ESRF	**Experiment title:** Hemoglobin engineering for blood substitution: how do site-directed mutations affect hemoglobin structural dynamics?	**Experiment number:** SC3013
Beamline: ID09B	**Date of experiment:** from: 23/09/2010 to: 28/09/2010	**Date of report:** 28/02/2010
Shifts: 15	**Local contact(s):** M.Wulff	*Received at ESRF:*

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Report:

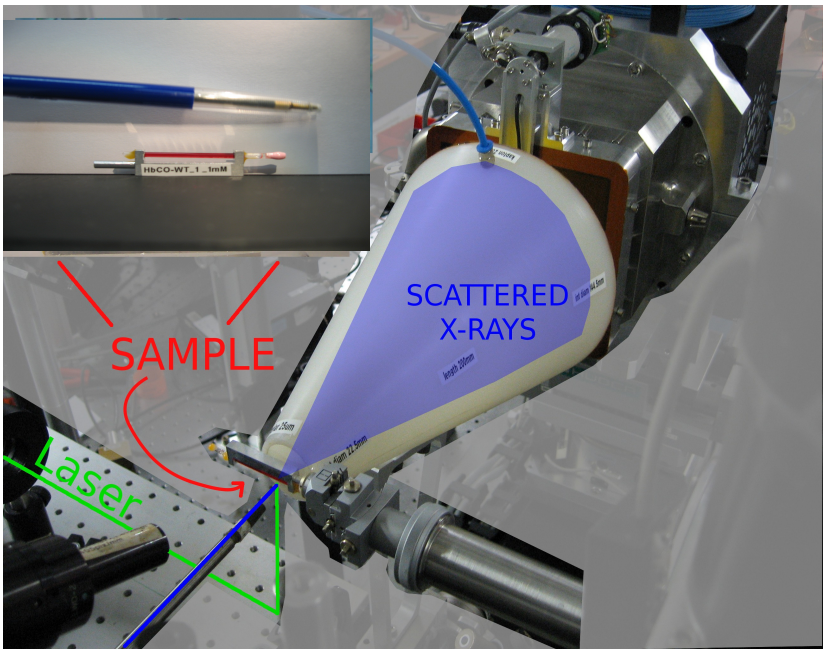

Figure 1: Experimental setup

The aim of this proposal was twofold:
1- to characterize the R-to-T transition in Hb mutants HbYQ HbaYQ and HbbYQ and compare it to the wt-Hb results.
2 - to reveal possible differences in the time scale of the local structural relaxation, so called tertiary relaxations.
Mutants Hb^{YQ}, $Hb\alpha^{YQ}$, $Hb\beta^{YQ}$ were prepared in prof. Vallone's lab using the decades of experience in protein expression and mutagenesis. Thanks to this experience we were able to prepare the amount of sample needed for this study.
Experimental parameters were be the same as those used in previous TR-WAXS studies on Hemoglobin (wild type) (1,2).
We used a 2 ns laser flash to get photolysis of Hbx-CO (x= YQ, αYQ, βYQ) inside a capillary previously equilibrated with 1 atm of CO and to acquire time-delayed structural snapshots of the solution as a function of time delay. The protein concentration was about 1 mM (tetramer).
The "Vibrant" laser available at ID09B was the most suited for such study; we planned to use about 0.5 mJ focussed to 0.2x1 mm^2 (at 530nm). An orthogonal laser pump / x-ray probe geometry was used to match the two different penetration depths of laser and x-ray.

Unfortunately the Vibrant laser stopped to work during the beamtime because of a technical problem. Nevertheless we managed to get some data on samples of Hb^{YQ}, $Hb\alpha^{YQ}$ and $Hb\beta^{YQ}$, obtaining promising results. The data have been reduced (from the CCD images to averaged difference curves) while collecting data using programs available at ID09B. In Figure 2 differential scattering signals at the time delays acquired are shown. The raw data suggest that significant differences in the structural time evolution are present. In particular, signals are different at longer time delays, when quaternary transition is expected to occur, thus suggesting that the main effect of mutation concerns quaternary structural changes. At ns time scale there is not any remarkable difference. However, a suitable number of time delays and a better signal-to-noise ratio is needed to obtain a complete description of structural kinetics.

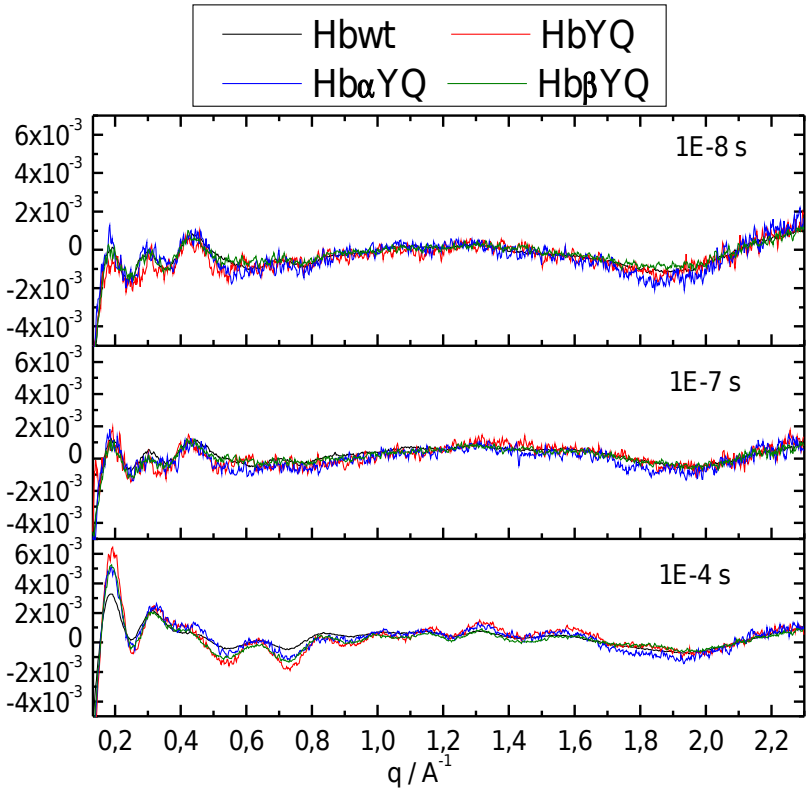

Figure 2: at fast time delays (1-100 ns) TR-WAXS signals are related to tertiary conformational changes of Hb induced by photolysis of CO ligand. At longer time delays (1 us to 100 us) TR-WAXS signal is related to the relative rotation of the two dimers hemoglobin is made of. At 100 ns time delay the amplitude of mutant and native hemoglobin is remarkably different suggesting a slower dimer rotation for HbYQ mutants.

Bibliography

1) M. Cammarata, M. Levantino, F. Schotte, P.A. Anfinrud, F. Ewald, J. Choi, A. Cupane, M. Wulff, and H. Ihee. *Tracking the structural dynamics of proteins in solution using time-resolved wide-angle X-ray scattering* Nature Methods 5: 881-886 (2008)

2) M. Cammarata, M. Levantino, M. Wulff and A. Cupane. *Unveiling the Timescale of the R–T Transition in Human Hemoglobin.* J. Mol. Biol. 400, 951–962 (2010)