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Effect of high pressure on the global and internal dynamics of a multimeric protein : case of Hemoglobin studied by quasielastic neutron scattering experiments

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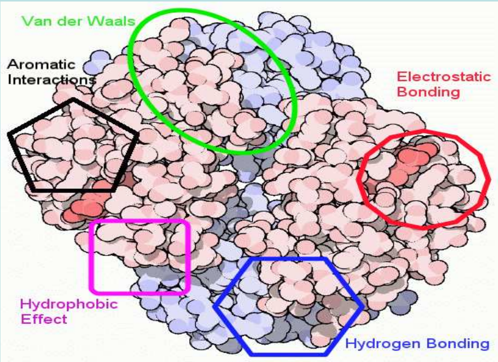
Effect of high pressure on the global and internal dynamics of a multimeric protein : case of Hemoglobin studied by quasielastic neutron scattering experiments

- Pressure and proteins : Thermodynamics of Unfolding
- Protein motions and quasielastic neutron scattering
- High Pressure cell description and Set-up of the experiment
- Hemoglobin description and previous results
- Results
- Conclusion
- Aknowledgements

Pressure and proteins : Thermodynamics of Unfolding

Pressure on protein interactions



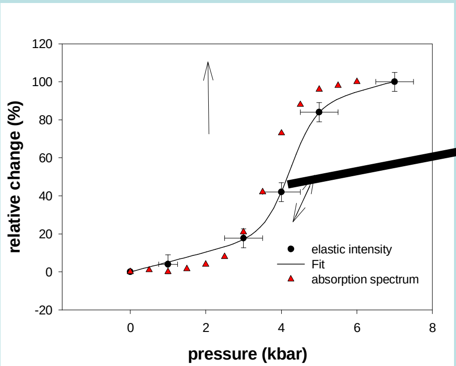
<u>**destabilized by pressure:**</u> decreasing volume

- **electrostatic interactions**: electrostriction
- **hydrophobic interactions:** water structure

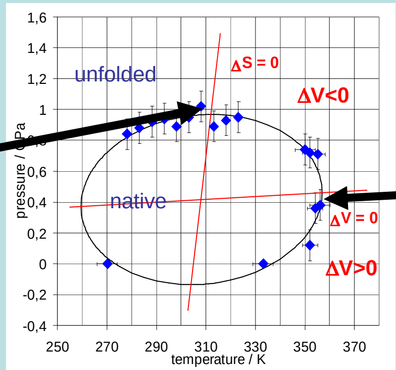
<u>**stabilized by pressure:**</u> decreasing distance

- **covalent bonds**: incompressible up to 1 GPa
- **van-der-Waals-interactions**
- **hydrogen-bonds**

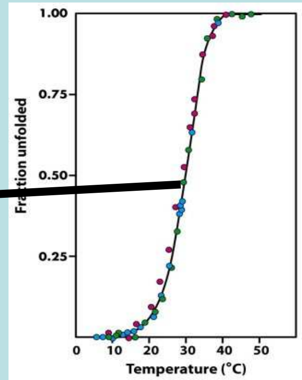
Pressure-Temperature phase diagram



Elastic neutron intensity and Absorbance for Metmyoglobin in solution. **(Doster W, Gebhardt R, 2003)**



d(ΔG) = - (ΔS) dT + (ΔV)dp
dp/dT= ΔS/ ΔV
(Lesch et al. Biophys.J. 2001)



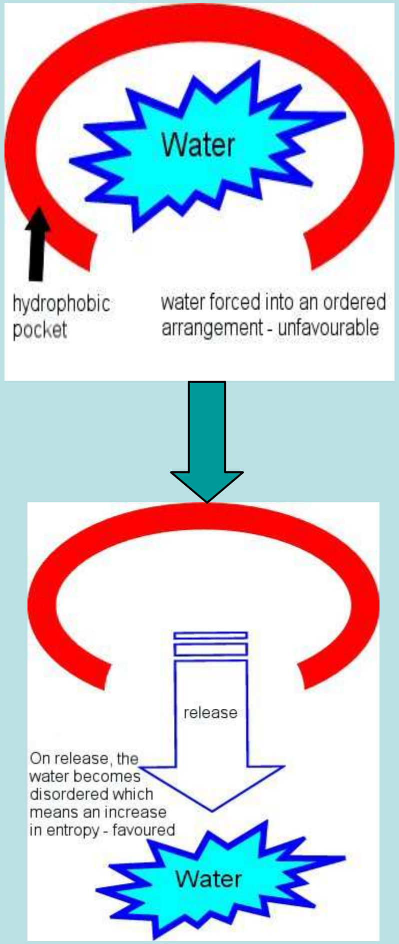
Absorbance, viscosity and optical rotation for Ribonuclease A solution. **(Stelea et al, 2001)**

