

*The Anomalous Properties of Water for the  
Dynamics and Glass Transition of Proteins*

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# *Acknowledgements*

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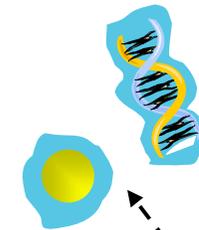
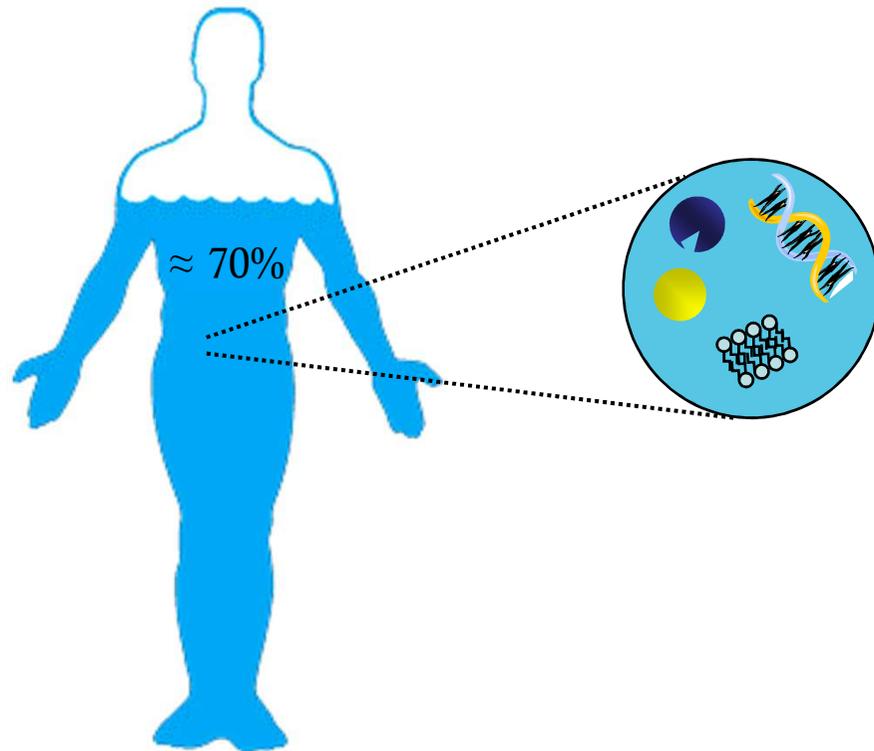
**Felix Fernandez-Alonso**

# *Outline*

- \* **Introduction to biological water and its role for proteins**
- \* **Experimental techniques we have used**
  - Dielectric spectroscopy
  - Differential scanning calorimetry (DSC)
  - Quasielastic neutron scattering (QENS)
- \* **Dynamics of confined supercooled water**
  - Why is there no calorimetric glass transition or  $\alpha$ -relaxation?
  - Is there a possible relation to supercooled bulk water?
- \* **Dynamics of myoglobin in water and water-glycerol**
  - The physical origin of the calorimetric glass transition
  - The role of the bulk solvent for protein dynamics and functions
  - The special role of hydration water for protein dynamics
- \* **Conclusions**

*Why study water in  
biological materials?*

# *Water in a body*



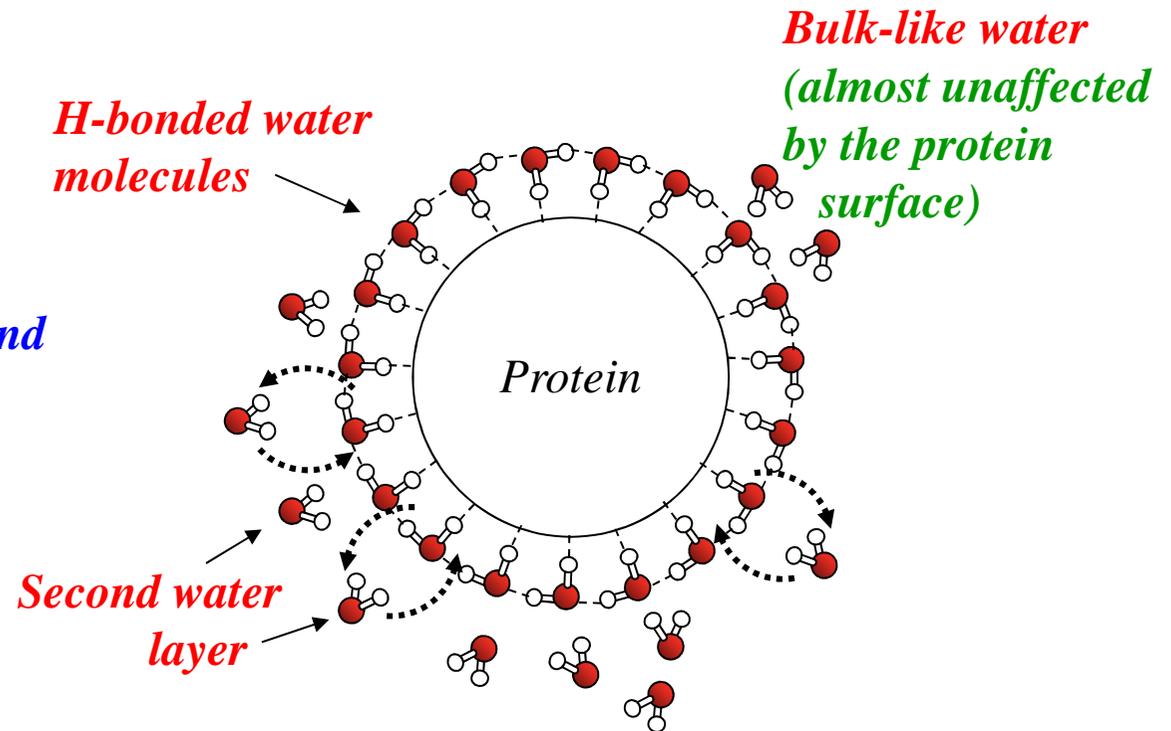
water molecules attached to  
or located in the vicinity of  
surfaces of different kinds

**interfacial/confined water**

# “Biological water”

## Hydration water

- ◆ *H-bonded water*
- ◆ *Less interacting water*
- ◆ *Dynamical exchange between the two molecular layers*
- ◆ *Hydration shell = first and second hydration layers*



# *The role of water for protein functions?*

Important for structure, stability, dynamics and function

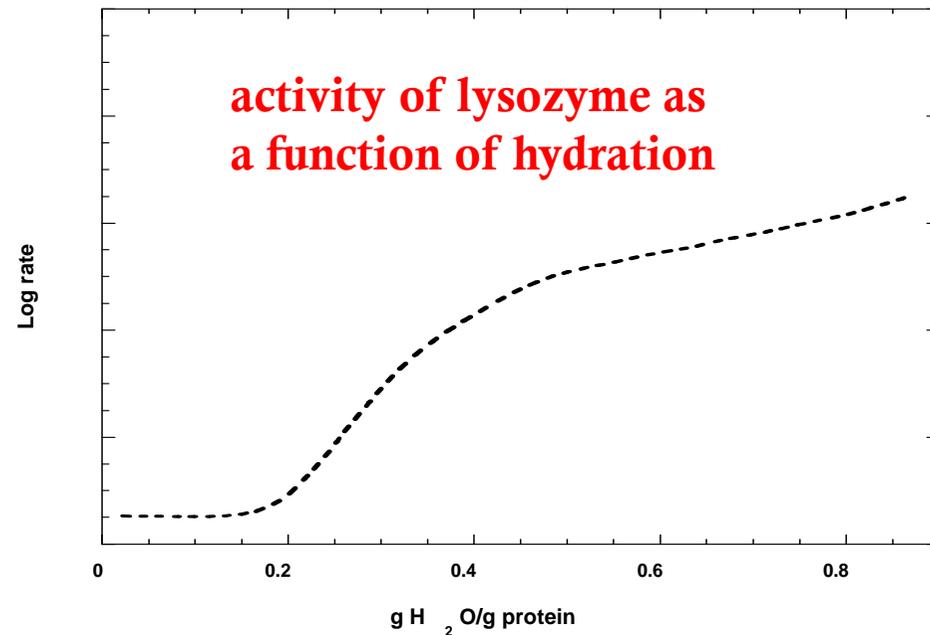
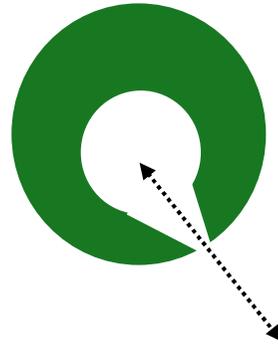
motions necessary for function

hydration necessary for function

“closed”



“open”



*Rupley et al. Trends. Biol. Sci. 1983*

*Relation between solvent and biomolecular dynamics;  
A way to understand the importance of water  
for biological functions*

**-Are dynamics and functions of biomolecules determined by solvent dynamics?**

**-How is the solvent affected by the biomolecules?**

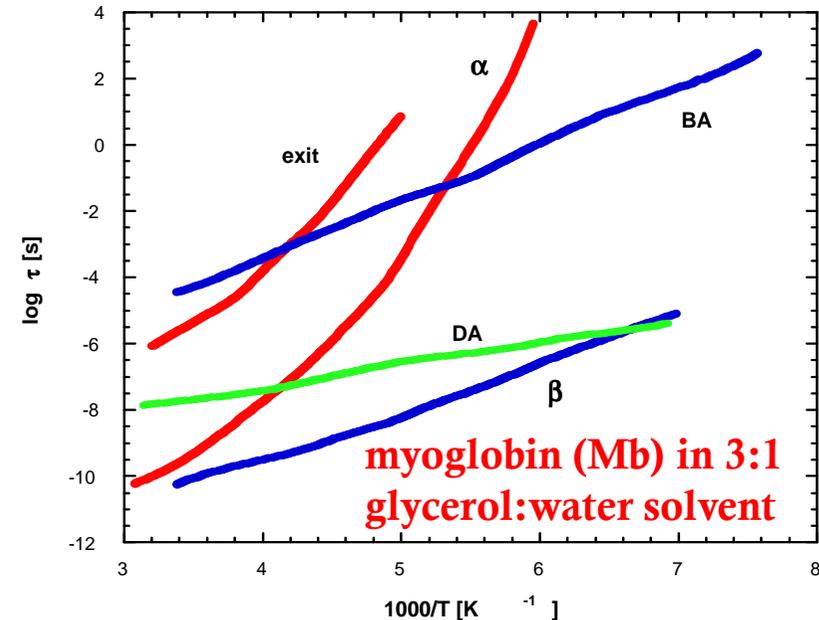
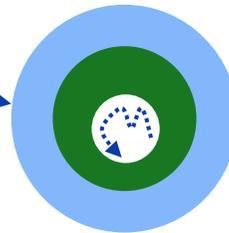
**-Does water exhibit unique properties as solvent?**

**These questions can only be answered by comparing biomolecular and solvent dynamics for different types of solvents.**

# The “slaving concept”

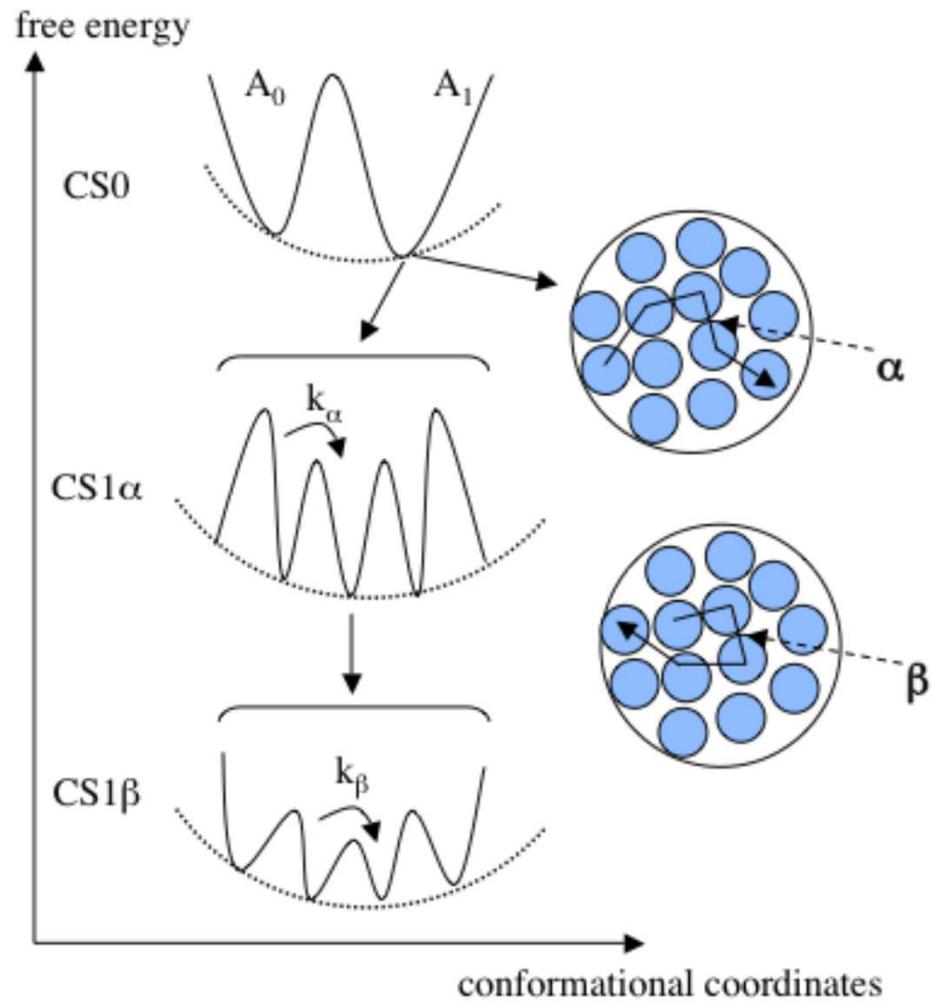
Protein dynamics and function are coupled to motions in the surrounding environment.

- **solvent-slaved**  
absent in dehydrated proteins  
and proteins in solid environments
- **hydration-shell-coupled**  
absent in dehydrated state
- **vibrational (non-slaved)**  
independent of the surrounding



**exit** (escape of CO from Mb) is determined by the **α-relaxation** in the solvent, whereas local CO migration (**BA**) within Mb is determined by the **β-relaxation**.

