optics were in place and operational, we uncovered excessive timing jitter in the firing of the regenerative amplifier. This problem was averted by supplanting the regenerative amplifier's internal triggering scheme with an external one. Within a few days of its arrival, the Hurricane laser was incorporated into the system and ready for use in time-resolved x-ray experiments.

Implementation of new synchronization scheme. Setting and maintaining the phase of the laser, x-ray, and synchronous chopper to the require precision for pursuing ps time-resolved pump-probe x-ray studies has always been time consuming. Because the frequency of each is a sub-harmonic of the synchrotron RF, a phase ambiguity must be resolved every time the synchronous chopper is restarted or the timing rack power is recycled. In the fall of 2001, the machine group routed a cable to ID09B to provide the single bunch frequency. In December 2001, Schotte modified the synchronization electronics to set the phase of the chopper according to the single bunch signal, thereby eliminating the need to rephase the chopper each time it is restarted. This innovation provides significant time savings when setting up time-resolved x-ray experiments.

Femtosecond photolysis. Compared to the previous regenerative amplifier, the newly installed Hurricane improved both the energy available for photolysis and the pulse-to-pulse stability. With the photolysis wavelength set to 607 nm, we acquired time-resolved x-ray diffraction data for WT and L29F MbCO on time scales from 100 ps to 3 ns. Unfortunately, the difference maps revealed negligible photolysis. In January 2002, fs time resolved optical studies of MbCO crystals performed at the National Institutes of Health revealed a strong, short-lived (decay time of 100 fs) excited state absorbance at the pump wavelength. Consequently, photolysis with 100 fs pulses suffers from shallow penetration into the crystal with photolyzed molecules absorbing multiple photons. By stretching the photolysis pulse to a duration that is long compared to the excited state lifetime, this problem can be minimized and significantly higher levels of photolysis can be achieved. An optical system is currently being designed to stretch the femtosecond optical pulse.

Nanosecond photolysis. Using a 2.2 ns optical pulse from an OPO, time-resolved diffraction data were collected for WT, V68F, L29F, and L104A at delay times ranging from 1 to 3000 ns. The WT data provided the greatest structural detail, as shown below. Ligand translocation from the distal docking site into the Xenon 1 docking site is evident as well as the correlated motion of protein side chains. Several mutant data sets revealed tantalizing differences from WT, but the quality of the diffraction data was inferior to the WT data, and those results to be repeated to determine if the differences observed are real or artifact.

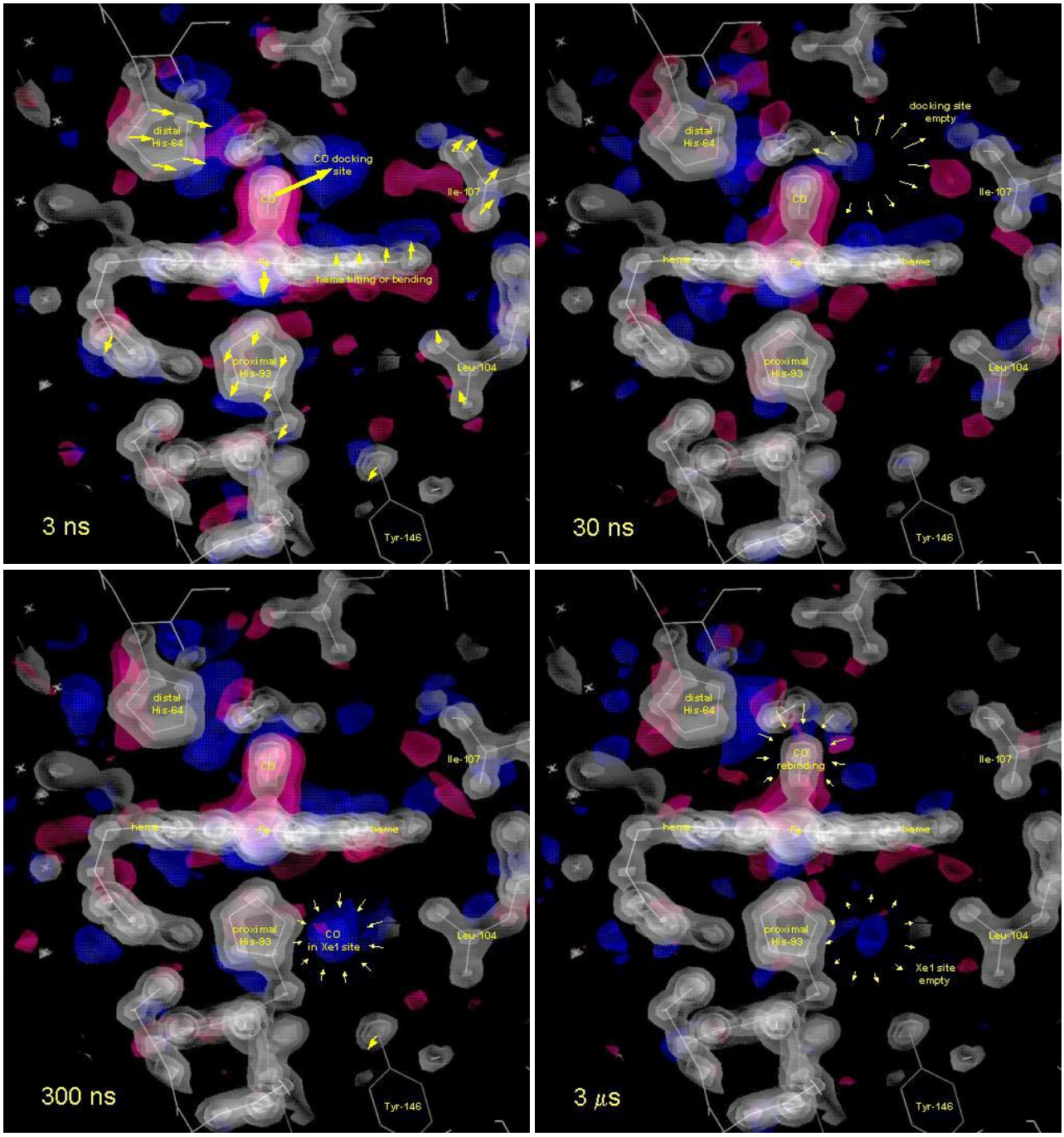

Figure 1. Difference map (Mb*CO –MbCO) recorded 3, 30, 300, and 3 μs after photolysis.