ΔV depends on temperature and pressure

0 -2 kbar: Dissociation of oligomeric proteins and large scale structures

3 - 8 kbar: Unfolding of monomeric proteins

Why does a compact protein structure unfold under pressure?



$$\Delta S > 0, \quad \Delta V <> 0 ?$$

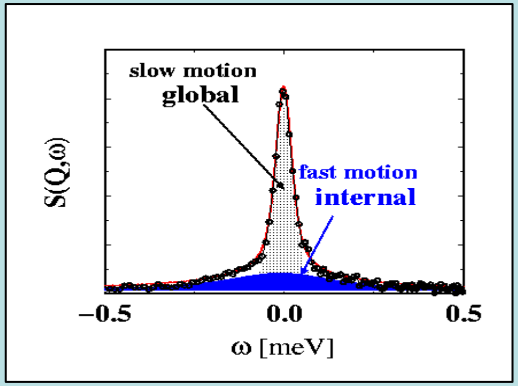
Protein motions and quasielastic neutron scattering

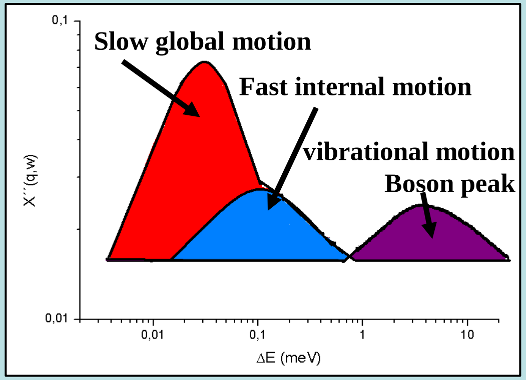
Motions in protein

Motion	Characteristic time (s)
Vibrations of bonded atoms	10^{-15}-10^{-13}
Side-group reorientations	10^{-12}-10^{-11}
Torsional libration of buried groups	10^{-10}-10^{-9}
Main-chain torsional reorientations	10^{-9}-10^{-8}
Relative motion of different protein regions	10^{-11}-10^{-7}
Main chain dynamics	10^{-7}-10^{-5}
Rotation of the whole molecule	10^{-7}-10^{-5}
Translation of the whole molecule	10^{-9}-10^{-3}

J.A. McCammon, S.C. Harvey, Dynamics of Proteins and Nucleic Acids, Cambridge University Press, Cambridge, 1988, p. 29.

quasielastic neutron scattering





The dynamic structure factor

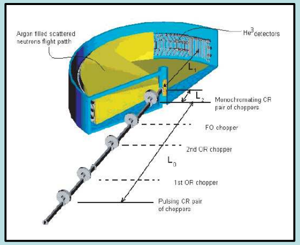
$$S_{inc}(q,\omega)=\sum_i \frac{1}{2\pi}\int_{-\infty}^{\infty} e^{i\omega t}(e^{-i\vec{q}.\vec{r}_i(0)}e^{-i\vec{q}.\vec{r}_i(t)})dt$$

The dynamic susceptibilty

$$\chi''_{inc}(q,\omega)=\frac{\hbar\omega S_{inc}(q,\omega)}{k_B T}$$

M. Bée, Quasi-Elastic Neutron Scattering, Principles and Applications in Solid State Chemistry, Biology and Materials Science, Adam Hilger, Bristol, 1988.

The time-of-flight neutron scattering spectrometer TOFTOF in FRM2 Garching

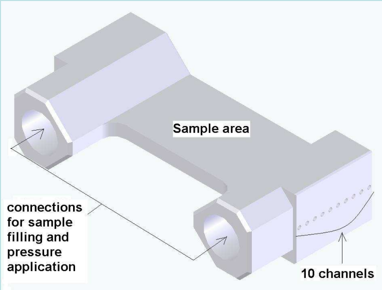


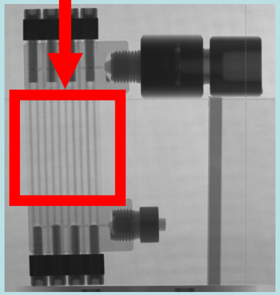
Wavelenght : 6 Å, Resolution = 60 μeV
time scale: 20 picosecondes

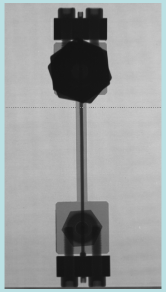
A.M. Gaspar, Methods for analytically estimating the resolution and intensity of neutron time-of-flight spectrometers. The case of the TOFTOF spectrometer, arXiv:0710.5319v1 [physics.ins-det] (2007)