# *The energy landscape*



*P. W. Fenimore, H. Frauenfelder et al., PNAS 110, 14408 (2004)*

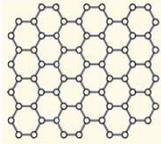
*Short introduction to supercooled  
and glassy materials*

# *Supercooled and glassy materials*

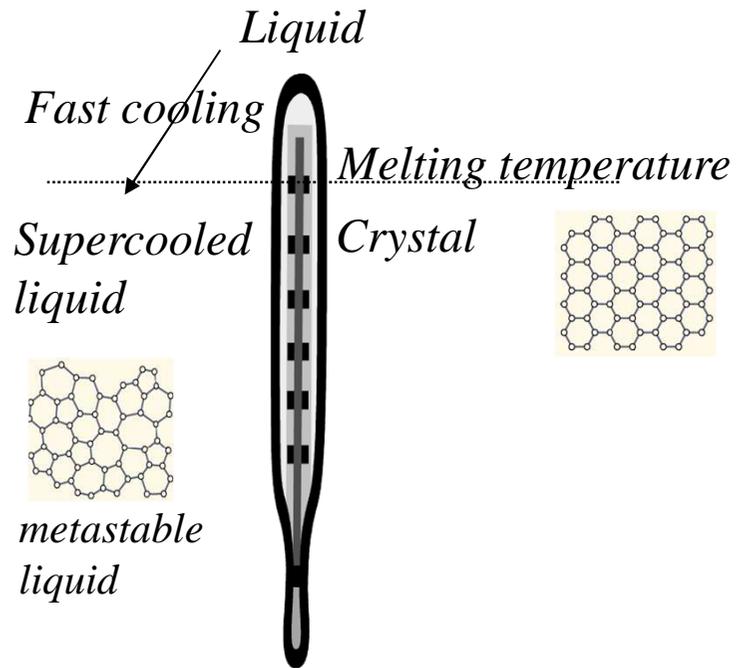
*Liquid*

*Melting temperature*

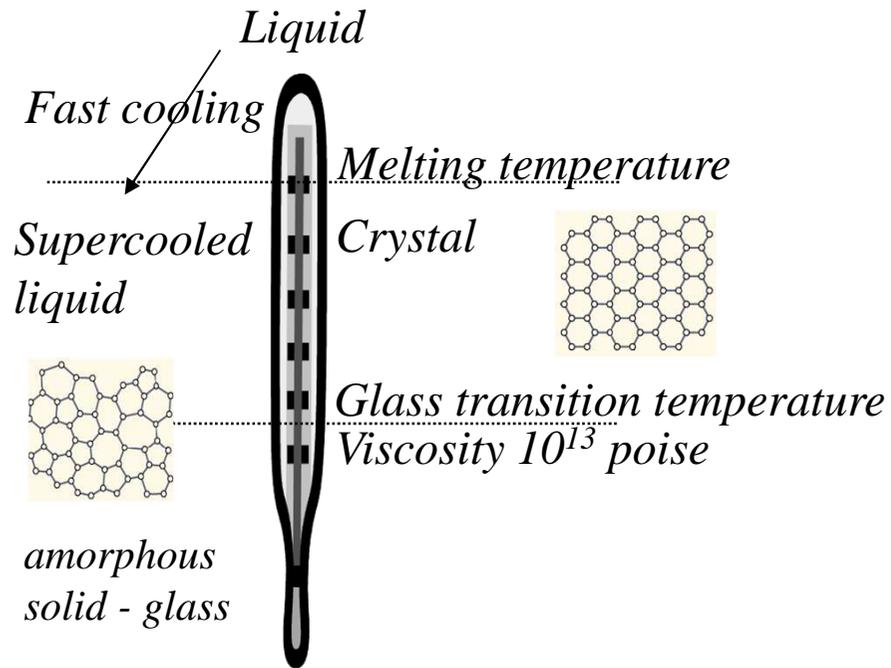
*Crystal*



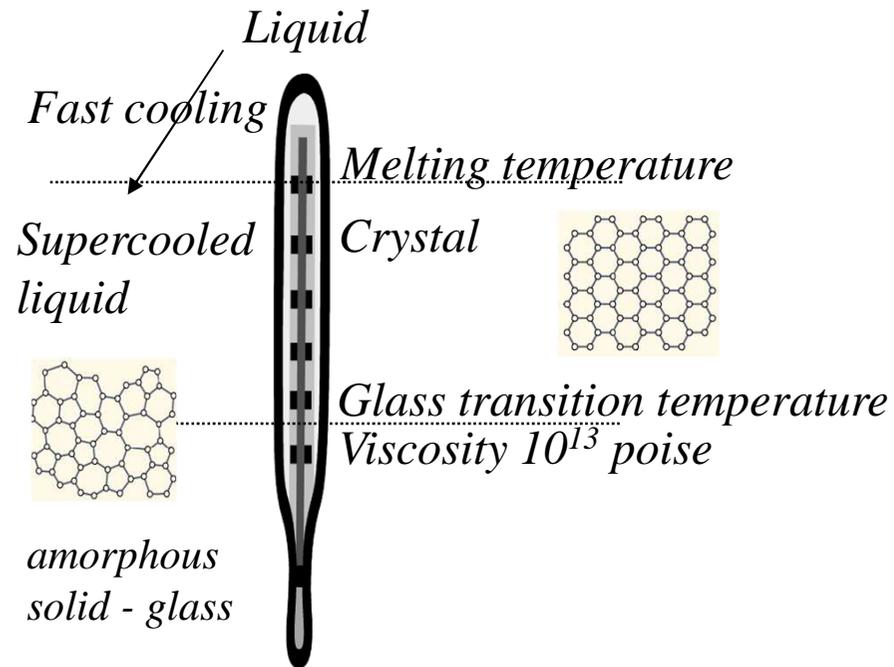
# *Supercooled and glassy materials*



# *Supercooled and glassy materials*



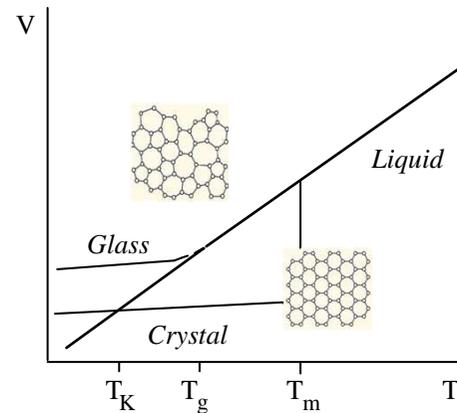
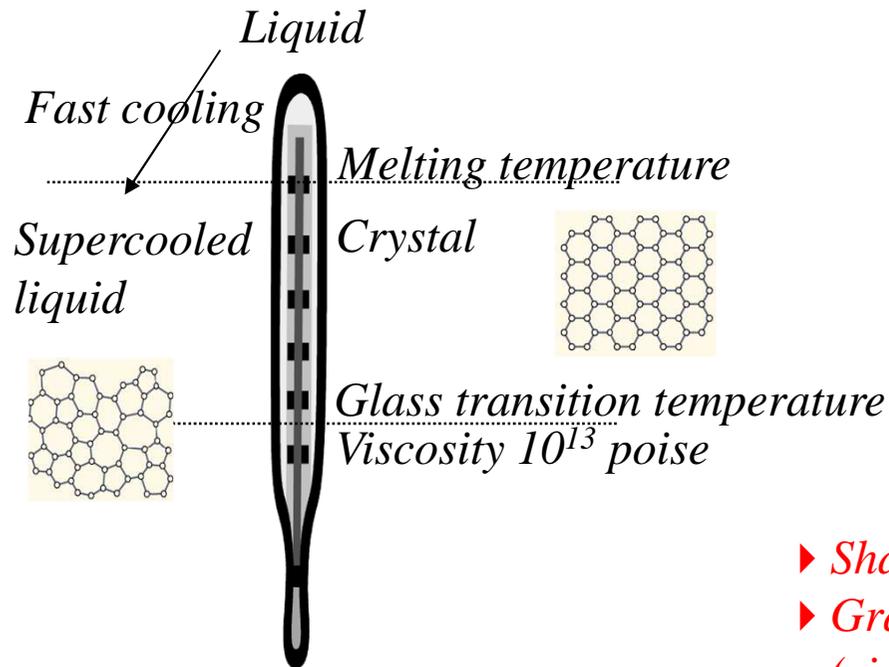
# *Supercooled and glassy materials*



## **Definition**

*A glass is an amorphous solid which exhibits a glass transition*

# Supercooled and glassy materials

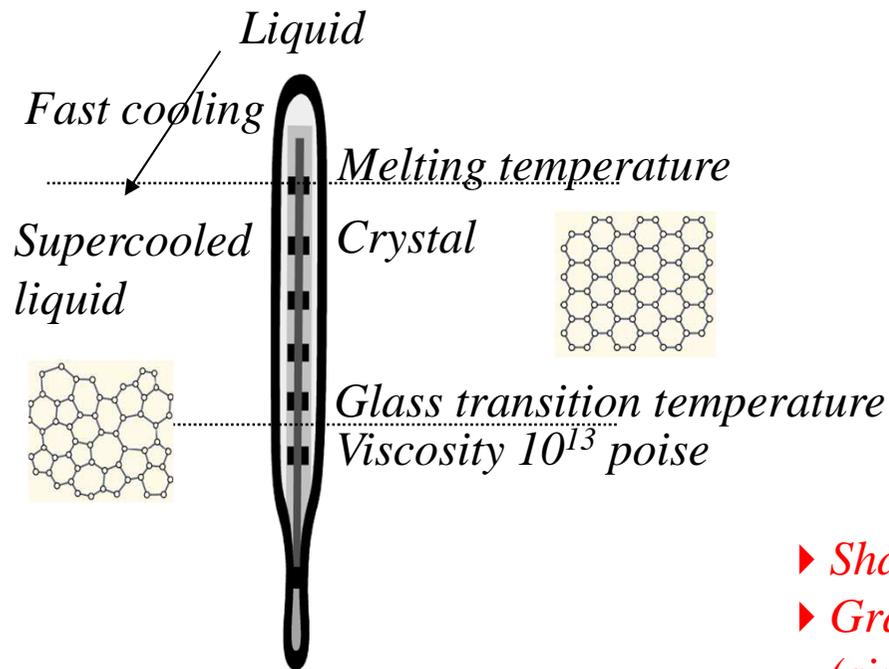


- ▶ Sharp drop in  $V$  at crystallization at  $T_m$
- ▶ Gradual change in  $V$  in the supercooled region  
(similar behaviour for other thermodynamic variables)

## Definition

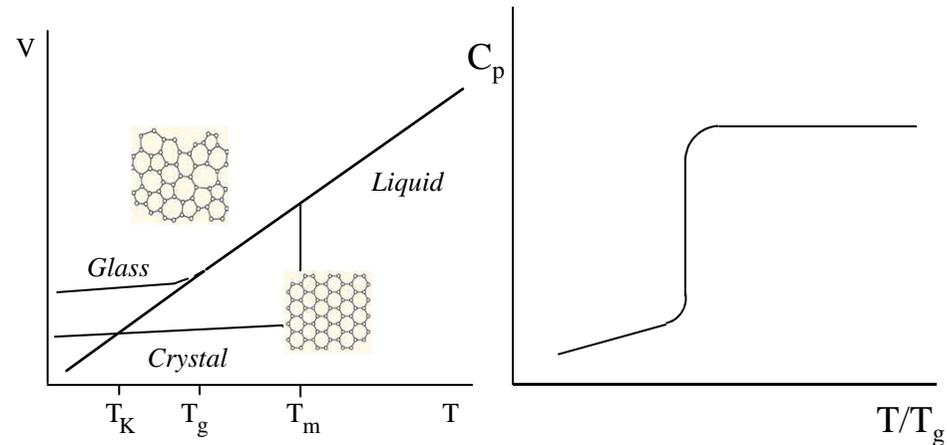
A glass is an amorphous solid which exhibits a glass transition

# Supercooled and glassy materials



## Definition

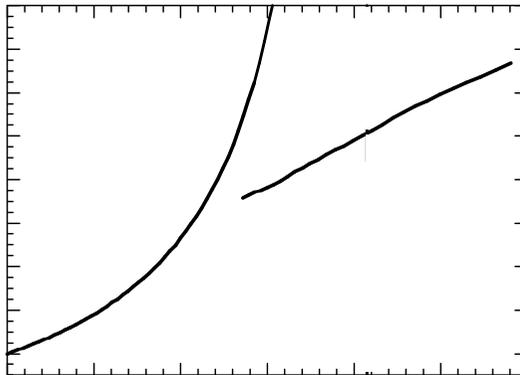
A glass is an amorphous solid which exhibits a glass transition



- ▶ Sharp drop in  $V$  at crystallization at  $T_m$
- ▶ Gradual change in  $V$  in the supercooled region (similar behaviour for other thermodynamic variables)
- ▶ Abrupt change in derivative thermodynamic properties such as heat capacity (or thermal expansivity) at  $T_g$

# *Supercooled and glassy materials*

*Liquid to glass transition  $\Rightarrow$  structural relaxation process*



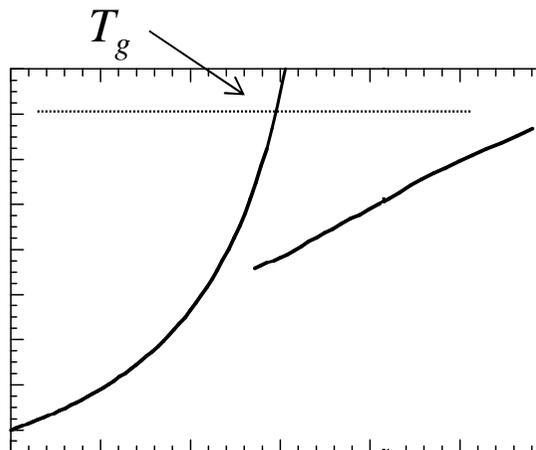
# Supercooled and glassy materials

*Liquid to glass transition  $\Rightarrow$  structural relaxation process*

## *The $\alpha$ -relaxation*

- *Due to cooperative rearrangement in supercooled region*
- *Directly related to the viscosity*
- *$T_g$  at  $\tau = 100$  s*
- *Vogel-Fulcher-Tamman (VFT) temperature dependence*

$$\tau = \tau_0 \exp\left(\frac{DT_0}{T - T_0}\right)$$



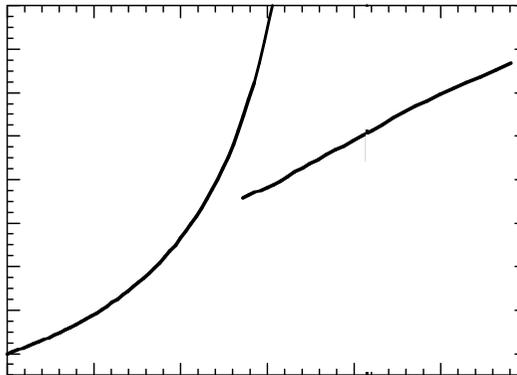
# Supercooled and glassy materials

Liquid to glass transition  $\Rightarrow$  structural relaxation process

## The $\alpha$ -relaxation

- Due to cooperative rearrangement in supercooled region
- Coupled to the viscosity
- $T_g$  at  $\tau = 100$  s
- Vogel-Fulcher-Tamman (VFT) temperature dependence

$$\tau = \tau_0 \exp\left(\frac{DT_0}{T - T_0}\right)$$



## The $\beta$ -relaxation

- Faster and more local than the  $\alpha$ -relaxation
- Persists below  $T_g$
- Arrhenius behaviour

