DYNAMICS OF WATER IN CONFINEMENT: FROM WATER IN SILICA HYDROGELS TO PROTEIN HYDRATION WATER

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Thanks to many collaborators and friends

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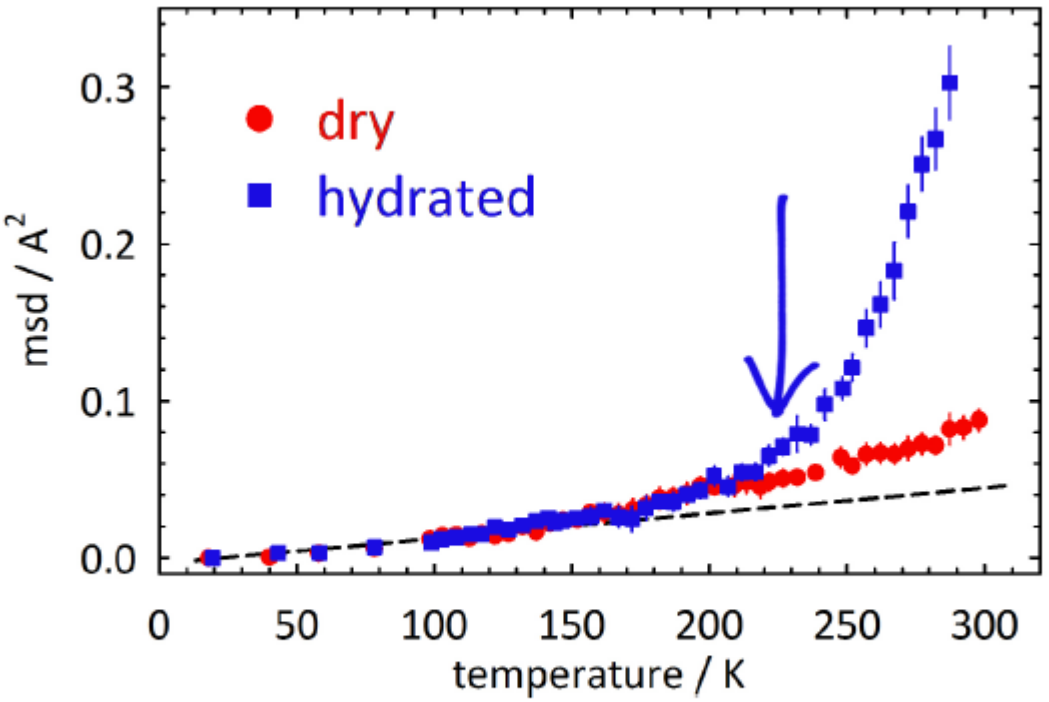
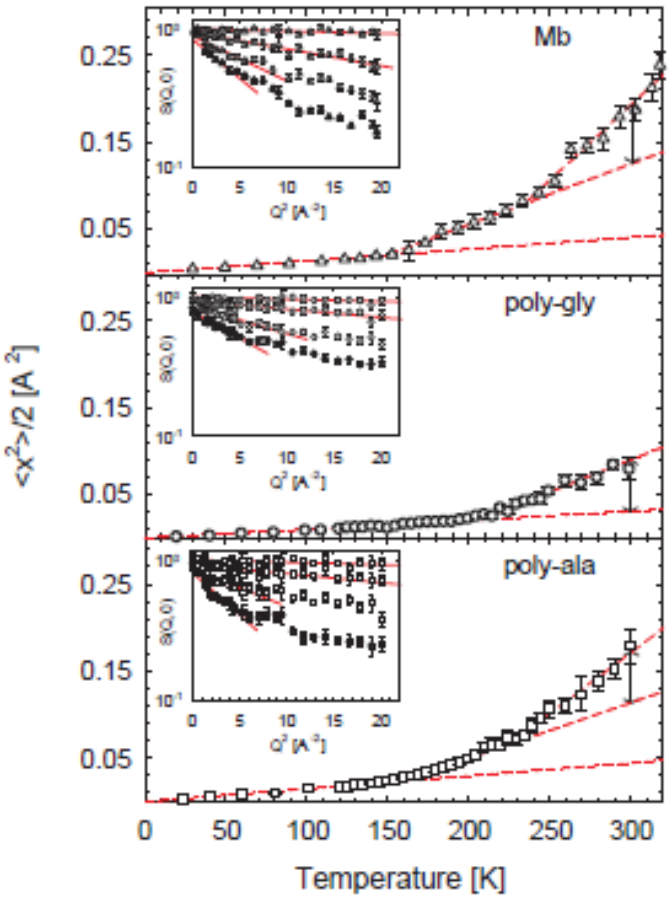
The so called «Protein dynamical Transition»



Doster et al. **Nature** 337 754, 1989



How do we measure MSD ?

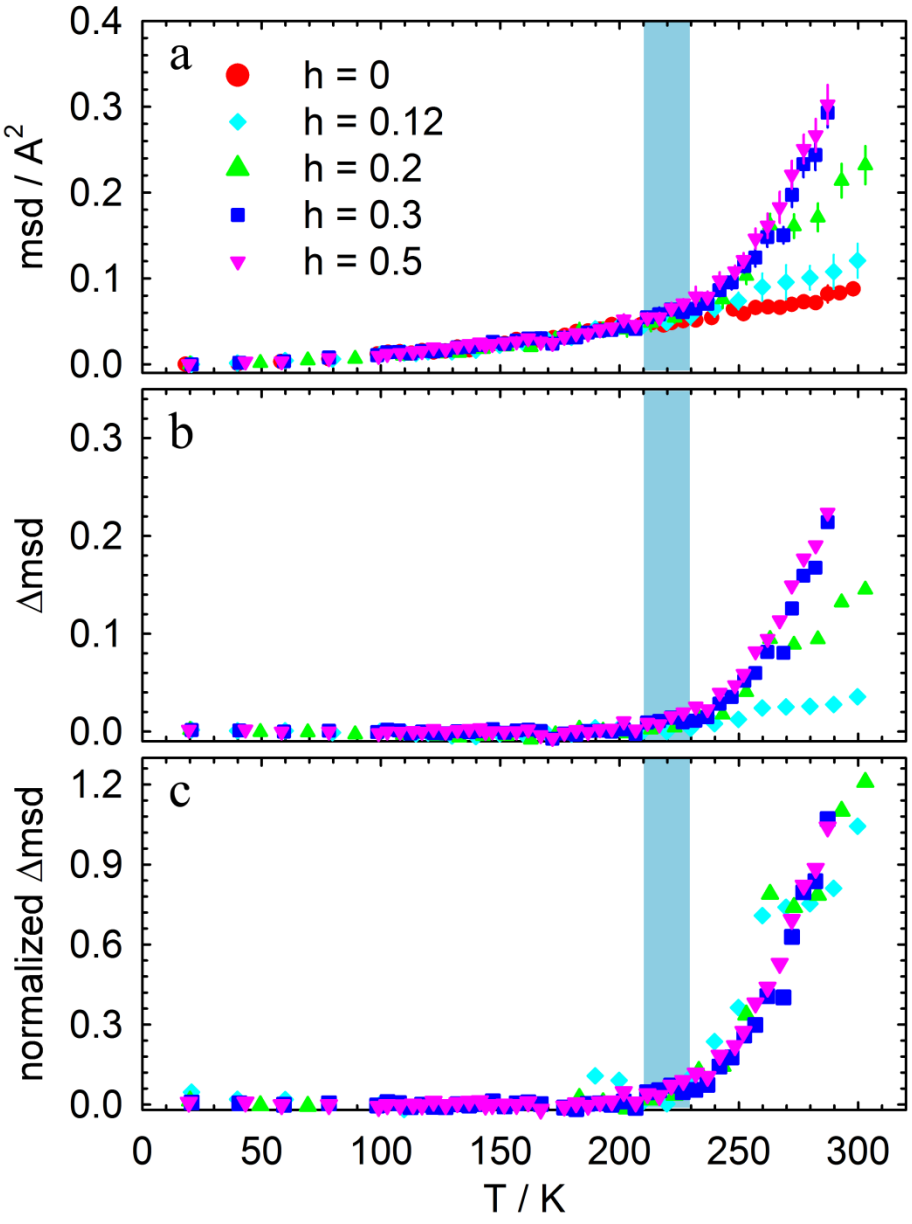
1) Measure elastic neutron scattering : $S(Q,\omega=0)$