High Pressure cell description and Set-up of the experiment

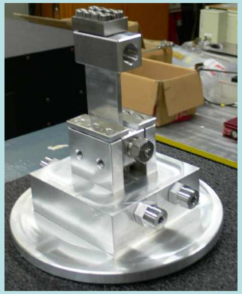
What is seen by the neutron beam





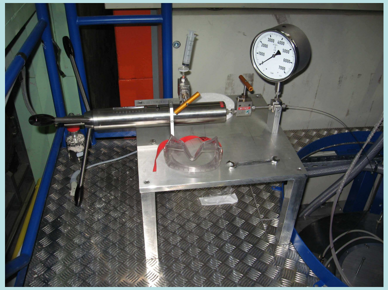


For temperature control



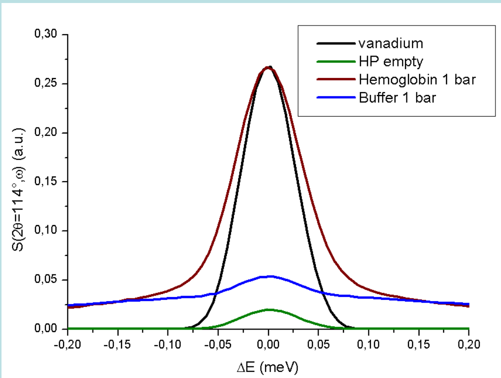
For protection

For inputing pressure

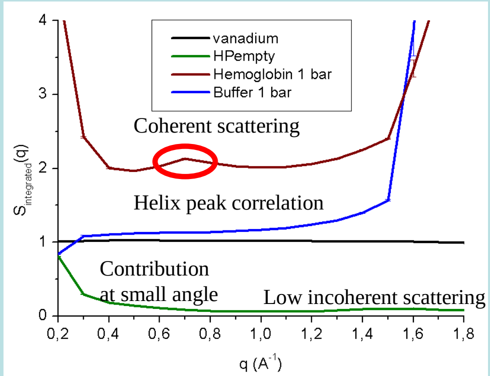


Aluminium alloy Al7075 ➔ pressure up to 2000 bar with a low scattering contribution!!!
Thickness sample (myoglobine solution) : 1,6 mm ➔ T = 82,6%
Thickness cell : 3,4 mm ➔ T = 93,9 %

Signals from the high pressure cell and the samples



Experimental dynamical structure factor



Integrated intensities

Neutronography measurements on ANTARES spectrometer at FRM2

Hemoglobin description and previous results

Description :

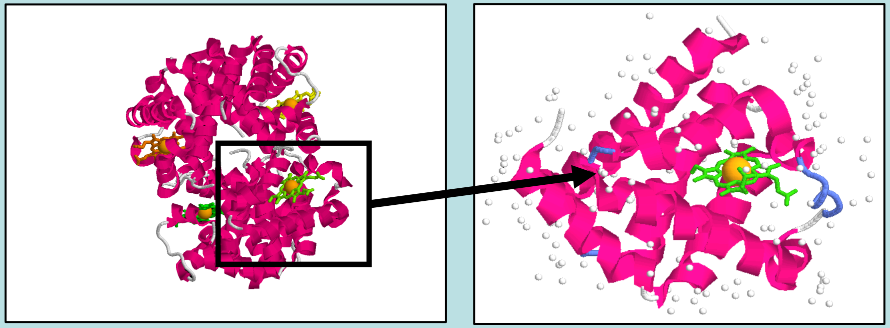
- 574 amino acid residues
- Molecular Weight = 61933,1 g.mol^{-1}
- Essentially constitued by helical structures (32 α helix)
- Composed by 4 subunits
  α 1 α 2 β1 and β2

with structures similar to myoglobin

- store oxygen from caught in the lungs and give to cells via the iron atom located in the heme

➔needs to diffuse

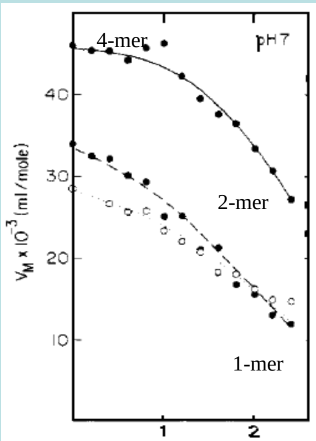
Hemoglobin **Myoglobin**



Experimental conditions :

