in-vitro AND *in-vivo* DIFFUSION OF PROTEINS IN CROWDED SOLUTIONS :

S. Longeville [1], W. Doster [2]

[1] Laboratoire Léon Brillouin (CEA-CNRS), CEA-Saclay, 91191 Gif-sur-Yvette cedex
[2] Technische Universität München, Physik Department, James Franck Strasse 1, D-85747 Garching

The interior of living cells is a complex, crowded environment, composed of a large number of molecules including proteins at high concentration. The respective volume fractions range up to 0.4. Under these conditions protein-protein interactions play a central role. The question is raised whether this environment could affect some physical, chemical or biological properties. A particular interest has been devoted to the study of diffusion mechanism in highly concentrated protein solutions, with the aim to address the question of transport properties and the possible diffusion limited kinetics of biochemical reactions. A particular aspect concerns the transport of small molecules like oxygen by protein diffusion. The transport of oxygen from the lung to muscle cells is performed by hemoglobin tightly packed in blood cells. Hemoglobin must catch the oxygen near the cell membrane. Thus the transport depends on a delicate balance between two opposing factors : High protein concentrations in the cells, which will enhance the quantity of stored oxygen, and crowding, which will depress the speed of oxygen carriage because strong protein interactions dramatically decrease hemoglobin mobility. In fact an optimum concentration for the oxygen flux is observed [1].

One central goal of our project is to clarify the question, whether the mobility of different components in a living cell can be understood based on their intermolecular interactions. To this end we studied the diffusion of myoglobin and hemoglobin molecules in-vitro, as a function of their volume fraction at concentration and temperatures corresponding to the physiological conditions. We also studied hemoglobin diffusion directly inside red blood cells.

As a first step, we perform a structural analysis of the solution, based on SANS data (small angle neutron scattering) and the molecular form factor measured on dilute protein solutions. The spectra were recorded as a function of the concentration corresponding to volume fraction ranging from F~0.05 and F~0.4. After some preliminary data treatment and background correction the measured quantity is accurately describable by the function,

$$I(Q) = F v_p (\Delta\rho)^2 . F(Q) . S(Q)$$

F is the volume fraction of the protein, v_p is the volume of the molecules $(\Delta\rho)^2$ is the coherent scattering length density contrast of the molecules with respect to the solvent in cm^{-1}. F(Q) is the normalized experimental molecular form factor and S(Q) the structure factor. The spectra were refined using the renormalized mean spherical approximation calculation (RMSA), the resulting experimental points and structure factor calculations are shown on figure 1.

In this theory, a Yukawa tail is used to account for the electrostatic potential, 3 parameters are relevant for this description, the Debye length L_D, the radius protein (assumed to be spherical) and the absolute protein charge. As can be seen on figure 1 reasonable description of the structure factor can be obtained over all refined concentrations with a diameter σ_p ~ 32±2 Å and the charge $|Z_p|$~1.7±0.2 e, L_D was computed from salt concentrations.

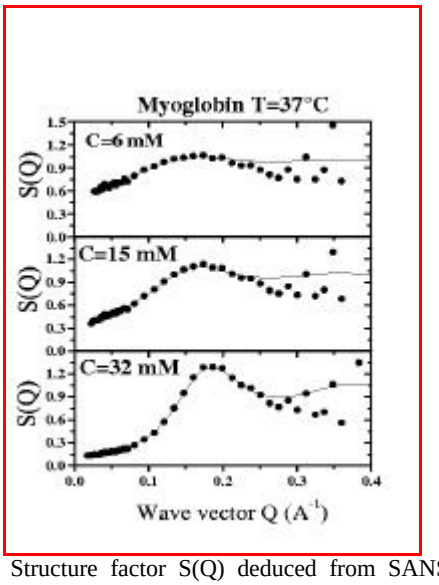

Figure 1. Structure factor S(Q) deduced from SANS data analysis (full circle). The lines are the results of the refinements using RMSA analysis (see text) .

In the second step we measure the time dependence of protein diffusion on the scale of the intermolecular distance using neutron spin echo spectroscopy. The spatial resolution provides insight into mechanistic aspects : How does the diffusion coefficient behave in the vicinity of the intermolecular structure factor maximum, where the interaction is most pronounced? How is hydrodynamic interaction between proteins affecting diffusion?

The Intermediate Scattering Function (ISF) was measured on the medium wave vector G1bis in the range of Q=0.05-0.3 $Å^{-1}$ and on IN15 for smaller Q values down to Q=0.02 $Å^{-1}$. Over the wave vectors and concentration under investigation the time decay doesn't seem to show any departure from the single exponential behaviour. The ISF was refined using $I(q,t) \sim \exp(-\Gamma t)$ with $D(q)=\Gamma/q^2$ D(q) is an apparent diffusion coefficient [2].

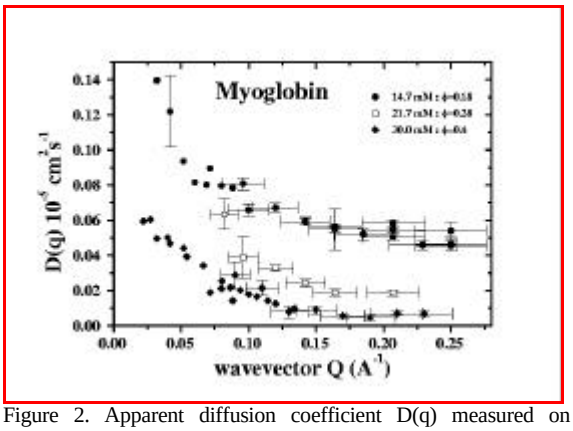

Figure 2. Apparent diffusion coefficient D(q) measured on 3 different myoglobin solutions with volume fraction F=0.18,F=0.28 and F=0.4. The plateau value corresponds to the self-diffusion coefficient.