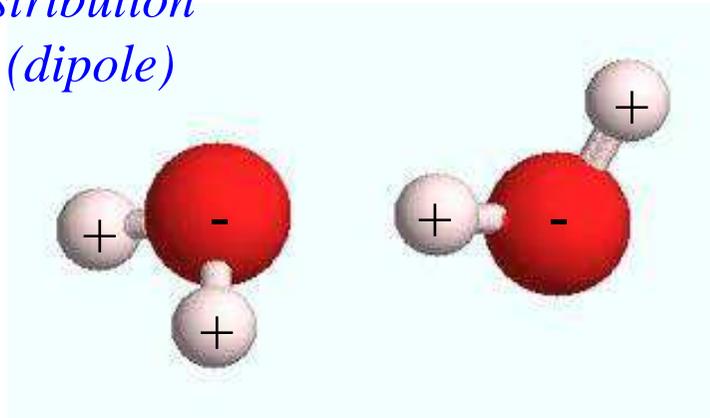
$$\tau = \tau_0 \exp\left(\frac{E_a}{k_B T}\right)$$

These relaxation processes tends to merges when  $\tau \approx 10^{-6}$  s

# *Water*

*Uneven charge distribution*

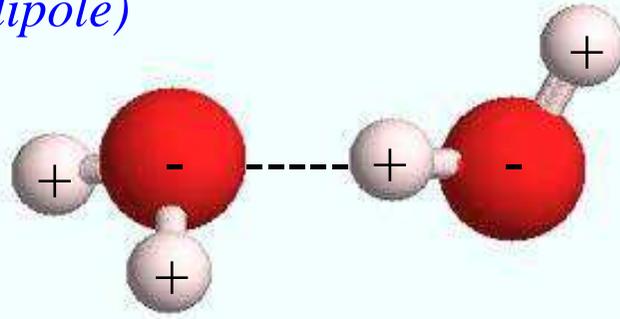
▲ *polar molecule (dipole)*



# *Water*

*Uneven charge distribution*

*▲ polar molecule (dipole)*

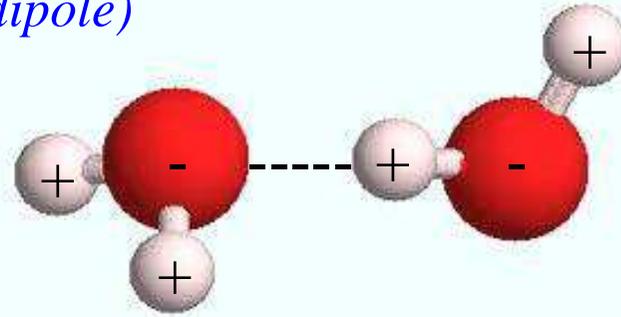


*Charge attraction ▲ hydrogen bonds*

# Water

*Uneven charge distribution*

▲ *polar molecule (dipole)*



*Charge attraction* ▲ *hydrogen bonds*

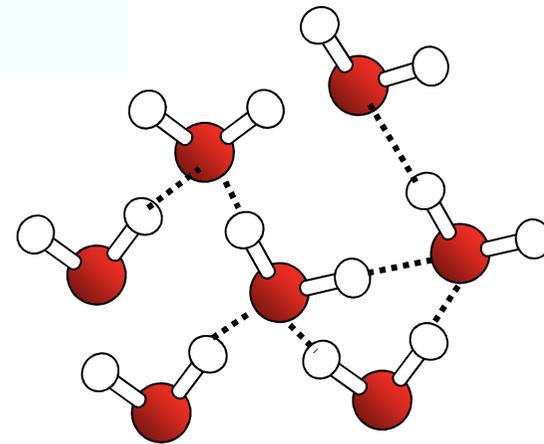
▲ *network of H-bonded molecules*

▲ *short life time of hydrogen bonds (ps)*

↑ *High boiling point*

↑ *Strong surface tension*

↑ *High specific heat....*



# *How does supercooled water behave in "no man's land" (150-235 K)?*

## • Fragile or strong?

R. S. Smith and B. D. Kay, *Nature* (1999)

K. Ito et al., *Nature* (1999)

J. Swenson & J. Teixeira, *JCP* (2010)

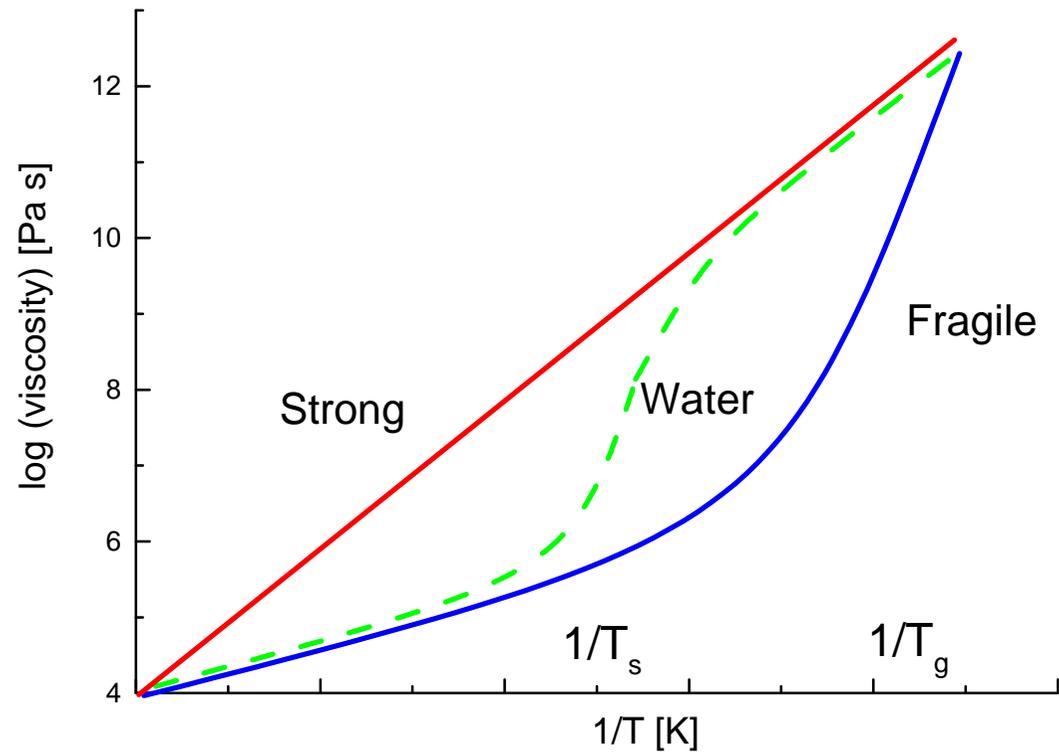
- Where is  $T_g$  located,  
~136 K or ~165 K or  
> 210 K or ~228 K?

G. P. Johari et al., *Nature* (1987)

C. A. Angell et al., *Science* (2001),  
*Nature* (2004)

M. Oguni et al., *AIP, conference  
proceedings* (2008)

J. Swenson & J. Teixeira, *JCP* (2010)



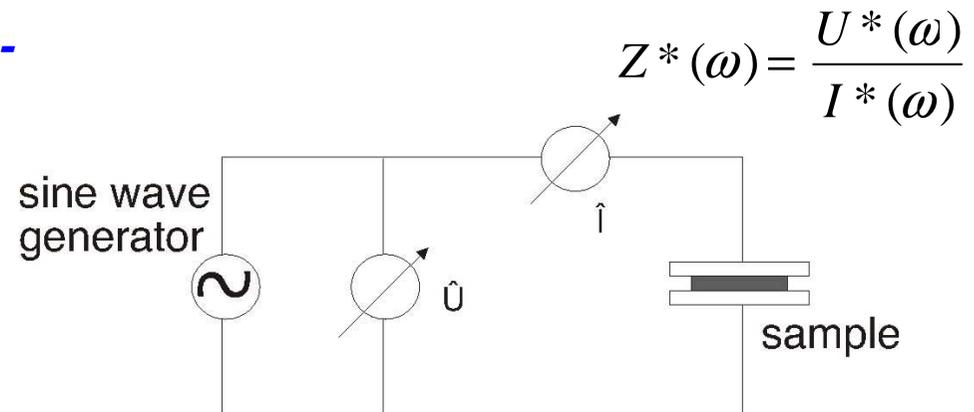
# *Experimental techniques*

- \* **Dielectric spectroscopy**
- \* **Differential scanning calorimetry (DSC)**
- \* **Quasielastic neutron scattering**

# Experimental techniques

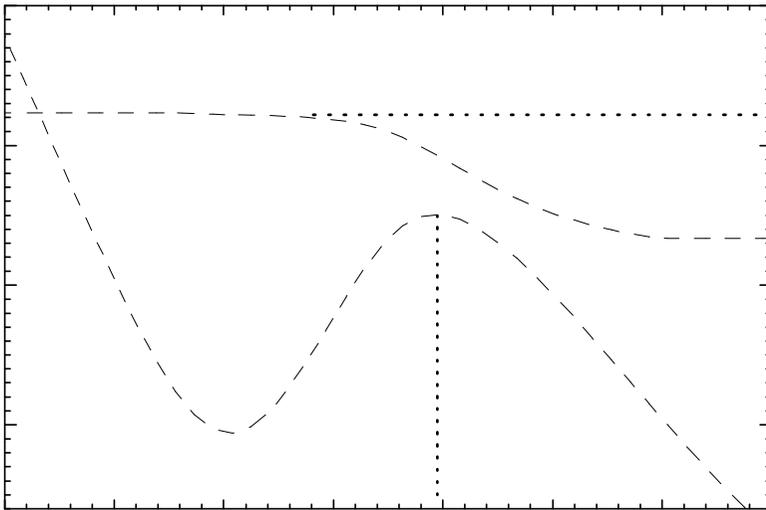
## Dielectric spectroscopy:

- \* Makes it possible to perform measurements over wide frequency ( $10^{-3}$ - $10^{12}$  Hz) and temperature ranges.
- \* Charge transport and dielectric relaxations can be measured.
- \* The time scale of a relaxation process is comparable to that probed by QENS at  $Q \approx 1 \text{ \AA}^{-1}$ .
- \* No length-scale dependence of the dynamics can be gained.



$$\epsilon^*(\omega) = \epsilon'(\omega) - i\epsilon''(\omega) = \frac{i}{\omega \epsilon_0 Z^*}$$

# Dielectric spectroscopy



$$\epsilon^*(\omega) = \epsilon'(\omega) - i\epsilon''(\omega) = -\frac{i}{\omega C_0 Z^*}$$

- *Dipolar relaxations*
  - ⇒ *peaks in the imaginary part ( $\epsilon''$ ) and steps in the real part ( $\epsilon'$ ) of the spectrum*
- *Conductivity and polarisation effects*
  - ⇒ *increase in  $\epsilon''$  (and  $\epsilon'$ ) at low frequencies*
  - ⇒ *pure dc conductivity invisible in  $\epsilon'$*

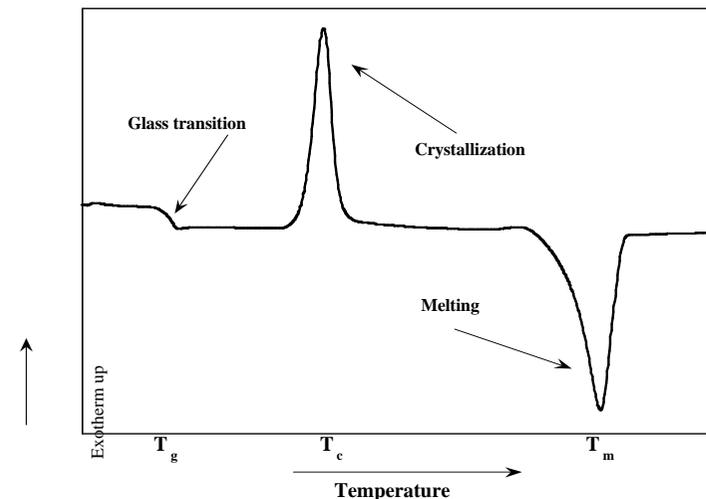
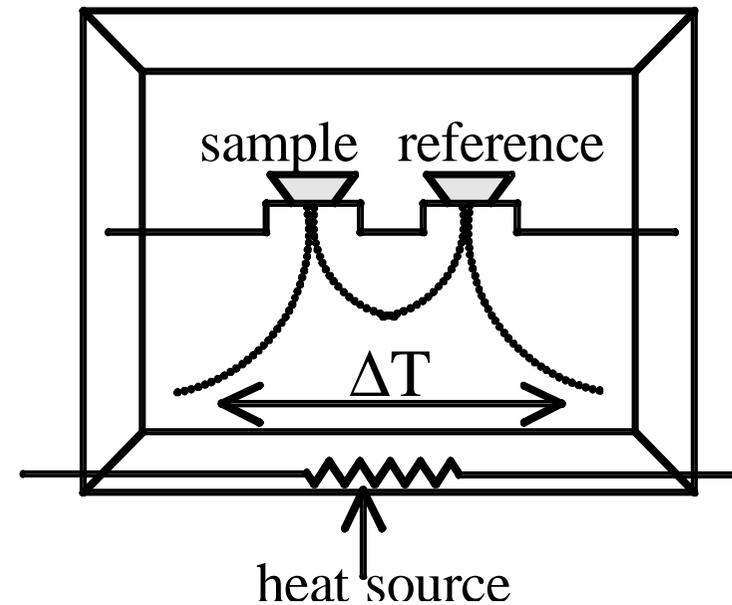
# Experimental techniques

## Differential scanning calorimetry (DSC):

\* Measuring the heat flow to/from the sample and a reference (empty sample pan).