- 320 mg/ml (like in physiological condition)
- Deuterated Phosphate buffer at pD = 7
- Temperature : 300 K

Fluorescence spectroscopy as a function of pressure on Hemoglobin



10^{-5} M

10^{-6} M

Gel formation at higher concentration

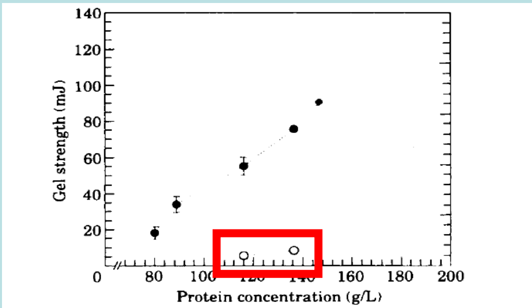
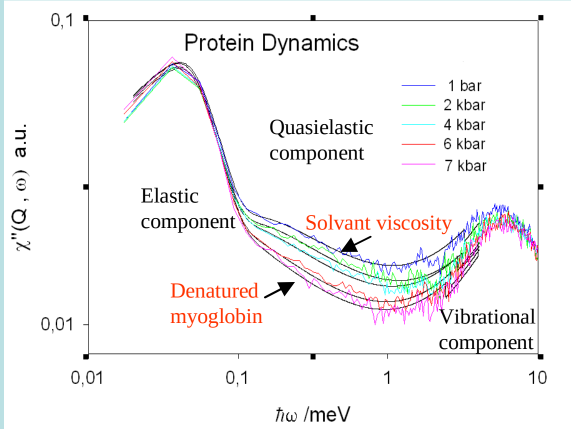


Fig. Comparison between heat and high pressure-induced HPC (● or ○) gels in function of the protein concentration. The pH was set to 7.0 in the presence of 0.05 mol/L phosphate buffer Gels were formed by heating for 30 min at 80 °C (●) or by pressurization for 30 min at 4 kbar (○).

Quasielastic neutron scattering study of myoglobin unfolding under pressure (7kbar)



Pin S et al, Biochemistry, 29, 1990, pp 9194-9202

Van Camp J. and Huyghebaert A., Lebensm.-Wiss u.-Technol., 28, 1995, pp 111-117

Wolfgang Doster, R. Gebhardt, Chemical Physics 292 (2003) 383–387

Dynamic susceptibility spectra for heavy water and protein solution as a function of pressure

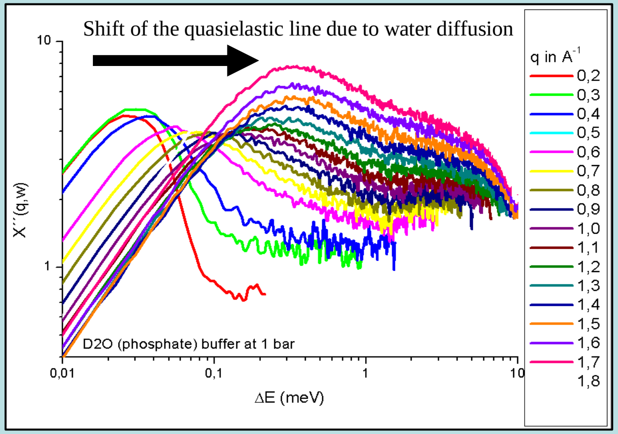
Heavy water

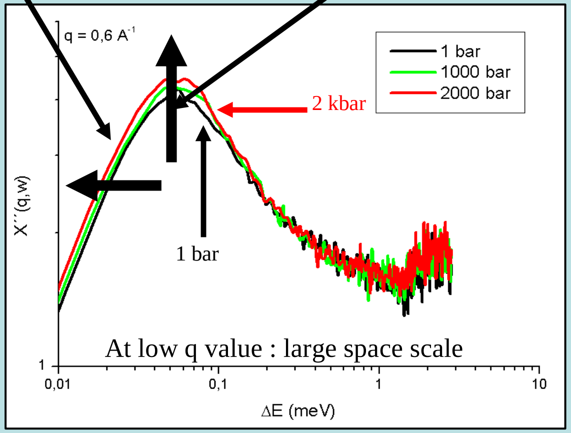
Lowering down of the water dynamics

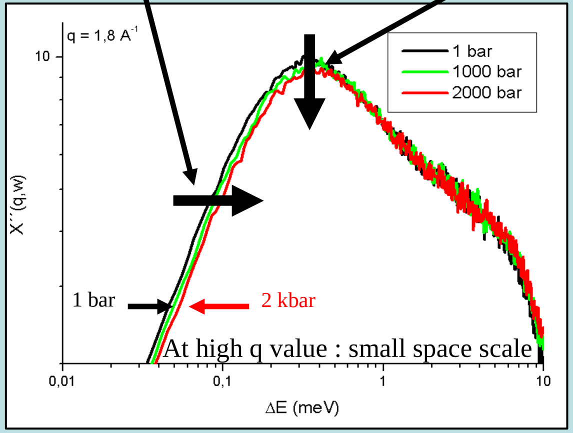
Intensity increase due to lowering down of the water dynamics

« Speeding up » of the water dynamics ?