The coherent scattering length density between protein and solution are strong enough to neglect any other contribution than the protein-protein one. In the wave vector range under investigation, polarisation analysis show that H incoherent scattering can be neglected and D_2O contribute only very little to the scattering intensity. Thus one should include all pair protein-protein contributions in the computation of the ISF and the apparent diffusion coefficient D(q) is similar to the one measured by light scattering. It corresponds to a collective diffusion coefficient, however in a completely different wave vector range. This diffusion coefficient measures the concentration fluctuations relaxations.

The wave vector dependence of the apparent diffusion coefficient D(q) is represented on figure 2 for 3 different concentrations. Whatever the myoglobin concentration the behaviour is similar, D(q) tends to a constant for wave vectors q ~ q*, whereas it present a pronounced increase for q < q*. The asymptotic value of D(q) for high q corresponds to the self diffusion coefficient D_s. We plot on figure 3 the concentration dependence of the self-diffusion coefficient measured on myoglobin.

The figure 4 presents the product D(q)S(q) myoglobin solution with volume fraction F~0.2. D(q) was measured by neutron spin echo spectroscopy and S(q) computed with the appropriate molecular fraction using analytical formulae of the RMSA analysis.

The results can be summarised as follow :

i- using neutron spin-echo spectroscopy one can investigate both individual and collective motions of the molecules over characteristic lengths around the mean interparticle distances,

ii- the reduction of the self-diffusion coefficient from infinite dilution solutions to physiological concentration is of the order of 25 for pure myoglobin and hemoglobin.

iii- Following Ackerson formula $D(q)=D_\infty H(q)/S(q)$, and combining structural and dynamical analysis one ca separate the effect of direct interactions (modelled by an hard sphere potential and a Yukawa screened type electrostatic interaction) and indirect (or solvent mediated) hydrodynamic interactions [3].

iv- The hydrodynamic factor H(q) oscillates in phase with the structure factor.

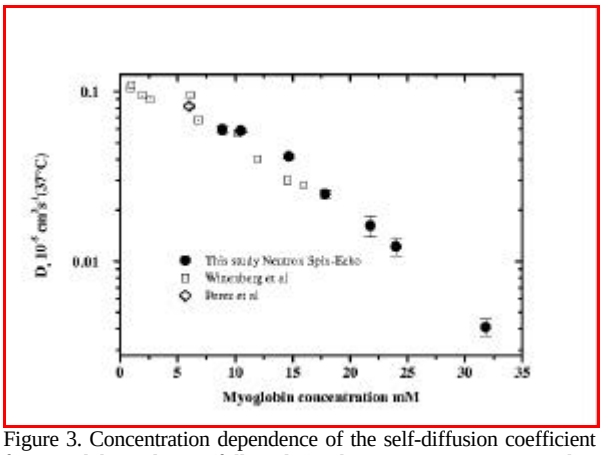

Figure 3. Concentration dependence of the self-diffusion coefficient for myoglobin solution (full circles). The open square correspond to macroscopic measurements by Wittenberg et al which have been corrected for temperature and different in solvent viscosity using Stokes-Einstein relation [2]

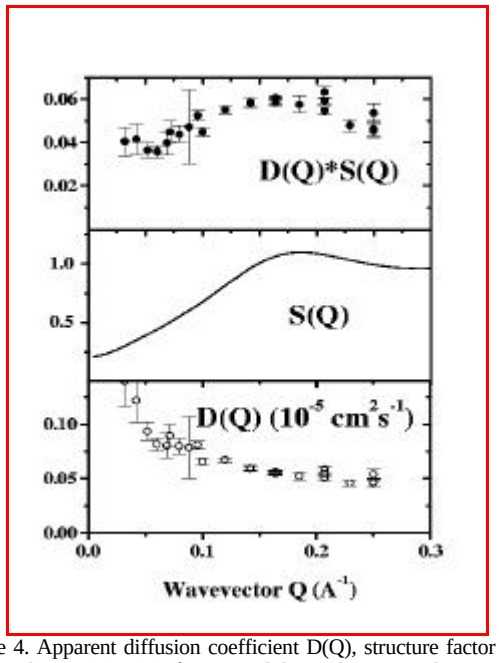

Figure 4. Apparent diffusion coefficient D(Q), structure factor S(Q) and product D(Q).S(Q) for myoglobin solution with a volume fraction F =0.18.

The oxygen transport is under investigation; in particular we focus on the mechanism of oxygen exchanged at the blood cell level with the aim to clarify the relative influences of self and collective motions. Therefore we performed measurements of hemoglobin diffusion directly inside the erythrocites.

References

[1] J.B. Wittenberg, *J.Biol.Chem. 241*(1966) 104-114.

[2] S. Longeville, W. Doster, M. Diehl, R. Gähler and W. Petry, *Neutron Spin Echo Spectroscopy, Basic Trends and Applications*, Lecture Notes in Physics, Vol. 601, Eds. F. Mezei, C. Pappas, T. Gutberlet. Springer, Berlin, 2002

[3] S. Longeville, W. Doster, and G. Kali, *Chem. Phys. (*Under press)