ESRF	**Experiment title:** Nanosecond crystallography: Probing the onset of the R-T transition in Hemoglobin	**Experiment number**: MX-77
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Shifts: 17	**Local contact(s)**: Dr. Michael Wulff	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Philip Anfinrud*, Friedrich Schotte*
National Institutes of Health, Bethesda, Maryland, USA
Michael Wulff*, Maciej Lorenc*
ESRF, Grenoble, France
George Phillips
University of Wisconsin, Madison, Wisconsin, USA
John S. Olson
Rice University, Houston, Texas, USA
Hyocherl Ihee*, University of Chicago, USA

Report:

During the allocated beam time of MX-77 we were able to collect seven data sets from hemoglobin-CO crystals showing photolysis, CO migration and rebinding from 3 ns to 1 µs.

The crystals grown by Jayashree Soman at the Rice University under CO atmosphere proved to be of excellent quality, showed no virtually no mosaicity before flash photolysis and gave diffraction data to 2.1 Å resolution (50% completeness at $I/\sigma>3$).

We used crystals of a size ranging from 200 to 400 µm of bipyramidal shape. The crystals where photolyzed by a 3-ns laser pulse of 580 nm and a power density of about 1 mJ/mm^2, delivered to the sample by fiber optics from the top side. The 100-µm X-ray beam was directed through the face of the crystal exposed to the laser, so it sampled only the 100-µm surface layer that is effectively photolyzed by light of this wavelength. Because of the spacegroup we had to collect data of a rotation range of 180º. We solved the problem of maintaining the pump-probe overlap over this range, given the crystal shape, by offsetting the rotation center as a function of orientation.

With the number of laser flashes received by the crystal, we saw a gradual increase of mosaicity of the crystal, leading to more streaky diffration spots and finally multiple diffration patterns as the crystals cracked. However, a crystal would tolerate about 2500 laser shots at this power density before the diffraction data became seriouly compromised, with the resolution limit dropping from 2.1 to 2.7 Å. This proved to be sufficient to collect three time points on a crystal, with 50 orientation per time point and 16 pulses per image.

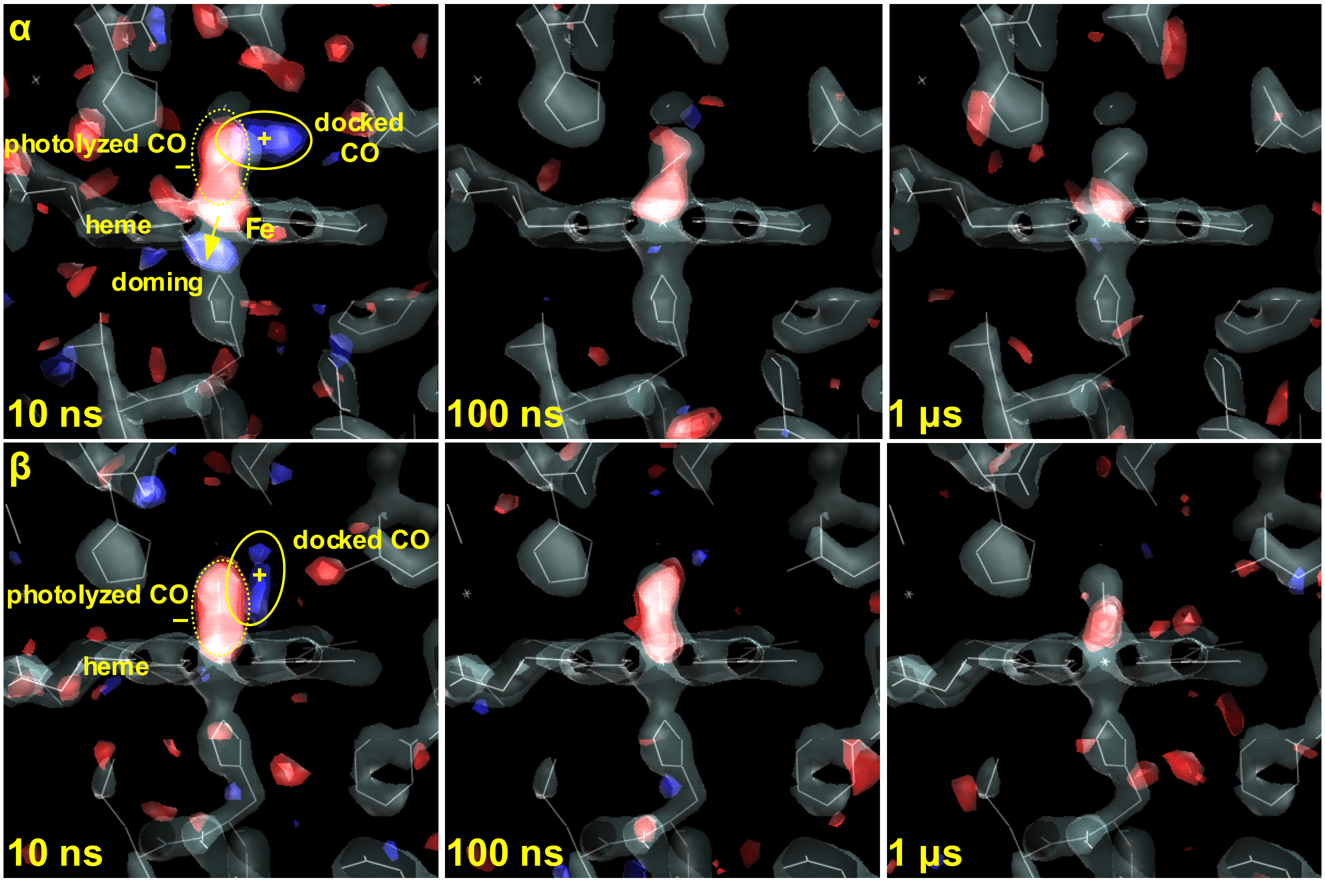

Figure 1: Photolysis and rebinding of CO in the α and β subunits of hemoglobin. This electron difference map is contoured a ±3.9, ±3.3 and ±2.6 σ, red indicates density decrease after photolysis, blue increase, grey is the unphotolyzed map ($2F_o$-F_c), white the T-state model (1AJ9). Note the different locations of the docking site in the α and β subunits. The CO seems to rebind faster in the α subunits. Heme doming is seen in the α subunit as an out-of-plane movement of the Fe. This feature is absent or much weeker in the β subunit. (data set hb8)