Temperature transitions of Hemoglobin and cytosolic water diffusion in human Red Blood Cells

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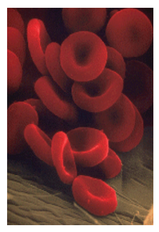
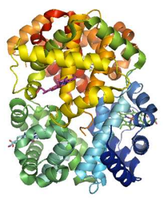
Background

Red Blood Cells are responsible for oxygen transport in the human body. Red Blood Cells (RBC) (diameter 5µm) consist of around 70% cytosolic water, 30% Hemoglobin (300mg/ml), all other substances contribute with less then 1%.

Hemoglobin is a tetrametric protein which structure is predominatedly alpha-helical. Each monomer holds a heme-group which carries oxygen or other small molecules.



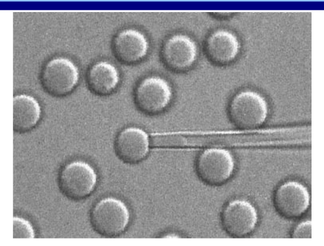
Red blood cells with discocyte shape



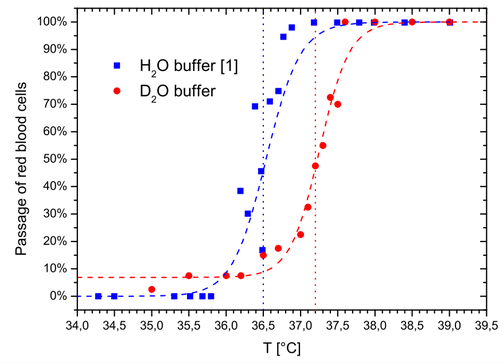
X-ray structure of hemoglobin (PDB 1G09) [3]

RBC which are aspirated by micropipettes at 20℃ undergo extreme deformation and the trailing sphere blocks the pipette. Above a critical temperature T_c=36.5℃ all RBC pass the pipette [1]. The critical temperature T_c is very close to human body temperature.

Micropipette aspiration experiments in H_2O buffer show a transition temperature T_c at 36.5℃ [1]. A similar behaviour was observed in D_2O buffer with a transition temperature T_c at 37.2℃ [2].



Red blood cell aspirated with a micropipette [1]

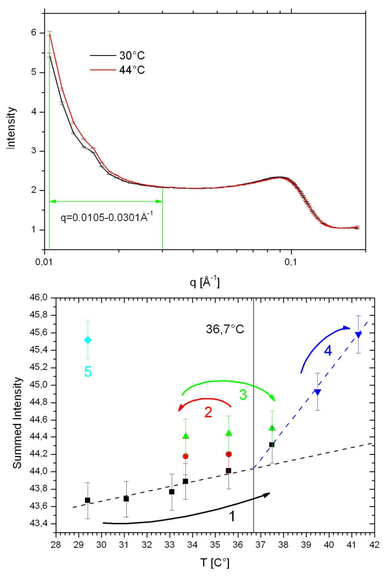


Hemoglobin interactions in red blood cells

By means of *Small Angle Neutron Scattering* on the instrument **D22 at ILL** we studied Hemoglobin interactions in native human Red Blood Cells in H_2O buffer.

At this high protein concentration the measured intensity I(q) is a product of molecular form factor F(q) and structure factor S(q): I(q) = S(q) * F(q)

The intensity at low q was summed and is an indicator of particle aggregation. **We found that Hemoglobin aggregation occurs at temperatures higher than 37℃.** Heating to 41℃ leads to irreversible aggregation.



Measured SANS data of RBC in H2O. The increase of intensity at low q-vector is a fingerprint of particle aggregation.

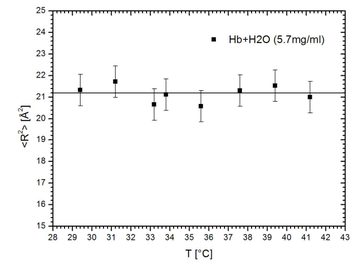
Integrated intensity at low q-vector range from SANS data. At 37℃ there occurs Hemoglobin aggregation. Heating to 41℃ provoces irreversible aggregation. The arrows indicate heating and cooling phases.

As a reference we also measured diluted Hemoglobin in H_2O (5.7mg/ml). Under these low concentrations we only obtain the form factor F(q) and we could determined the Guinier radius $<R^2>$.

We found that $<R^2>$ of Hemoglobin remains constant in the investigated temperature range. This proves that the effects shown above result only from interparticle interations.

$$< R^2 >= \frac{-3 \cdot \ln I(q)}{q^2}$$

$$< R^2 > \cdot q^2 \leq 1$$



Radius of Gyration $<R^2>$ of Hemoglobin determined from SANS. No changes of $<R^2>$ could be found in the temperature region.

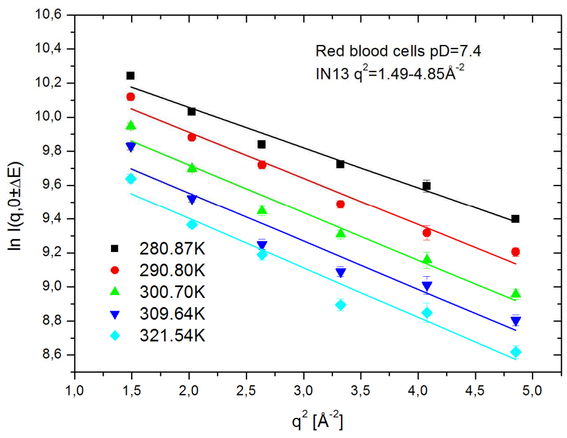
Hemoglobin dynamics in human Red Blood Cells

We measured *Elastic Incoherent Neutron Scattering* of human RBC in D_2O buffer on the backscattering instrument **IN13 at ILL**. Only hemoglobin dynamics are being measured by the use of D_2O and the energy resolution and q-vector range of **IN13**.

