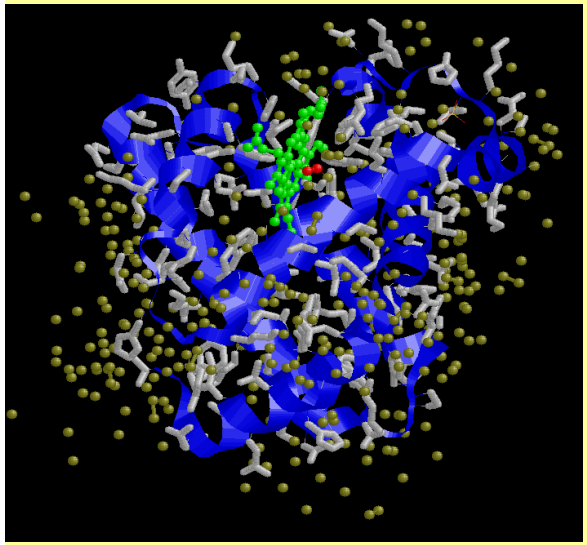
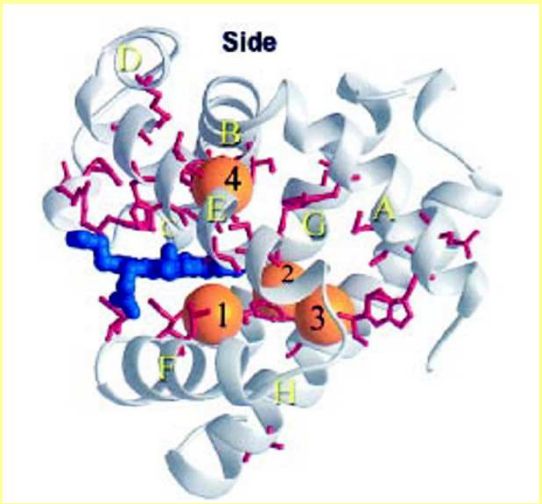
On Masters and Slaves: Solvent control of protein motions and function





Wolfgang Doster
Technische Universität München
Physik Department and FRM 2

Topics:

1) Properties of „Biological Water“
2) Solvent control of protein function, „slaving“
3) Transitions: glass-liquid (GT), liquid-liquid (FST), protein dynamical transition (PDT)

Methods:
Neutron Scattering and Flash Photolysis

Myoglobin

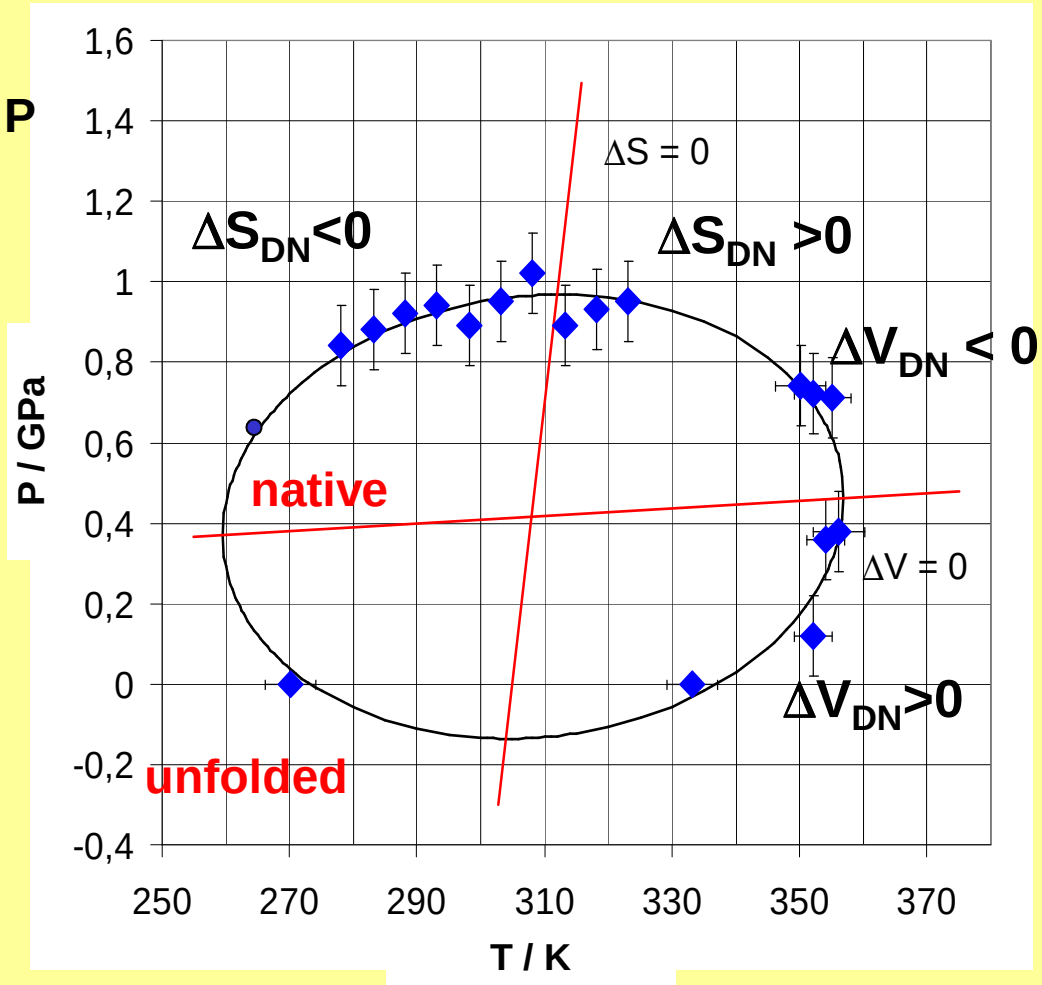
„on cavities and cages“

Literature:
http://www.e13.physik.tu-muenchen.de/Doster

Does water stabilize protein structures?

reentrant phase diagram of cytochrome C

P-T phase diagram of cytochrom C



Folded (N) $\leftrightarrow$ Unfolded (D)

Entropy of unfolding: ΔS_{DN}

Unfolding volume: ΔV_{DN}

Phase boundary: CC equation

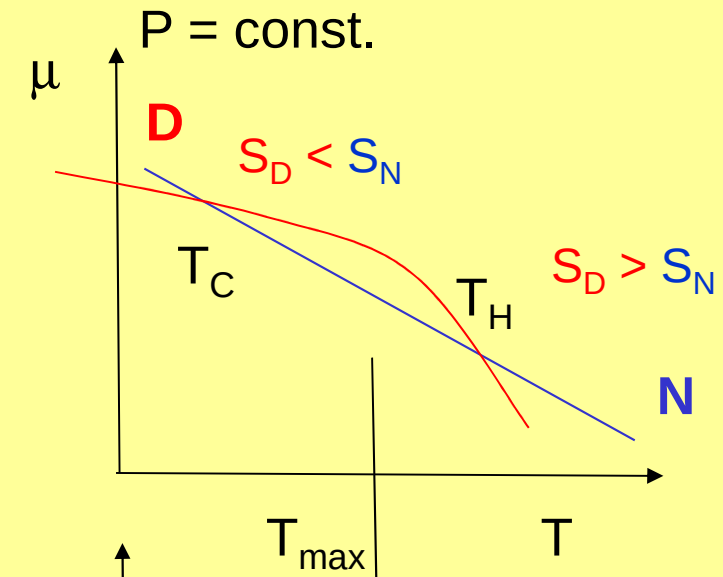
$dP/dT = \Delta S_{DN}(T, P) / \Delta V_{DN}(T, P)$

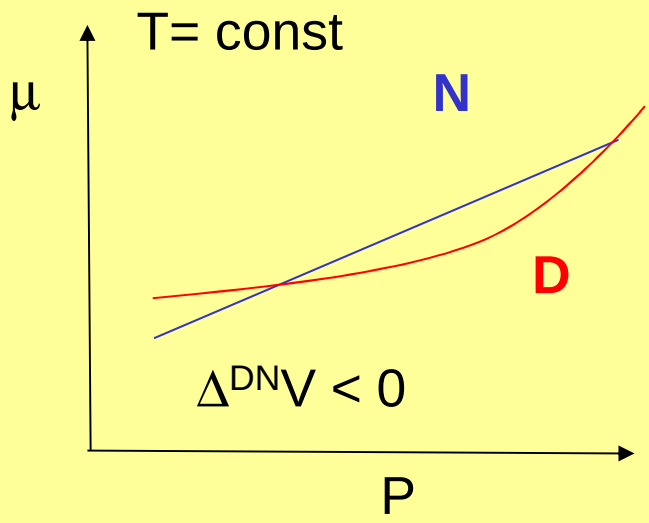
ΔS_{DN}, ΔV_{DN} depend on temperature and pressure due to hydration water

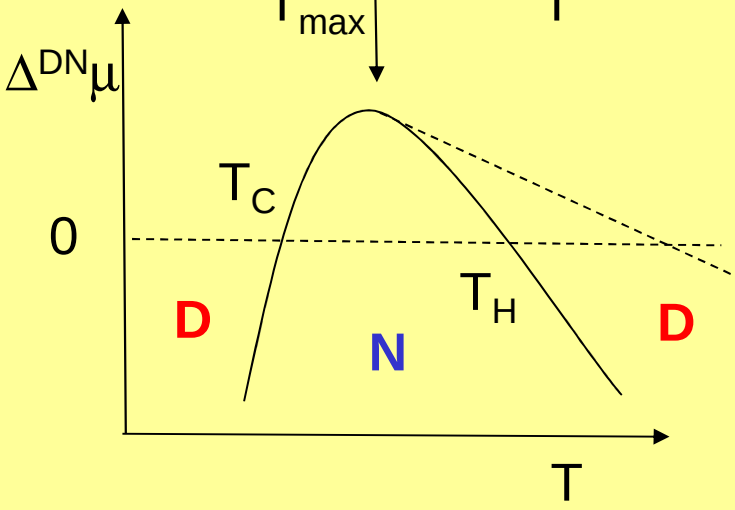
Doster and Friedrich in: Protein Folding Handbook Part 1 (Wiley- VCH 2005)

Water destabilizes protein structures: cold denaturation

Chemical potential: $d\mu = - S\, dT + V\, dP$ $\quad S > 0,\ V > 0$







Entropy of D-HW varies with T, P

Solvent is more ordered in D than in N at low T

T_{max}: maximal stability

$\Delta S_{DN}\ (T_{max}) = 0$

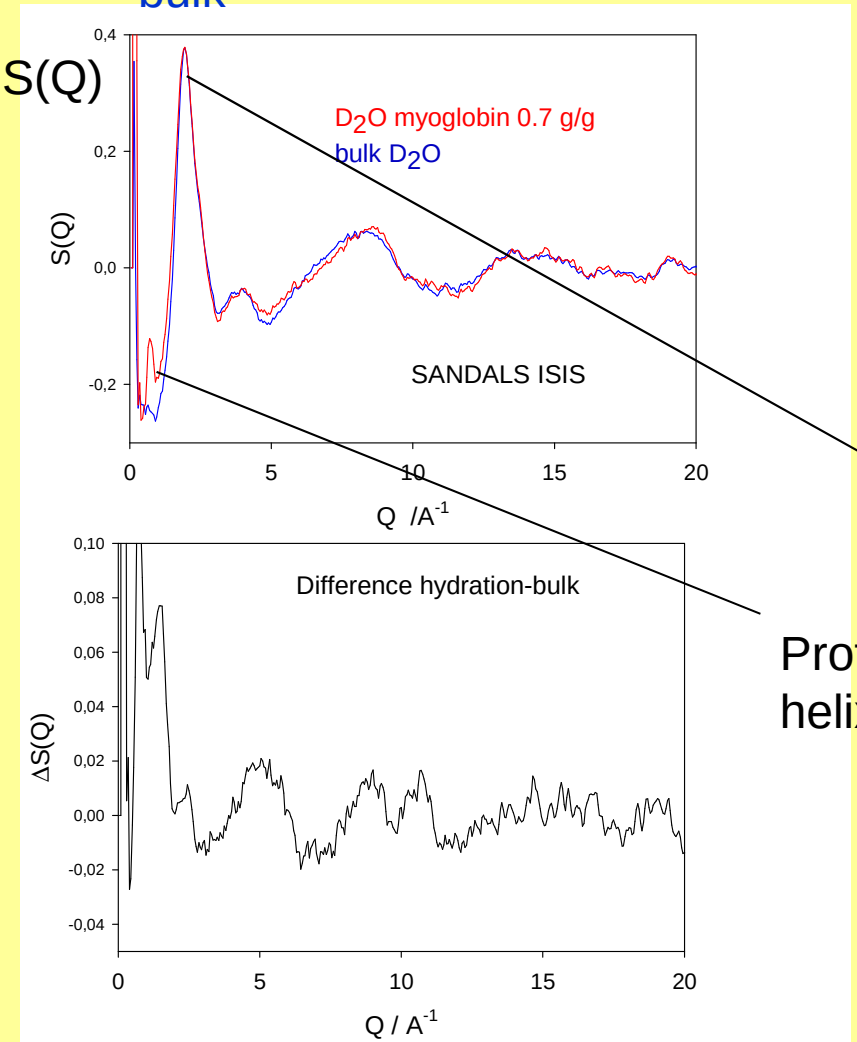
Doster and Friedrich in: Protein Folding Handbook Part 1 (Wiley VCH 2005)

Structure of hydration water: D_2O (myglobin)

coherent neutron scattering ISIS (SANDALS) with D. Bowron

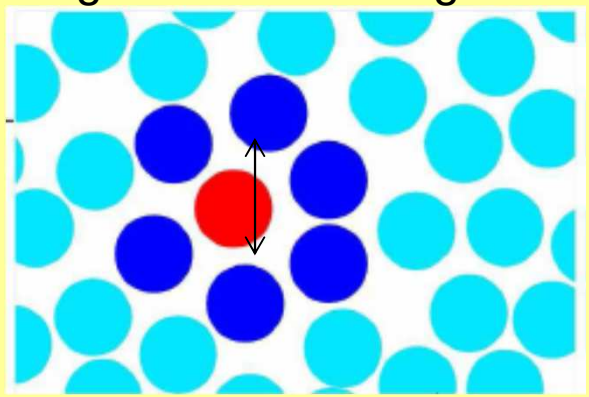
Hydration water

bulk



Protein helix

cage of nearest neighbors

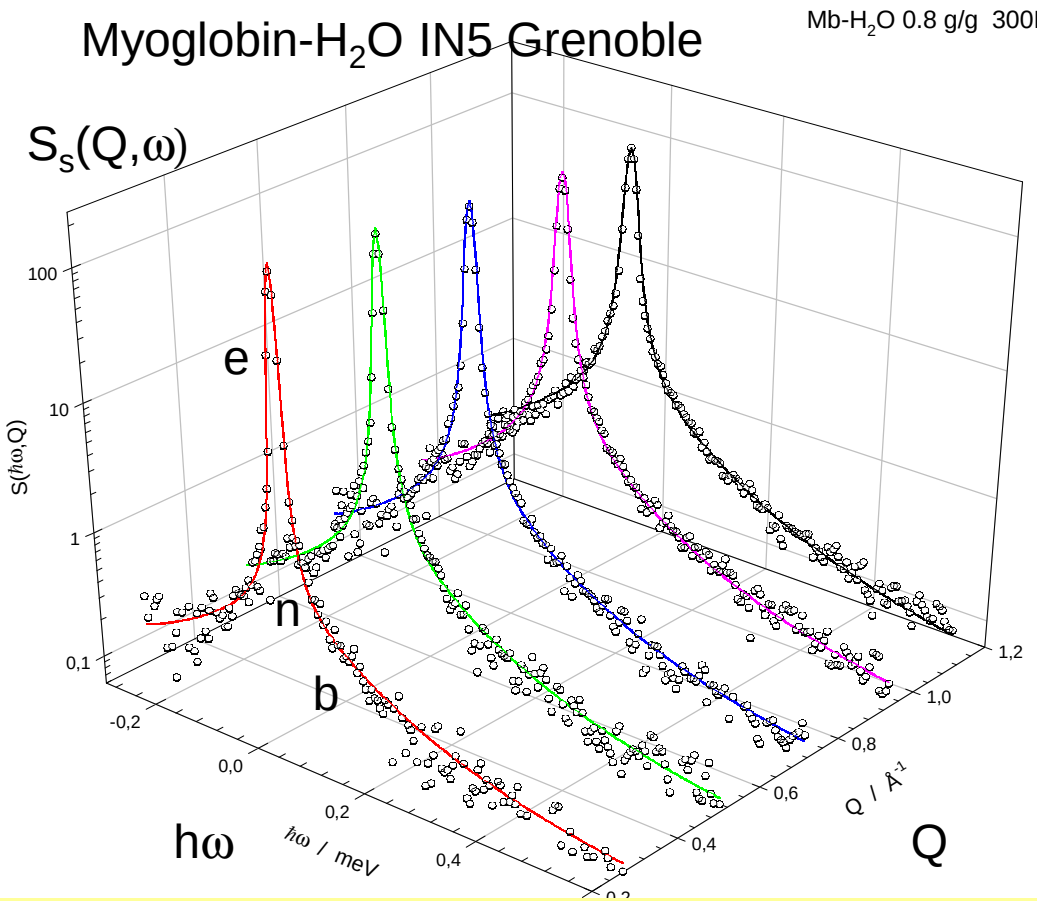


cage size: $d \approx 2\pi / Q_{max} \approx 0.3$ nm

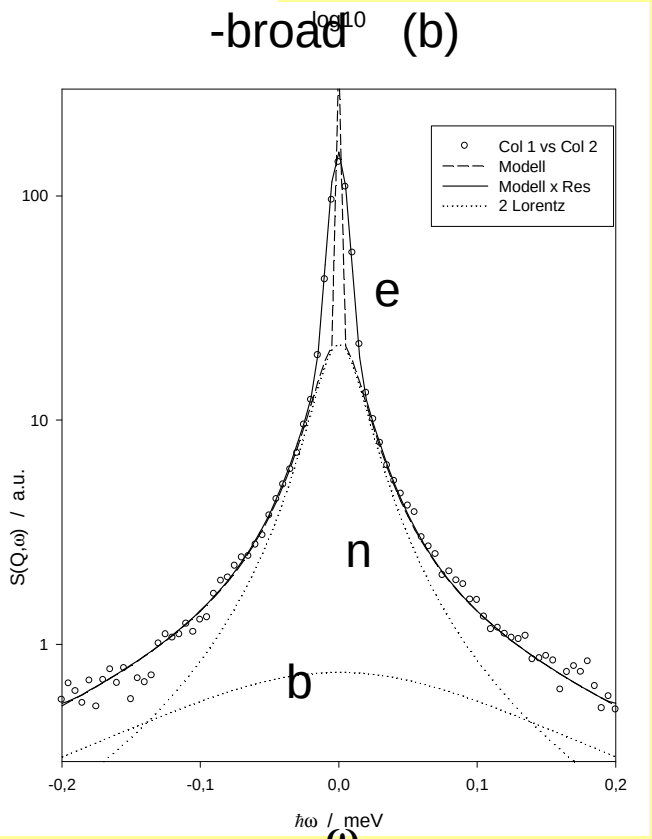
..structure of simple liquids..is largely determined by geometric factors, associated with the packing of the molecular hard cores (Hansen,McDonald)