\* Can determine:

- glass transitions
- crystallisations
- meltings
- other phase transitions, such as the gel-to-liquid transition of liposomes and the denaturation of proteins
- chemical reactions



# Experimental techniques

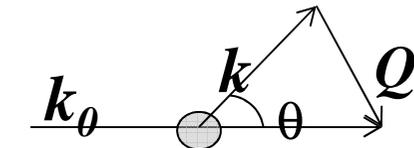
## Neutron scattering:

### Neutrons

uncharged  $\Rightarrow$  scattered by the nuclei  
wavelength  $\approx$  interatomic distances

### Neutron - nucleus interaction

$\Rightarrow$  Changes in  
direction and energy



Incident  
neutron

Nucleus

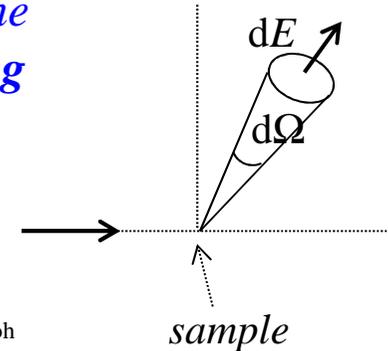
$$Q = k_0 - k$$

$$\Delta E = E_0 - E$$

$$= \hbar\omega = \frac{\hbar^2}{2m}(k_0^2 - k^2)$$

In a scattering experiment the  
double differential scattering  
cross section is measured

$$\begin{aligned} \frac{d^2\sigma}{d\Omega dE} &= \left( \frac{d^2\sigma}{d\Omega dE} \right)_{\text{coh}} + \left( \frac{d^2\sigma}{d\Omega dE} \right)_{\text{incoh}} \\ &= \frac{k}{\hbar k_0} N \left\{ \langle b \rangle^2 S_{\text{coh}}(Q, \omega) + (\langle b^2 \rangle - \langle b \rangle^2) S_{\text{incoh}}(Q, \omega) \right\} \end{aligned}$$



The scattering cross section  $\sigma$  is different  
for different isotopes and depends on the  
scattering length ( $b$ )

$$\sigma = \sigma_{\text{coh}} + \sigma_{\text{incoh}} = 4\pi \langle b \rangle^2 + 4\pi (\langle b^2 \rangle - \langle b \rangle^2)$$

$\Rightarrow$  2 types of neutron scattering

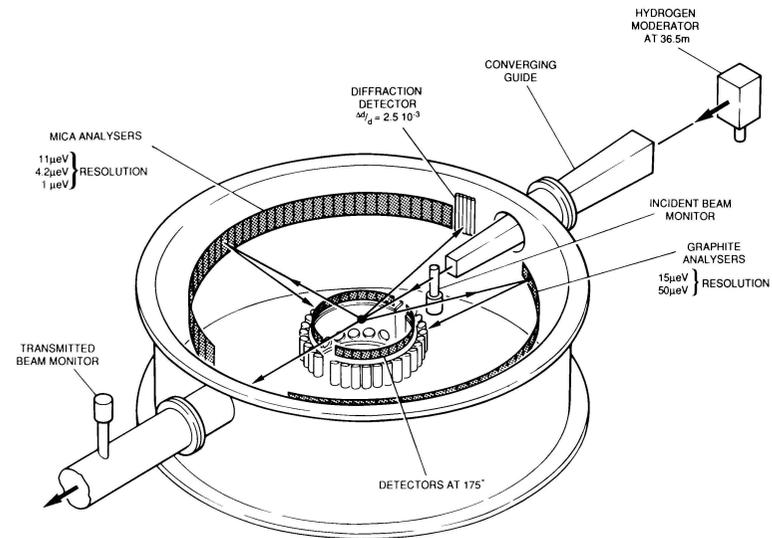
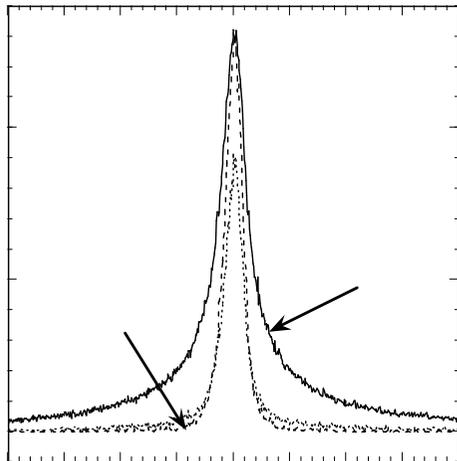
- coherent - correlated positions or motions
- incoherent - single particle motion

# Quasielastic neutron scattering (QENS)

- \* Dynamic time range 1 ps - 100 ns.
- \* Length-scale (Q) dependence of the dynamics gives the physical nature of motions.
- \* Contrast variation (H/D isotope exchange) can be used.

Coherent cross section ( $\sigma_{coh}$ ):  $H \approx 2$  and  $D \approx 6$

Incoherent cross section ( $\sigma_{incoh}$ ):  $H \approx 80$  and  $D \approx 2$

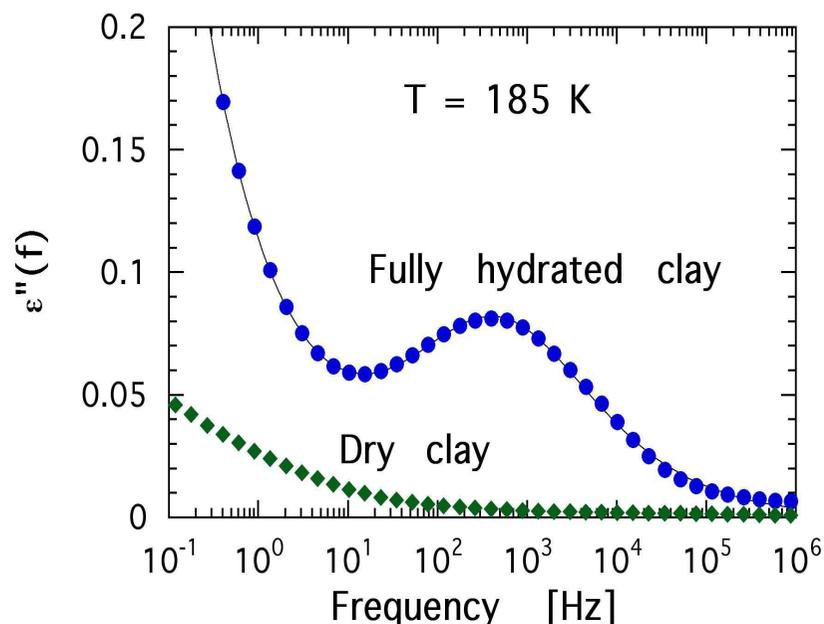


## The IRIS spectrometer at ISIS

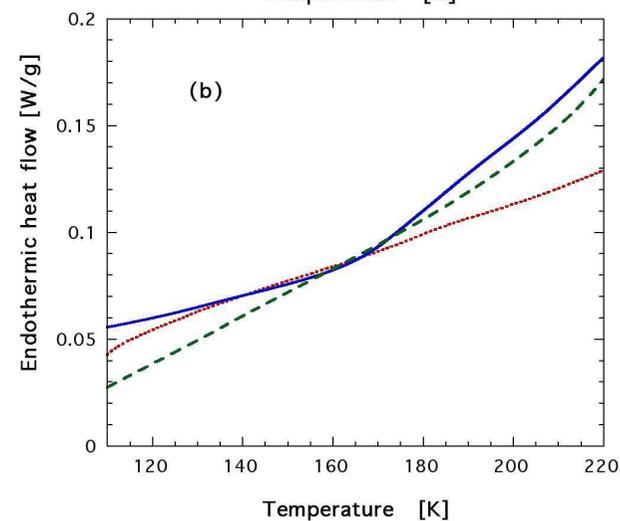
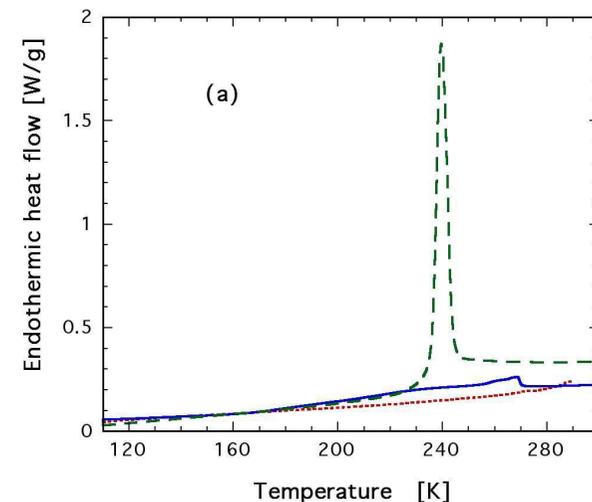
- PG002 analyser and  $\lambda \approx 6.6 \text{ \AA}$
- Energy resolution (FWHM) =  $17 \mu\text{eV}$
- Total energy window  $\pm 0.5 \text{ meV}$
- 51 detectors grouped into 17 groups  
⇒ Q-range  $0.46 - 1.84 \text{ \AA}^{-1}$

# *Studies of confined water*

## *Dielectric and DSC data on confined water*

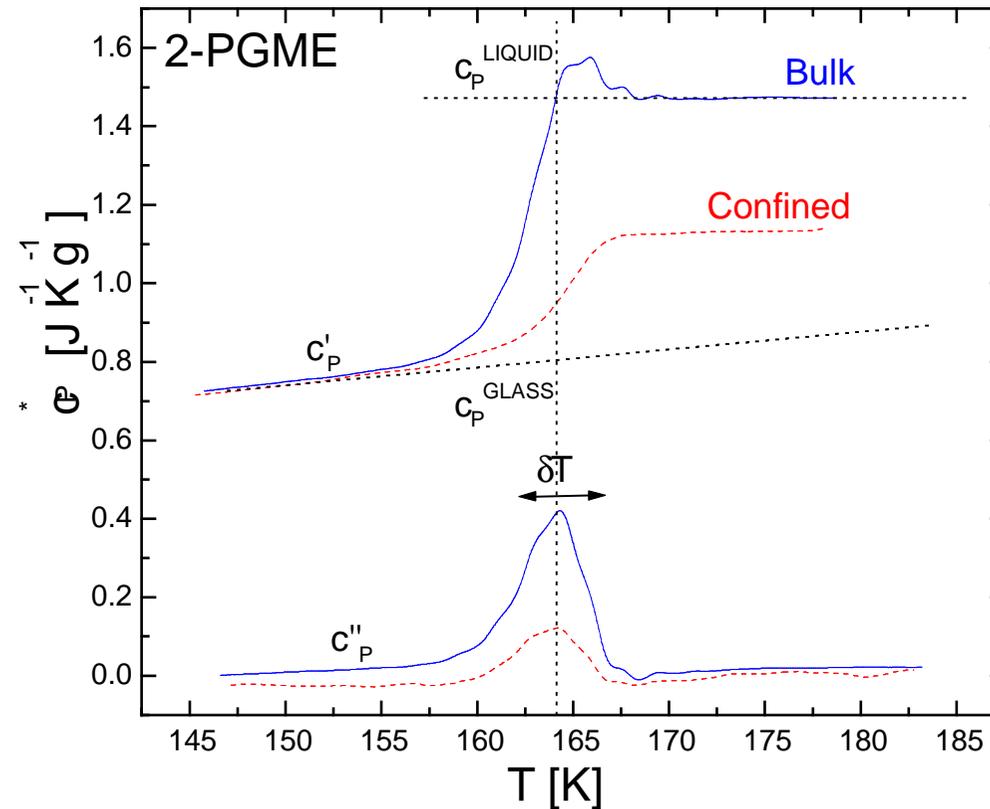


**Imaginary part of the dielectric permittivity of vermiculite clay at 185 K. A clear dielectric loss peak is observed for the fully hydrated clay.** *R. Bergman and J. Swenson, Nature 403, 283 (2000).*



**DSC measurement of endothermic heat flow during reheating (10 K/min) of fully hydrated clay and MCM-41 with pore diameters 21 Å and 36 Å. No clear glass transition can be observed.**

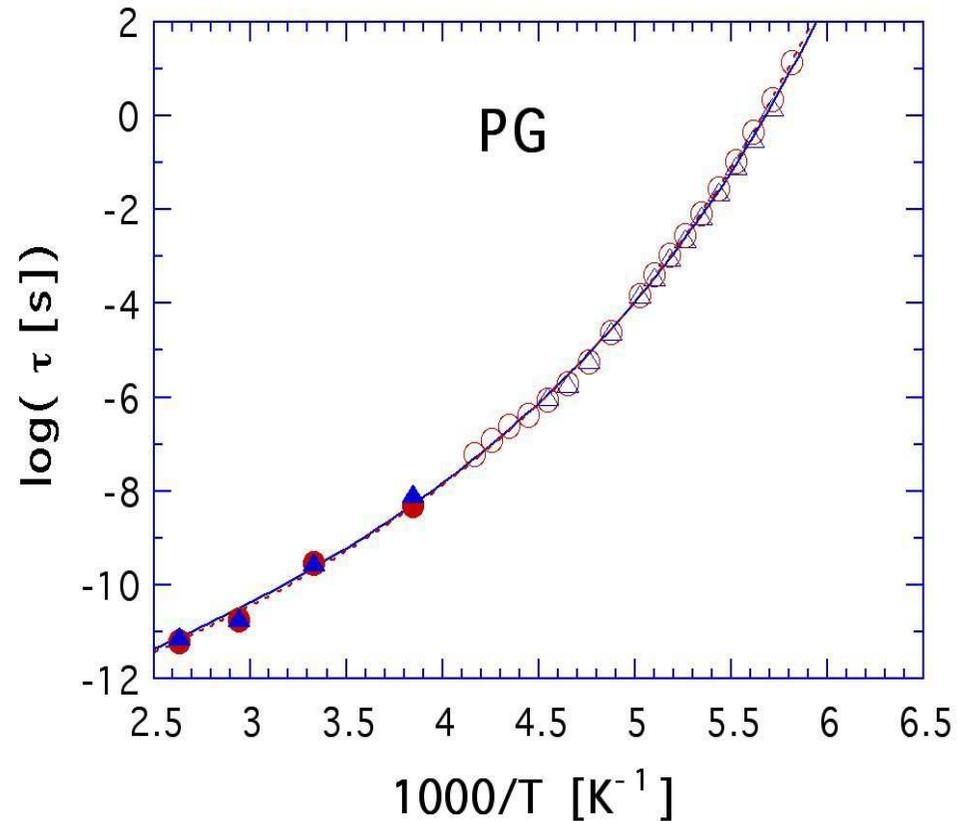
# Typical glass transition for confined liquids



Calorimetric  $T_g$  for di-(proylene glycol monomethyl ether) (2-PGME) confined in a Na-vermiculite clay.

