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| ESRF | **Experiment title:**<br>Conformational transitions of hemoglobin inside intact red blood cells: a time-resolved Wide Angle X-ray Scattering Study | **Experiment number**:<br>SC 3010 |
| **Beamline**:<br>ID09B | **Date of experiment**:<br>from: 24/11/2010 to: 27/11/2010 | **Date of report**:<br>28/02/2010 |
| **Shifts:**<br>12 | **Local contact(s)**:<br>M. Wulff | *Received at ESRF:* |

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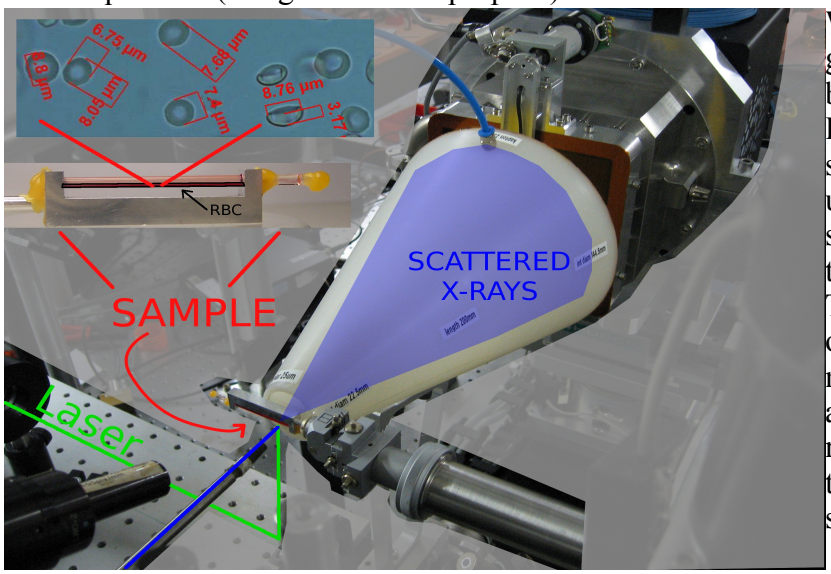
## Report:

The aim of this experiment was to study the Hemoglobin (Hb) structural dynamics following ligand dissociation in its 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 used a sample of densely packed red blood cells obtained by centrifugation and we put it in our liquid cell (designed for this purpose).




We used an orthogonal pump-probe beam geometry with the laser beam coming from below (see Fig.1)
Because of the high concentration of the sample, we aligned the x-ray beam at 100 um from the
surface of the sample holder in order to get the maximum possible extent of photolysis. The new design
of our liquid cell (shaped as a small cylinder made of mylar) allowed to get stable signals and good S/N
ratio at different time delays (4 delays per time decade in the range 300ns-10ms) as shown in figure2.

**Figure 1: Experimental setup.**

The shape of TR-WAXS signal in us time scale it is representative of quaternary transition [1-2], and from figure2 it is evident that we were able to track qatermary transition in vivo in intact red blood cells (see also figure3). Disappearance of the peak at around 0.45 Å-1 can be taken as fingerprint of the quaternary transition of Hb [1-2]. The kinetic of quaternary transition is affected by the ligation state of hemoglobin subunits after photolysis: from a raw inspection of the data it is not possible to understand if the difference observed in figure 2 is due to a different extent of photolysis in the two samples or to av packing effect in RBC slowing down the hemoglobin dynamics. To compare the results of in vivo measurements with the solution case we had to take into account the presence of different ligation states after laser photolysis and the relative effect on the quaternary transition rate. We used a simple kinetic model reported in [2] (see figure4), and fixed the same kinetic parameters obtained for hemoglobin in solution [2] to check if RBC data are consistent with our previous results or if there are remarkable differences in vivo.
The results of the analysis clearly show that quaternary transition rate is very similar to the solution case: we can reproduce our experimental data in RBC using the same kinetic parameters of hemoglobin

in solution. Even if the hemoglobin is densily packed in the red blood cells the rotation of its dimers is not affected (figure5).