2) Plot ln $S(Q,\omega=0)$ vs. Q^2

3) Slope ~ MSD
(Gaussian approximation)

Several open questions:

- Hydration (h = gr H_2O / gr dry protein) dependence of PDT
- Molecular origin of MSD (heterogeneity)
- How to locate the PDT onset temperature in an «objective » way
- Resolution dependence of the PDT onset temperature
- Physical origin of PDT



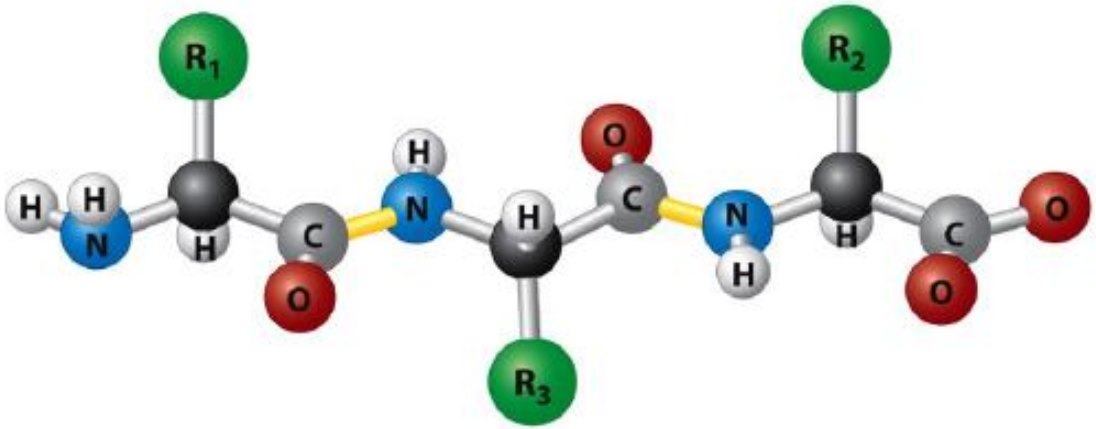
Three steps:

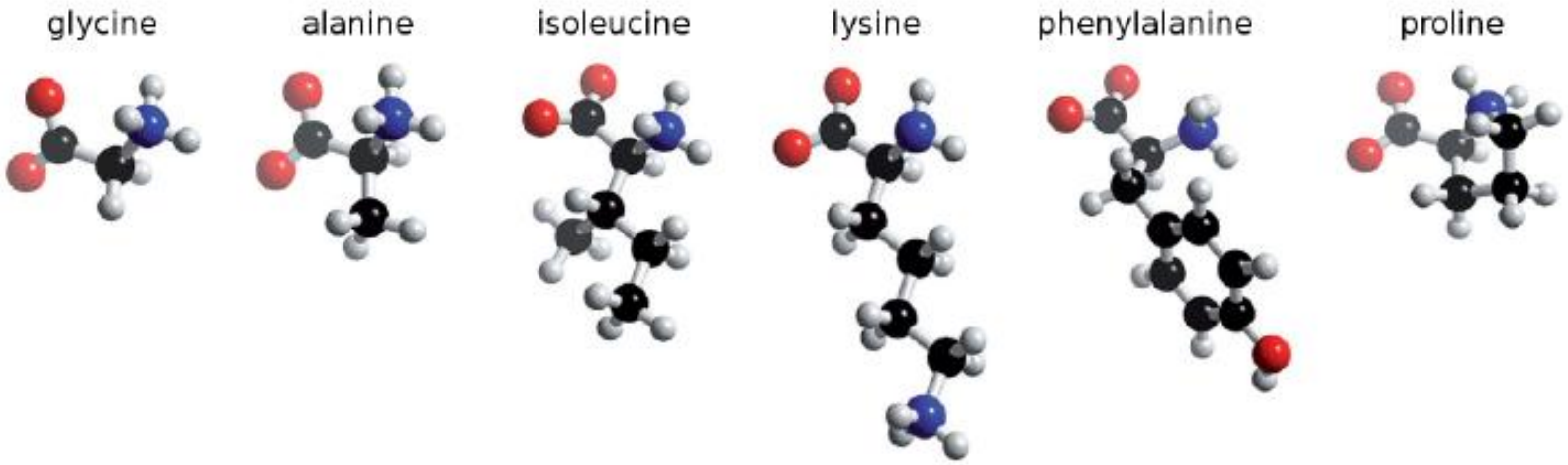
a) Measure MSD at various h
b) Subtract the h=0 data
c) Normalize to high T