*S. Cervený et al., J. Phys. Chem. B 108, 11596 (2004).*

# *Relaxation times of PG confined in clay*



$\Delta$ =confined

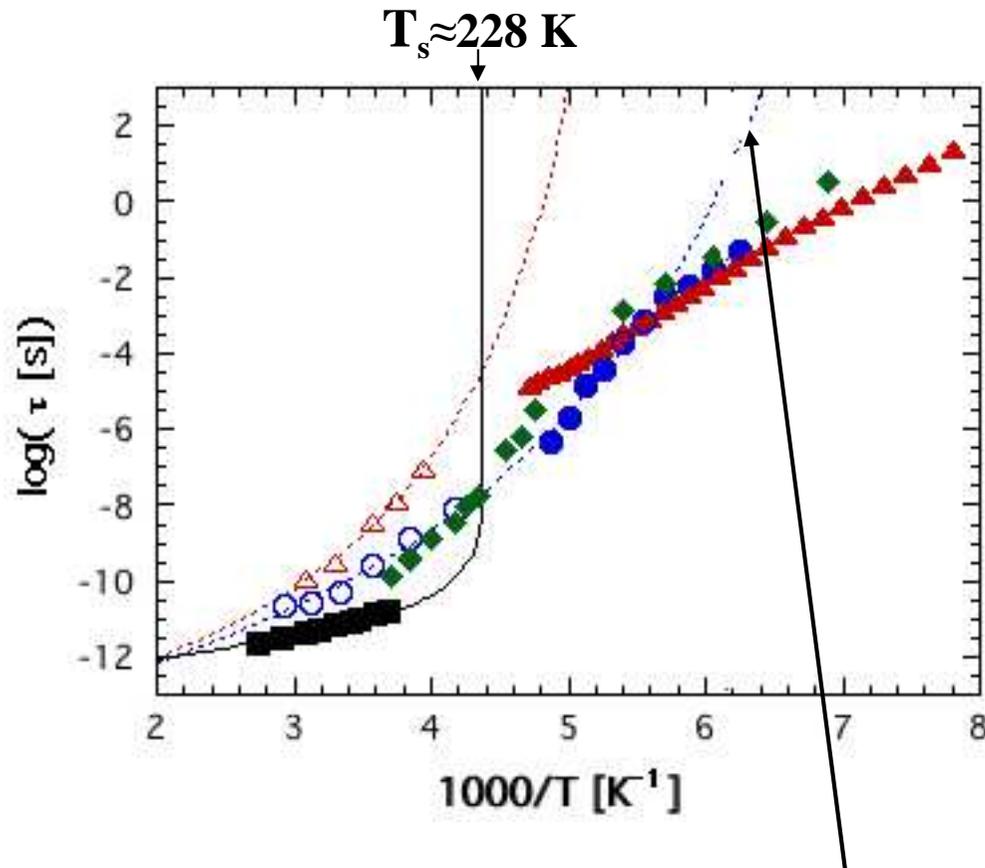
$O$ =bulk

J. Swenson et al. Eur.  
Phys. J. E12, 179 (2003)

- Average relaxation time from QENS ( $Q=1 \text{ \AA}^{-1}$ )  $\approx$  dielectric  $\alpha$ -relaxation time.
- These relaxation times are almost unaffected by the confinement.

# ”Typical” relaxation times of interfacial water

## Fragile-to-strong transition? **No!**



O = molecular sieve (diameter=10 Å)

Δ = vermiculite clay

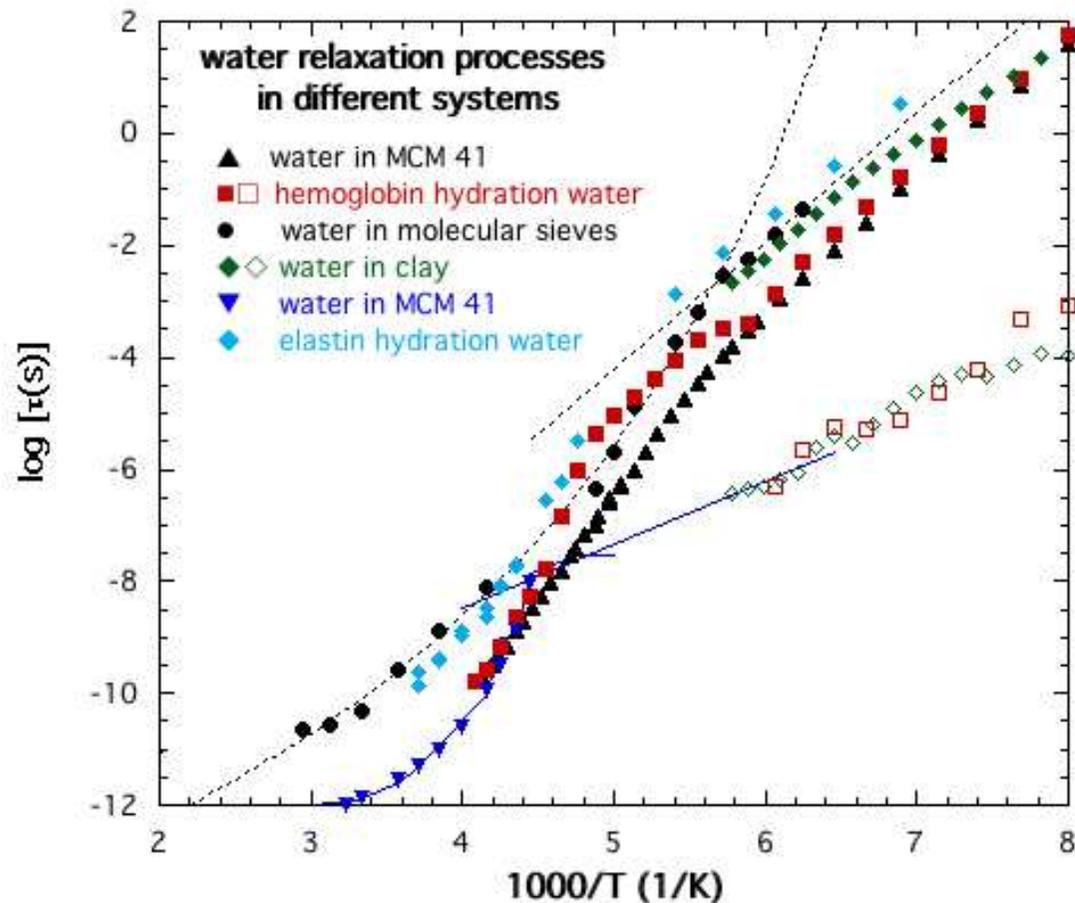
Green symbols = <sup>2</sup>H NMR data of hydrated elastin (*M. Vogel, Phys. Rev. Lett. 101 (2008) 225701*)

Squares = dielectric data of bulk water (*C. Rønne et al., Phys. Rev. Lett. 82 (1999) 2888*)

The solid line represents the power law behaviour of bulk water proposed by *C. A. Angell et al.*

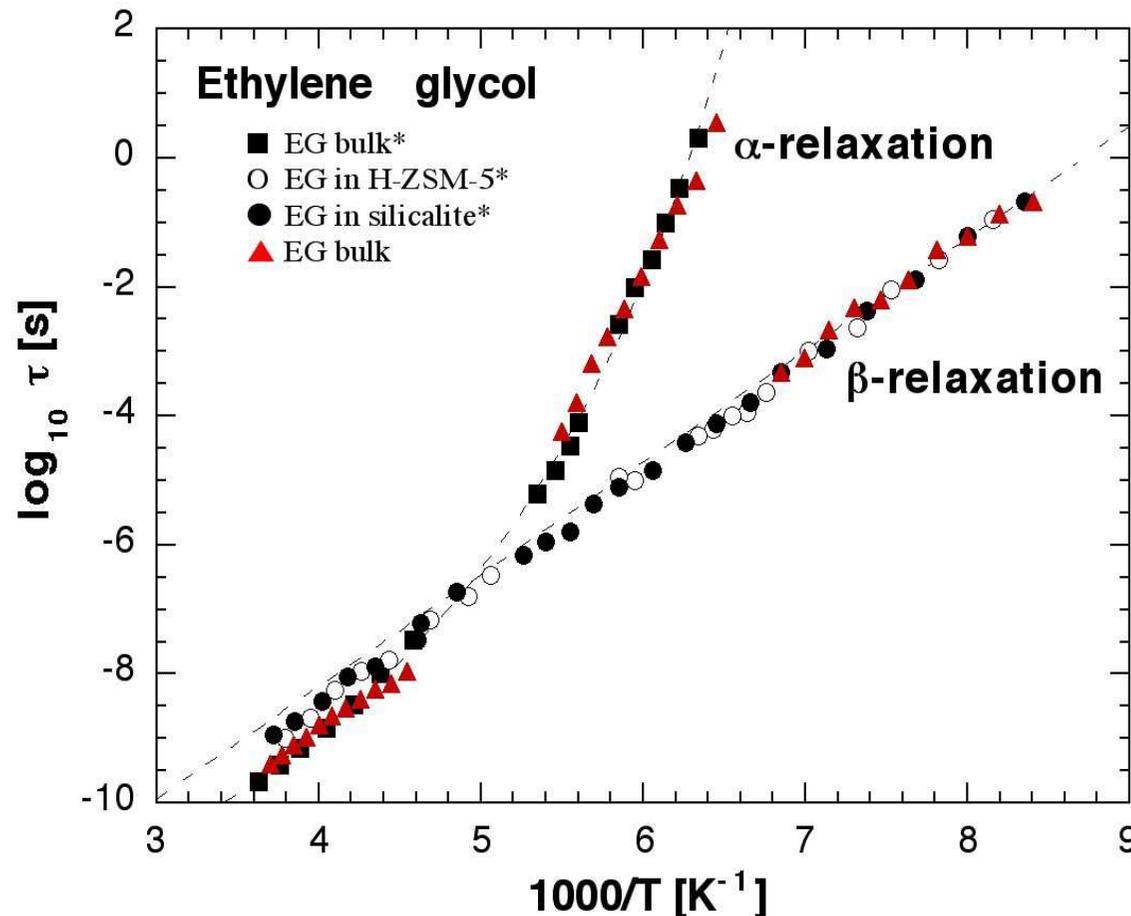
**T<sub>g</sub> at 160 K? (From adiabatic calorimetry of 1.1 nm pores, M. Oguni et al. Chem. Asien J. 2, 514 (2007)).**

*In fact, there are two "universal" water relaxations in the deeply supercooled regime*



**Relaxation times for hydration water of different proteins and water confined in various host materials.**

# Can confinements make the $\alpha$ -relaxation disappear?

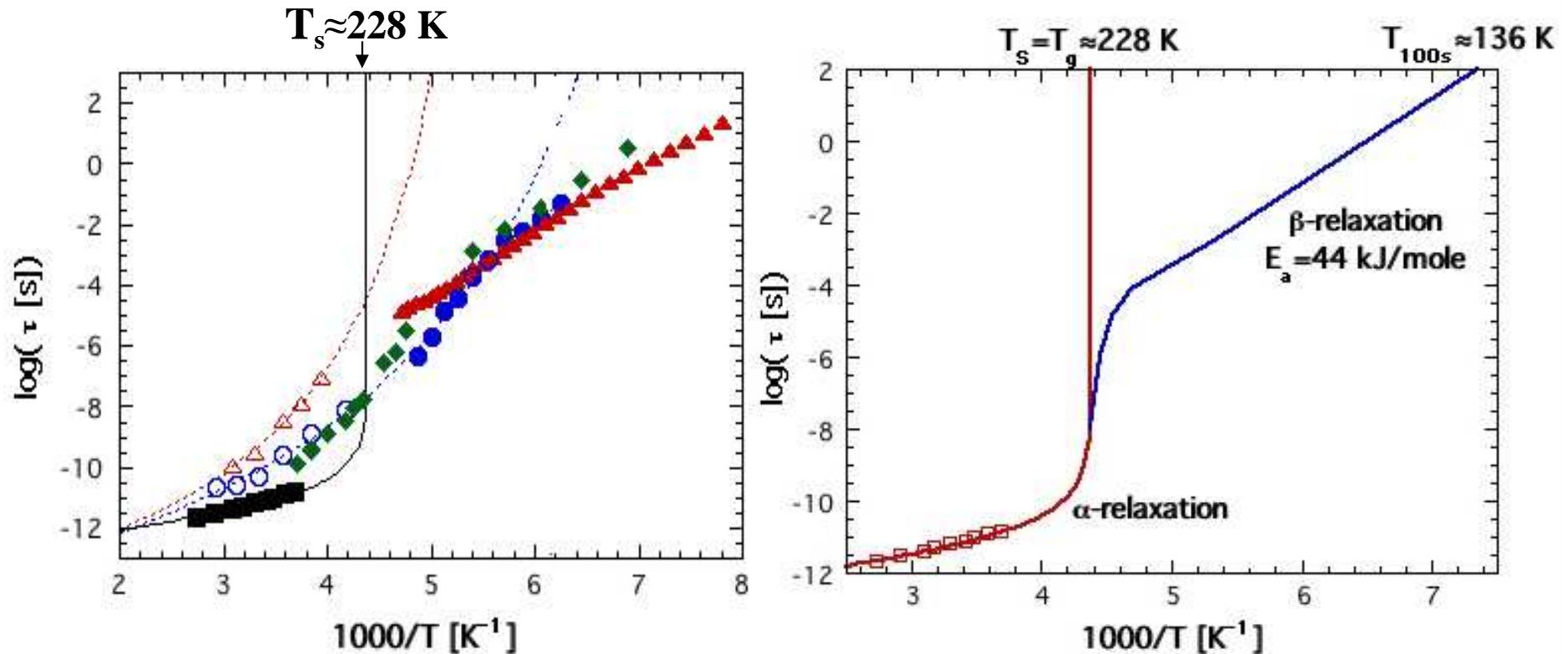


Dielectric relaxation times of ethylene glycol (EG). Both the  $\alpha$  and  $\beta$  relaxations are present for bulk EG, whereas only the local  $\beta$  relaxation can be observed for EG in the very severe confinements (pore diameter approximately 5.5 Å) of the zeolitic host materials silicalite and H-ZSM-5.

\*Black and white data points from Huwe *et al.* J. Phys. IV France 10, 59 (2000).