First order:
HW structure close to bulk water
peak of S(Q) at Q = 2 A^{-1}
Second order:
Distortions of tetrahedral stucture

dynamics of hydration water
incoherent neutron scattering H_2O

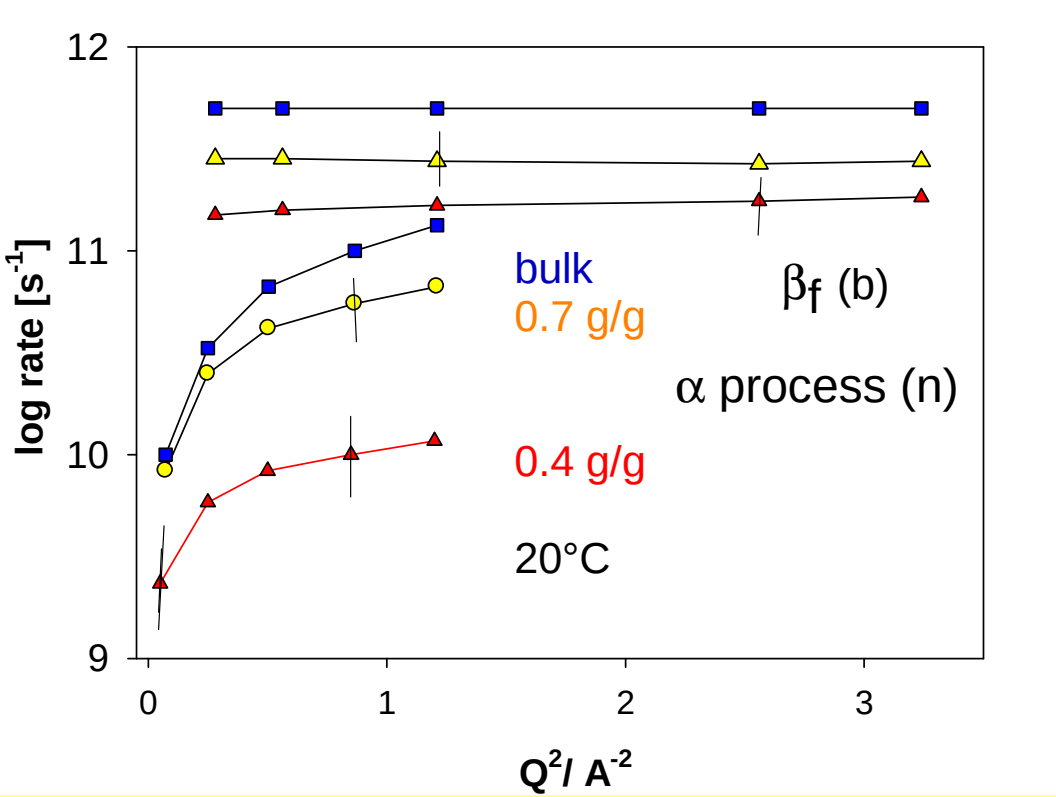


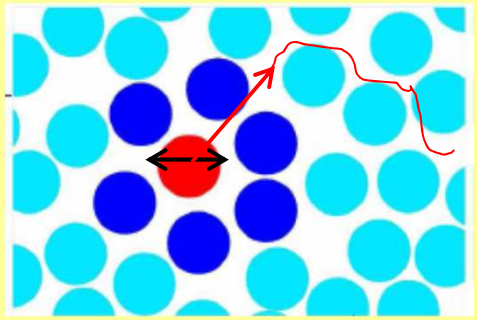
Three component fit
-elastic line (e)
-narrow (n)
-broad (b)



Two spectral components+elastic:
1) narrow (n): width, increases with Q
2) broad (b): width independent of Q

Relaxation rates of hydration water versus degree of hydration





2 time scales:

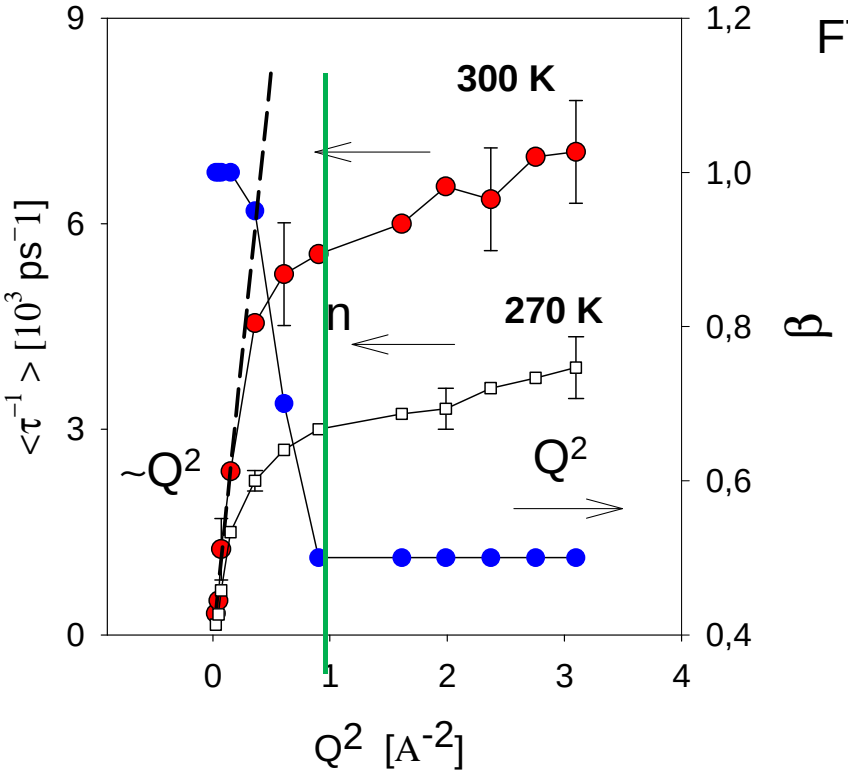
1) fast local in cage β_f (b) 5-10 ps
independent of Q

2) Slow process α (n) 50-100 ps
varies with Q, diffusion

Γ_b (Q) = Q^2 D_s at low Q
$\rightarrow \Gamma_\alpha$ high Q: cage effect

Hydration water dynamics: two processes, slow (n), fast (b) depends on degree of hydration

Log rate



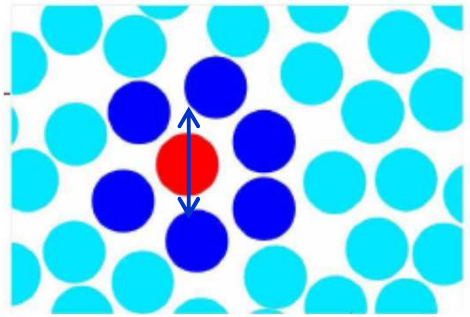
perdeuterated phycocyanin
D-CPC H_2O h = 0.3 g/g

Doster et al. PRL (2010)

$FT\{S(Q,\omega)\} = I(Q,t) = \exp[-(t / \tau(Q))^{\beta}]$

Stretching parameter
β depends on Q

onset peak of S(Q) at Q = 2 A^{-1}



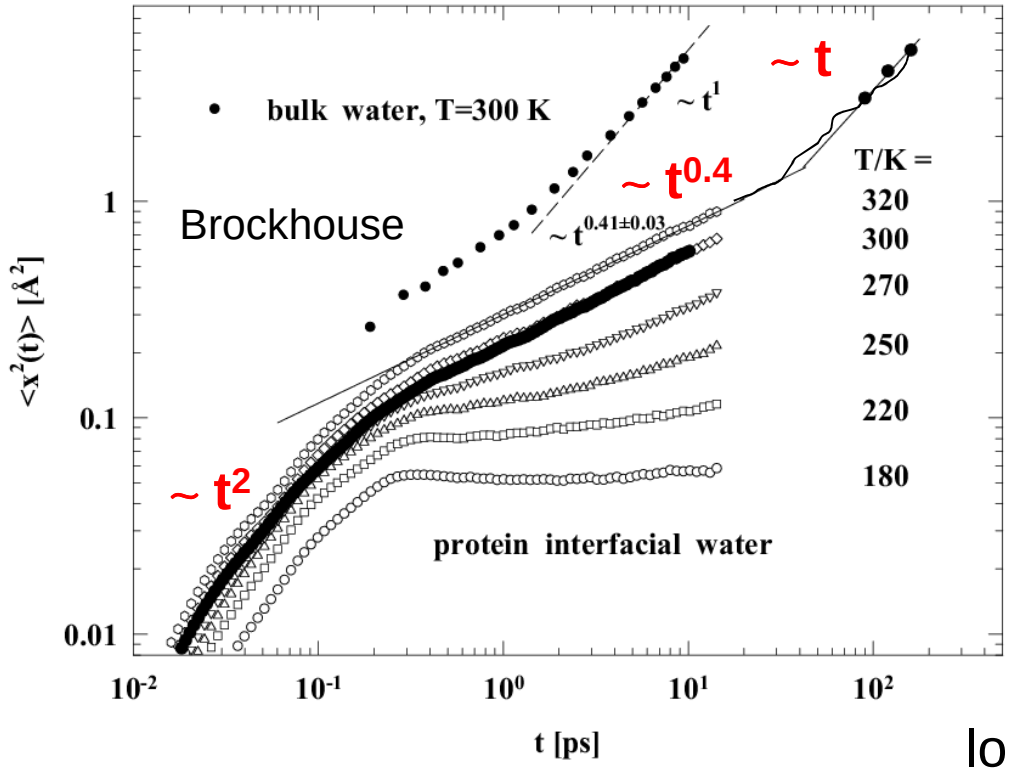
Narrow line (n) slow
Low Q:
Long range diffusion
High Q:
cage effect: α-relaxation

Time-resolved mean square displacements

$$I_s(Q,t) = 1 - 1/6\ Q^2 \langle r^2(t) \rangle + 1/120\ Q^4 \langle r^4(t) \rangle - .....$$

Moment Expansion of the Scattering Function: Placzek expansion

$<r^2(t)>/3$



log time

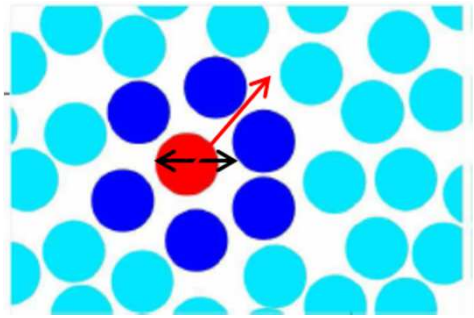
free flight **cage effect** **free diffusion**

Time resolved displacements:

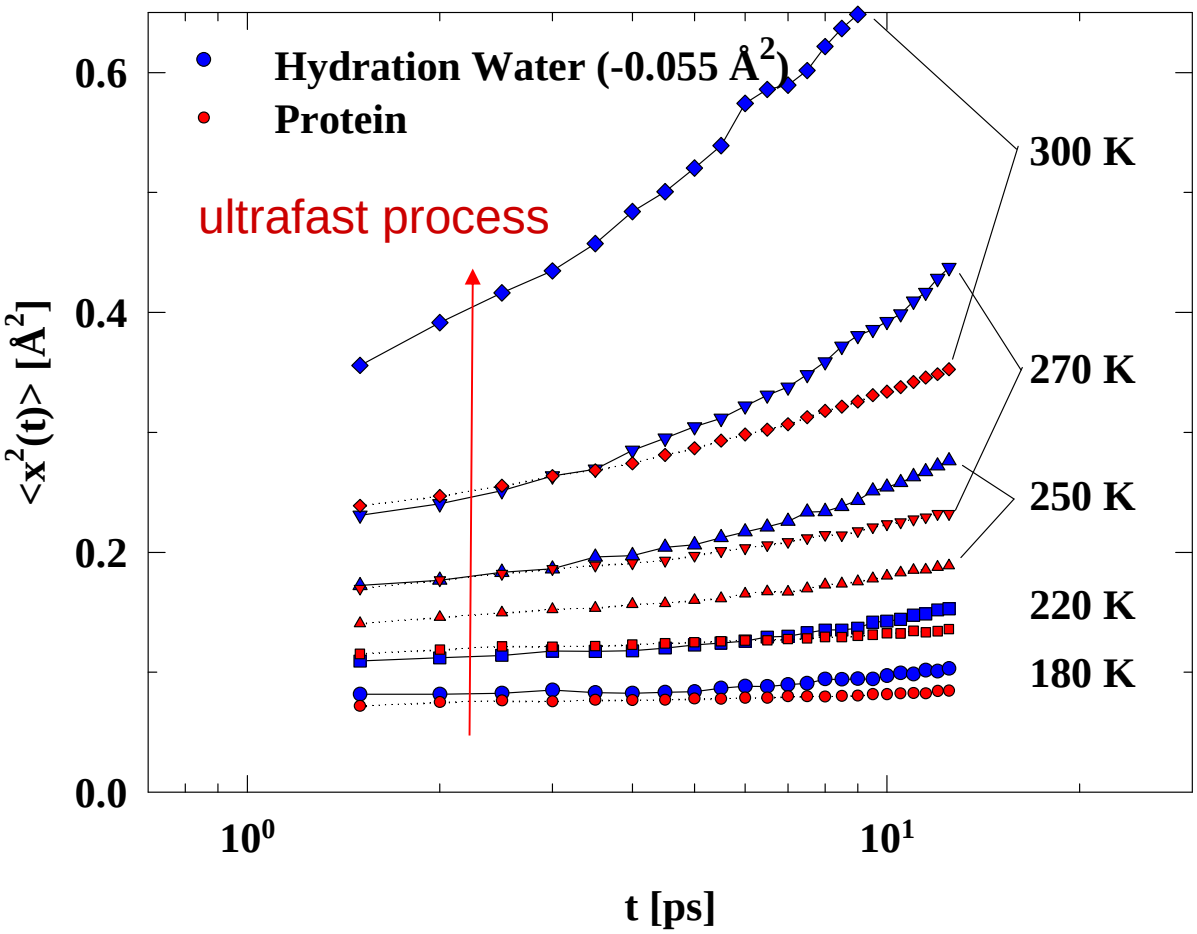
Anomalous Diffusion

Doster, Settles
Faraday Disc. 103(1996)
BBA (2005)

Hydration water myoglobin



protein-water displacements versus temperature



H_20/D_2O hydrated myoglobin 0.4 g/g

neutron scattering IN6

same time scale and temperature dependence

different amplitudes

Settles, Doster
Biochim. Biophys.Act.
2005

Protein-Water Dynamics from femtosecond fluoresence spectroscopy

1) Two water time scales: (Zhang et al. JACS (2009)131,10677

τ_β: 5-10 ps

Exchange with bulk

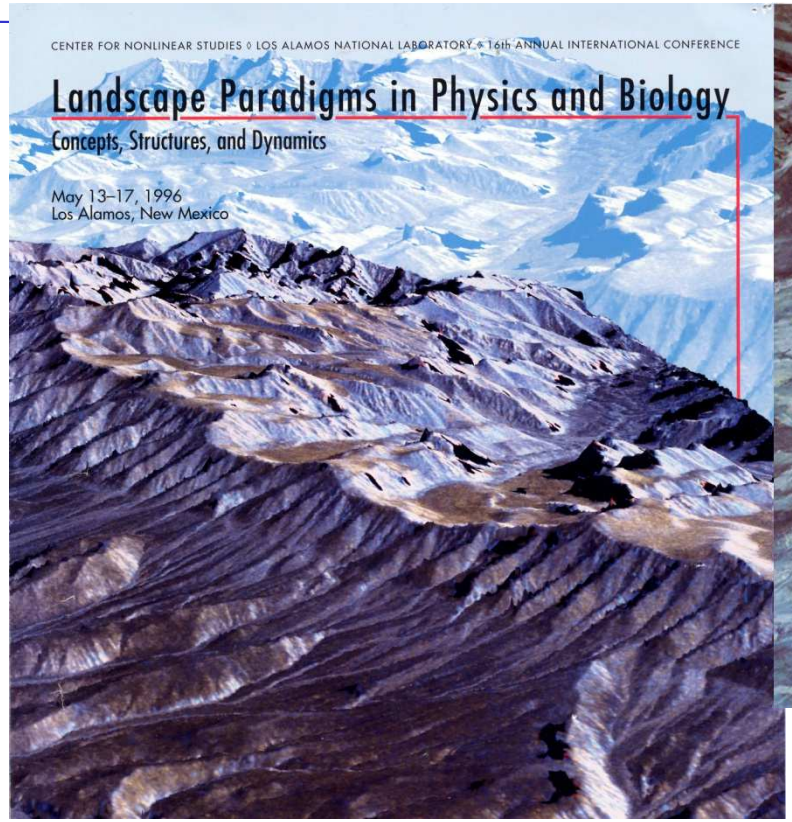
$\tau_\alpha \cong \tau_{prot}$ 20–100 **ps**

fast local reorganisation of water H-bond network, reorientation, libration, site specific