Intensity lowering due to shift of the intermolecular water correlation







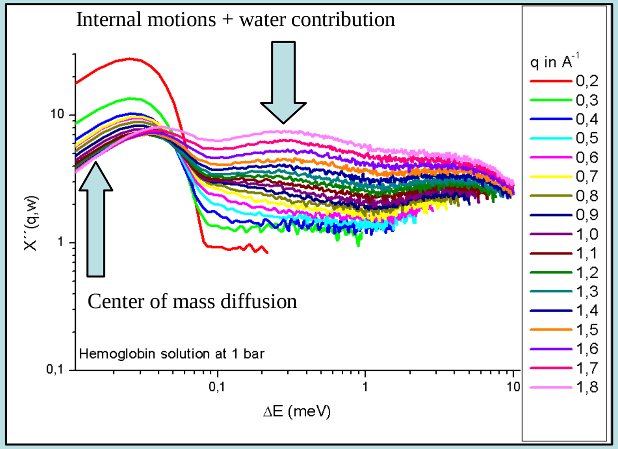
Opposite effect on water dynamics between large and small scale

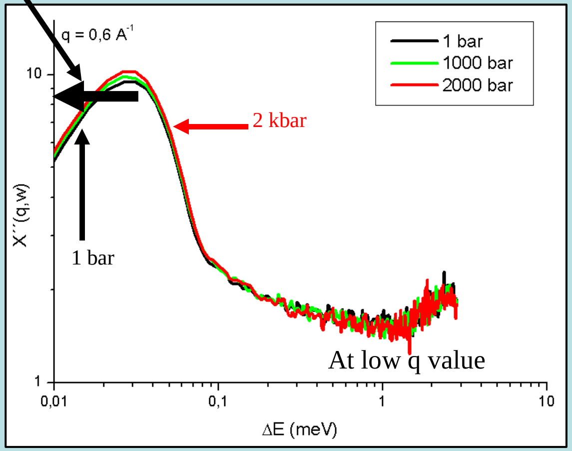
Protein solution

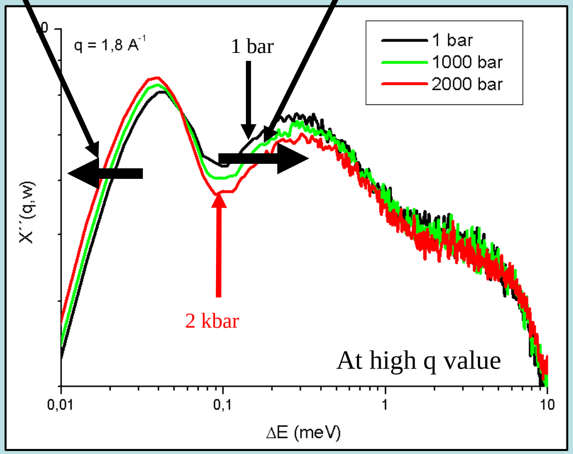
Lowering down of the dynamics (protein and water)

