Electronic Supplementary Material (ESI) for Soft Matter

SUPPLEMENTARY ONLINE MATERIAL

For manuscript:

Probing *in cell* protein structural changes with time-resolved X-ray scattering

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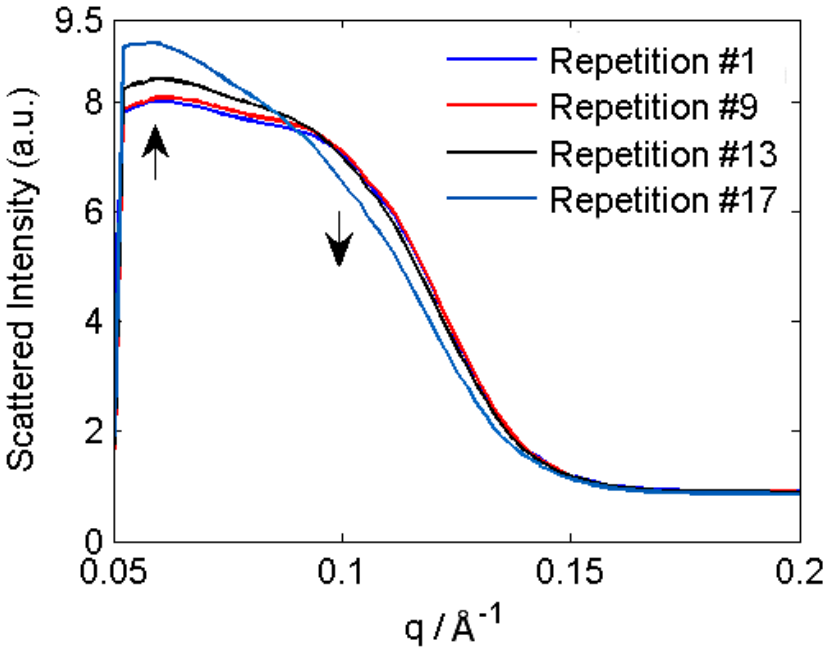
This supplement contains:

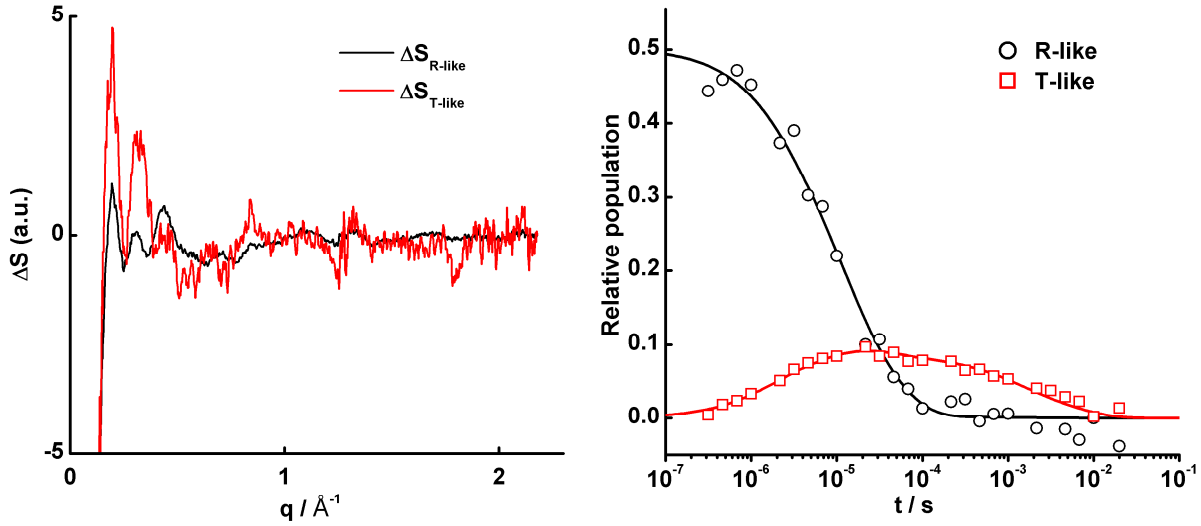
3 Supplementary Figures
Materials and Methods