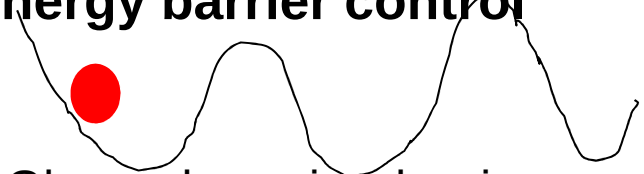
Lateral structural relaxation of hydration shell coupled to protein fluctuations, same time scale

2) Third time scale: Slow protein motions vary with surface viscosity: 1000 ps

The protein-solvent interface
solid-liquid

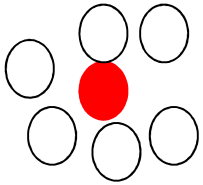


energy barrier control

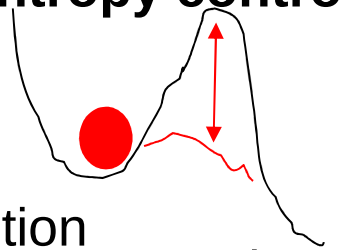


Glass transition

⇔



entropy control

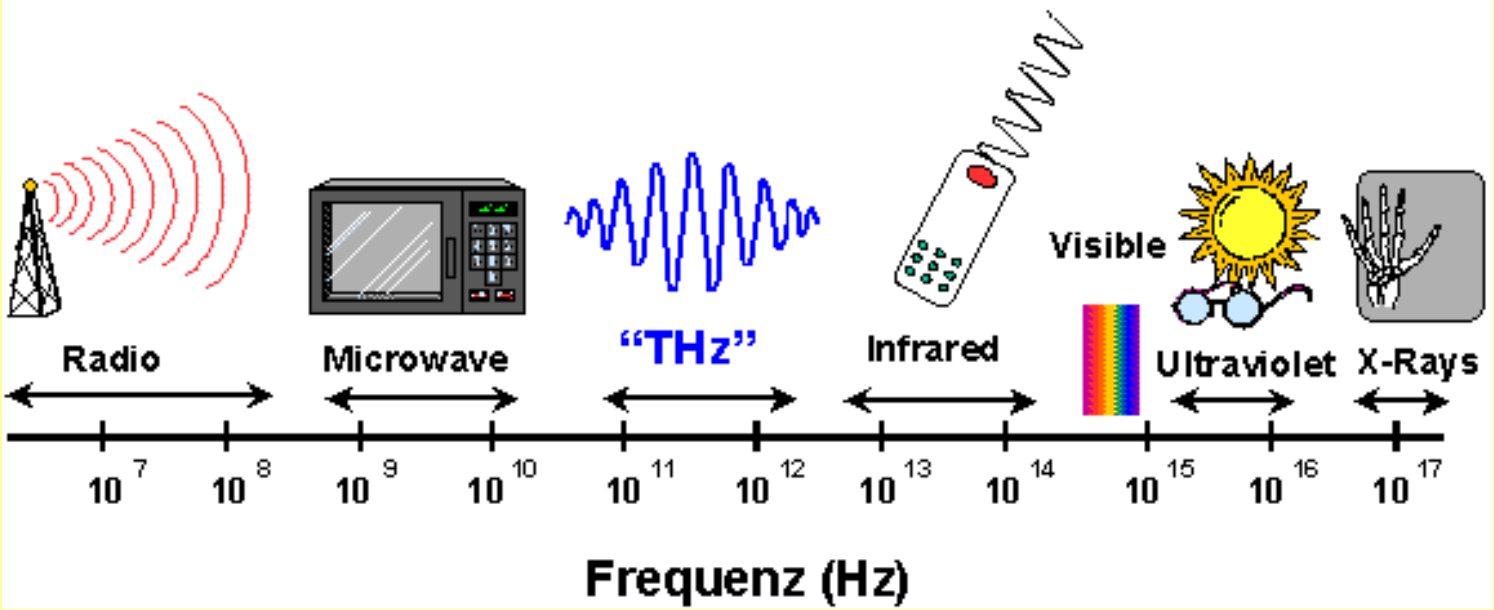


collective motion
free volume, entropy
Liquid:α- relaxation

barriers
fluctuate

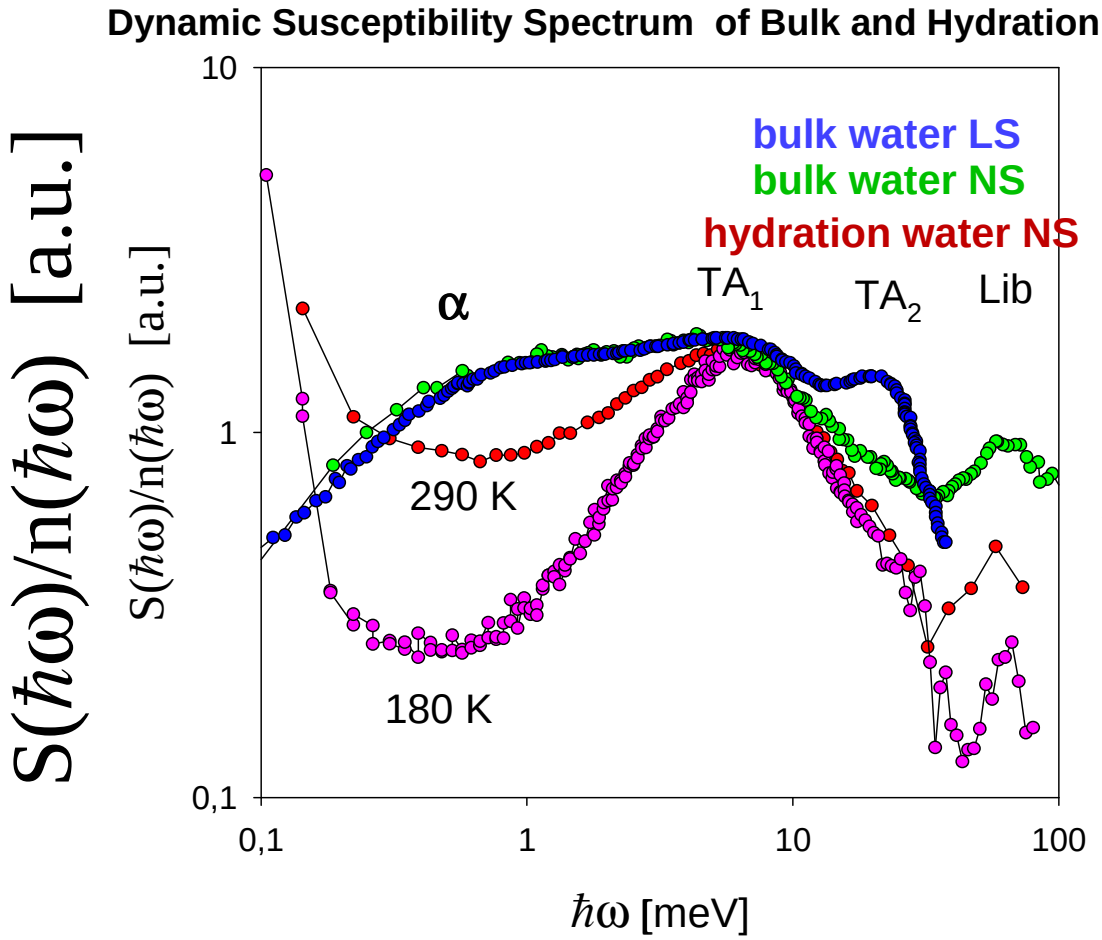
Glassy dynamics: barrier crossing:
β-relaxation , energy controlled

Dynamic Neutron Scattering and Spectroscopy



Collective Dynamics
Far Infrared: 1-100 cm^{-1}
Terahertz

Bulk- Hydration Water: Terahertz vibrations



1 meV = 8 cm^{-1}

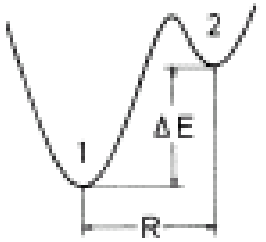
Bulk and hydration water same spectral peaks
only α-relaxation retarded

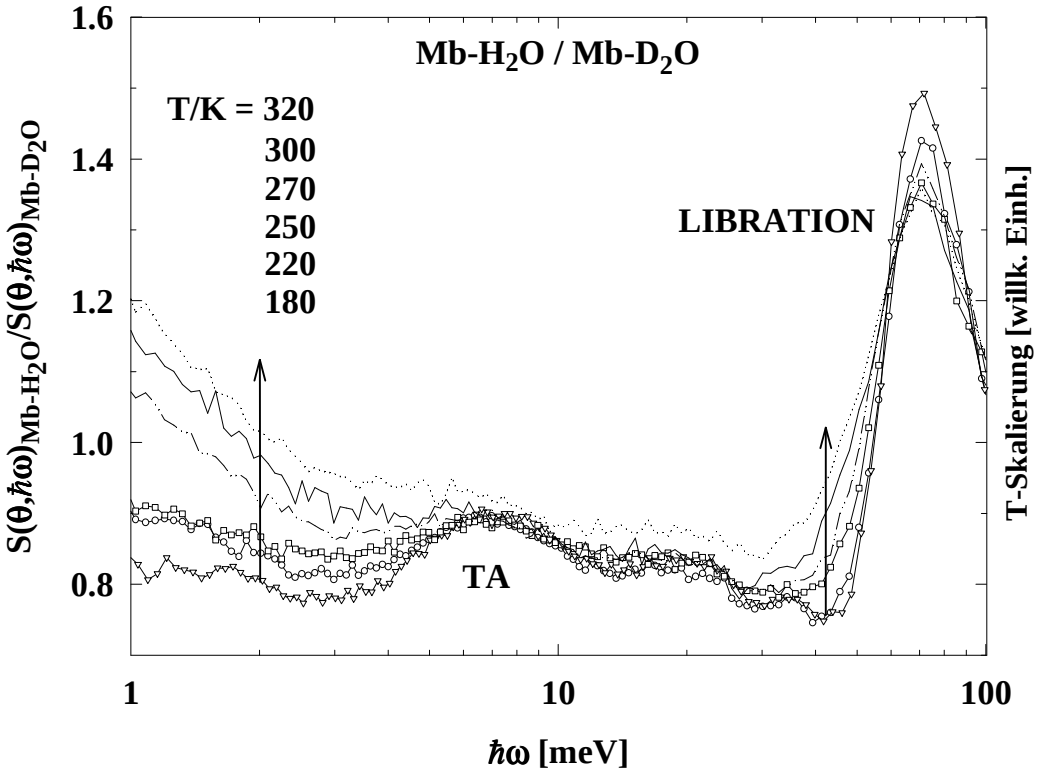
TA1 T-independent
fast structural relaxation
of H-bonds

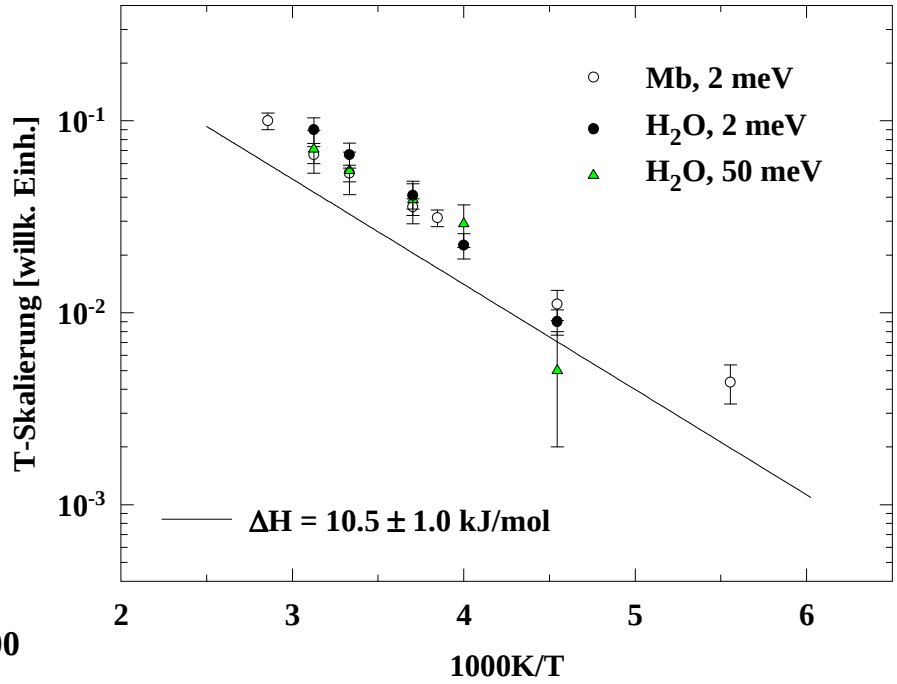
Short time diffusive dynamics is dominated by hydrogen bond fluctuations : β_f-process

Water librational mode (H-bond braking) determines fast diffusive dynamics of protein-water system

Doster BBA 2010 , Tarek,Tobias, PRL 2002

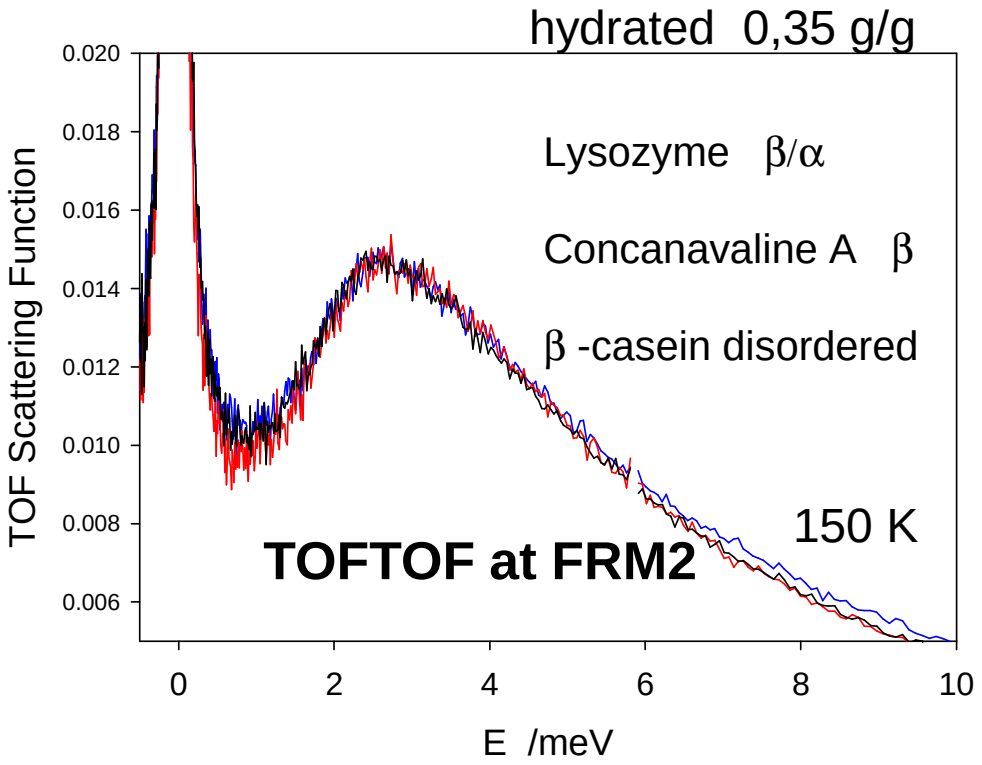


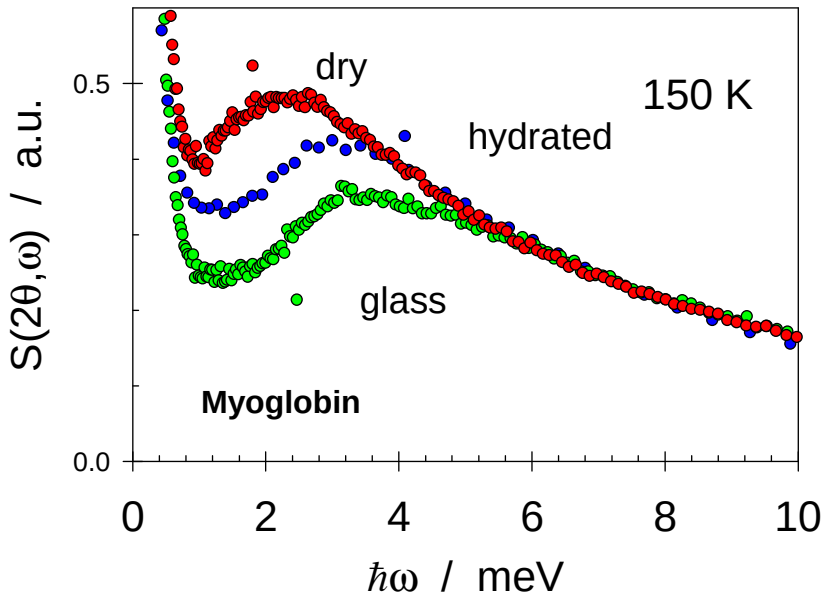




Protein Low frequency vibrations: Boson peak

Doster et al. Nature (1989), Diehl et al. Biophys.J **73,(**1997), Tobias, Tarek PRL 2002)



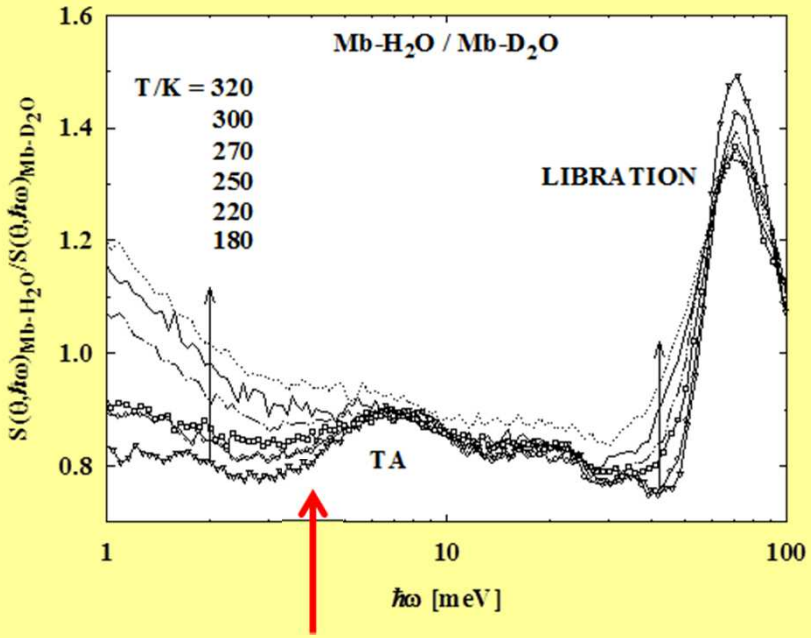


Boson- Peak is not secondary structure sensitive

but depends strongly on environment

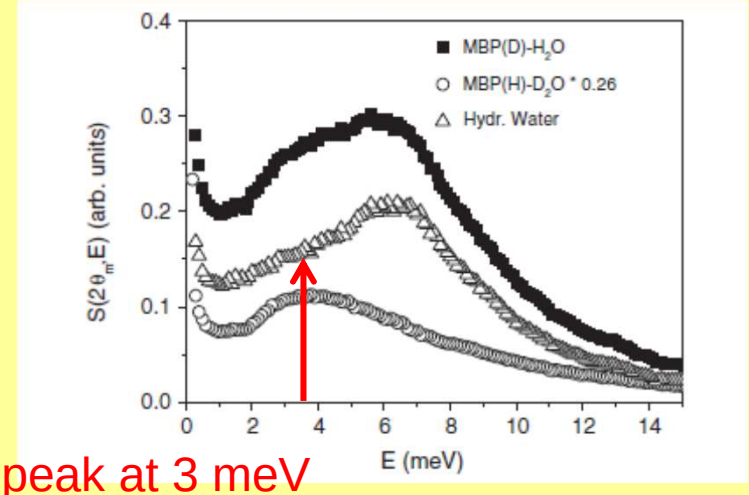
Does hydration water have a protein boson peak?