*J. Swenson et al., Phys. Rev. Lett. 96, 247802 (2006)*

# *A relaxation scenario for supercooled and glassy bulk water in analogy to confined water*

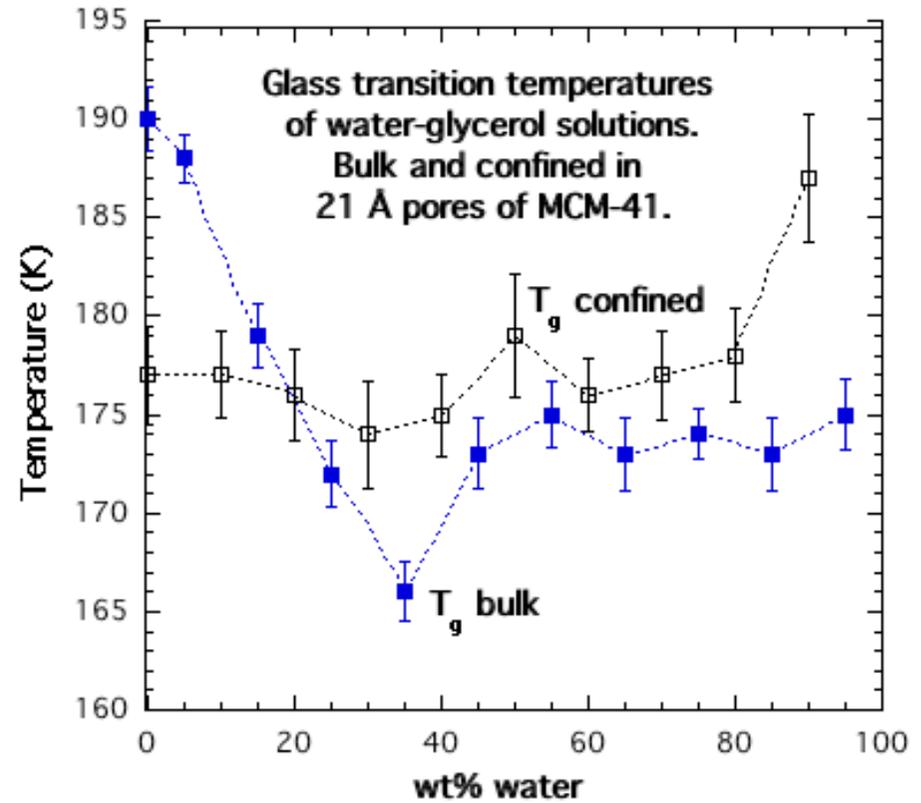
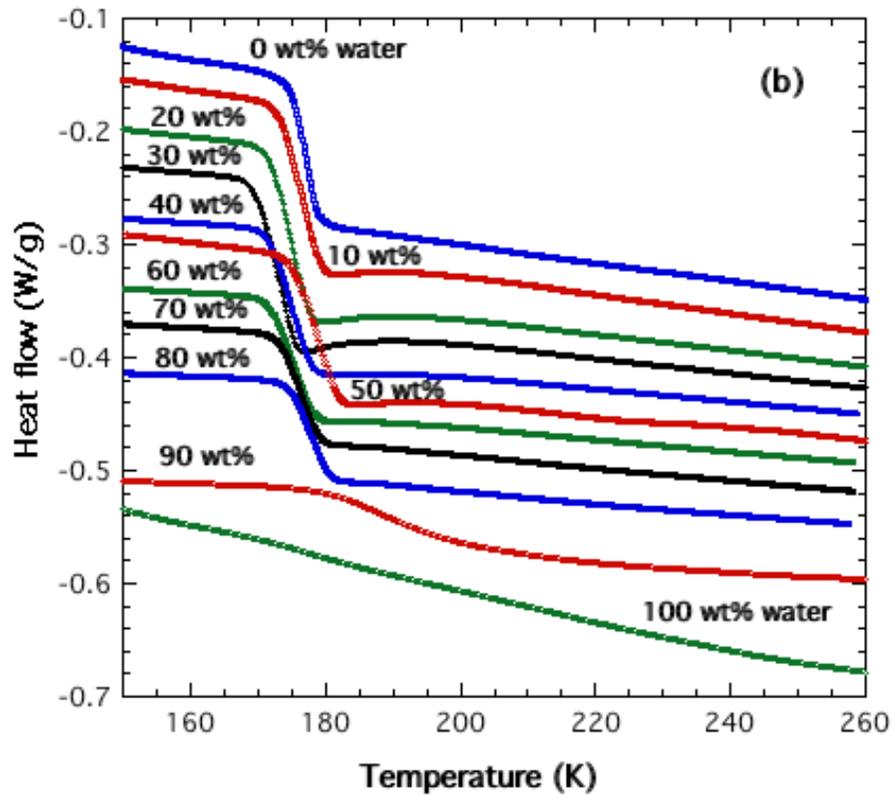


A sharper crossover from merged  $\alpha$ - $\beta$  to  $\beta$ -relaxation in bulk water due to a more completed hydrogen bonded network. The change in heat capacity at 136 K is of typical magnitude for a local relaxation, but considerably smaller than is typical for a true glass transition (e.g. only 2% of typical aqueous solutions).

J. Swenson and J. Teixeira, *J. Chem. Phys.* 132, 014508 (2010)

*Can we understand the  
anomalous properties of  
supercooled interfacial water?*

# *Water-glycerol solutions confined in 21 Å pores of MCM-41*



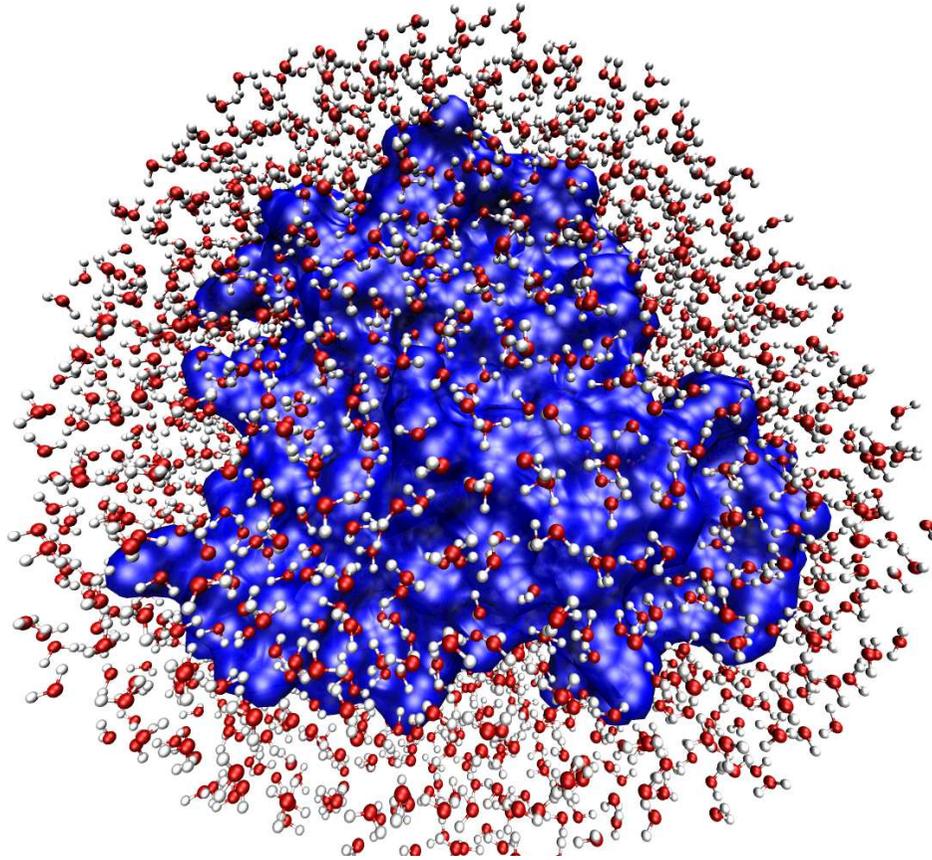
**Results from DSC measurements**

# *Concluding indications*

- \* The glass transition related dynamics of bulk water seems to involve an exceptionally large volume of cooperatively rearranging regions at temperatures close to  $T_g$ . In confinements, where ice formation can be prevented, no similar relaxation process can occur and no true  $T_g$  is consequently observed.**
- \* The structure and dynamics of water at high and low solute concentrations seem to be different. Water goes from a plasticizer to a non-plasticizer with decreasing solute concentration, due to the formation of a rigid ice-like network structure close to  $T_g$  at high water concentrations.**
- \* These findings indicate that  $T_g$  of bulk water is located above 200 K.**

*What are the biological  
implications of the anomalous  
properties of supercooled  
interfacial water?*

# *Dynamics of myoglobin in water and water-glycerol*



**Myoglobin surrounded by water molecules.  
The hydration level  $h \approx 2$  (g water/g protein).**

**Figure taken from H. Frauenfelder *et al.*, Proc. Natl. Acad. Sci. USA 106, 5129 (2009)**

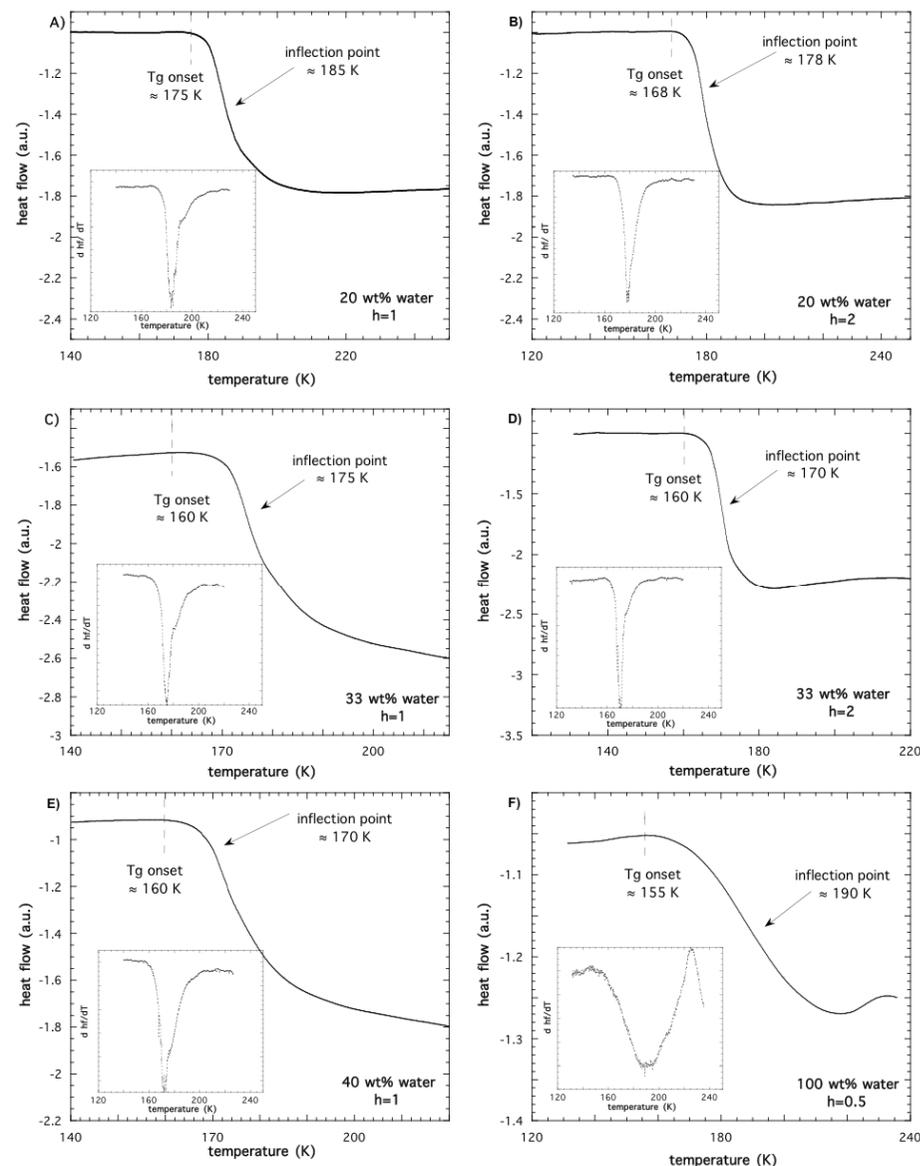
# *DSC data on myoglobin in water-glycerol mixtures*

Samples of different hydration levels ( $h = g$  solvent/ g protein) and weight fractions of water in the solvent.

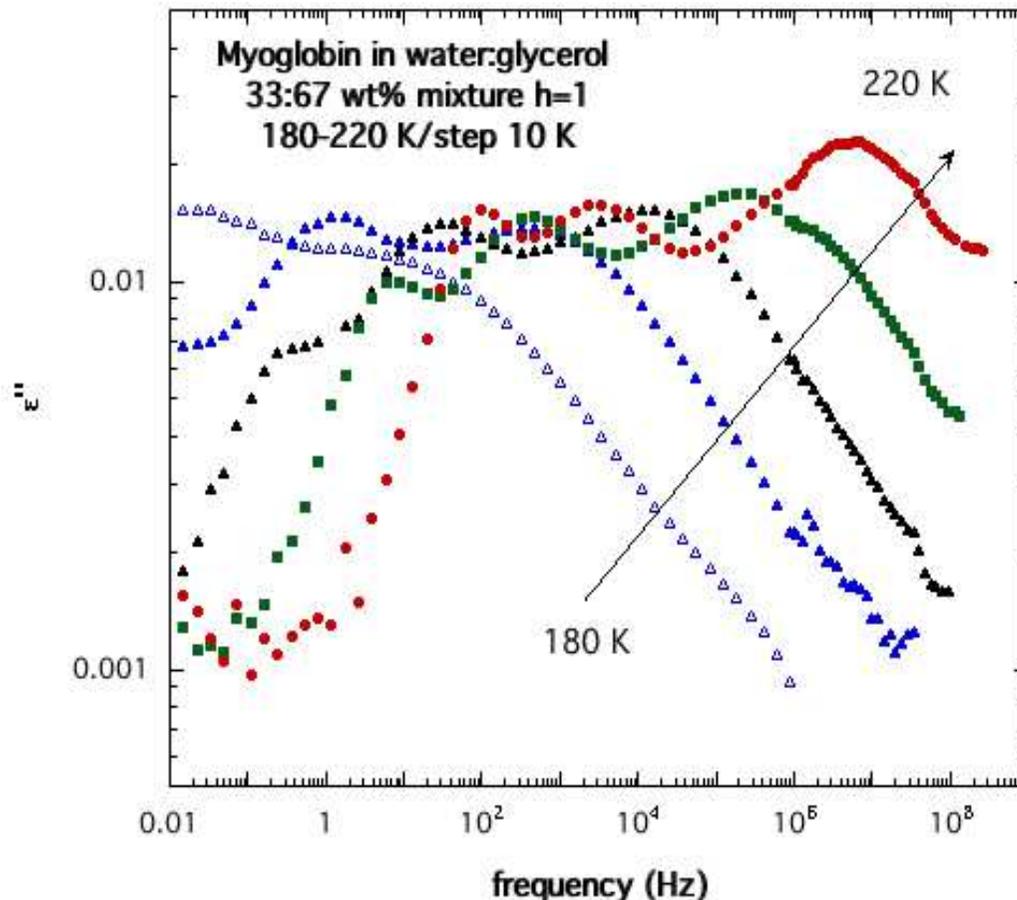
When glycerol is present in the solvent the solvent relaxation dominates the calorimetric glass transition, but with only hydration water there is no contribution from this water.

