 <b>ESRF</b>	<b>Experiment title:</b> Conformational transitions of hemoglobin inside intact red blood cells: a time-resolved Wide Angle X-ray	<b>Experiment number:</b> SC-2542
<b>Beamline:</b> ID09B	<b>Date of experiment:</b> from: 03/12/2008                      to: 09/12/2008	<b>Date of report:</b> 11 <sup>th</sup> December 2008
<b>Shifts:</b> 18	<b>Local contact(s):</b> Marco Cammarata	<i>Received at ESRF:</i>
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#### Report:

The aim of this experiment was to study the Hemoglobin (Hb) structural dynamics following ligand dissociation in their natural environment: intact Red Blood Cell (RBC).

RBC have been prepared and characterized at the University of Palermo. “Smooth” and “healthy” cells have been obtained by properly choosing the salt concentrations. To avoid problems with the precipitations of the cells during the data collection we have waited for the cell to sediment and used an orthogonal pump-probe beam geometry with the laser beam coming from below (see fig.1 This ensured a stable concentration of cells during the experiment. At the beginning of the experiment we had some problem with a fast x-ray induced deterioration of the cells resulting in a loss of signal to the Hb. This has been solved changing the preparation protocol (see figure in the next page)

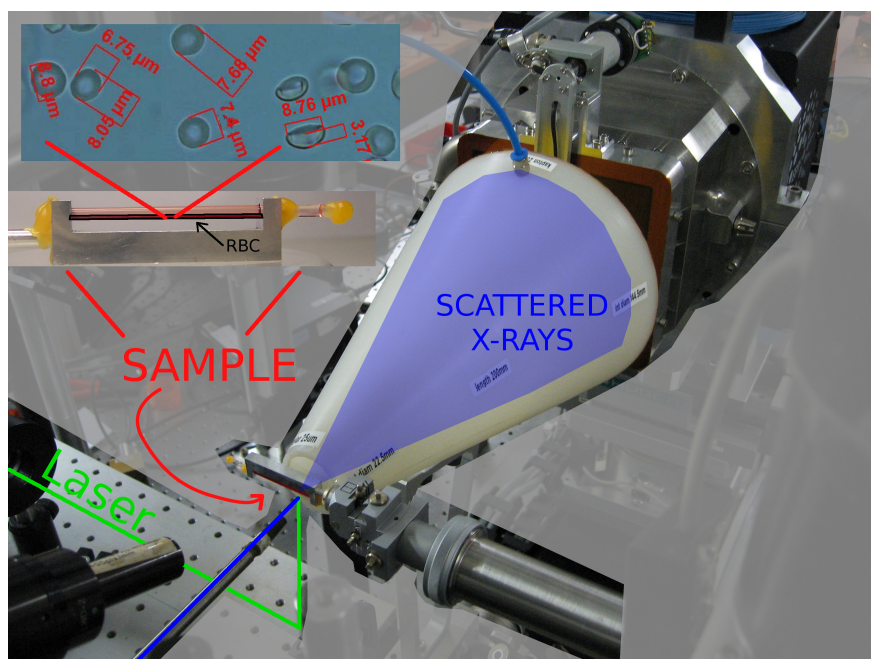
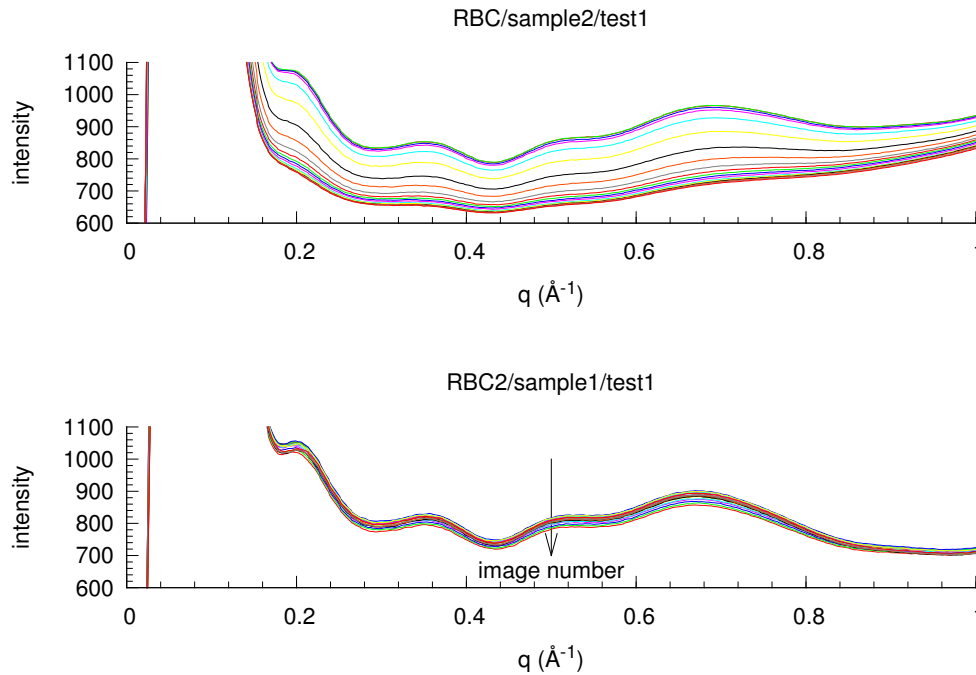


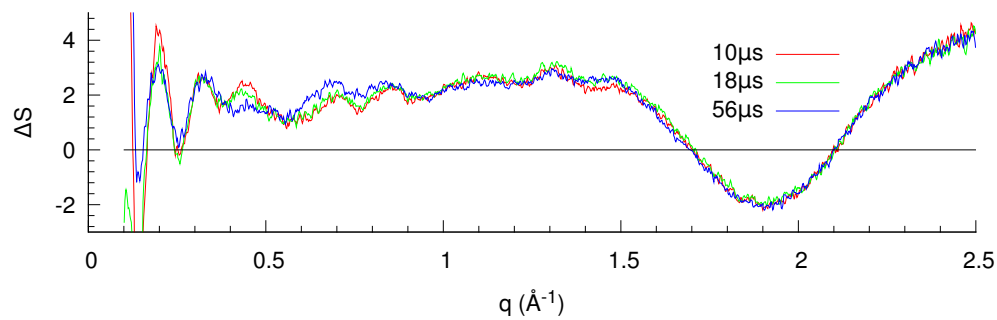
Figure 1: Experimental Setup

The data have been reduced (from the CCD images to averaged difference curves) while collecting data using programs available at ID09B.



Stability of the scattering pattern as function of the accumulated dose each image has been obtained with about  $4 \cdot 10^{12}$  incident photons. Every 4<sup>th</sup> images is shown. Upper panel: original preparation; Lower panel: modified one

In spite of the difficulties of exciting an highly concentrated solution (with the extra complication that being the RBC relatively big object they scatter visible significantly) we managed to get good S/N ratio at different time delays (4 delays per time decade in the range 100ns-10ms) as shown in the figure below.



Indeed the disappearance of the peak at around  $0.45 \text{ \AA}^{-1}$  can be taken as fingerprint of the quaternary transition of Hb. According to this very rough analysis it seems to happen later with respect to the solution case. The lower photolysis fraction could be a partial reason for it and a more detailed analysis will follow.

## 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)