The MSD amplitude depends on hydration

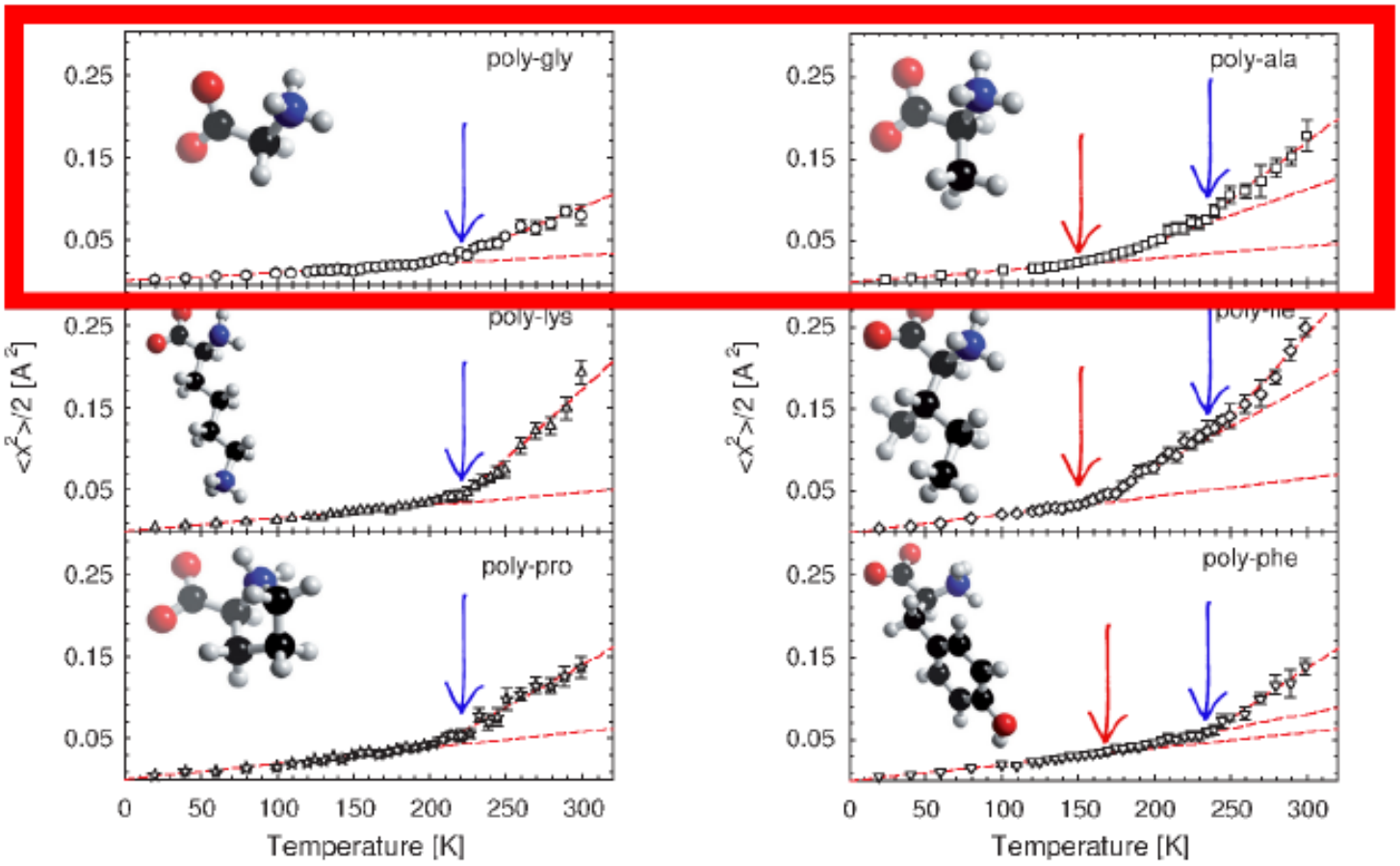
The PDT onset temperature doesn't

Homopeptides to overcome heterogeneity





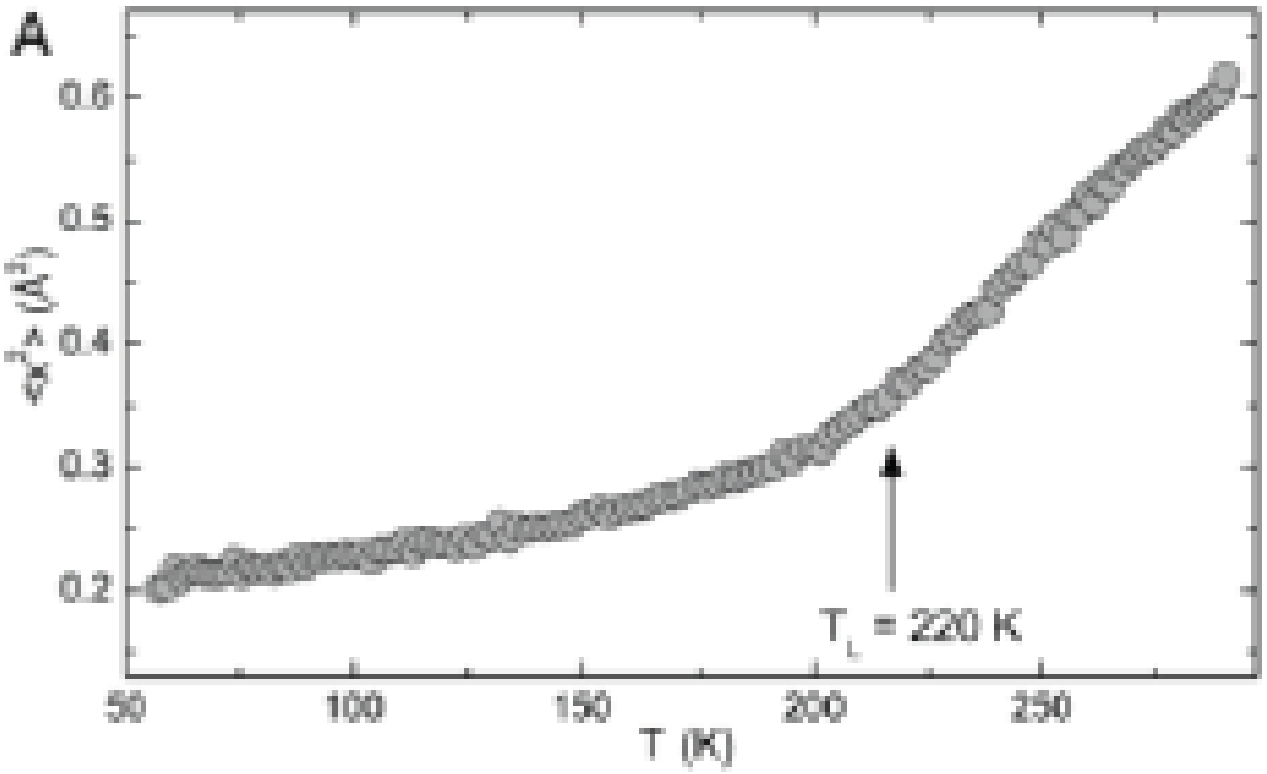
Methyl groups are the main contributors to the low-T onset



Low temperature anharmonic onset: Methyl Groups Activation (MGA)

High temperature anharmonic onset: Protein Dynamical Transition (PDT)

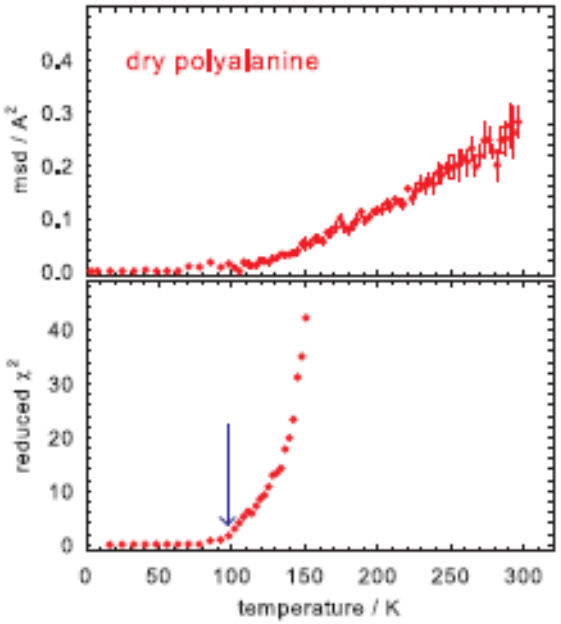
How to locate the onset temperatures in an «objective » way?
Purpose: study the dependence of the PDT onset temperature on the spectrometer energy (time) resolution



Determination of anharmonic onset temperatures

MGA onset temperature:

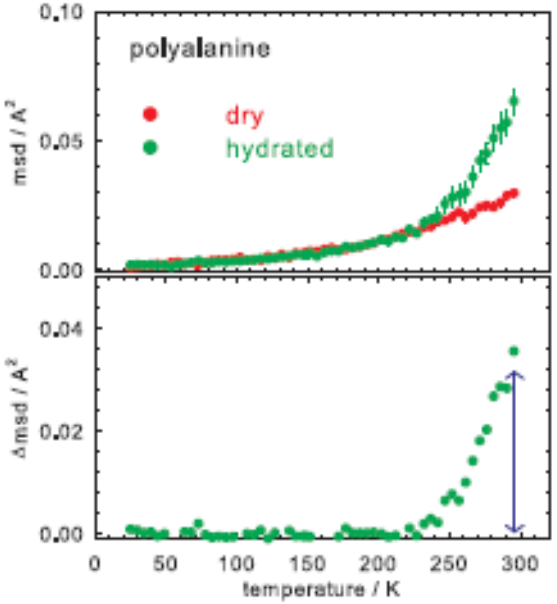
reduced χ^2 of msd(T) fitting with a linear dependence from low T up to a given T_f: sharp increase ⇒ harmonic-to-anharmonic transition



PDT onset temperature:

1. $\Delta msd = msd_{hydrated} - msd_{dry}$
2. Δmsd normalized to room T

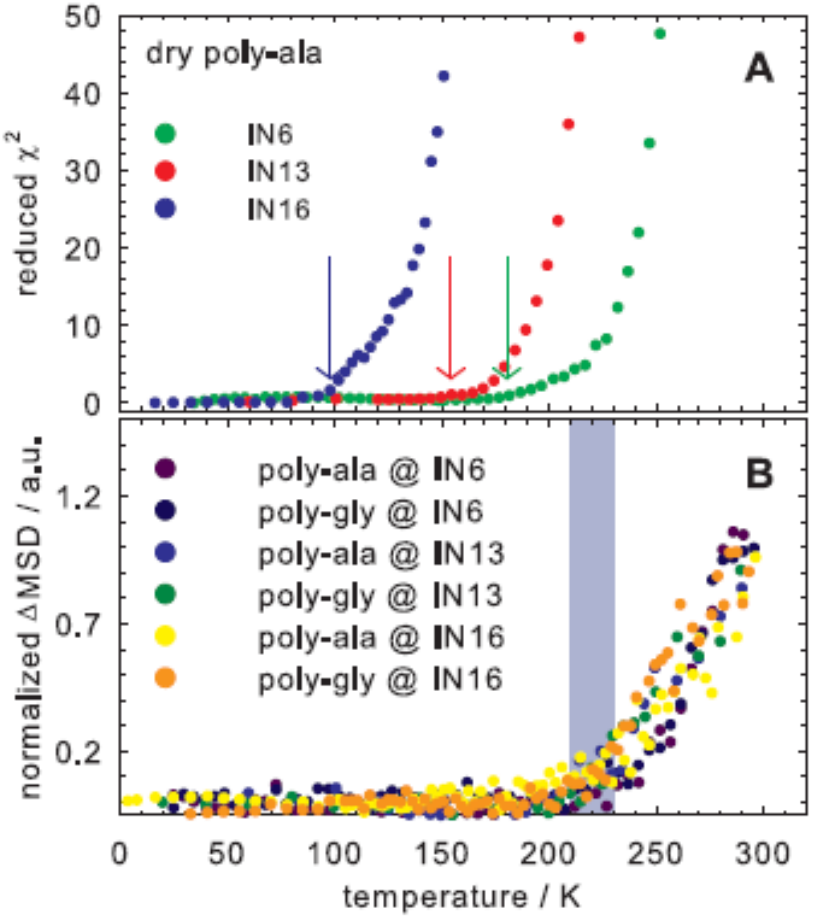
deviation from zero ⇒ PDT onset
(comparison of data from different spectrometers or different systems independent of msd amplitude)