Lowering down of the protein dynamics

« Speeding up »of the water dynamics





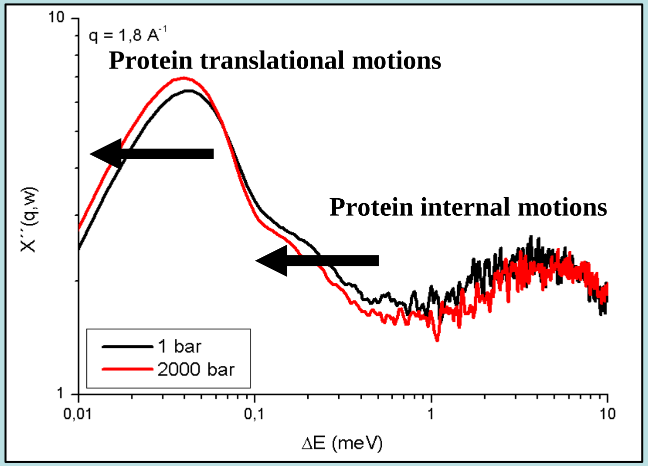


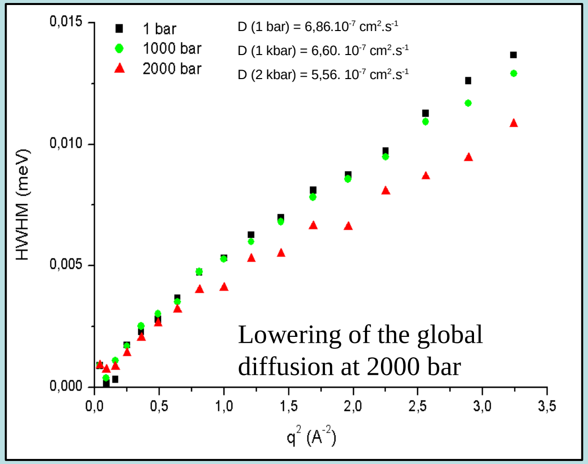
Main effect on water dynamics

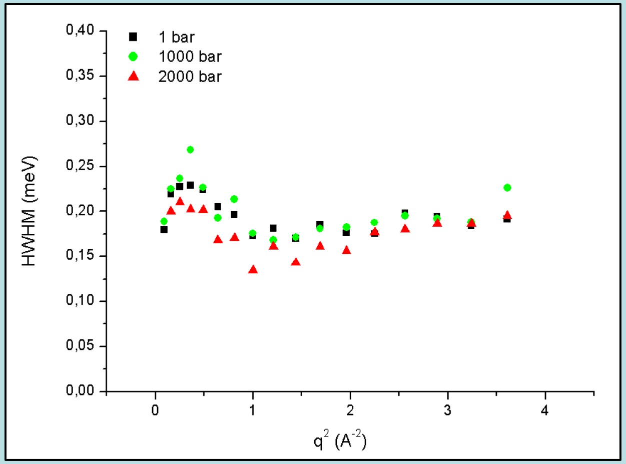
Dynamic susceptibility spectra for Haemoglobin protein after solvent spectra subtraction from the protein solution spectra

$$S(q,\omega)_{protein} = S(q,\omega)_{protein\ \ solution} - \varphi_{Bulk\ D_2O} * S(q,\omega)_{buffer\ solution}$$

$$S(q,\omega)_{protein} = P(q) * \{L_{dif}(q,\omega) \otimes [A_0.\delta(\omega) + (1-A_0).L_{int}(q,\omega)] + B(q)]\} \otimes S_{res}(q,\omega)$$







Dynamic susceptibility spectra for Haemoglobin protein after solvent spectra subtraction from the protein solution spectra

D_{trans} close to theoretical values and to those from litterature

Pressure effect is essentially on the global motion and affects the translationnal diffusion since 2000 bar.

→essentially due to water viscosity increase (η_{D2O}(2kbar) = 1,2 cp)

→Not reversible : pressure induced association ? (R_H = 32,9 Å at 1 bar to 38,4 Å at 2 kbar)

- HWHM is constant for each pressure value

→ typical of confined motions

- Pressure has a small lowering effect on the internal motions in this range of pressure

→ Due to rearrangement of the water molecules at the surface of the protein leading to more constraints on side chain residus libration.

R. Gebhardt et al, in: Advances in High Pressure Science and Biotechnology II p. 33, Springer 2003, Ed. R. Winter

Conclusion :

- Our high pressure cell allows us to perform dynamic neutron scattering studies on protein in solution as a function of pressure up to 2 kbar : we have a low scattering contribution due to the choice of the material

- From quasielastic neutron scattering experiment on concentrated solution of haemoglobin (4,37 mM), we used susceptibilities calculation in order to differentiate pressure effect on water dynamics.

- Water dynamics is lowered by pressure at large scale whereas it is speeded up at lower scale. Because of the shift of the intermolecular water correlation peak of D2O at 2 Å^{-1} but also maybe due to hydrogen bond rearrangement (already seen by NMR by Prielmeir F.X., Lang E.W., Speedy R.J. and Lüdemann H.-D., Phys. Rev. Lett., 59, pp 1128-1131, 1987.)

-A slowing down of global diffusion of the protein in solution due to the increase of the solvent viscosity and maybe by another kind of association induced by the pressure. We should check it by Small Angle Neutron Scattering measurement under pressure.

- In this range of pressure, there is a slowing down of internal motion probably due to the rearrangement of the hydration water shell at the surface of the protein. It is possible to verify by doing SANS with D2O and H2O as buffer and X-ray scattering.

