ESRF	**Experiment title:** Nanosecond Crystallography: Probing the onset of the R-T transition in Hemoglobin (Continuation)	**Experiment number**: MX-212
Beamline: ID09B	**Date of experiment**: from: 18/05/2004 to: 25/05/2004	**Date of report**: 28/02/2005
Shifts: 9	**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*, Marco Cammarata*, Manuela Lo Russo*
ESRF, Grenoble, France
Beatrice Vallone*, Guiliano Sciara*, Università di Roma "La Sapienza", Roma, Italy
Alessandro Arcovito*, Stockholms Universitet, Stockholm, Sweden
George Phillips, University of Wisconsin, Madison, Wisconsin, USA
John S. Olson, Jayashree Soman, Rice University, Houston, Texas, USA

Report:

During the allocated beam time we were able to collect three data sets from hemoglobin-CO, covering 5 decades in time, from 100 ps to 10 μs.

Unfortunately, high-quality crystals of the double mutant α-PheB10/β-IleE11 were not available at the time of the experiment. The expression system our collaborator John Olson used produces protein with a modified N-terminus, preventing the crystallization under the previously established conditions. Therefore, we instead used crystals of native HbCO grown by Jayashree Soman at the Rice University in March 2003.

To photolyze the crystal, we used laser pulses of about 100 ps duration, obtained by stretching of 100-fs pulses. The TOPAS OPA generated 100-fs pulses of 520 nm wavelength. These were stretched by passing through two 300-mm long fused silica slabs, and then focused into a 200-μm optical fiber of 2 m length. The propagation through the fiber caused the wavelength of the pump light to be shifted from 520 to 540 nm, and the bandwidth to be broadened from 12 to 40 nm (FWHM). The fiber output was imaged by a 2:1 reducing lens assembly onto the sample in spot of 125x400 μm (FWHM). The focus was deliberately elongated by a cylindrical lens in the X-ray beam direction, in order to match the sample size. 47 μJ of light could be delivered to the sample, resulting in a flux density of 1.2 mJ/mm^2. The crystals were able to receive about 2000-3000 flashes before the diffraction images showed signs of cracking.

Based on the occupancy of the CO binding site in the electron density maps, we estimated that we obtained 15% photolysis. This allowed us to calculate maps that extrapolated to 100% photolysis, which are easier to interpret than difference maps. We were able to improve the quality of the maps by collecting three datasets from different crystals, but each with the same time points (off, -10 ns, 100 ps, 1 ns, 10 ns, 100 ns, 1 μs, 10 μs). Merging of these data sets gave us a high-redundancy set with 630,000 observations per time point and 2.5 Å resolution (Table 1). The resulting maps (Figure 1) show more subtle features besides the CO hole and

the docking site, which were the only prominent features seen in MX-77. At 100 ps the heme doming is visible with the Fe pushing the proximal histidine and the F helix. The distal histidine relaxes toward the cavity left behind by the photolyzed CO. The B helix is moving in the opposite direction of the F helix.

date	time	name	pulses / image	collected images	usable images	N_{obs}	N_{uniq}	redundancy	d_{min} begin	d_{min} end	R_{cryst}	R_{sym}
21 May 2004	22:46	hb12	8	73	63	301666	19610	15.4	2.5	2.9	0.251	0.341
22 May 2004	03:18	hb13	8	73	62	293047	19637	14.9	2.5	3.0	0.255	0.432
25 May 2004	05:23	hb1	32	24	9	39306	16481	2.4	2.1	2.5	0.247	0.137
total				170	134	634019	19691	32.2			0.242	0.347

Table 1: Data collection statistics, all counts for one (of eight) time points

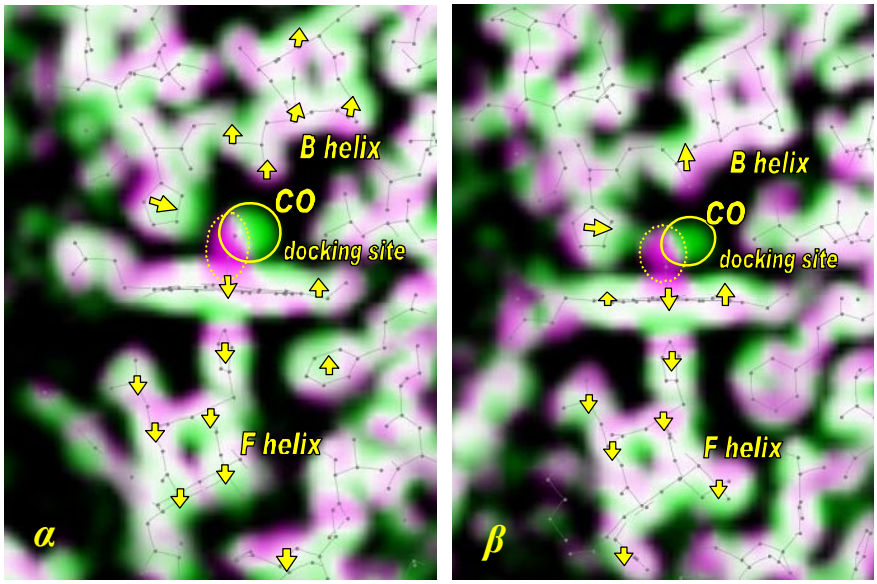

Figure 1: α and β subunits of hemoglobin, 100 ps after photolysis. The color indicates the photolysis-induced change of the electron density. This image is a superposition of two $2F_o$-F_c maps, unphotolyzed in green, photolyzed in magenta. Where the density remains unchanged both maps blend to white. The color gradient magenta-white-green indicates the direction of motion.