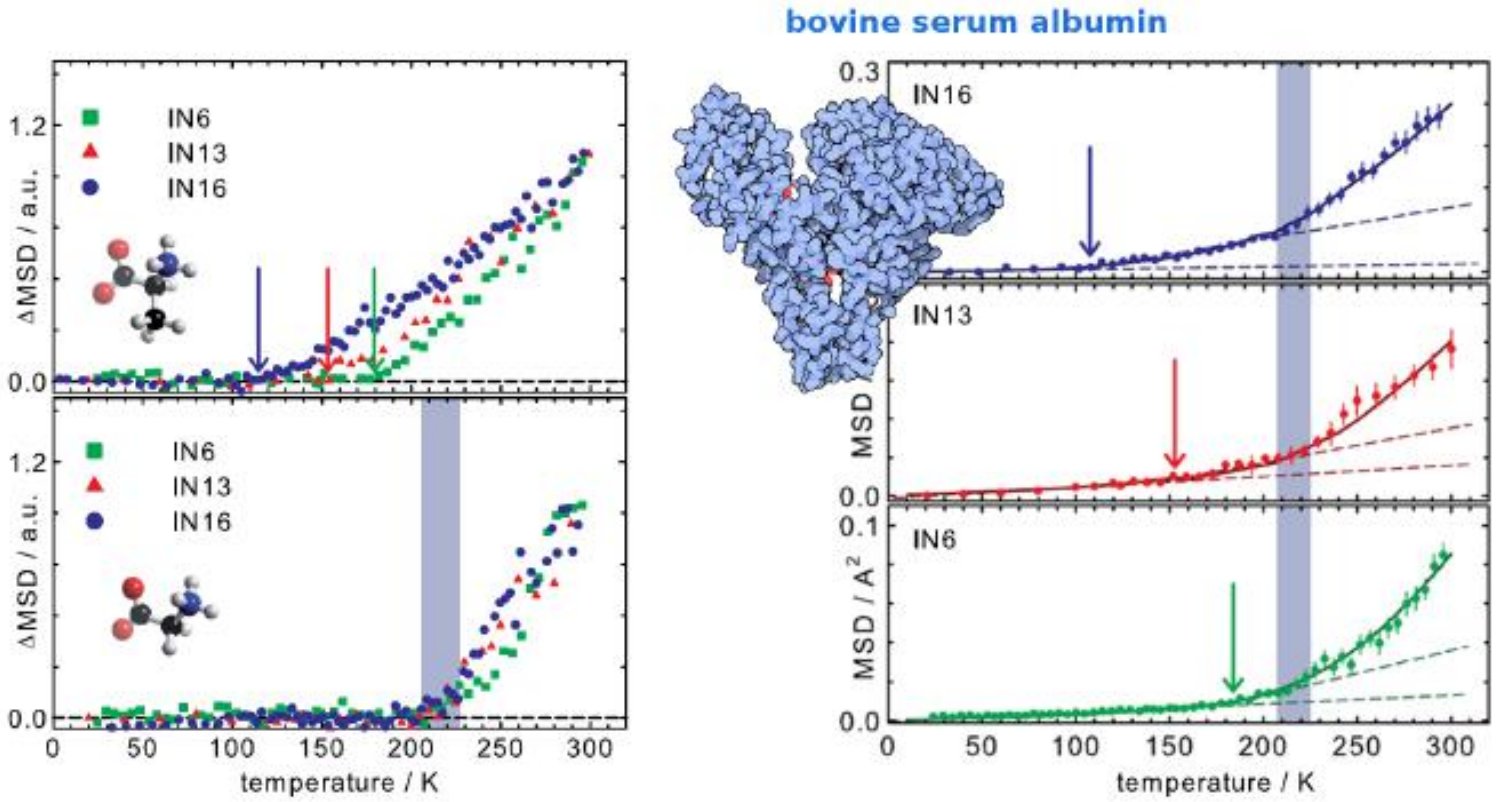
Onsets of anharmonicity and energy resolution

- 3 different spectrometers: IN16, IN13 and IN6 at ILL, Grenoble
- 2 ord. of magnitude in E resolution: 0.9 (IN16), 8 (IN13) and 70 μeV (IN6) $\rightarrow$ 100 ps$\div$10 ns time range

- MGA: evident resolution dependence
  $\sim$100 K at IN16
  $\sim$150 K at IN13
  $\sim$180 K at IN6
- PDT: no evidence of resolution dependence of the onset temperature 220$\pm$10 K



Exploring energy landscape by energy resolution dependence



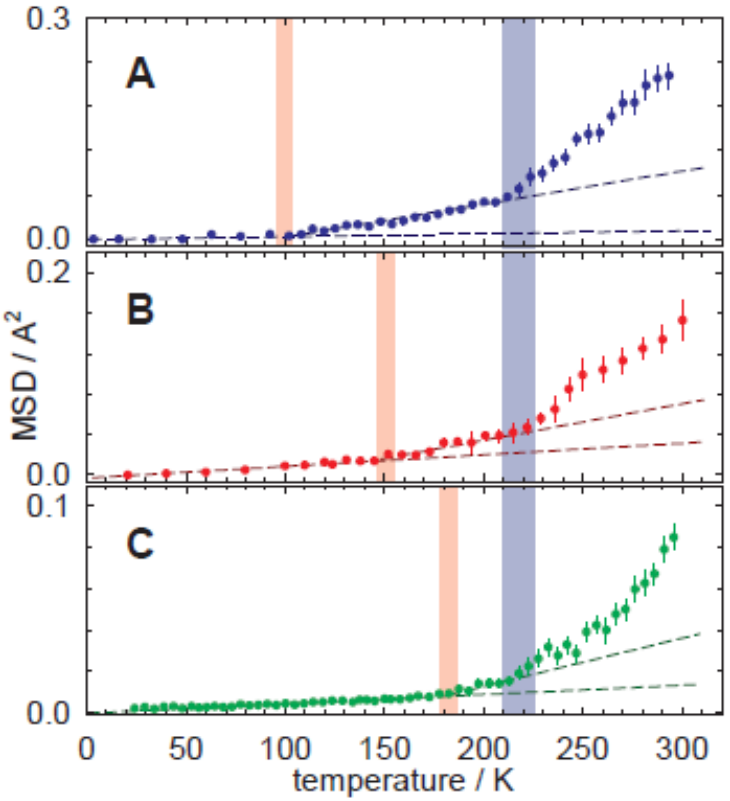
Just to show that the procedure works also for real proteins

Onsets of anharmonicity and energy resolution

analogous results in globular proteins:
hydrated Bovine Serum Albumin (BSA)

- MGA dependent on E resolution
- PDT independent of E resolution
- amplitudes dependent on E resolution

A: IN16
B: IN13
C: IN6



Proteins and polypeptides: two main onsets of anharmonicity

onset at ∼100-150 K:

- thermally activated motions of CH_3 methyl groups (Sokolov, Weik&Zaccai, Doster, Schiro&Cupane)
- independent of presence and amount of hydration water
- hereon we call it methyl groups activation, MGA

Onset temperature dependent on the energy resolution

onset at ∼220 K:
protein dynamical transition, PDT

- abrupt change of structural flexibility in response to the glass transition of hydration water (Doster)
- protein response to a liquid-liquid transition from a low density to a high density form (dynamics: fragile-to-strong crossover) in the hydration water (Chen)
- result of the protein structural relaxation reaching the limit of the experimental frequency window (Sokolov, Magazú)
- protein internal motions induced by β-fluctuations in the hydration shell (Frauenfelder)
- a change in the thermodynamic resilience of the water-protein system (Zaccai)