Svergun D.I., Richard S., Koch M.H.J., Sayers Z., Kuprin S., et Zaccaï G., *P.N.A.S.*, **95**,1998, pp 2267-2272.

- The effect is not reversible in terms of global diffusion. We form a gel after pressurization.

We would like to thank very much:





Wolfgang Doster   Sebastian Busch   Ana Gaspar   Reinhold Funer   Joachim Dörbecker

Tobias Unruh and Jandal Ringe from **TOFTOF :**
High Resolution Direct Geometry Time-of-Flight Spectrometer

Elbio Calzada and Martin Mühlbauer from ***Antares*** : **Advanced Neutron Tomography And Radiography Experimental System**

Lichtinduzierte Dynamik von Biopolymeren – Light-induced dynamics of biopolymers –
SFB 533

DFG

E 13

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And I would like to thank you very much for your attention…

Pressure effect on water viscosity and dynamics

Even between atmospheric pressure and 2000 bar

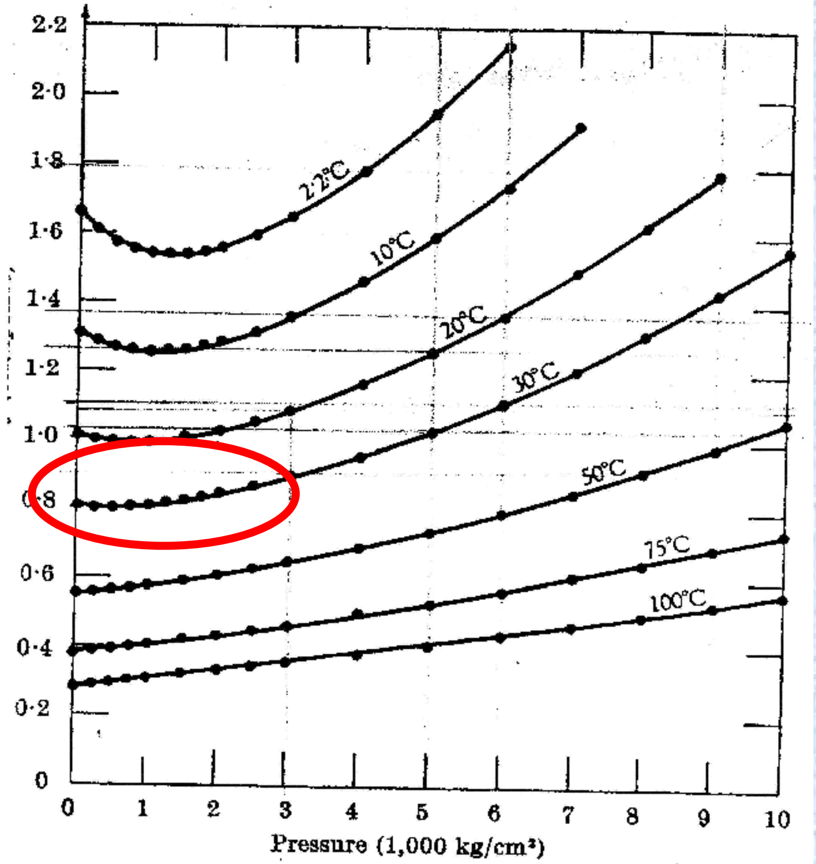


Fig. 2. Absolute viscosity of water

Water's pressure-viscosity behavior [1] can be explained by the increased pressure (up to about 150 MPa) causing deformation, so reducing the strength of the hydrogen-bonded network, which is also partially responsible for the viscosity. This reduction in cohesivity more than compensates for the reduced void volume. It is thus a direct consequence of the balance between hydrogen bonding effects and the van der Waals dispersion forces [2] in water; hydrogen bonding prevailing at lower temperatures and pressures. At higher pressures (and densities), the balance between hydrogen bonding effects and the van der Waals dispersion forces is tipped in favor of the dispersion forces and the remaining hydrogen bonds are stronger due to the closer proximity of the contributing oxygen atoms [3]. Viscosity, then, increases with pressure. The dashed line (opposite) indicates the viscosity minima