Settles, Doster, Faraday Disc1996, BBA 2010
H_2O/D_2O hydrated myoglobin

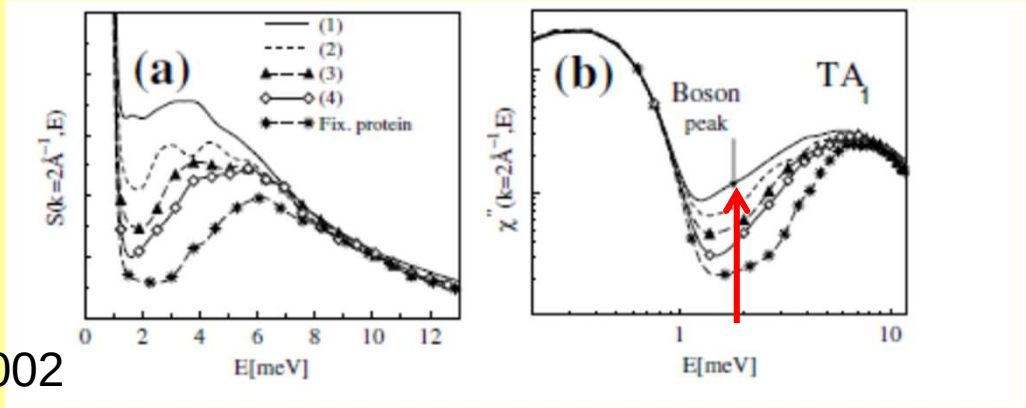


No Boson peak

Paciaroni et al. PRL 2008
Phys. Rev E 1999
Maltose binding protein



peak at 3 meV

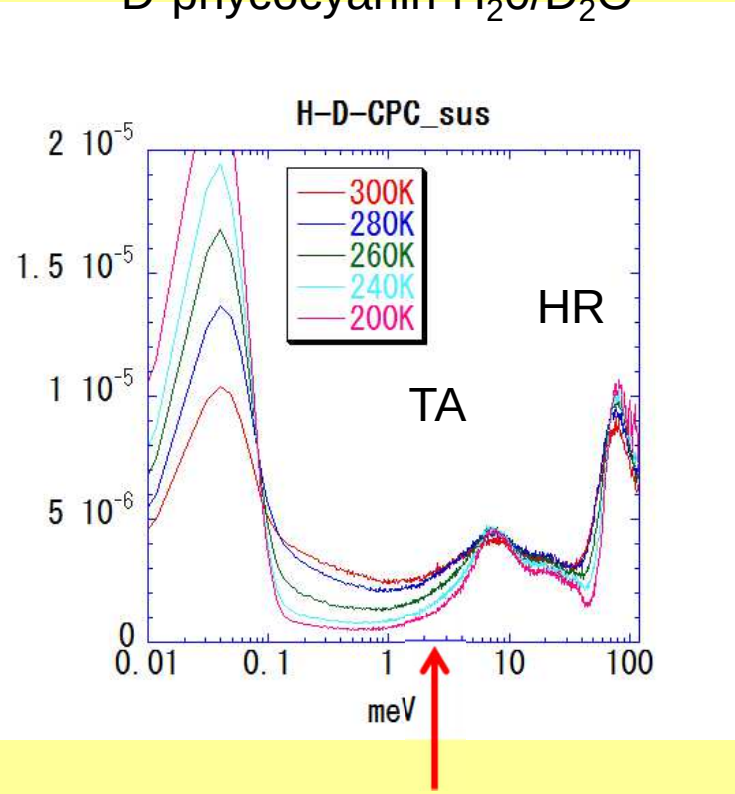


Tarek, Tobias
Simulation PRL 2002

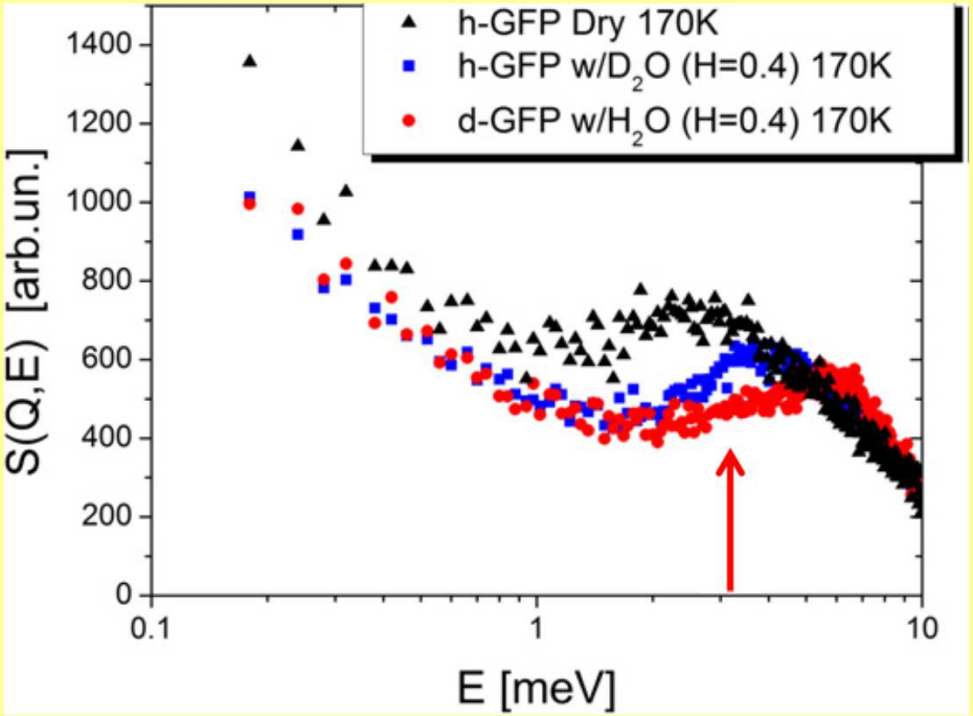
Does hydration water have a protein Boson peak?

Neutron Scattering spectra of hydration water
with perdeuterated proteins H2O-D20 hydrated

Doster et al. 2012
D-phycocyanin H_20/D_2O

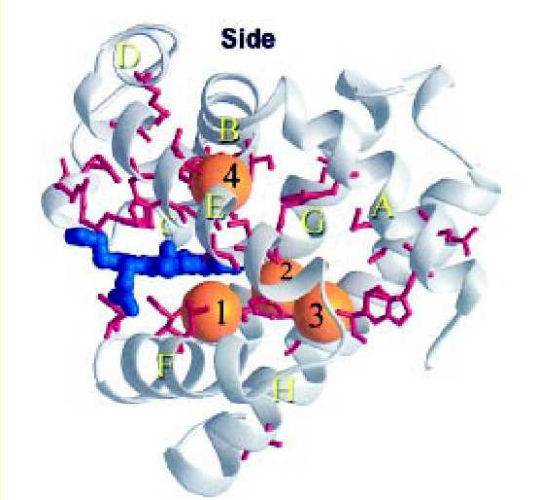


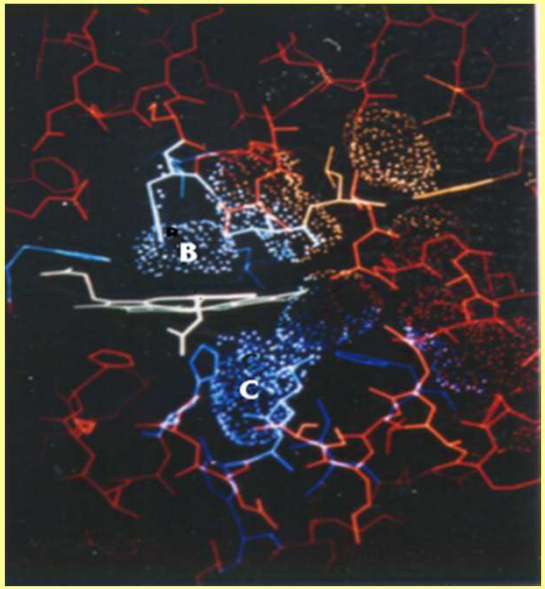
Nickels et al. Bioph.J. 2012



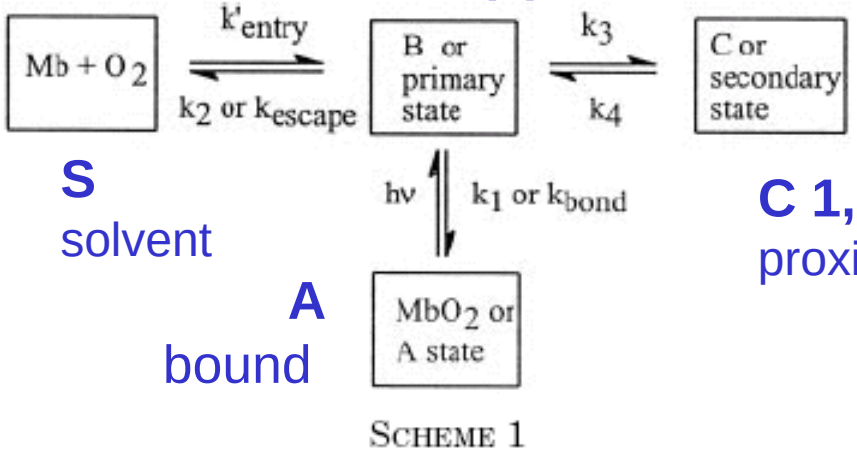
No evidence of Boson peak in hydration water spectrum

Protein function: CO- binding to myoglobin,
elementary steps, flash photolyis





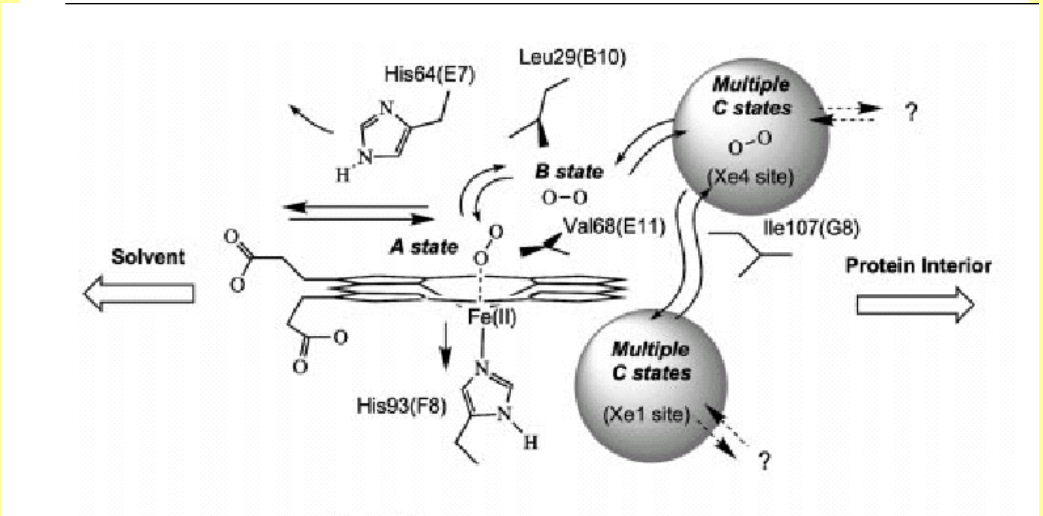
B (4) distal



S
solvent

A
bound

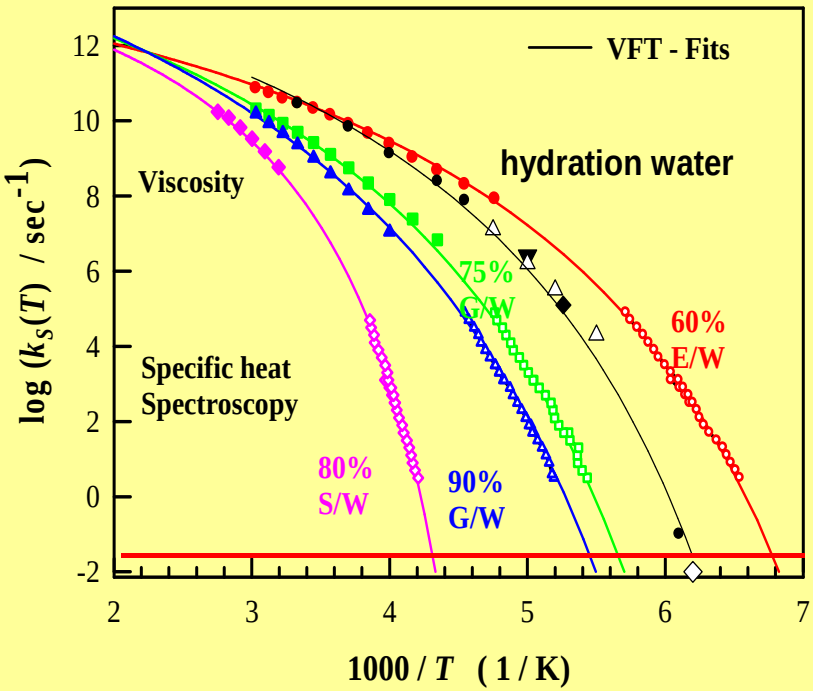
C 1,2,3
proximal



Relaxation rates and viscosity of glass forming solvents

Hws

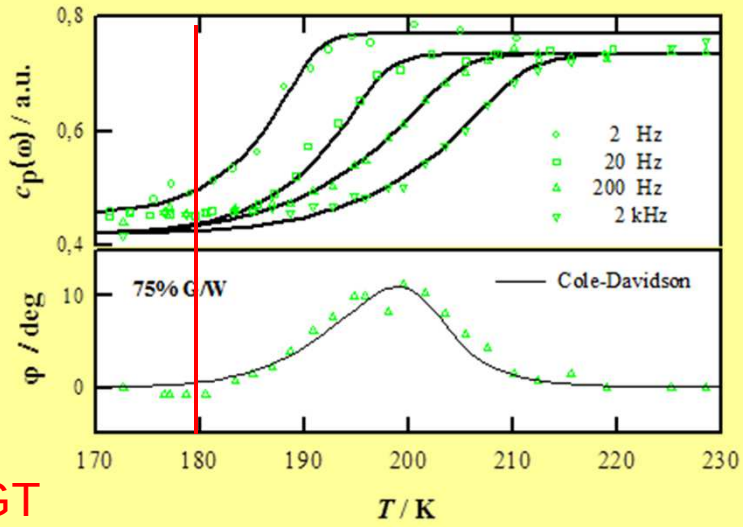
Log ($k_s \sim \eta^{-1}$)



cGT 100 s

Method: Specific heat relaxation

$C_p(\omega,T)$ (75% glyerol/water)



glass transition temperature, step of specific heat varies with frequency !!