- all models: key role of hydration water

Onset temperature independent on the energy resolution

Physical Origin of Anharmonic Dynamics in Proteins: New Insights From Resolution-Dependent Neutron Scattering on Homomeric Polypeptides

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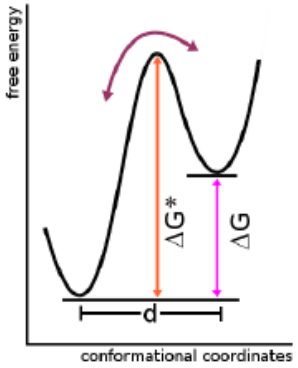
(Received 4 May 2012; published 19 September 2012)

Toward a quantitative description of protein anharmonicity

a simple model of anharmonicity

$$S(E,Q,T)=e^{-Q^2 u_V^2}\left(S_0\,\delta(E)+S_1\,\frac{\frac{\tau_0}{\pi}\,\frac{e^{\frac{\Delta G^*}{RT}}}{1+e^{\frac{\Delta G}{RT}}}}{1+E^2\tau_0^2\,\frac{e^{\frac{2\Delta G^*}{RT}}}{\left(1+e^{\frac{\Delta G}{RT}}\right)^2}}\right)$$

$$S_0=1-2\,\frac{e^{-\frac{\Delta G}{RT}}}{\left(1+e^{-\frac{\Delta G}{RT}}\right)^2}\left(1-\frac{\sin(Qd)}{Qd}\right);\qquad S_1=1-S_0$$



scattering function convoluted with resolution function R(E;ΔE):

$$S_{model}(E,Q,T)=S(E,Q,T)\otimes R(E;\Delta E)$$

msd_{model} calculated as:

$$msd_{model}=-\partial ln[S_{model}(E=0,Q,T)]/\partial Q^2$$

onset temperatures change or not with the resolution depending on $\Delta G^*/\Delta G$:

- $\Delta G^*/\Delta G \gg 1$: T-dependent broadening of q.e. width vs. T-independent resolution width ⇒ decrease of "elastic" counting
- for $\Delta G^*/\Delta G \sim 1$: thermal population of higher site ⇒ decay of S_0

Toward a quantitative description of protein anharmonicity

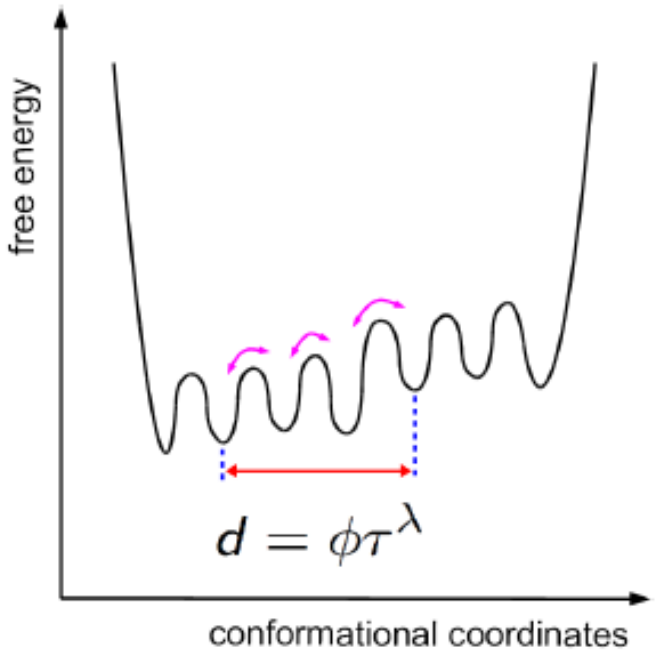
an oversimplified model for proteins:

1. homogeneity of non-exchangeable H nuclei vs. chemically and structurally heterogeneous proteins
2. two energy wells vs. complex multi-minima landscape (Brownian-like motion within protein/water conformational space)

our strategy to refine it:

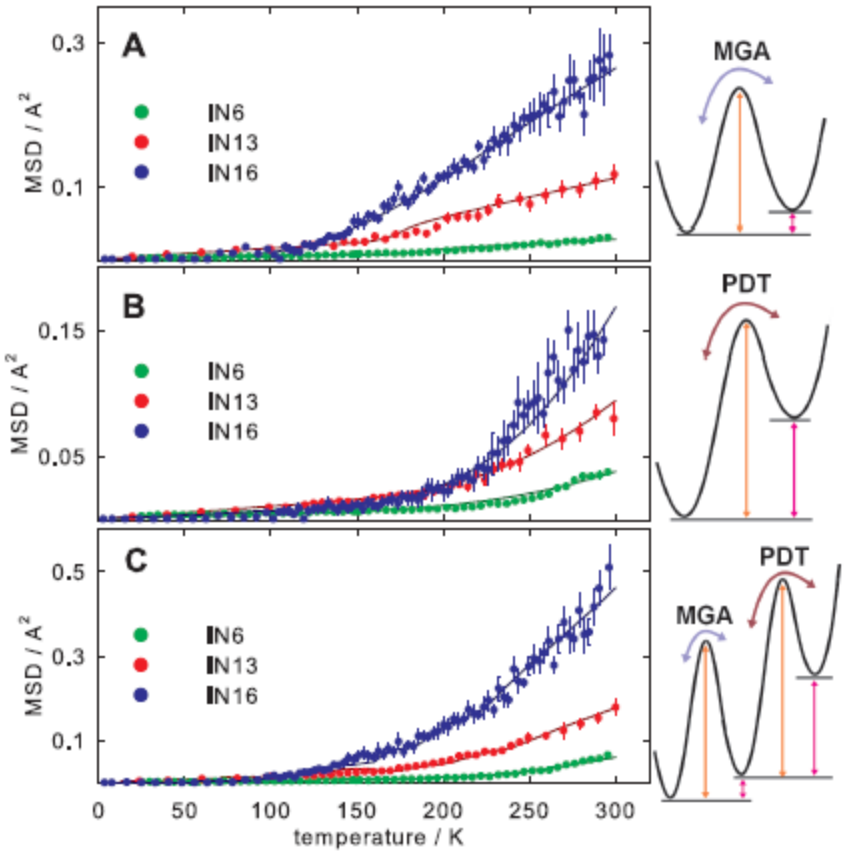
1. homomeric polypeptides reduce heterogeneity
2. "effective distance" d: length scale explored by protein H atoms in the time scale $\tau = h/\Delta E$ accessible with resolution ΔE

$$d = \phi\tau^{\lambda}$$



the model works if experimental msd from different spectrometers are reproduced by msd_{model} with same parameters but ΔE

Toward a quantitative description of protein anharmonicity



A: dry polyalanine
B: hydrated polyglycine
C: hydrated polyalanine

	ΔG	τ_0	ΔG^*	ϕ	λ	d_{IN16}	d_{IN13}	d_{IN6}
poly-ala	5.0	2.1	44	$2.0{\cdot}10^3$	0.35	2.9	1.4	0.7
poly-gly	13.8	2.1	21	$5.0{\cdot}10^2$	0.23	7.5	4.7	2.9
BSA^{MGA}	5.0	2.1	44	$2.0{\cdot}10^3$	0.37	2.1	1.0	0.5
BSA^{PDT}	13.8	2.1	21	$5.0{\cdot}10^2$	0.21	9.0	5.8	3.7