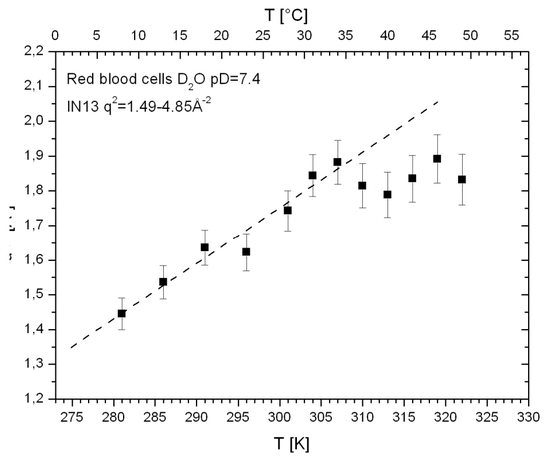
Mean square displacements of Hemoglobin hydrogen atoms were calculated according to

$$< u^2 >= \frac{-6 \cdot \ln I(q)}{q^2}$$

We found a linear increase of $<u^2>$ below human body temperature (37℃) and a flattening of $<u^2>$ starting at body temperature. **This is caused by aggregation of Hemoglobin molecules. The occuring interparticle forces stabilize protein dynamics**.



Measured data as a function of q^2-vector and temperature. Mean square displacements were obtained from the indicated linear fits.



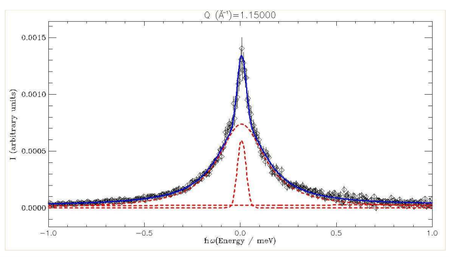
Mean square displacements $<u^2>$ of Hemoglobin hydrogens. A onset of flattening of $<u^2>$ is clearly visible around 37℃.

Diffusion of H_2O in Red Blood Cells

We studied if changes of Hemoglobin dynamics influence water diffusion in RBC. We didn‘t find that Hemoglobin dynamics are connected to cytosolic water diffusion.

We measured *Incoherent Quasi-Elastic Neutron Scattering* on the time-of-flight spectrometer **FOCUS at PSI** with an energy resolution of 50µeV.
Data could be fitted with a delta function plus one Lorentz function and background.

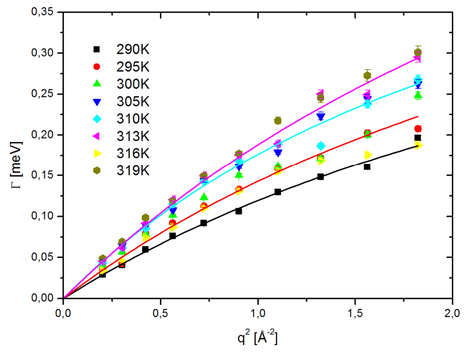
$$S(q,\omega)_{measured} = \left(\delta(q) + L(q,\omega) + C\right) \otimes S(q,\omega)_{resolution}$$



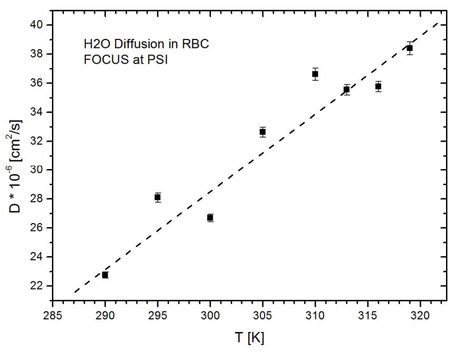
Data of H_2O in Red Blood Cells at 290K and q=1.15Å^{-1}
The blue line is the fit to the data, the red lines show the contribution of the delta and Lorentz function plus background.

The Half-Width at Half-Maximum Γ(q) of the Lorentz function contains information about the diffusion coefficient D of H_2O. We fitted Γ(q) best with a jump-diffusion model:

$$\Gamma(q) = \frac{Dq^2}{1 + Dq^2\tau}$$



The resulting diffusion coefficient D of H_2O shows no correlation to Hemoglobin dynamics. Compared to H_2O diffusion in buffer measured on TofTof at FRM2 with an energy resolution of 100µeV (unpublished results) we find very similar values. **We conclude that most water in cells resemble bulk water and doesn’t feel the influence of protein surfaces and dynamics**.



Literature

[1] Artmann G., Kelemen Ch., Porst D., Bueldt G., Chien S. (1998) Temperature Transitions of Protein Properties in Human Red Blood Cells. Biophysical Journal 75:3179-3183
[2] Artmann et al. Hemoglobin senses body temperature, in preparation
[3] Mueser TC., Rogers PH., Arnone A. (2000) Interface Sliding As Illustrated by the Multiple Quarternary Structures of Liganded Hemoglobin. Biochemistry 39:15353-15364