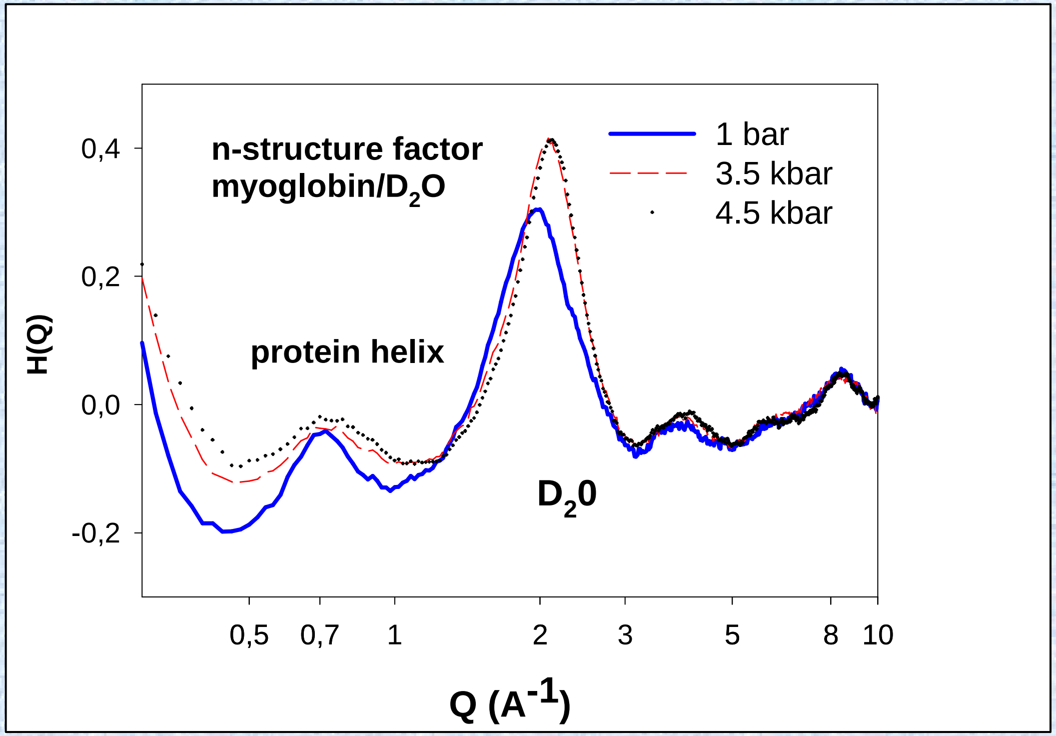
Martin Chaplin
http://www.lsbu.ac.uk/water/

[1] K. E. Bett and S. B. Cappi, Nature, 207,. 620 (1965).

[2] H. Tanaka, A new scenario of the apparent fragile-to-strong transition in tetrahedral liquids: water as an example, *J. Phys.: Condens. Matter* 15 (2003) L703-L711.

[3] T. Kawamoto, S. Ochiai and H. Kagi, Changes in the structure of water deduced from the pressure dependence of the Raman OH frequency, *J. Chem. Phys.* 120 (2004) 5867-5870.

Explaination for the speeding up of the water dynamics



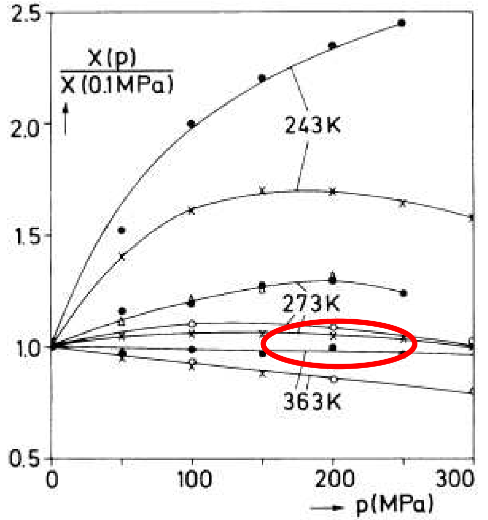


FIG. 2. The effect of pressure on the transport properties of water at three temperatures. The value of $X(p)$ at pressure p, relative to its atmospheric pressure value $X(0,1\ \text{MPa})$ is plotted against pressure. $X = D, x$; $1/\tau_2$ (Ref. 5), filled circles; $1/\tau_1$ (Ref. 2), triangles; $1/\eta$ (Ref. 3), open circles; where D is the self-diffusion coefficient, τ_2 is the relaxation time for rotational diffusion, τ_1 is the dielectric relaxation time, and η is the shear viscosity.

W. Doster, R. Gebhardt and A. Soper in: Advances in High Pressure Science and Biotechnology II p. 29, Springer 2003, Ed. R. Winter

Prielmeir F.X., Lang E.W., Speedy R.J. and Lüdemann H.-D., Phys. Rev. Lett., 59, pp 1128-1131, 1987.

Pressure effect on bulk water dynamics : our results

Fit of S(q,w) with a single Lorentzian   Fit of $\Gamma(q^2)$ with $\Gamma(q^2) = \dfrac{Dq^2}{Dq^2\tau_0 + 1}$