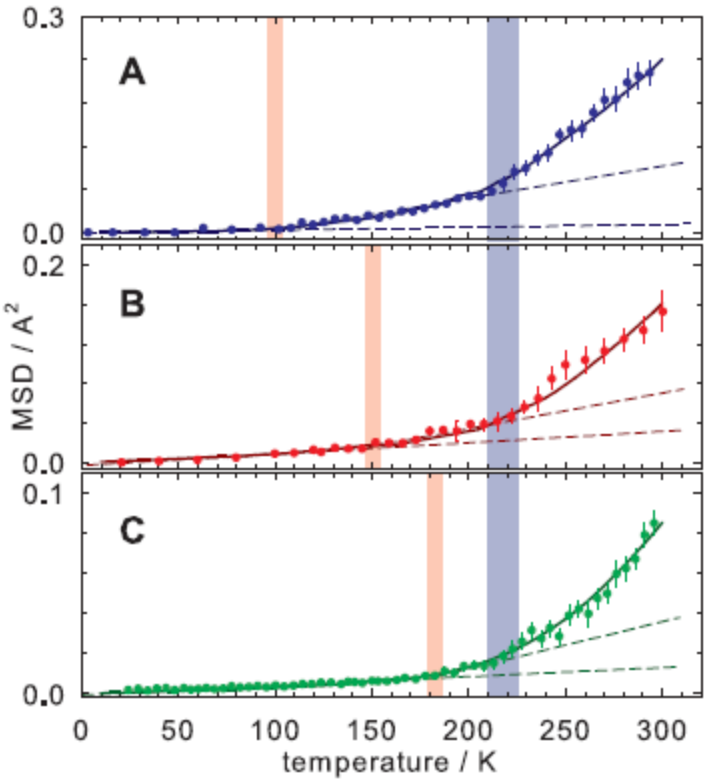
Energies are reported in KJ/mol, τ_0 in 10^{-20}s, ϕ in $A/s^{2\lambda}$, d in A.

for hydrated systems:

$$S_{model}(E,Q,T) = S^{MGA}_{model}(E,Q,T) \otimes S^{PDT}_{model}(E,Q,T)$$

Toward a quantitative description of protein anharmonicity

BSA



A: IN16
B: IN13
C: IN6

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Energies are reported in KJ/mol, τ_0 in 10^{-20}s, ϕ in $Å/s^{2\lambda}$, d in Å.

for hydrated systems:

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«Protein» dynamical transition ?

THE JOURNAL OF PHYSICAL CHEMISTRY Letters

LETTER

pubs.acs.org/JPCL

The "Protein Dynamical Transition" Does Not Require the Protein Polypeptide Chain

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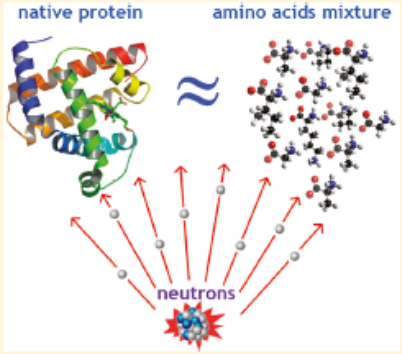
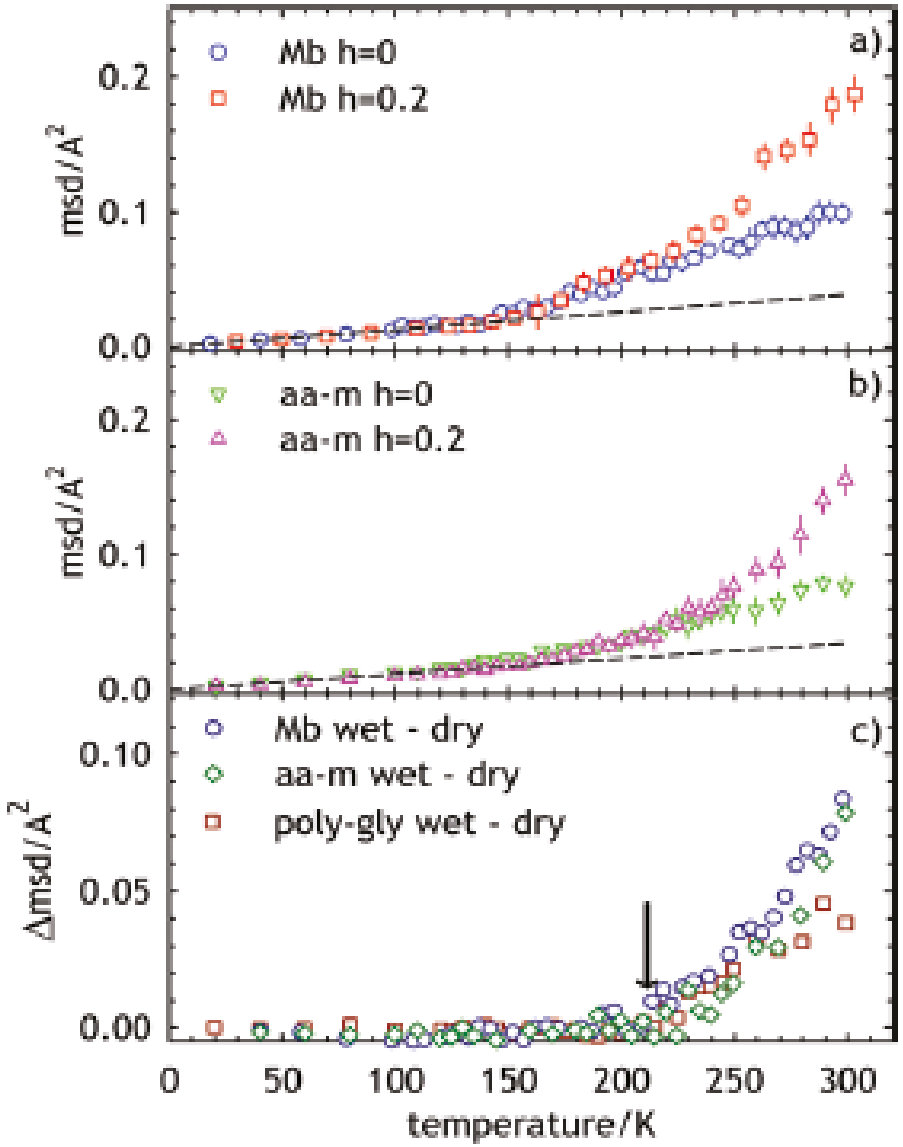
Supporting Information

ABSTRACT: We give experimental evidence that the main features of protein dynamics revealed by neutron scattering, i.e., the "protein dynamical transition" and the "boson peak", do not need the protein polypeptide chain. We show that a rapid increase of hydrogen atoms fluctuations at about 220 K, analogous to the one observed in hydrated myoglobin powders, is also observed in a hydrated amino acids mixture with the chemical composition of myoglobin but lacking the polypeptide chain; in agreement with the protein behavior, the transition is abolished in the dry mixture. Further, an excess of low-frequency vibrational modes around 3 meV, typically observed in protein powders, is also observed in our mixture. Our results confirm that the dynamical transition is a water-driven onset and indicate that it mainly involves the amino acid side chains. Taking together the present data and recent results on the dynamics of a protein in denatured conformation and on the activity of dehydrated proteins, it can be concluded that the "protein dynamical transition" is neither a necessary nor a sufficient condition for active protein conformation and function.



SECTION: Biophysical Chemistry



The same PDT is observed in Mb and in the aa mixture

Physical origin of the PDT

Observation of fragile-to-strong dynamic crossover in protein hydration water

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municated by H. Eugene Stanley, Boston University, Boston, MA, March 28, 2006 (received for review March 11, 2006)

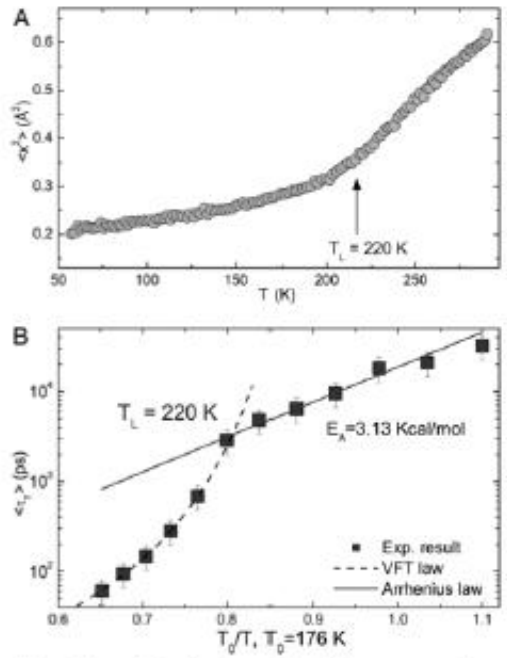


Fig. 3. Evidence for the dynamic transition. (*A*) The temperature dependence of the mean-squared atomic displacement of the hydrogen atom at 2-ns time scale measured by an elastic scan with resolution of 0.8 μeV. (*B*) Temperature dependence of the average translational relaxation times plotted in $\log(\langle\tau\rangle)$ vs. T_0/T, where T_0 is the ideal glass transition temperature. Here, there is a clear and abrupt transition from a Vogel–Fulcher–Tammann law at high temperatures to an Arrhenius law at low temperatures, with the fitted crossover temperature T_L = 220 K and the activation energy E_A = 3.13 kcal/mol extracted from the Arrhenius part indicated in the figure.