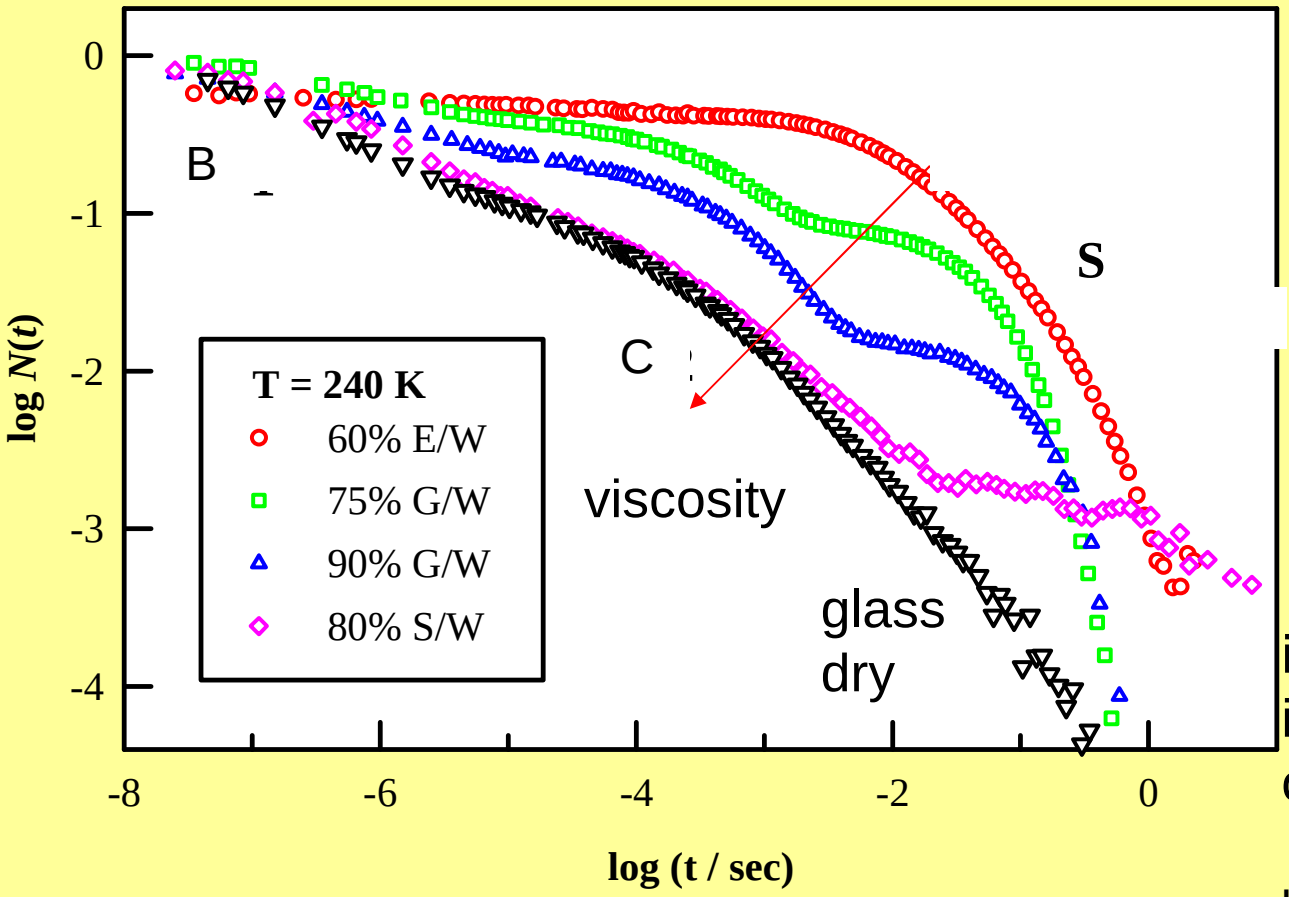
Arrhenius plot of solvent α-relaxation rates

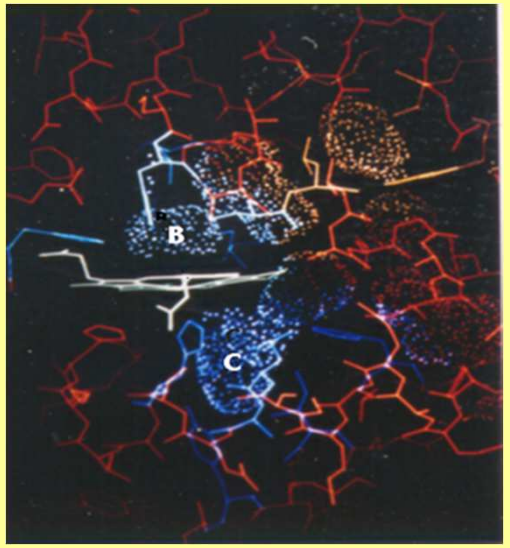
$k_s^{-1} = \tau_\alpha = G\,\eta$ Maxwell relation

Kleinert Biochem.1998, Doster BBA 2005

Kinetics of CO- binding to myoglobin
Flash photolysis experiments
different solvents, hydration

Log(CO unbound) (Kleinert et al. Biochem. 1998)



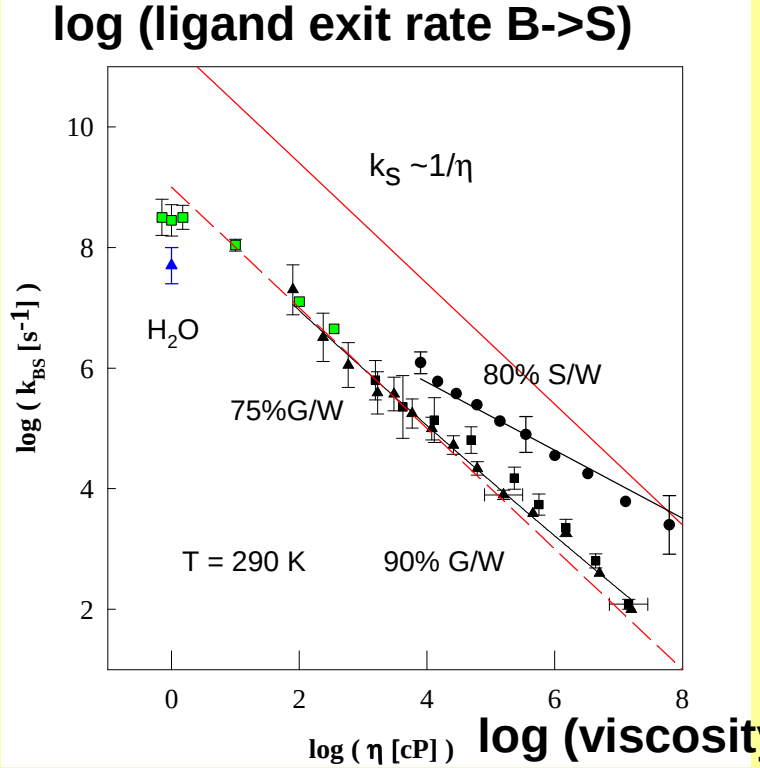


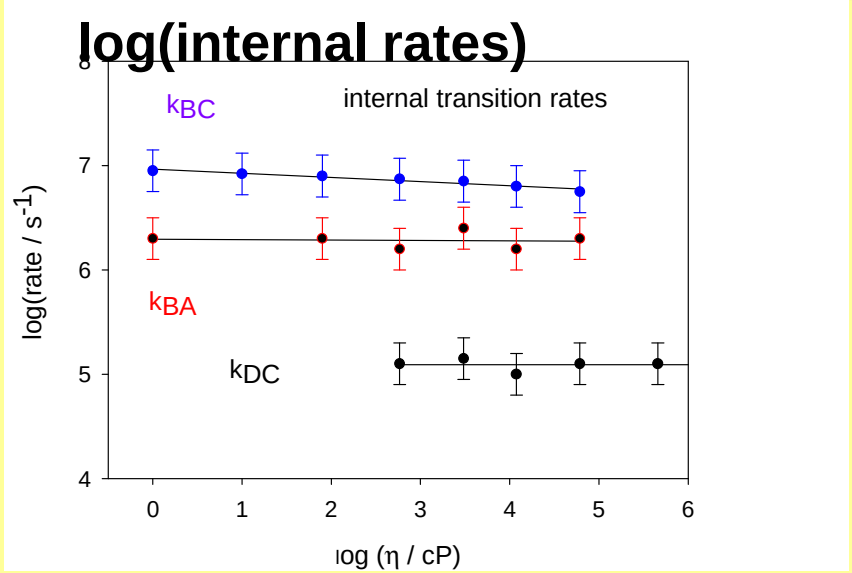
internal displacements
independent of viscosity
or degree of hydration

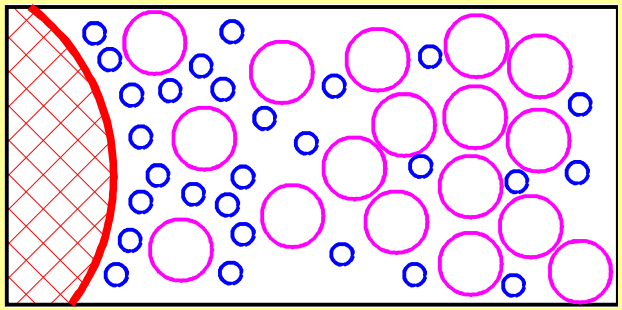
ligand escape S decreases
with viscosity

Viscosity Effect on Rate Constants

(Kleinert et al, Biochem. 1998)







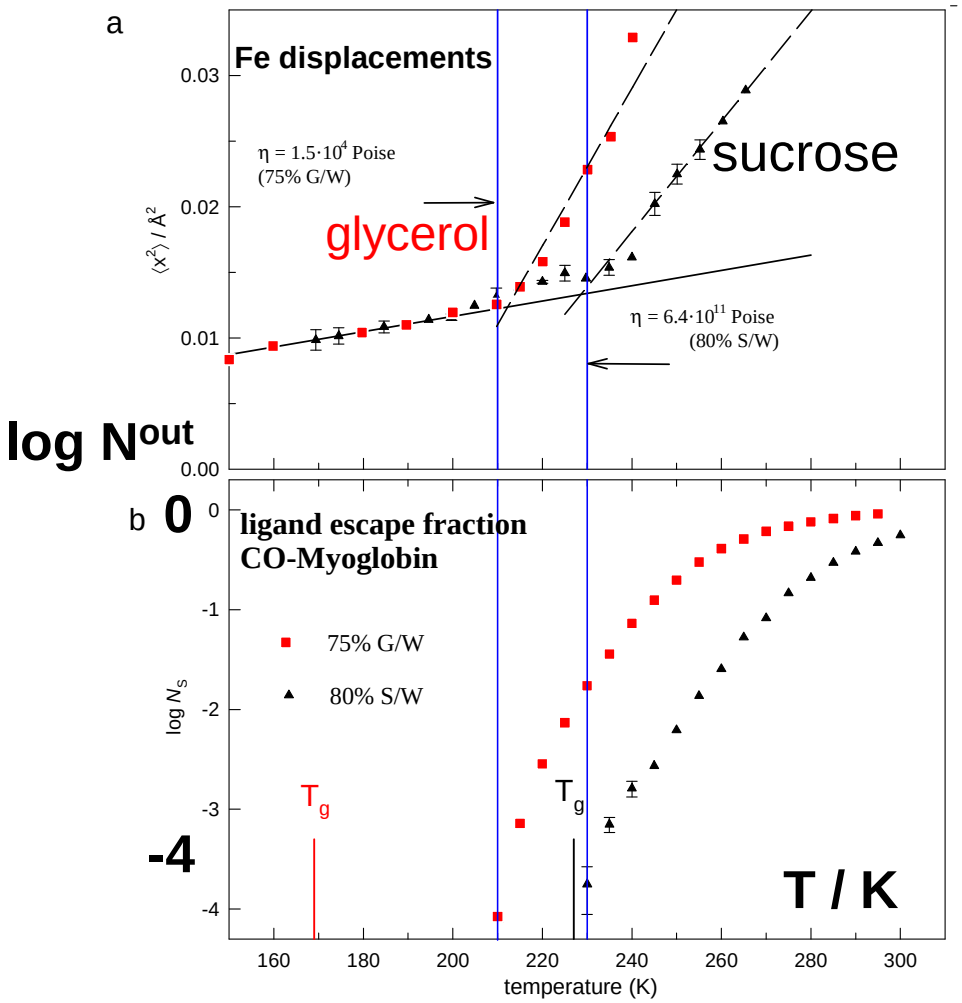
Sucrose 80% S/W
Preferential
Hydration
Timasheff
Lower surface viscosity than bulk

Result:
Two classes **of functional motions**
Type I: internal,decoupled from solvent viscosity, hydration
Type S: coupled to protein surface viscosity, hydration

Heme dynamics and functional motions

Solvent- dependence, function below transition?

$\langle \Delta x^2 \rangle$ heme displacements in myoglobin



Mössbauer effect:
heme iron displacements
75 % glycerol-water
80 % sucrose-water

flash photolysis:
ligand escape fraction N_{out}

common solvent viscosity effect on heme motions and ligand motions

Lichtenegger et al. Biophys.J. 76 (1999) 414
Kleinert et al. Biochem. (1998) 37:717,
Srajer et al. Biochem. (2001)