SUPPLEMENTARY FIGURE 1



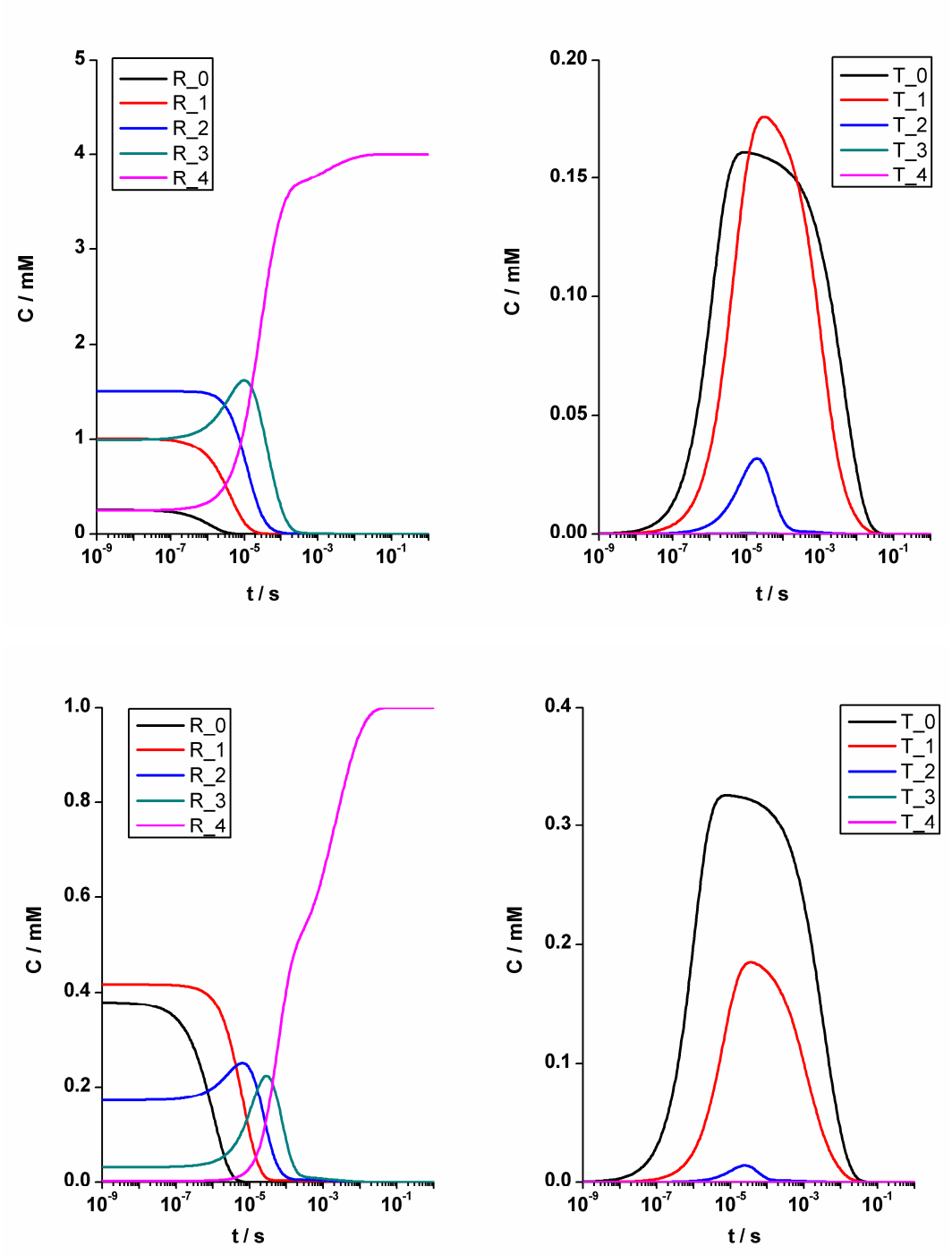
Supplementary Figure 1. Effect of X-ray irradiation on packed RBCs. Absolute scattering patterns measured in the absence of the photolysis laser (laser off images) are reported in the 0.05-0.1 $Å^{-1}$ region after normalization at 2.1 $Å^{-1}$. The scattering signal in this region is sensitive to Hb intermolecular distance and to RBC membrane structure[14]. A continuous monitoring of this region can be used as a real time check of radiation damage on RBCs. Only data showing less than 5% variation in the scattered intensity have been retained for data analysis.

SUPPLEMENTARY FIGURE 2



Supplementary Figure 2. Basis patterns and relative populations obtained from the analysis of TR-WAXS data in terms of the kinetic model schematically depicted in Figure 2c. Left panel: $\Delta S_{R\text{-like}}$ (black curve) and $\Delta S_{T\text{-like}}$ (red curve) basis patterns. Right panel: R-like (black symbols) and T-like (red symbols) coefficients obtained from a linear decomposition of the data in terms of the two basis patterns reported in the left panel. Continuous lines have been calculated from the model using best fit kinetic parameters; the agreement between the symbols and the lines represents a measure of the fitting quality.

SUPPLEMENTARY FIGURE 3



Supplementary Figure 3. Time dependence of the different Hb ligation states concentration as obtained by fitting the TR-WAXS data according to the kinetic model shown in the main text. Upper panels refer to packed RBCs while lower panels to Hb in solution. Left panels: Hb molecules in the R state having i ligands bound (R_i with i=0,..,4). Right panels: Hb molecules in the T state having i ligands bound (T_i with i=0,..,4). Note the ordinate scale differences between upper and lower panels.

MATERIALS AND METHODS

Sample preparation and handling. A fresh human blood sample was collected using 0.02 M EDTA as anticoagulant. Plasma was removed by repeated washing with 0.9% NaCl solution at 5 ℃. In order to ensure Hb equili bration with CO while minimizing bacterial contamination, the sample was equilibrated with humidified CO by gentle bubbling the supernatant solution for 20 minutes. Immediately before the experiment the RBCs were suspended in CO-saturated 130 mM Na-phosphate buffer at pH 7.4, which guarantees nearly isotonic condition (290 mOsm)[20]. Suitable aliquots of the sample were pelleted by centrifugation at 10000 g for 20 minutes (at 5 ℃) and deposited into the sample holder.