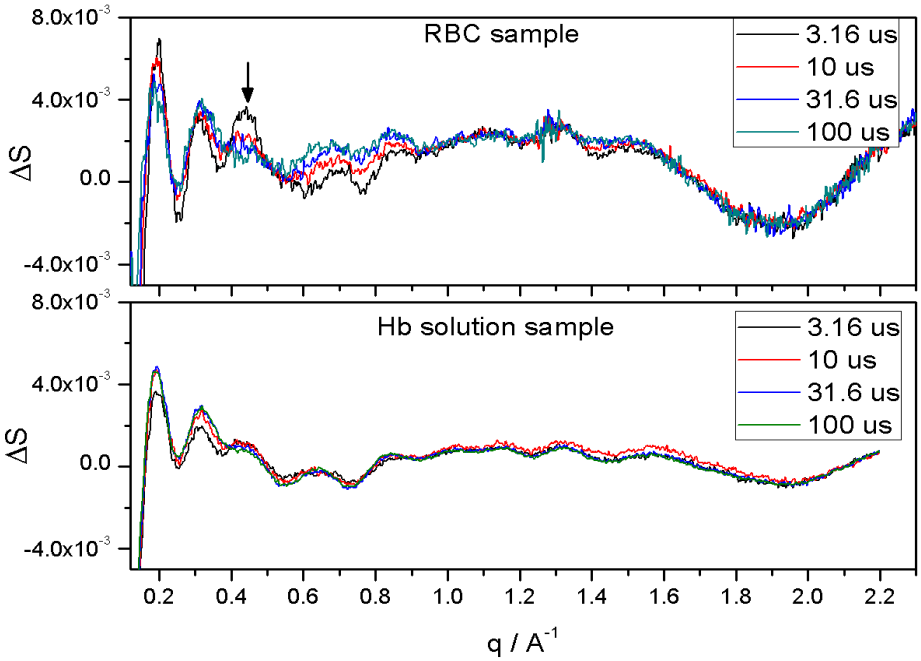



**Figure 2: Data in us time scale and comparison with Hemoglobin in solution.**

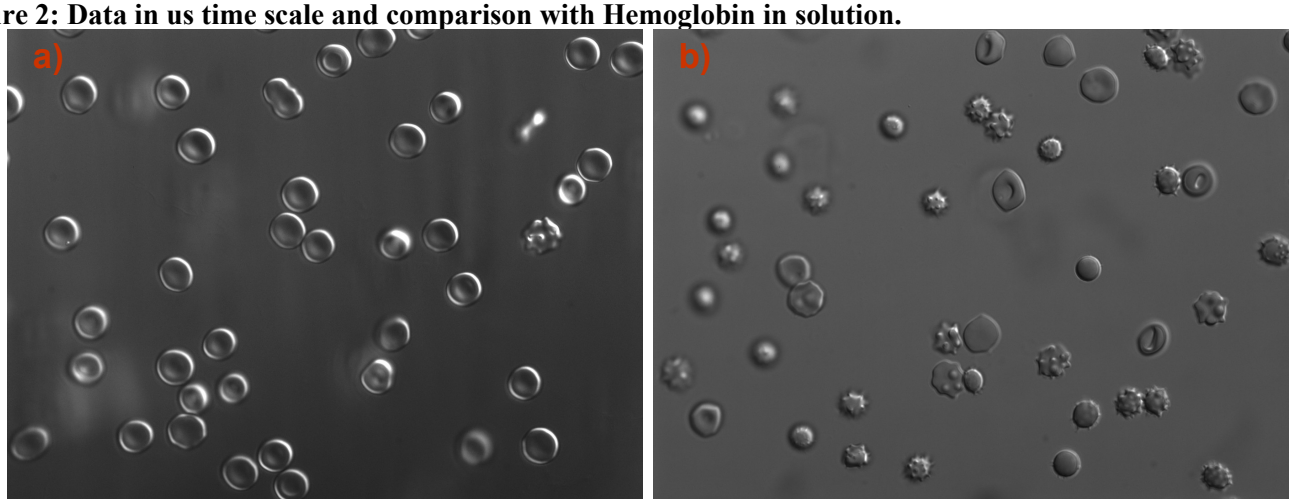



**Figure 3: Panel a: RBC sample before experiment; Panel b: RBC sample after experiment. The mean number of intact RBC is the same.**

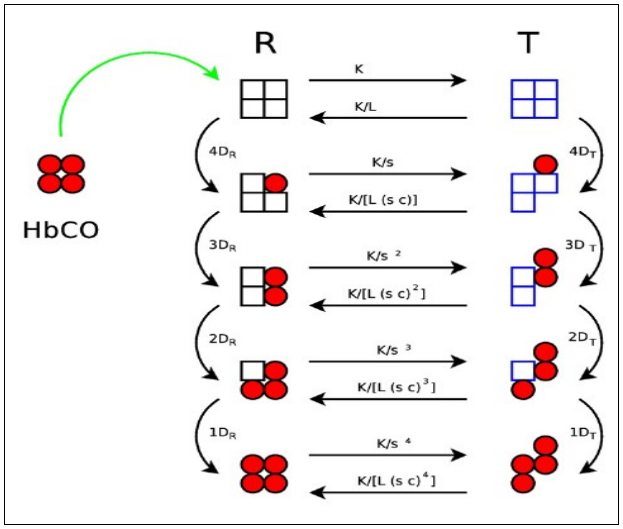



**Figure4: schematic sketch of the kitetic model used to analyze both in vivo measurements and solution data to compare the results.**