water at ambient pressure (15). The Widom line is originated from the existence of the second critical point of water and is the extension of the liquid–liquid coexistence line into the one-phase region. Therefore, our observation of the FSC at ambient pressure implies that there may be a liquid–liquid phase transition line in the protein hydration water at elevated pressures. This dynamic crossover, when crossing the Widom line, causes the layer of the water surrounding a protein to change from the "more fluid" high-density liquid form (which induces the protein to adopt more flexible conformational substates) to the "less

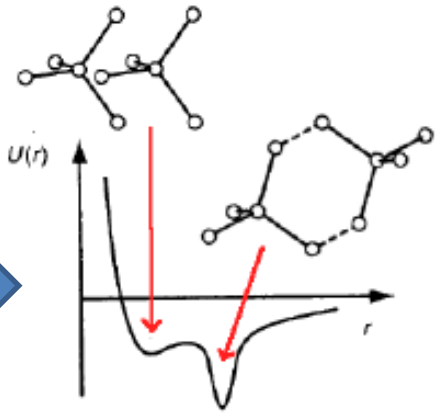
same PDT temperature (∼220 K) in hydrated peptide systems chemically and structurally different:

1. native and denaturated proteins
2. homomeric polypeptides (even poly-gly → "pure backbone")
3. amyloid fibrils (Schiró et al. 2012)
4. amino acid mixtures lacking the polypeptide chain (Schiró et al. 2011)

PDT onset independent of hydrati

ΔG value, that sets the PDT temperature, determined by hydration water

LDL → HDL transition at ∼220K: two-wells water interaction potential



H E Stanley *et al*
J. Phys.: Condens. Matter 21 (2009) 504105

Experimental evidence on the connection between PDT and FSC in protein hydration water using different experimental techniques

THE JOURNAL OF CHEMICAL PHYSICS **139**, 121102 (2013)

Communication: Protein dynamical transition vs. liquid-liquid phase transition in protein hydration water

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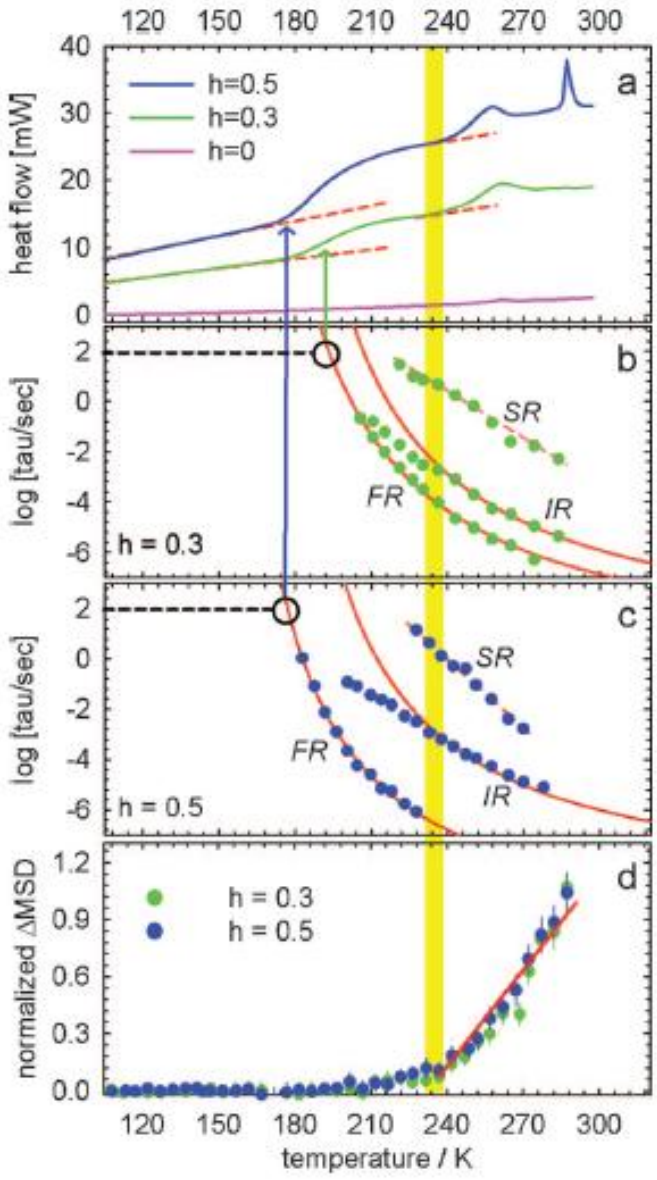
(Received 7 August 2013; accepted 11 September 2013; published online 27 September 2013)

In this work, we compare experimental data on myoglobin hydrated powders from elastic neutron scattering, broadband dielectric spectroscopy, and differential scanning calorimetry. Our aim is to obtain new insights on the connection between the protein dynamical transition, a fundamental phenomenon observed in proteins whose physical origin is highly debated, and the liquid-liquid phase transition (LLPT) possibly occurring in protein hydration water and related to the existence of a low temperature critical point in supercooled water. Our results provide a consistent thermodynamic/dynamic description which gives experimental support to the LLPT hypothesis and further reveals how fundamental properties of water and proteins are tightly related. © *2013 AIP Publishing LLC*. [http://dx.doi.org/10.1063/1.4822250]