Flash Photolysis at high pressure: Myoglobin-CO

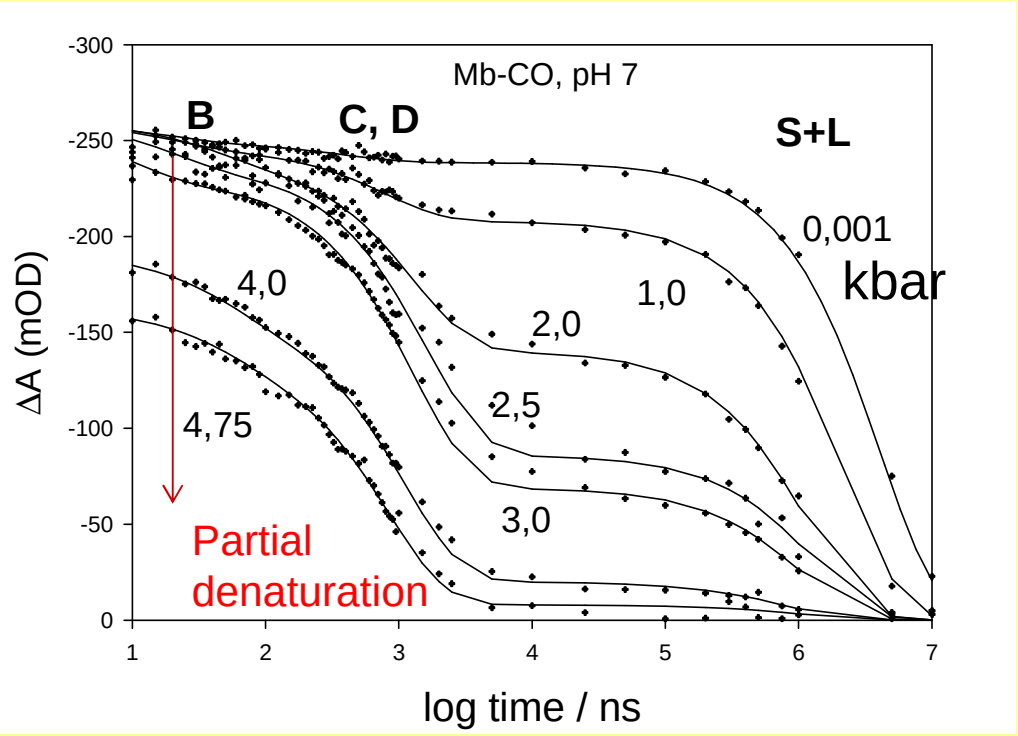
Solvent primary interior sites

$S \Leftrightarrow B \Leftrightarrow C_i$

$\Downarrow$

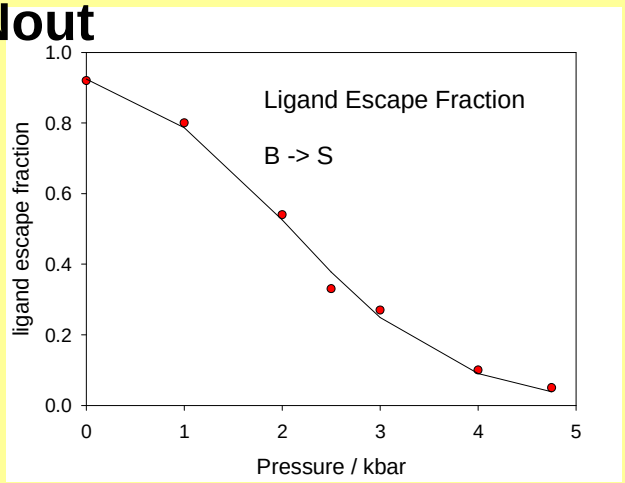
A ligated

Binding kinetics

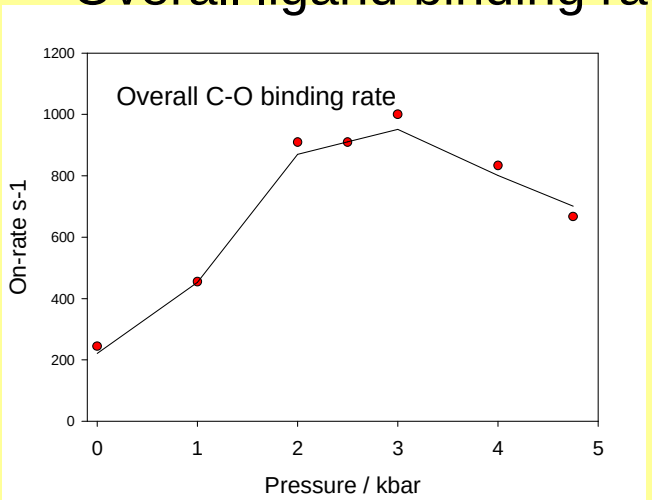


All or none transition: D<->N

Ligand escape fraction Nout

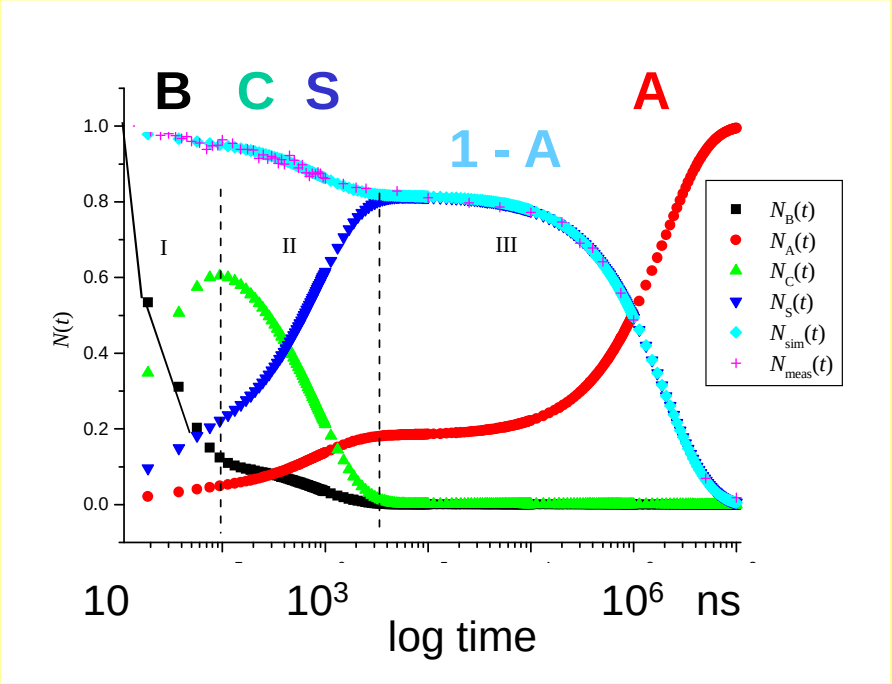


Overall ligand binding rate

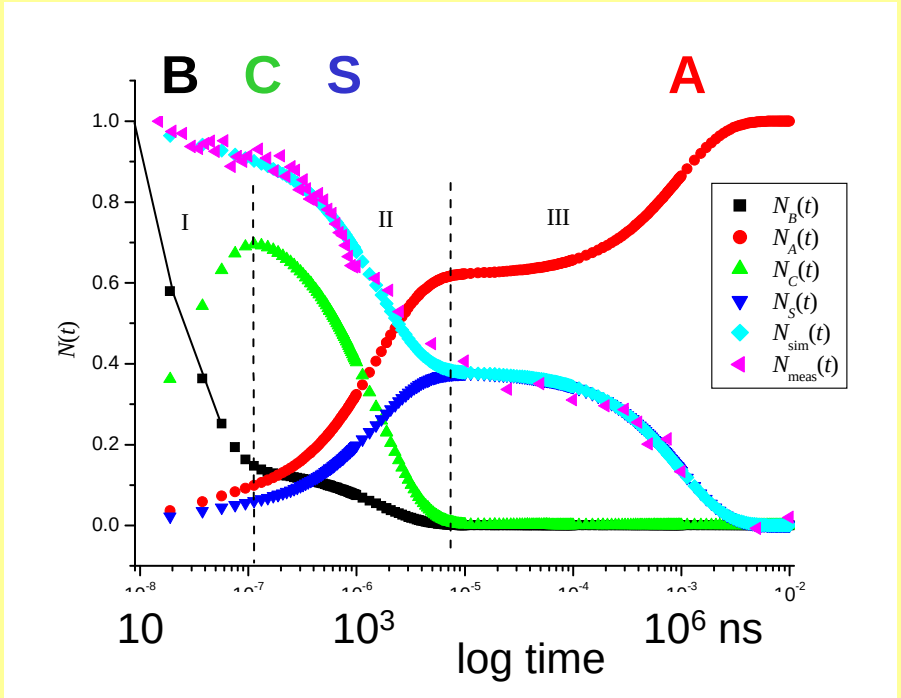


Flash photolysis at high pressure: population evolution

1 kbar



2.5 kbar



Solvent primary interior sites

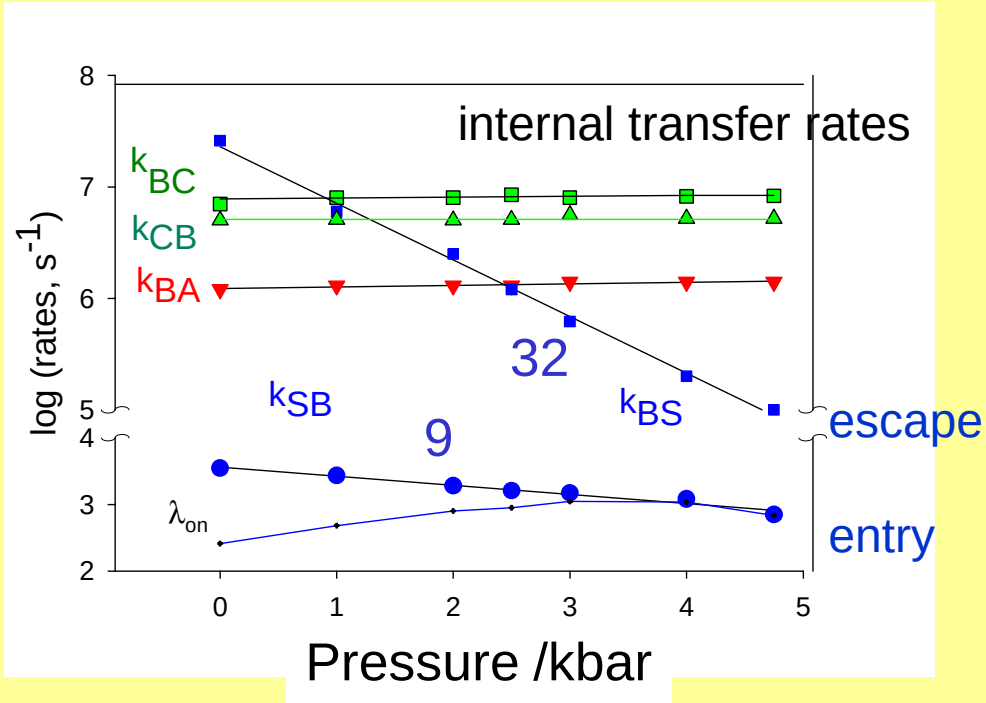
S ⇔ B ⇔ C_i

hν ⇓

A ligated

Triangles: experiment

Flash Photolysis at high pressure: Mb-CO



Microscopic rates

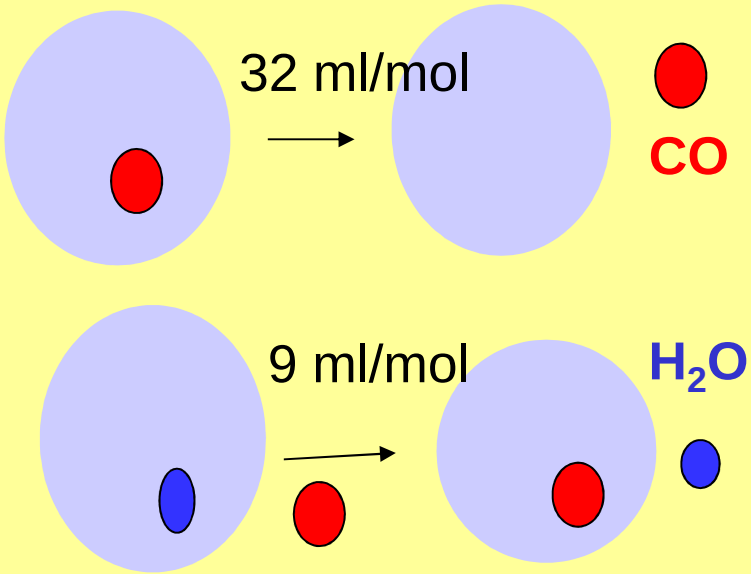
$k(P) = k(0) \exp(-\Delta V^* P / RT)$

Solvent primary interior sites

S ⇔ B ⇔ C_i

hν ⇑

A ligated



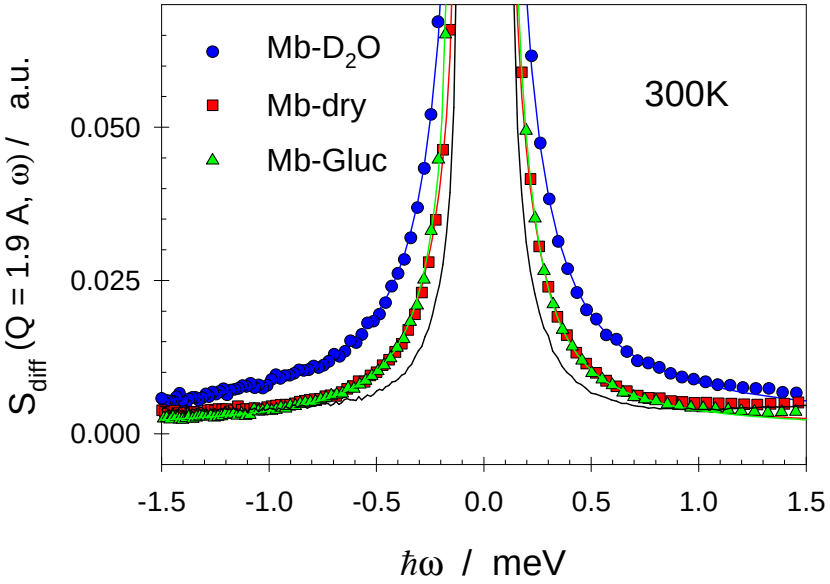
1) internal rates independent of P
vacancies stay empty up to D←>N

2) recombination rate depends on rate of internal water replacement

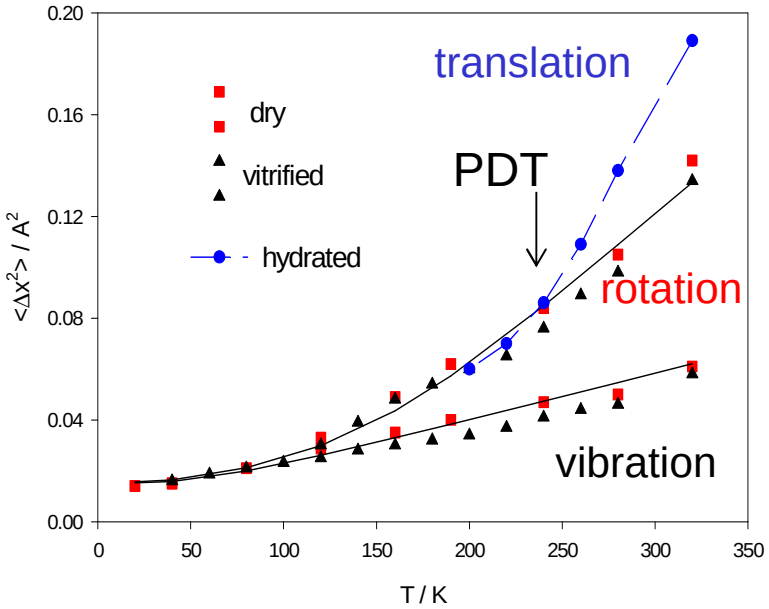
2 classes of functional motions I and S
2 classes of protein motions ??

Doster, Settles BBA 2005

myoglobin in various solvents



Proton Mean Square Displacements



IN13

quasielastic broadening:

myoglobin
dry, glassy, hydrated

PDT: protein dynamical transition

Two types of structural transitions:

internal and solvent-coupled motions

$$\langle \Delta x^2 \rangle_{tot} = \langle \Delta x^2 \rangle_{vib} + \langle \Delta x^2 \rangle_{rot} + \langle \Delta x^2 \rangle_{trans}$$

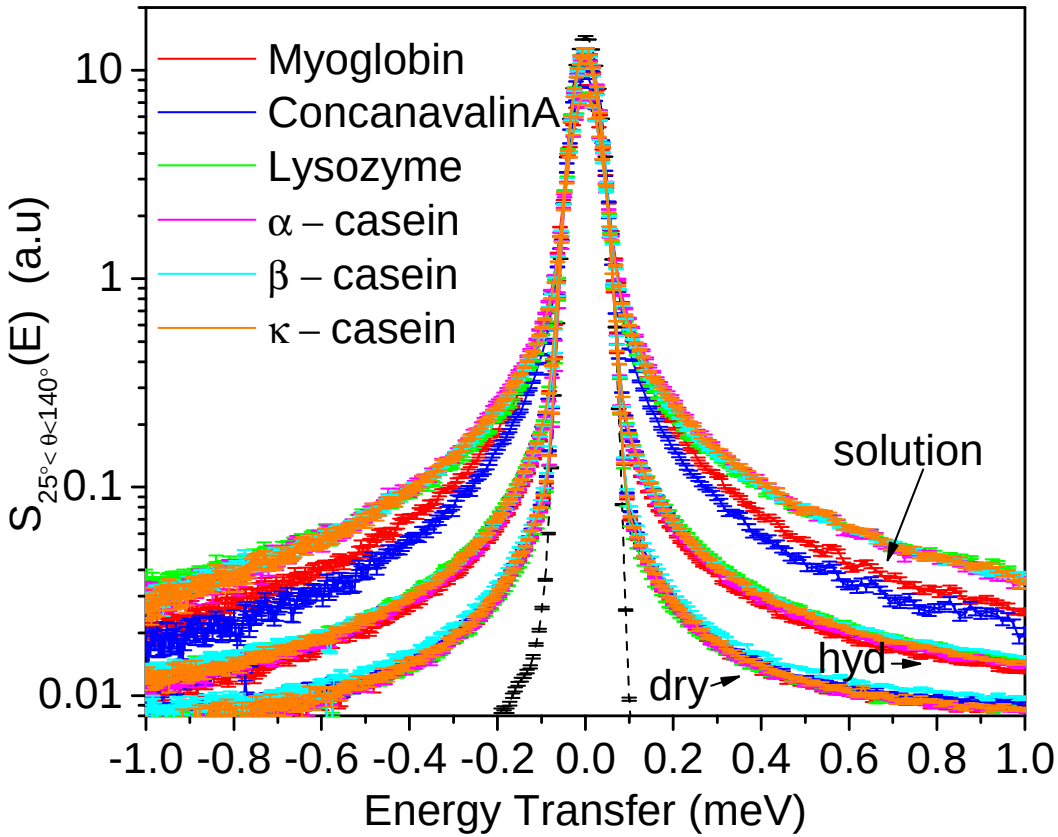
hydrated/solvated protein spectra

Gaspar et al. Eur.Biophys.J. (2008)

FRM 2 Munich

„Benshmark“ proteins with different structure and environment

Log(S(Q,w))



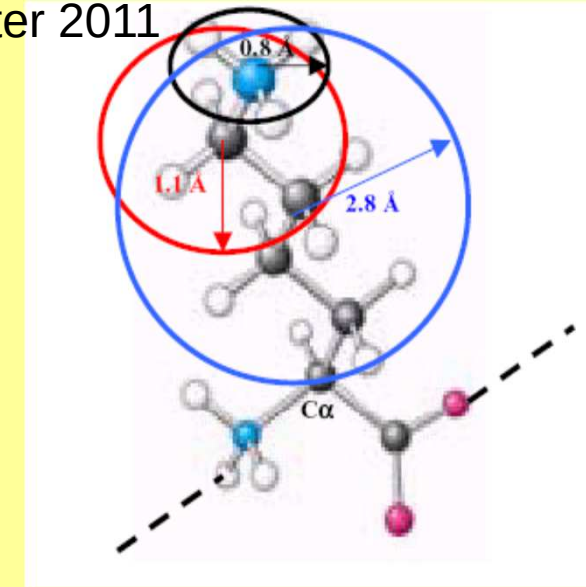
marked differences only in solution:

elastic scattering depends on structural rigidity

largest differences due to protein global diffusion

protein dynamics and function: 2 major components I, S

Doster, Settles BBA 2005
Doster in Dynamics of Soft Matter 2011
Smith, Sokolov PRL 2012

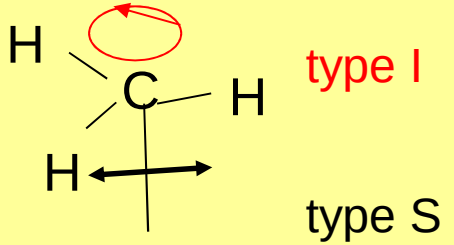


1) rotation, type I: independent of solvent

non-Gaussian, discontinuous,
torsional jumps of side chains, methyl groups and main chain
little dependent on environment
internal ligand migration

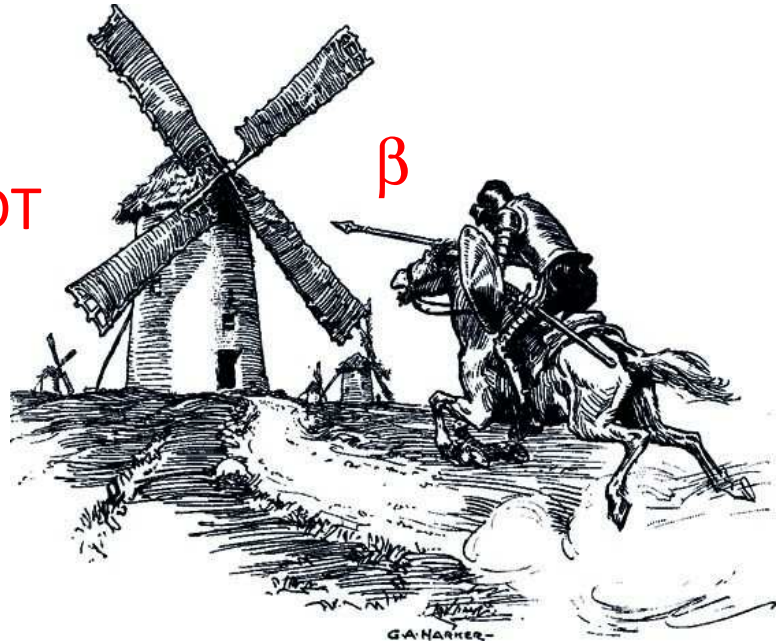
2) local translation, type S: coupled to viscosity

Gaussian, water-assisted, small scale
continuous translational displacements
coupled to α-(β)relaxation of hydration water
and the **entry and exit of protein ligands**