Is this odd? No, as expected from the confinement studies

H. Jansson, R. Bergman and J. Swenson,  
J. Phys. Chem. B 115, 4099 (2011).



# *Dynamics of myoglobin in water-glycerol solvents by dielectric spectroscopy*



At least three relaxation processes can be observed.

Relaxation times were determined by curve fitting, using a conductivity term and one Havriliak-Negami function for each relaxation process.

$$\epsilon''(\omega) = \left( \frac{\sigma}{\epsilon_0 \omega} \right)^n + \text{Im} \left[ \frac{\epsilon_s - \epsilon_\infty}{(1 + (i\omega\tau)^\alpha)^\beta} \right]$$

H. Jansson, R. Bergman and J. Swenson,  
J. Phys. Chem. B 115, 4099 (2011).

# Dynamics of myoglobin in water-glycerol solvents

Relaxation times for different solvent and protein processes in samples of different hydration levels ( $h = \text{g solvent/ g protein}$ ) and weight fractions of water in the solvent.

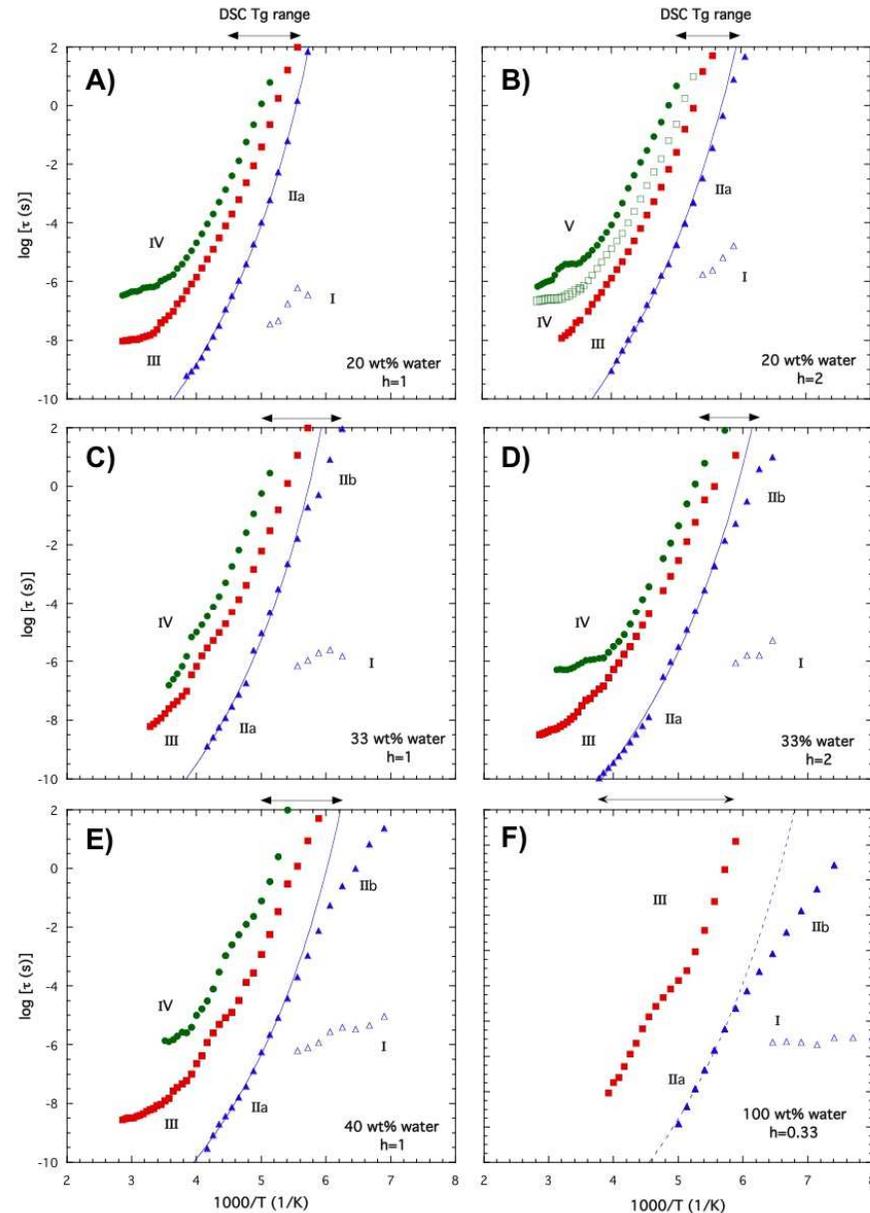
## Interpretations:

Blue symbols = solvent relaxations

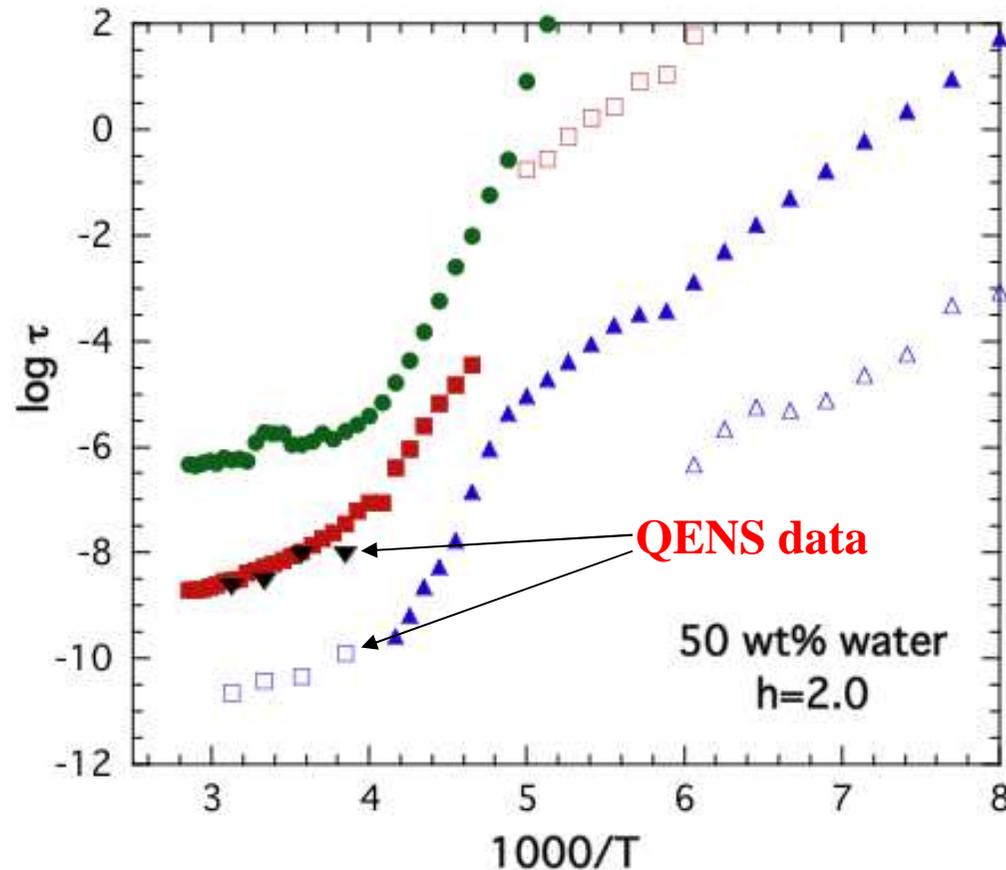
Solid red symbols = motions of polar side groups

Solid green symbols = conformational protein fluctuations

- Similar T dependence for both protein and solvent.
- Crossover in the solvent relaxation at about 170 K from a solvent  $\alpha$ -relaxation to a water  $\beta$ -relaxation.



# *Crossover in the solvent dynamics causes the protein glass transition*



## Interpretations:

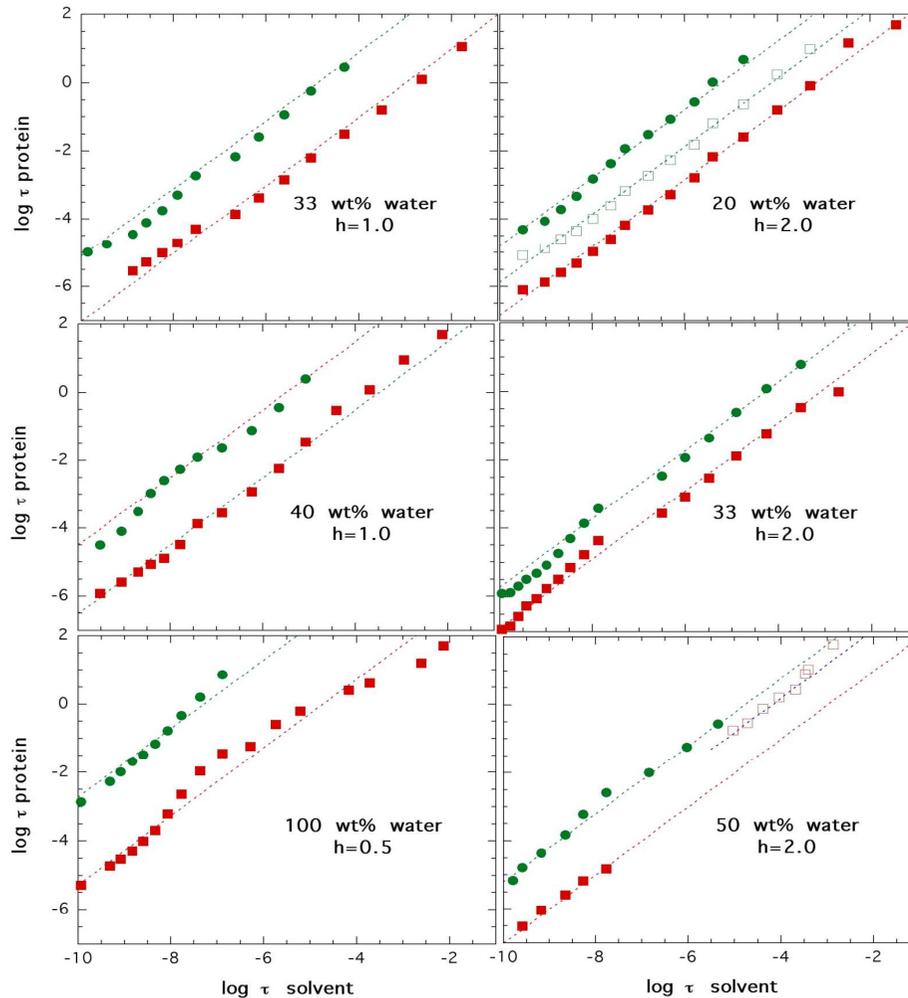
Blue symbols = solvent relaxations

Solid red symbols = motions of polar side groups

Solid green symbols = conformational protein fluctuations

Only local protein processes seem to be present below the crossover temperature where the solvent relaxation is given by the  $\beta$ -relaxation of interfacial water.

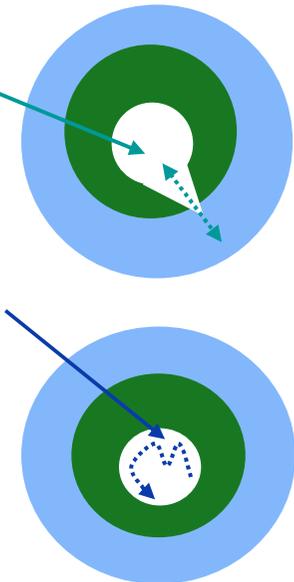
# Relation between the main solvent process and the observed protein processes



- \* Linear dependence between solvent  $\alpha$ -relaxation and the observed protein processes
- \* For the sample with 50 wt% water an additional local protein process is related to water  $\beta$ -process

• Bulk solvent ( $\alpha$ )-slaved  
Do not need water, but a low viscous environment.

• Hydration shell ( $\beta$ )-slaved  
Do not need a low viscous bulk solvent, but a water hydration shell.

