ESRF

**Experiment title:** Investigation of photo-stimulated structural changes with ns time resolution in protein crystals.

**Experiment number:** LS427

**Beamline:** BL3/ID9

**Date of Experiment:** from: 15-May-96 to: 21-May-96

**Date of Report:** 25-August-96

**Shifts:** 9sb + 8 1/3 fill

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**Report:**

After very successful ns time resolved studies of carbonmonoxy myoglobin (MbCO) photolysis in April 95, results of which have been presented in Bourgeois et al., 1996[1] and Srajer et al., 1996[2], the objectives of the May 96 experiments were the following:

1) to improve crystallographic data quality by signal averaging in order to more accurately quantify the small tertiary structural changes that occur upon CO photodissociation. Although data collected in April 95 experiment were of good enough quality to reveal the most substantial structural changes, such as the CO loss and heme relaxation, features resulting from smaller tertiary changes still could not be confidently distinguished from noise in the difference Fourier maps. To improve the signal to noise ratio of the data, in May 96 experiment we increased the signal averaging from 3-fold employed in April 95 to 10-15 fold. In addition, two insertion devices were used in series, a hybrid wiggler and an undulator, to yield a 3-fold increase in photon flux at the sample as compared to the hybrid wiggler alone used in April 95. Also, in the May 96 experiment we tested a new, faster mode of data collection in which the repeated X-ray exposures are accumulated on the CCD detector before the final single readout. This protocol allows a much more efficient data collection than saving separate exposures and summing them later for data processing, as done in April 95. A data set consisting of about 50 images could be collected in a total time of l-2h.

2) collect more crystallographic data in the ns to $10\mu s$ time domain. Due to the short duration of the single bunch experiment in April 95 and relatively slow data collection method, we collected only two data sets with 4ns laser/X-ray time delay at that time in the single bunch mode. Data for remaining time delays of $1\mu s, 7\mu s, 50\mu s, 350\mu s$ and 1.9ms were collected in April 95 using super pulses of $1\mu s$

duration in the 1/3 filling mode. However, a significant fraction of Mb molecules rebinds the CO molecule in $10\mu s$ (about 50% of molecules that had CO photodissociated) and the rebinding is completely over in about $100\mu s$. In addition, most important tertiary structural changes seem to occur in less than $10\mu s$, based on our April 95 data[2](also see our ESRF report of 29-Feb-96) and spectroscopic studies in solution[3]. Therefore, we needed to collect more data with ns time resolution in the 10ns to $10\mu s$ time domain. During the May 96 run we collected data at laser/X-ray delay times of ins, 4.9ns, 7ns, 18ns, 19ns, 45ns, 90ns, 182ns and 360ns in single bunch mode. In addition, we collected data at $1.4\mu s, 2\mu s, 5\mu s$ and $24.5\mu s$ delay times in 1/3 filling mode. This better sampling of the important time domain should yield a better understanding of the time evolution of tertiary structural changes.

3) improving the extent of crystal photolysis. Due to the plate-like habit of the monoclinic $P2_1$Mb crystals, it is advantageous to rotate the laser beam with crystals during the data collection in order to preserve the relative laser-crystal orientation and thus be able to preserve the optimum conditions for maximum crystal photolysis, where the laser beam pathlength through the crystal is short and constant. The laser light in these experiments is delivered to crystals via optical fiber. In the new, improved experimental set-up, another rotation stage, coaxial with the $\phi$ rotation of the crystal, is added to rotate the optical fiber when crystal orientation is changed. Due to the mechanical constraints of the very crowded environment oft he X-ray camera around the sample, we were able to rotate the laser fiber for only 90°, while crystal has to be rotated for 180° during a complete data set collection. Nevertheless, the fiber rotation helped to reduce the laser beam pathlength through the crystal as much as possible.

The analysis of the data collected in May 96 is in progress and it will enable us to assess how much these experimental changes helped to improve the data quality, to maximize the difference signal, to extract more accurate structure factor amplitudes and to enhance our confidence in the structural interpretation of the relatively small tertiary structural changes we see in the globin.

[1]Bourgeois, D., Ursby, T., Wulff, M., Pradervand, C., LeGrand, A., Schildkamp, W., Laboure, S., Srajer, V., Teng, T. Y., Roth, M. and Moffat, K. (1996) *J. Synch. Rad. 3, 65-74.*

[2]Srajer, V., Teng, T. Y., Ursby, T., Pradervand, C., Ren, Z., Adachi, S., Schildkamp, W., Bourgeois, D., Wulff, M. and Moffat, K., (1996) submitted to Science.

[3]Ansari, A., Colleen, J. M., Henry, E. R., Hofrichter, J. and Eaton, W. E. (1992) Science 256, 1796-1798; Tian, W. D., Sage, J. T., Srajer, V., Champion, P. M. (1992) *Phys. Rev. Lett. 68408-411.*