Temperature Dependence

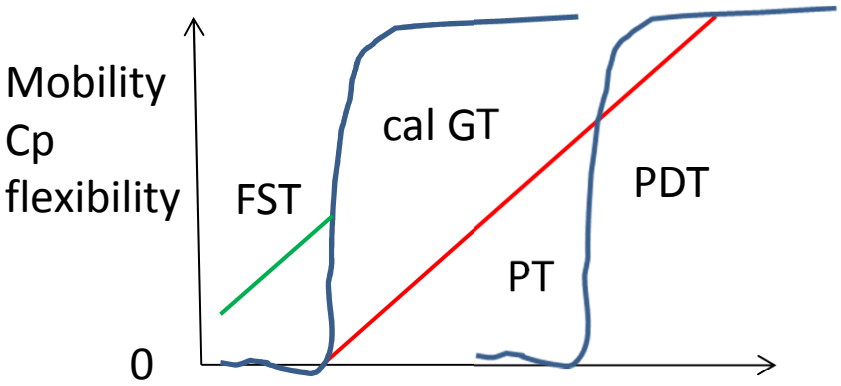
Dynamical Transitions: mobile → rigid (GT, PC, FST, PDT)
at nearly constant structure



$$D_s = \lim_{\omega \to 0, Q \to 0} \left(\frac{\omega}{Q}\right)^2 S_s(Q,\omega)$$

D(T, P, ω ..) -> 0 Diffusion vanishes

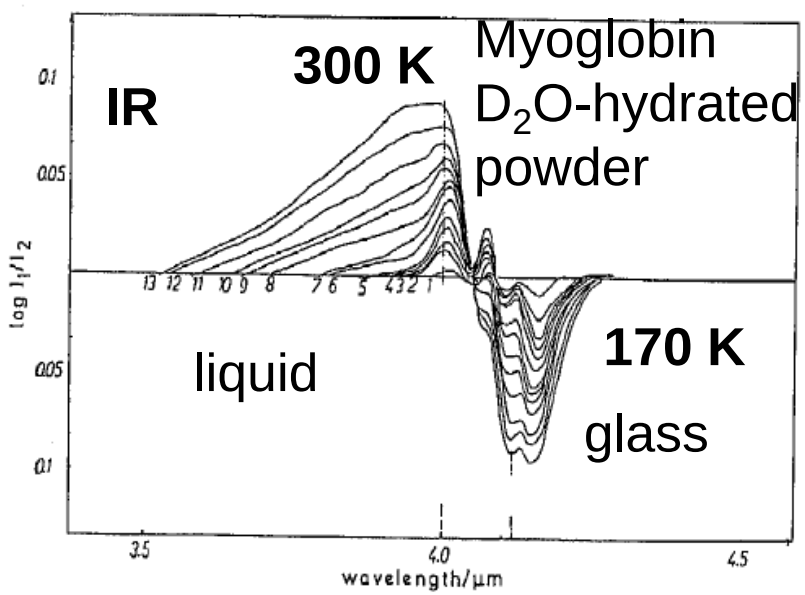
1) Percolation Transition
   Trapping-detrapping, energy landscape
   continuous
2) Liquid-glass transition
   discontinuous, ergodic-nonergodic
   specific heat, thermal expansion
3) protein dynamical transition:
   GT at shorter time scales



FST: fragile strong transition due to structural change
Static transition!

Glass Transition of Protein Hydration Water was defined originally by IR O-D spectroscopy

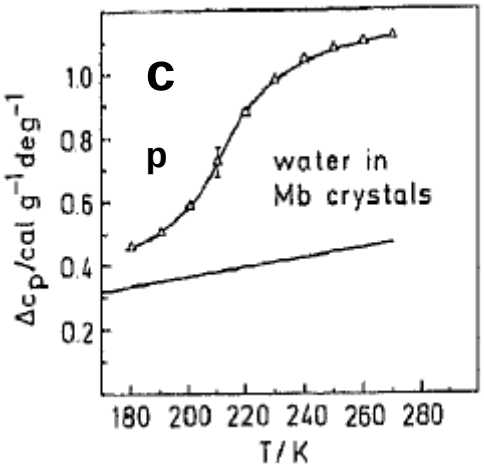
, W.D. Biophys.J. 50 (1986)



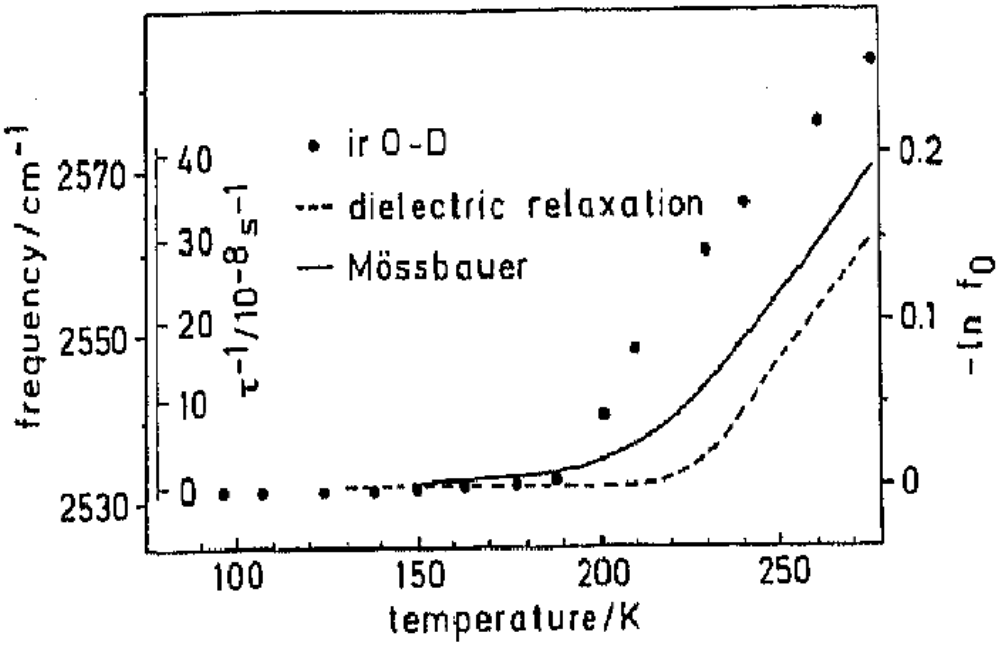
O-D stretching difference spectra: 100 - 300 K
liquid-- > amorphous ice structure

Glass transition depends on time scale of experiment:
T_G(IR) < T_G(Möss) < T_G (diel)

100 s 140 ns 1 ns
(Doster BJ 1986)



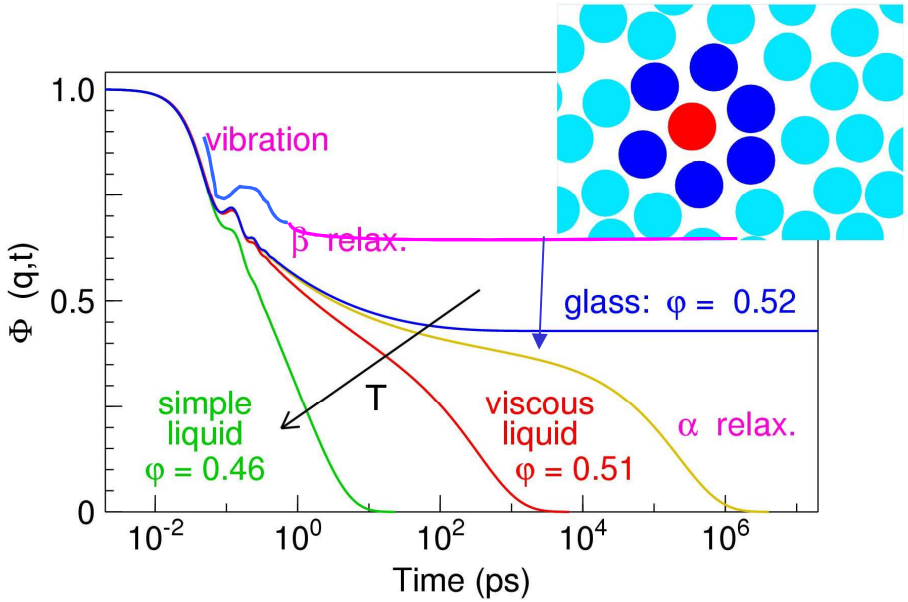
Step of Specific heat of hydration water in myglobin crystals



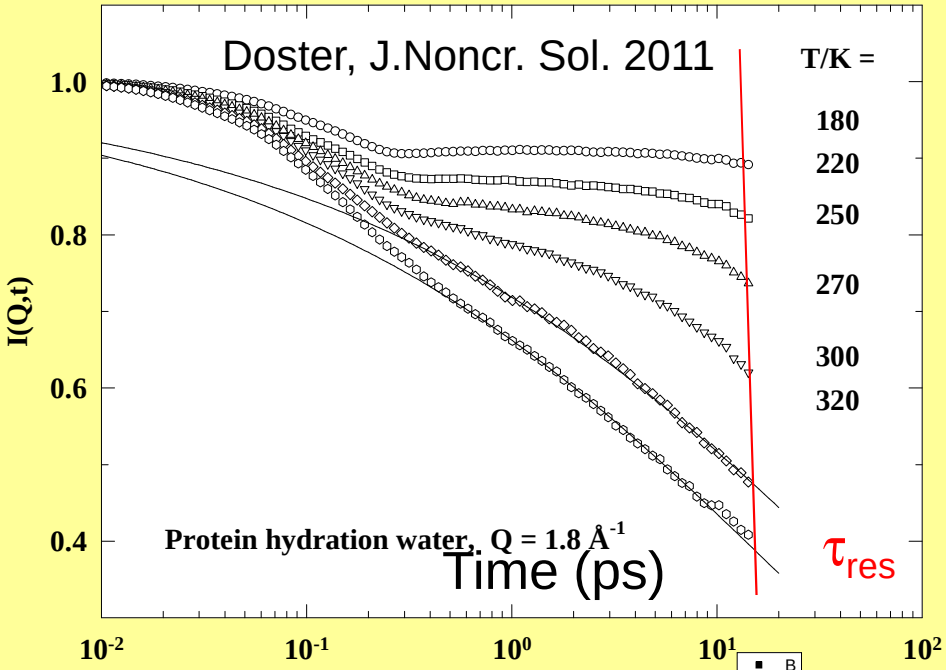
ideal liquid to glass transition: cage becomes a trap at a critical density

mode coupling theory of hard sphere liquid (Götze et al.)

Density correlation function



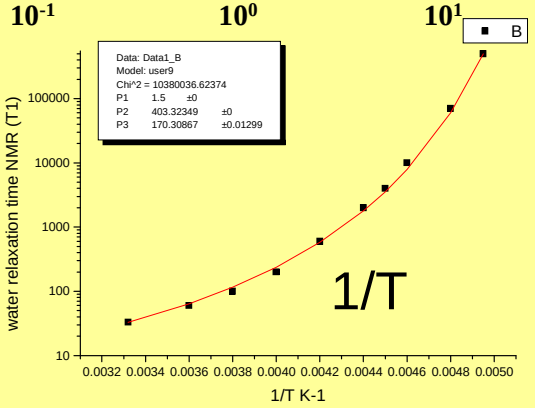
protein hydration water



correlations persist, nonequilibrium

$\Phi(Q,t\rightarrow\infty) = f_C \sim I(Q,t\rightarrow\infty) = EISF(Q)$

Log τ



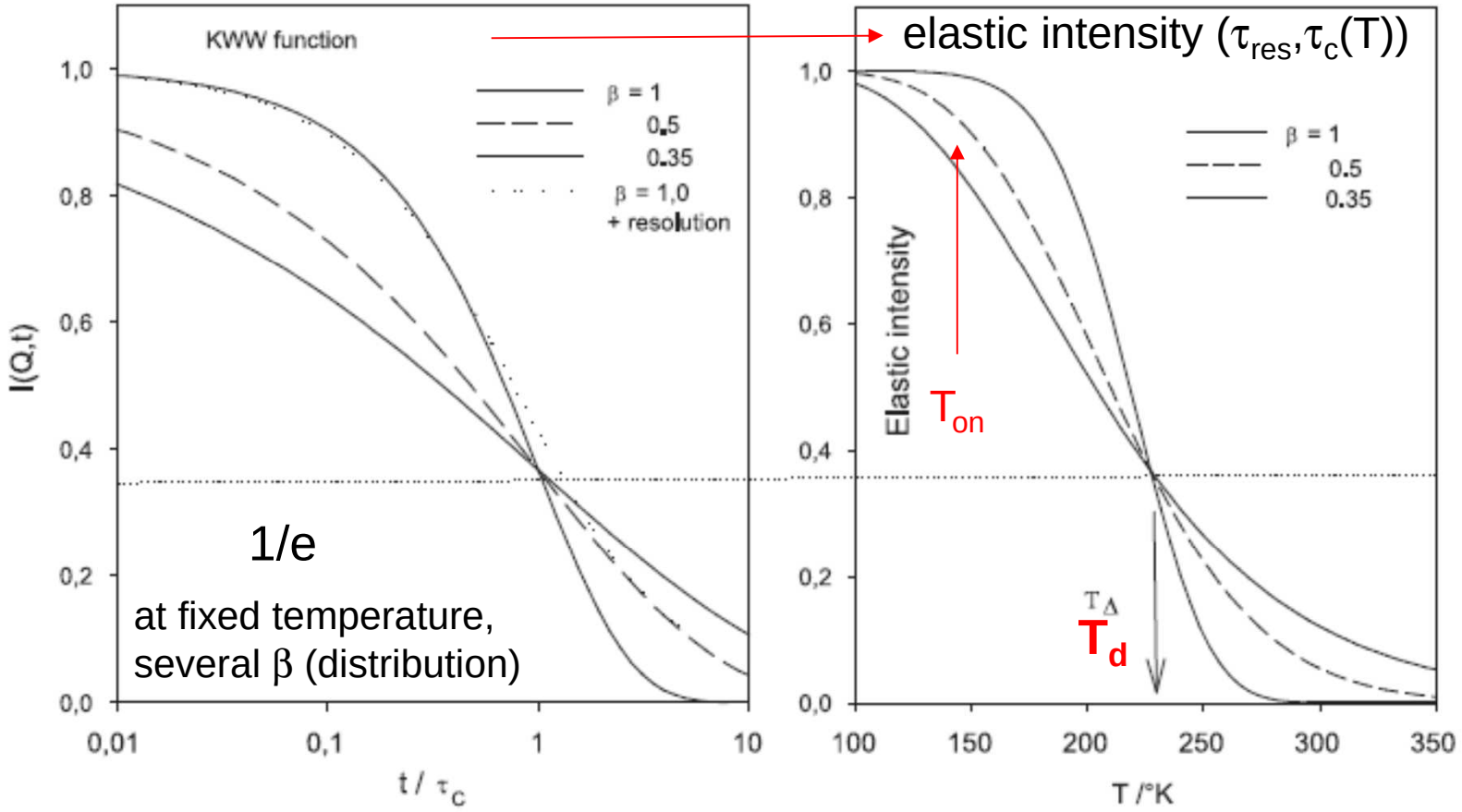
$f_C(Q)$ glass form factor

$f_C = 0$ liquid state

$f_C > 0$ glass, correlations persist: D_s (Q-> 0)= 0

Definition of dynamic transition temperature T_d
$I(Q,\tau_{res},\tau_c(T),\beta) = I_{el}(T)$: Effect of distribution

Doster JNCSol. 2011



$I(Q,t) = F(Q) \exp[-(\tau_{res}/\tau_c(T))^{\beta}]$ $\quad \tau_{res} / \tau_c(T_d) = 1$ independent of β

Effect of finite resolution
The elastic scattering intensity scaling relation

$I_{el}(Q) = S_{el}(Q,\omega=0,\Delta\omega) = \int_{-\infty}^{\infty} \mathrm{Re}\, s(Q,\omega',\Delta\omega)\cdot S(Q,\omega')d\omega'$

$\approx \exp(-\langle\Delta x^2\rangle Q^2)$ Gaussian approximation in low Q limit

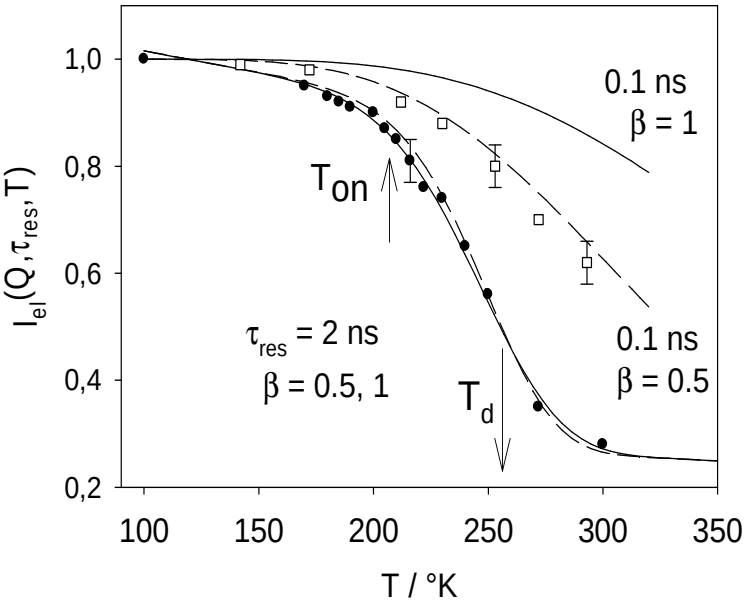
$$I_{el}^{N} = EISF(Q) + QEISF(Q)F(\tau_{res}/\tau_c(T))$$