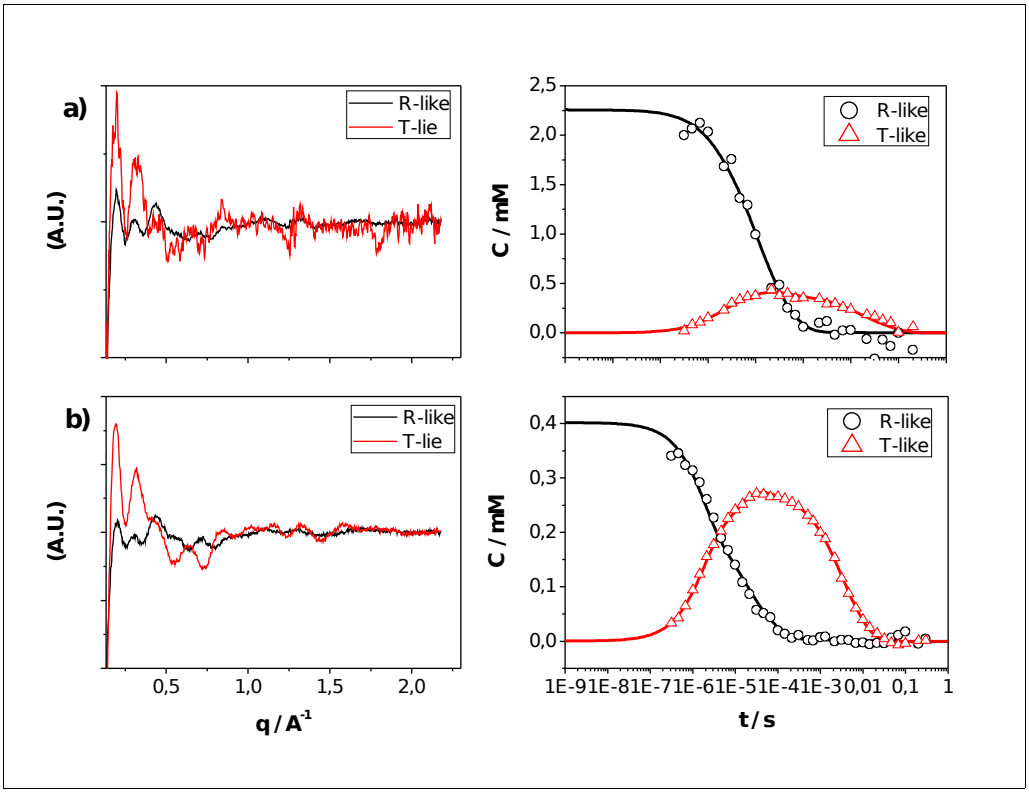



**Figure5: results of data analisys. Panel a) RBC data have been decomposed in terms of two basis pattern (left) representing an R-like specie produced after photolysis and a T-like specie produced after quaternary transition. In the right side of panel a) results of the decomposition are reported. Panel b) same analysis have been done on Hb data in solution.**
**The formation of the T state has a time constant of 2us for both RBC and solution experiment.**

**Table 1.** Parameters obtained by the fitting procedure described in [2], in parentheses (minimum:maximum)

| | Hb (solution) | RBC |
|---|---|---|
| Fraction excited | 0.80 (0.80:0.81) | 0.50 (0.50:0.51) |
| $\tau_{R-T}$ (us) | 1.9 (1.8:2.1) | 1.9 |
| L (x $10^{-3}$) | 8.85 | 8.85 |
| c (x $10^{3}$) | 2.92 (2.63:3.23) | 2.92 |
| s | 14 (13:15) | 14 |
| $D_R$ ($uM^{-1}$ $s^{-1}$) | 7.9 (7.6:8.2) | 7.9 |
| $D_T$ ($uM^{-1}$ $s^{-1}$) | 0.031 (0.030:0.032) | 0.03 |

**Bibliography**

1. M. Cammarata, M. Levantino, F. Schotte, P.A. Annrud, F. Ewald, J. Choi, A. Cupane, M. Wul, and H. Ihee. Tracking the structural dynamics of proteins in solution using timeresolved wide angle Xray scattering. Nature Methods 5: 881886 (2008).
2. M. Cammarata, M. Levantino, M. Wulff, A. Cupane. Unveiling the Timescale of the RT Transition in Human Hemoglobin. Journal of Molecular Biology 400: 951962 (2010).