Data acquisition. TR-WAXS pattern were acquired at the beamline ID9B in the European Synchrotron Radiation Facility in Grenoble (France) while the machine was running in 7/8+1 hybrid mode. Packed RBCs samples were photolyzed with a circularly polarized 527 nm laser pulse having a time duration of 180 ns (full width at half maximum) at an energy density of ~2.5 mJ/mm^2 . Laser pulses incident on the bottom surface of the mylar sample holder were followed by delayed quasi-monochromatic X-ray pulses (100 ps, FWHM) extracted from the synchrotron with a high speed chopped and a millisecond shutter. X-ray pulses penetrated the mylar sample holder at ~80 um from its bottom edge so that an orthogonal geometry between X-ray and laser pulses was realized. To dilute any radiation damage over a large sample volume, the sample holder was translated back and forth along its long axis over a ~20 mm range. To ensure that successive pulses in the 10 Hz pulse train excited adjacent but spatially separated sample volumes, the holder was translated by 0.2 mm after each probe pulse. Scattered X-rays were recorded in the forward direction by a sensitive FReLoN camera (ESRF). Each image was azimuthally averaged and converted into a one dimensional q-pattern using λ = 0.6793 Å corresponding to the peak of the U17 undulator. After normalization (in the q-region 2.0-2.2 $Å^{-1}$), a reference scattering pattern ("laser off" image), which probed the unexcited sample, was subtracted from the scattering pattern at each time delay. Intensity differences acquired at the same time delay were averaged.

Kinetic model and fit. The kinetic model depicted in Figure 4a of the main text corresponds to a set of differential equations describing how the population of the different

molecular species of the system evolves with time. The model, similar to that originally introduced by Sawicki and Gibson[24] and already successfully used for TR-WAXS data analysis on Hb solutions[13,15], assumes the presence of Hb molecules in two quaternary conformations, R and T, and five ligation states (R_i and T_i, where i=0..4 indicates the number of bound ligands). Transitions from one state to the other are accomplished by bimolecular rebinding or by interconversion between quaternary states (unimolecular ligand dissociation is neglected in view of the high CO-iron bond stability). A single bimolecular ligand rebinding rate (apart from statistical factors) is introduced in the model for each quaternary state (the microscopic bimolecular rates to R and T species are indicated as D_R and D_T, respectively). The complete set of R-T transition rates are assumed to be proportional to the R_0-T_0 transition rate and to scale down with the number of bound ligands through the s parameter[15,25]. The inverse T-R transition rate are linked to the direct R-T rates through thermodynamic equilibrium relations that make use of the allosteric constant $L=[T_0]/[R_0]$ and the affinity ratio $c=K_T/K_R$, where K_T and K_R are the equilibrium association constants to T and R species, respectively. To solve this set of differential equations the following initial conditions have been used:

$$[T_i] = 0 \qquad \text{for i = 0,...,4} \qquad (1)$$

$$[R_i] = [Hb]\binom{4}{i}(1-N_0)^i N_0^{4-i}$$

where N_0 is the fraction of deoxyhemes after the pump pulse. Starting from initial guess values, the fitting program calculates all $[R_i](t)$ and $[T_i](t)$ and the concentration of CO in solution, [CO](t). In analogy with previous analyses[13,15], TR-WAXS patterns have been decomposed in terms of two basis patterns, $\Delta S_{R\text{-like}}(q)$ and $\Delta S_{T\text{-like}}(q)$:

$$\Delta S(q,t) = \text{R-like}(t)\times\Delta S_{R\text{-like}}(q) + \text{T-like}(t)\times\Delta S_{T\text{-like}}(q) \qquad (2)$$

where R-like(t) and T-like(t) are weighted sum of the $R_i(t)$ and $T_i(t)$ populations:

$$\text{T-like}(t) = \frac{1}{[Hb]}\left([T_0](t) + [T_1](t) + [T_2](t) + [T_3](t) + [T_4](t) \right)$$

$$\text{R-like}(t) = \frac{1}{[Hb]}\left([R_0](t) + \frac{3}{4}[R_1](t) + \frac{1}{2}[R_2](t) + \frac{1}{4}[R_3](t) \right) \qquad (3)$$

The results of the fitting procedure is illustrated in Figure 4b. The basis patterns and the time evolution of their population (R-like and T-like) are reported in Supplementary Fig. 2. The time changes in the R_i and T_i concentration are given in Supplementary Fig. 3 and compared with those obtained for a 1 mM Hb solution.

Supplementary References

24. Sawicki, C, Gibson, Q. H. *J. Biol. Chem.* 251,1533-1542 (1976).

25. Eaton, W. A., Henry, E. R. and Hofrichter, J. *Proc. Nati. Acad. Sci. USA* 88, 4472-4475 (1991).