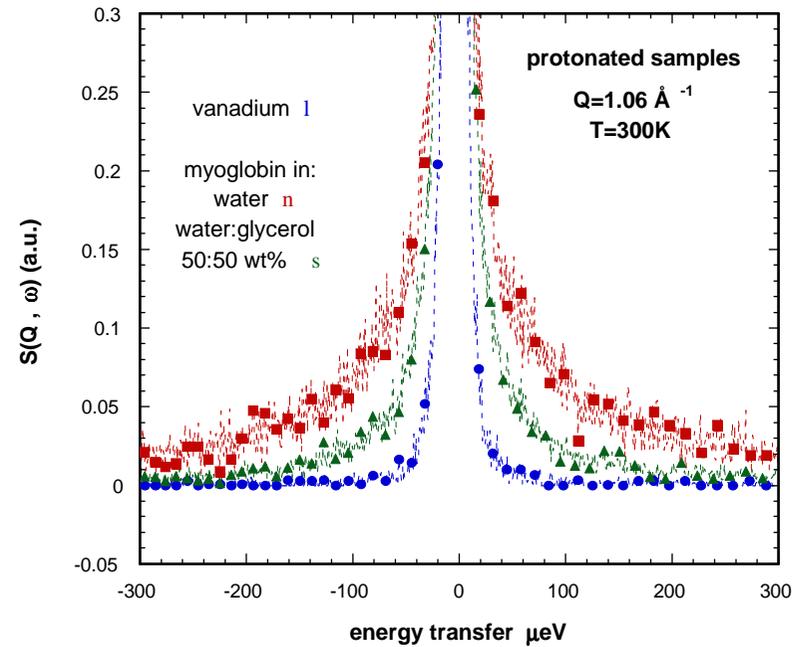
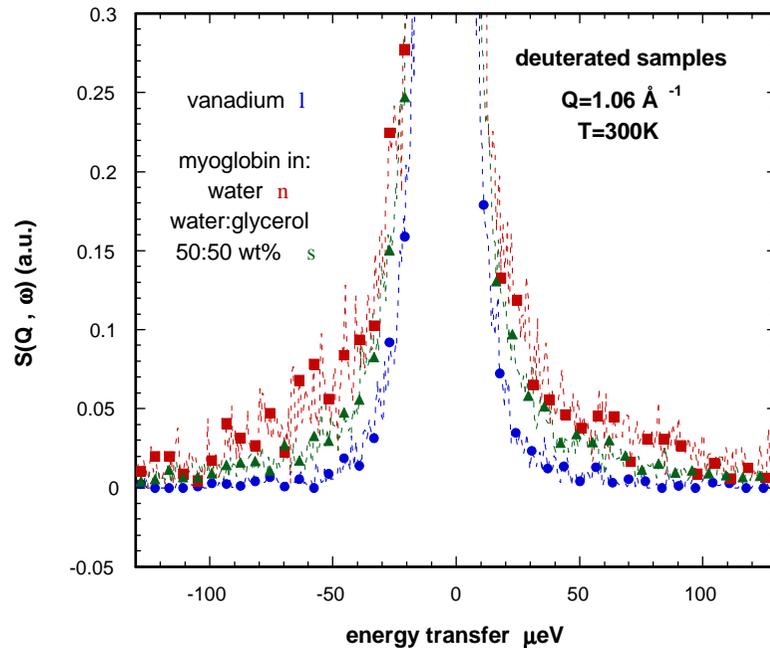


Solid red symbols = motions of polar side groups  
Solid green symbols = conformational protein fluctuations

H. Frauenfelder *et al.*, Proc. Natl. Acad. Sci. USA 106, 5129 (2009).

H. Jansson, R. Bergman and J. Swenson, J. Phys. Chem. B 115, 4099 (2011).

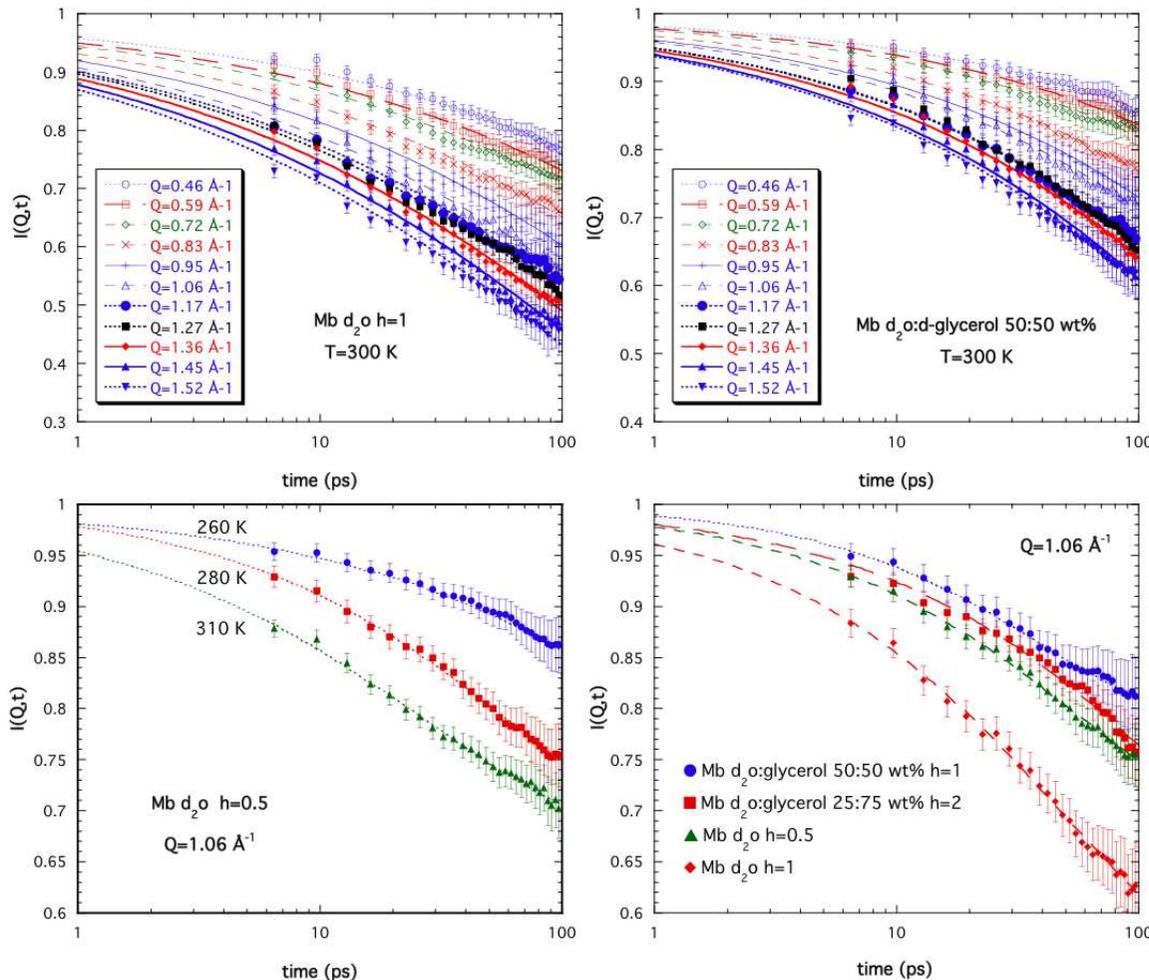
# *QENS data on myoglobin in water-glycerol solvents*



**Raw data normalized to 1 at zero energy transfer.**

H. Jansson et al., J. Chem. Phys. 130, 205101 (2009).

# $S(Q, \omega)$ Fourier transformed to $I(Q, t)$

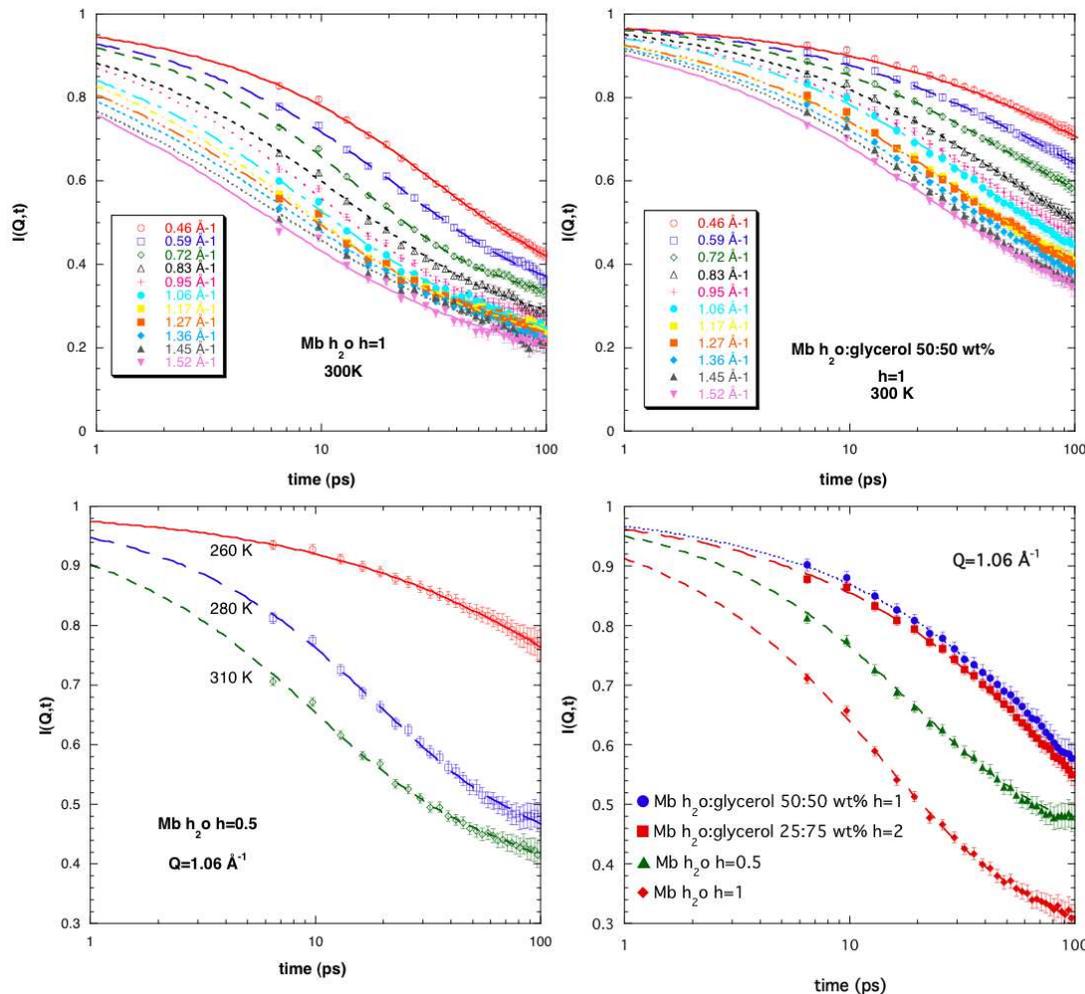


**Kohrausch-William-Watts (KWW) function:**

$$I(Q, t) = \exp \left[ - \left( \frac{t}{\tau_p} \right)^{\beta_{KWW}} \right]$$

$I(Q, t)$  of Mb samples in  $D_2O$ . The data have been described by a single KWW function with  $\beta_{KWW} = 0.4-0.5$ . The fastest possible relaxation time averaged over all protein dynamics is obtained.

# *I(Q,t) of corresponding Mb samples in H<sub>2</sub>O*

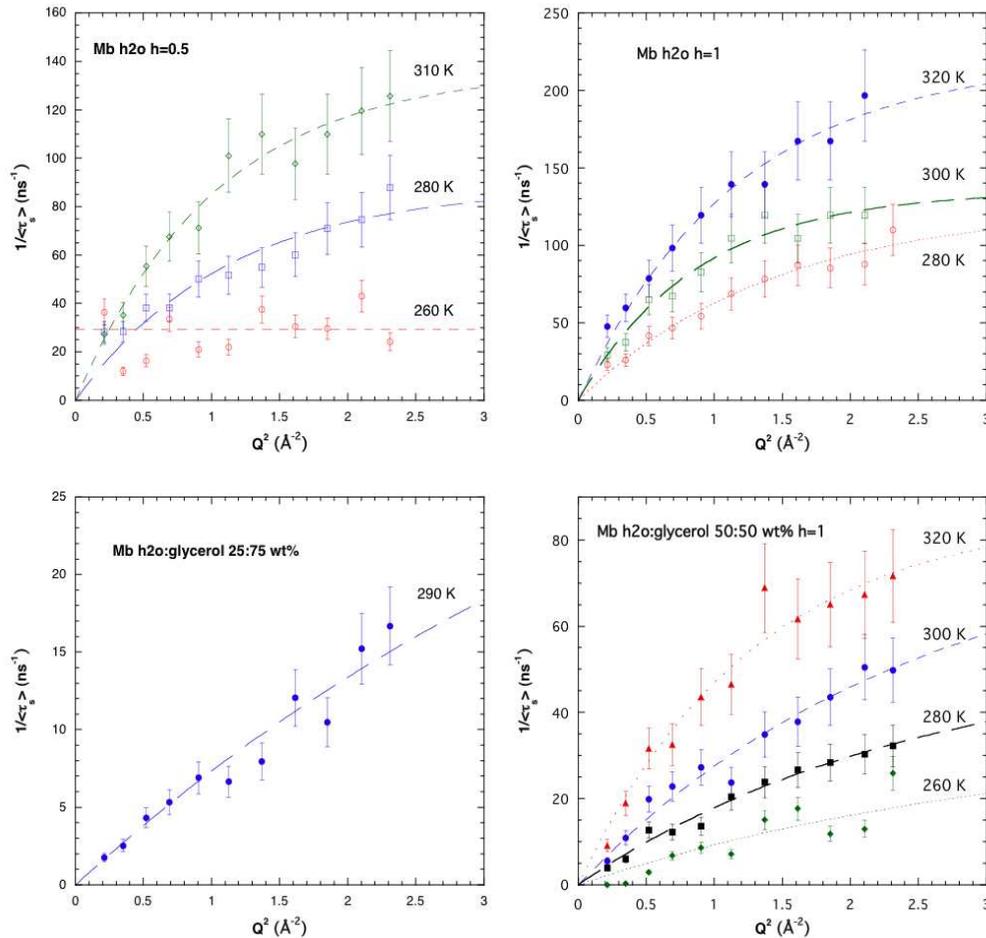


Two KWW functions were used to describe the data; one for the protein dynamics, with the same  $\tau_p$  and  $\beta_{KWW}$  as obtained for the corresponding samples in  $D_2O$ , and one for the solvent dynamics.

$$I(Q,t) = (1 - A) \exp \left[ - \left( \frac{t}{\tau_p} \right)^{\beta_{KWW_p}} \right] + A \exp \left[ - \left( \frac{t}{\tau_s} \right)^{\beta_{KWW_s}} \right]$$

In this way the solvent dynamics could be extracted.

# The extracted $Q$ -dependence of the reciprocal average relaxation time of the solvent



The Gaussian jump-length diffusion model:

$$\frac{1}{\langle \tau \rangle} = \frac{1}{\tau_{res}} \left[ 1 - \exp \left( - \frac{Q^2 \langle r^2 \rangle}{6} \right) \right]$$

The diffusion constant can be extracted from the mean square jump-length and the residence time between the jumps.

$$D_s = \langle r^2 \rangle / 6\tau_{res}$$

At 300 K the water diffusion is a factor 2 slower at  $h=1$  and a factor 2.6 slower at  $h=0.5$  compared to bulk water. However, the protein dynamics is even more affected by the hydration level.

The  $Q$ -dependence is generally well described by the Gaussian jump-length diffusion model.

# *Conclusions*

- \* The more large scale protein motions are very much dependent, or even controlled or slaved by the viscosity related dynamics of the solvent.**
- \* More local internal protein motions seem to be determined by the hydration water, although also the local structure and dynamics of the protein affect the hydration water, to some extent.**
- \* An apparent dynamic crossover occurs when the merged  $\alpha$ - $\beta$  process of the hydration water transforms to a pure  $\beta$  process. This causes a glass transition of the protein.**
- \* In contrast to other solvents, hydration water does not give any direct contribution to the calorimetrically observed glass transition of the protein system.**
- \* Both DSC and QENS studies of myoglobin in water-glycerol mixtures show that not only the time-scale of the solvent dynamics is important for the protein dynamics, but also the total amount of solvent.**