ESRF	**Experiment title:** Tracking protein structural changes in solution using 100 ps resolved wide angle X-ray scattering (WAXS)	**Experiment number:** MX 653
Beamline: ID09B	**Date of experiment:** from: 10/09/2008 to: 14/09/2008	**Date of report:** 10^{th} December 2008
Shifts: 12	**Local contact(s):** Marco Cammarata	*Received at ESRF:*

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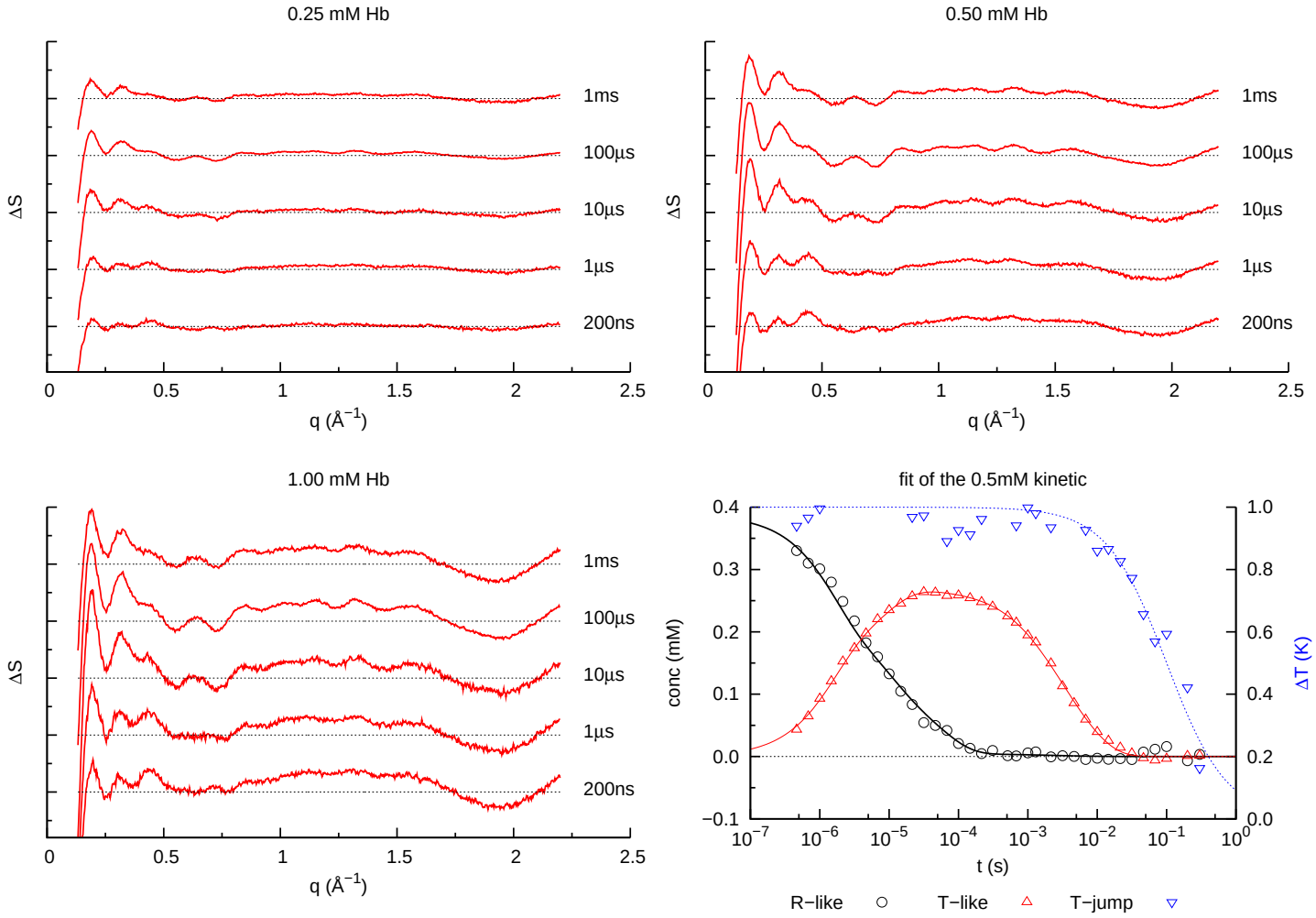
Report:

This experiment was initially proposed to investigate the Hemoglobin (Hb) structural dynamics with the highest possible time resolution (100ps). For a variety of reasons we have then preferred to focus our attention on the longer time scale (t > 200ns) to confirm the results published in our recent paper (1). Indeed the much faster formation of the unbound Hb conformation (T state) has been confirmed over a variety of conditions. The sample have been prepared using state of the art procedures. The Hb has been extracted by healthy person and purified in the Cupane's laboratory. This stock can be kept under liquid nitrogen for years. Immediately before the experiment the Hb has been complexed with carbon monoxide (CO) and prepared at different concentrations with the idea of varying the T-jump induced by the laser pulse. No significant dependence has been found. The optimized data collection strategy and the fact that we knew already the experimental parameters we wanted to use allowed us to collect essentially continuously allowing us to collect in 12 shift very good data for 6 time delays per decade.

The status of the sample was checked before and after the experiment using the spectrophotometer available at ID09B. While before the experiment an exceptionally good CO derivative was observed, after about 12 hours of data collection a small ($\sim$ 5%) of MetHb was found.

The data have been reduced (from the CCD images to averaged difference curves) while collecting data using programs available at ID09B and written by one of us (MC). Also the data analysis has been done in realtime allowing to stop the data collection for a given sample when the fit was found to converge in a robust way.



In the above figure we show the experimental data we collected. Indeed they can be described as the best ever collected with time resolved protein solution scattering. The analysis similar to the one described in (1) clearly reveal a time scale for the formation of the T-like conformation of $\sim 2\mu s$ independent on the concentration and T-jump. We are currently prepare a manuscript that would describe the Hb kinetics following photolysis.

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)