F(x) scaling function

$$\tau_c = \tau_0 e^{\Delta H^*/RT}$$

$$\Delta H^* = RT_d \ln\left(\frac{\tau_{res}}{\tau_0}\right)$$

$$\frac{d}{dT} I_{el}^{NLL}(T_d) = \frac{1}{2}\frac{\Delta H^*}{RT_c^2}$$



CPC hydration water
ΔH^* = 17 kJ /mol
$\tau_0 = 10^{-13}$ s
β = 0.5

different β values

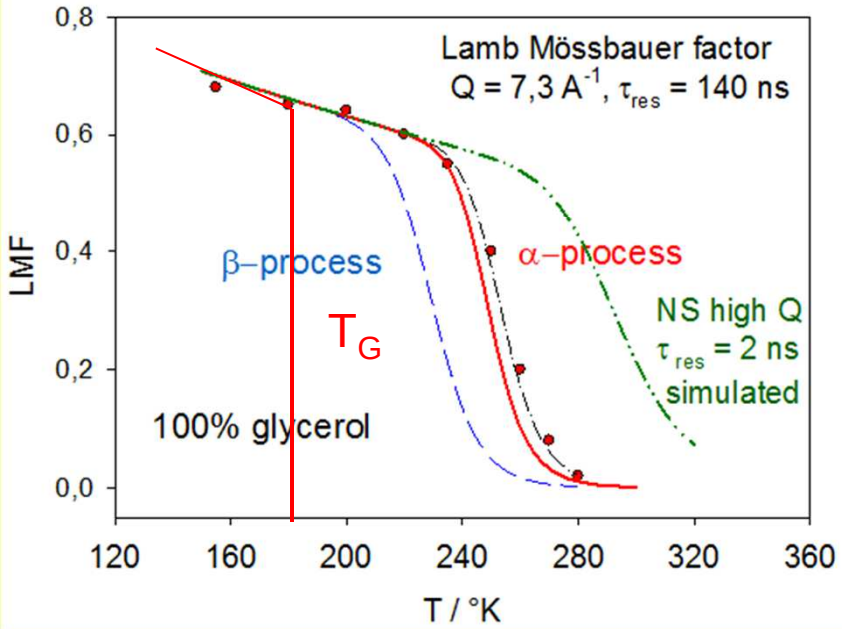
Doster, J. Noncryst. Sol. (2011) and Doster et al. JCP 2013 submitted

Lamb Mössbauer factor of pure glycerol

$$I_{el}^{N}(\tau_{res},\tau_c) = \frac{\tau_c + \tau_{res}^{-1}}{(\tau_c + \tau_{res}^{-1})^2} = \frac{\tau_{res}}{1+\tau_{res}/\tau_c(T)}$$

Q = 7.3 A^{-1}

Elastic intensity transition: GT at 140 ns



elastic intensity: Lorentz-Lorentz model

^{57}Fe in 100 % glycercol τ_{res} = 140 ns

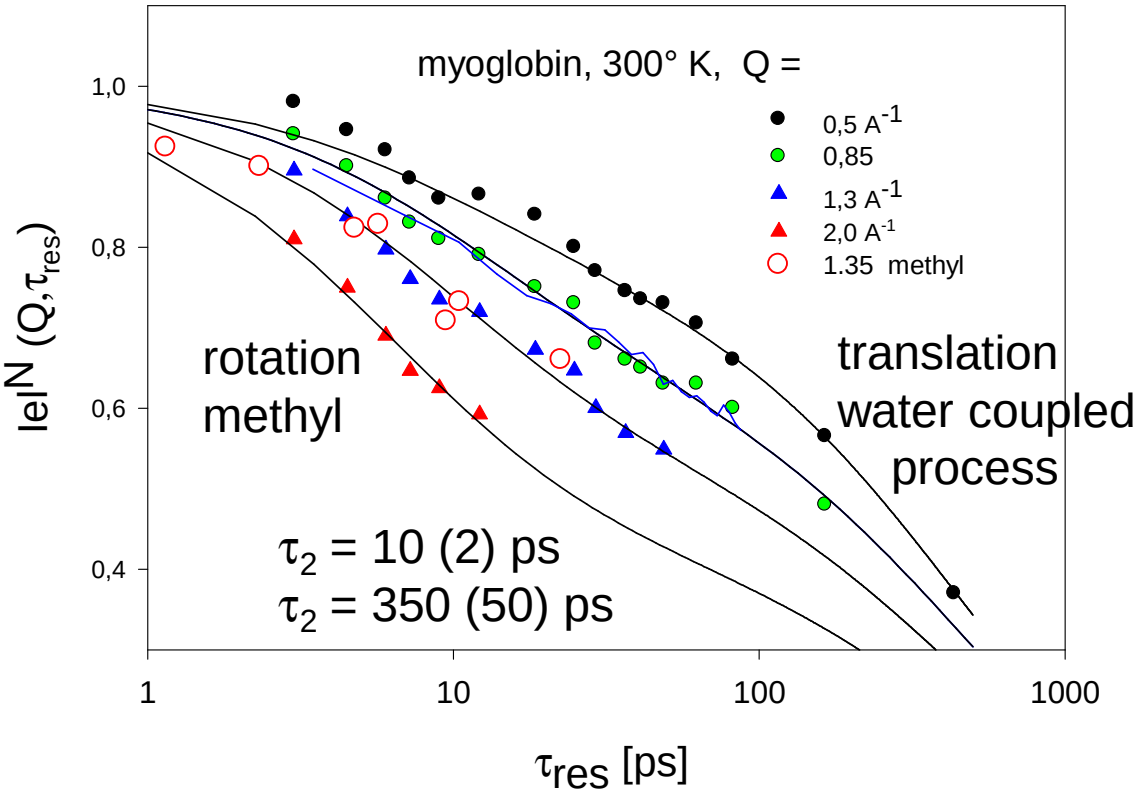
α, β relaxation data taken from:
Cappacioli et al. J.Phys. Chem B 2012
Mössbauer:
Champeney, Woodham
J. Phys. B. 1968

LMF transition due to α-relaxation not beta in contrast to Cappacioli et al.

Protein Dynamical Transition at room temperature

$I_{el}(Q,\tau_{res}) \approx I(\tau_{res}, Q)$ intermediate scattering function

elastic intensity: change resolution instead of temperature!



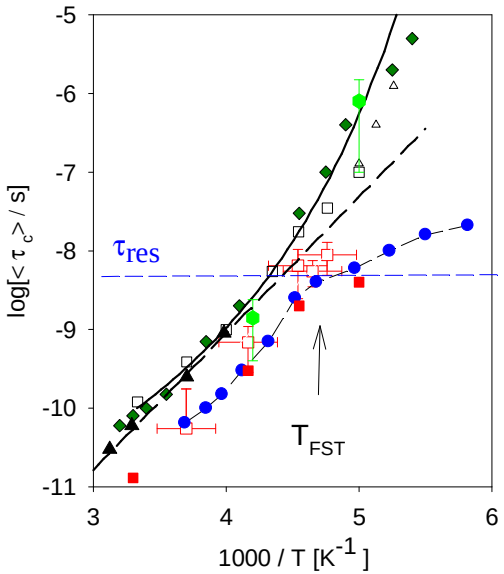
Time of flight IN5

hydrated myoglobin
0.4 g/g
25 mg protein!!

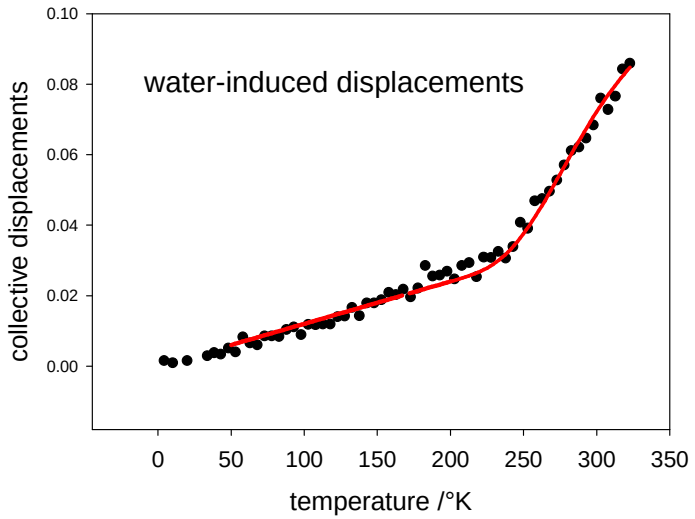
Elastic Resolution Spectroscopy: Doster, Diehl, Petry Ferrand
Physica B (2001) 301, 65 and Chem. Phys. 292 (2003) 487

Protein-Dynamical Transition: α-relaxation of the protein + hydration water system

water relaxation time α-process



protein displacements



$\tau_{hw} = \tau_0 \exp(-\Delta H_{hw}/(R(T-T_0))$

super-Arrhenius T-dependence

Doster et al. PRL 2010

$$\langle \Delta x^2 \rangle = \langle \Delta x^2 \rangle_{vib} + \langle \Delta x^2 (\tau_{res}/\tau_{hw}) \rangle_{conf}$$

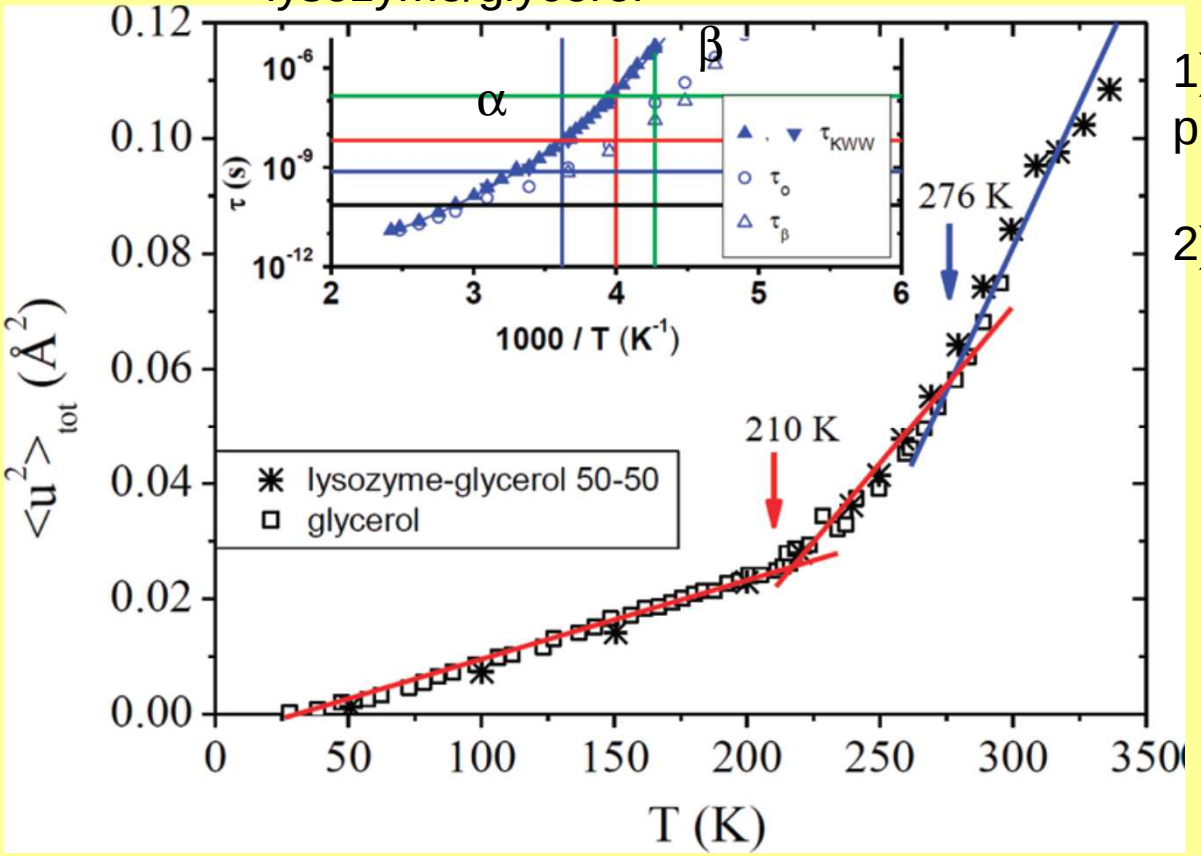
onset effect of resolution

Doster (2008) EBJ

Strong Protein-Solvent Coupling in Glycerol
the α / β controversy

Capaccioli, Ngai, Ancherbak Paciaroni, JPCB 2012

lysozyme/glycerol

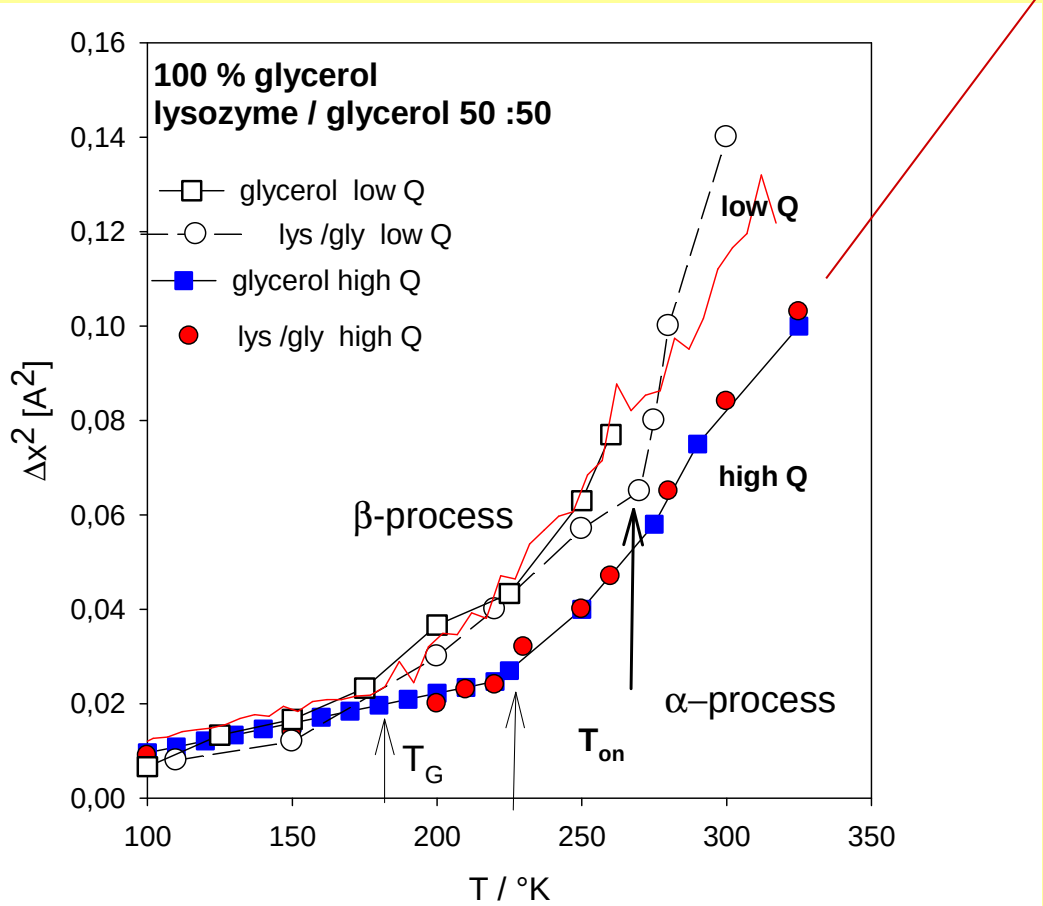


1) Perfect agreement of protein-solvent displacements

2) Insert show Arrhenius plot of dielectric relaxation of glycerol with α and GJβ process

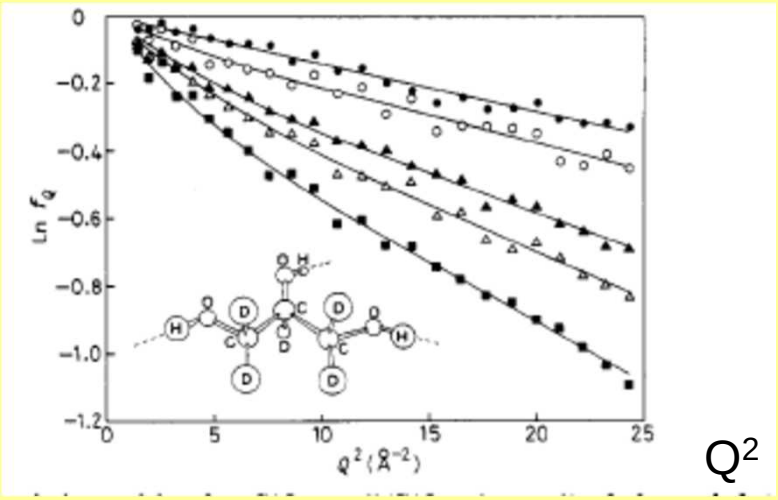
Lysozyme in glycerol: Solvent Slaving of Protein Motions?

The displacements depend on the Q-range: Cappacioli et al.; only high Q.



100 % glycerol

ln $I_{el}(Q)$ Fujara et al.



Q^2

High Q versus low Q displacements

$\langle \Delta x^2 \rangle = Q^{-2}$ d (ln I_{el})/dQ^2

Result: low Q displacements different from high Q, beta processes emerge only at low Q, ignored by Cappacioli et al.

Conclusions: Solvent Effects on Protein Function

„Biomolecules feel and respond only to those water molecules that are in close contact and not to bulk water“ (N. Dencher et al. 1998 Les Houches)

1) Hydration water shows two relaxation processes: fast local reorientation (beta) and slow long range diffusion (alpha)
2) Two classes of protein functional motions exist:
- (S) Surface viscosity coupled protein displacements
- (I) truely internal (solvent decoupled) displacements
  pressure cannot push water into protein voids
3) dynamical transition is induced by alpha-relaxation of the solvent, role of beta-relaxation unclear
4) Protein-water vibrations: hydration water does not exhibit a protein Boson peak, hindered rotation of water couples to low frequency diffusive motion: bond splitting

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