a) DSC upscans

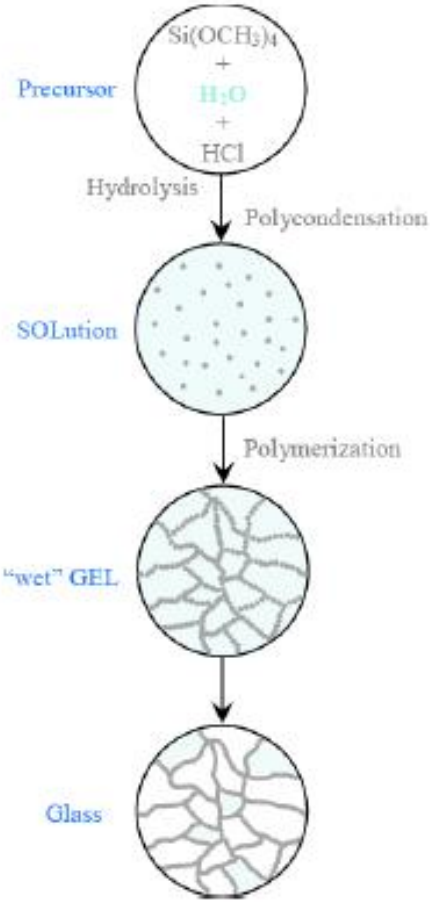
b) Dielectric spectroscopy

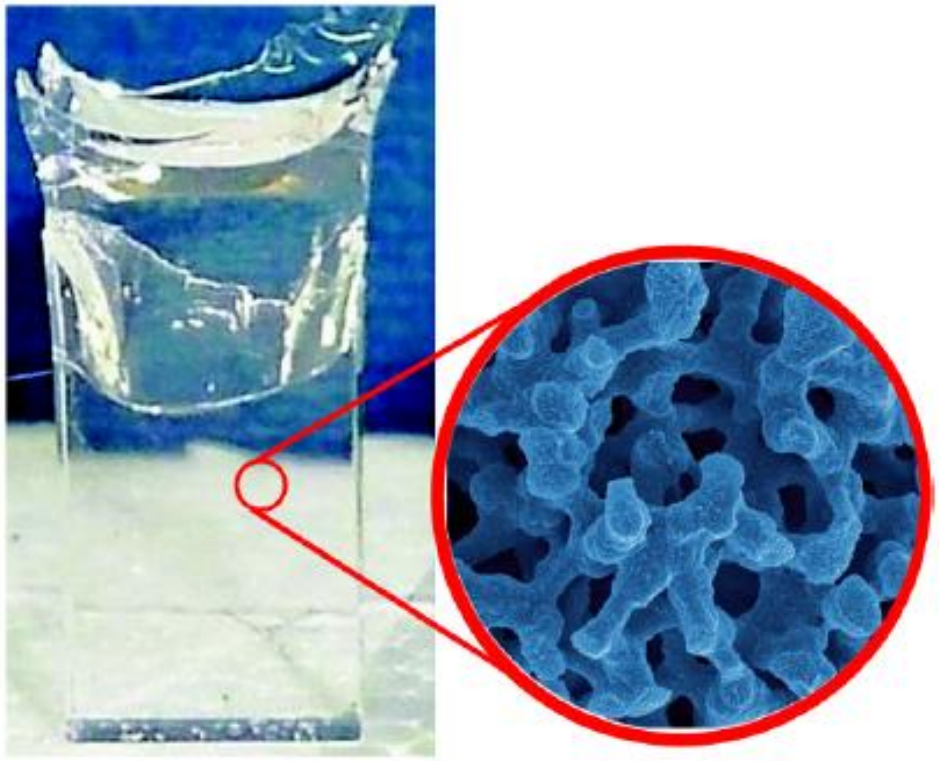
c) Dielectric spectroscopy

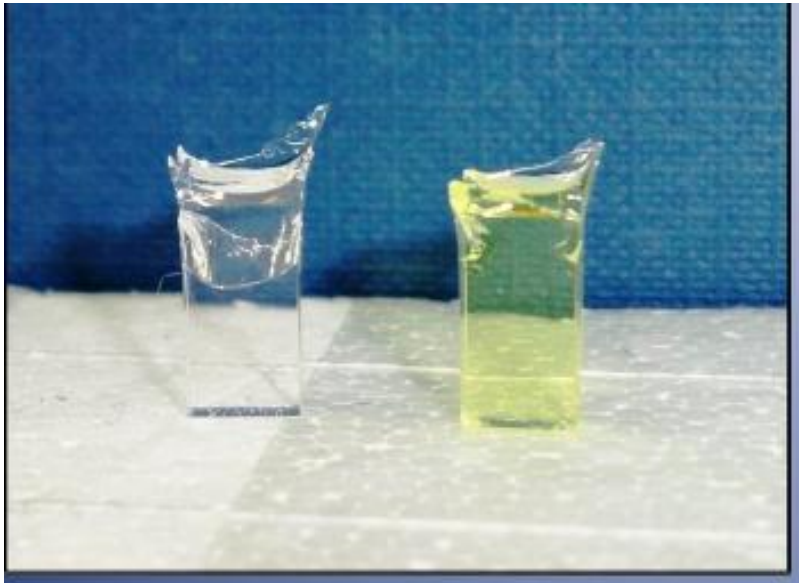
d) Elastic neutron scattering

Note: same samples; same experimental protocol

Silica hydrogel as a model of hydrophilic confinement







Low cost

Easy to prepare/handle

Hydration $h = gr[H_2O] / gr[SiO_2]$ can be easily varied

Experimental Evidence for a Liquid-Liquid Crossover in Deeply Cooled Confined Water

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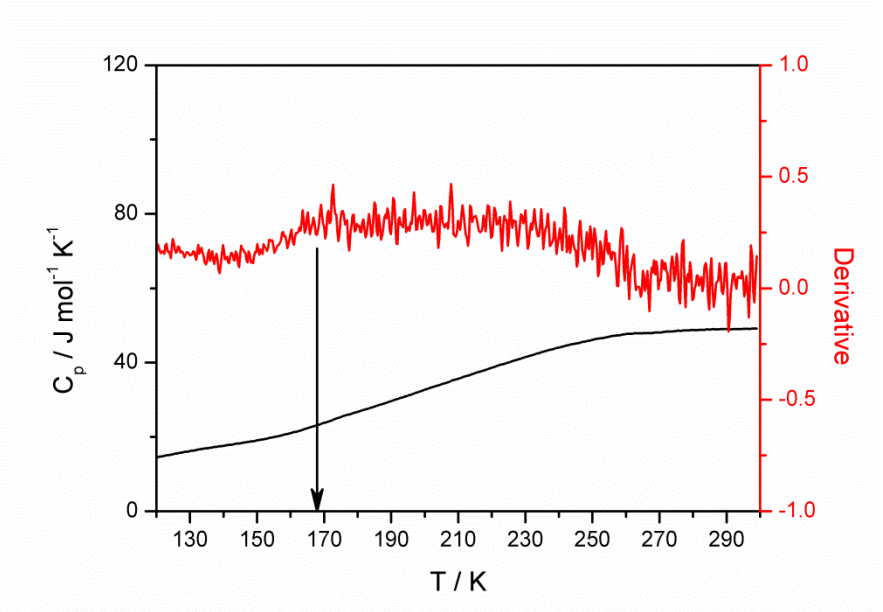
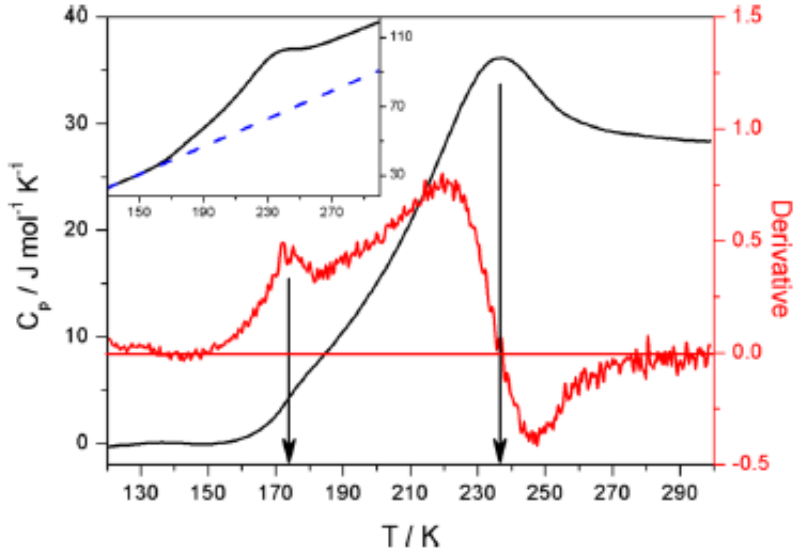
(Received 16 June 2014; published 21 November 2014)

Water confined in silica Xerogels: DSC upscans

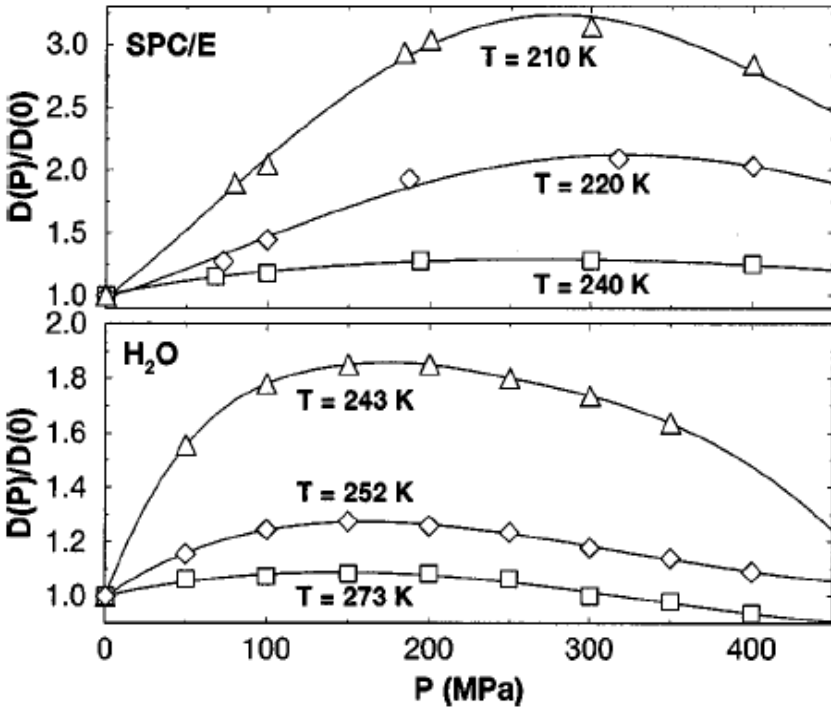


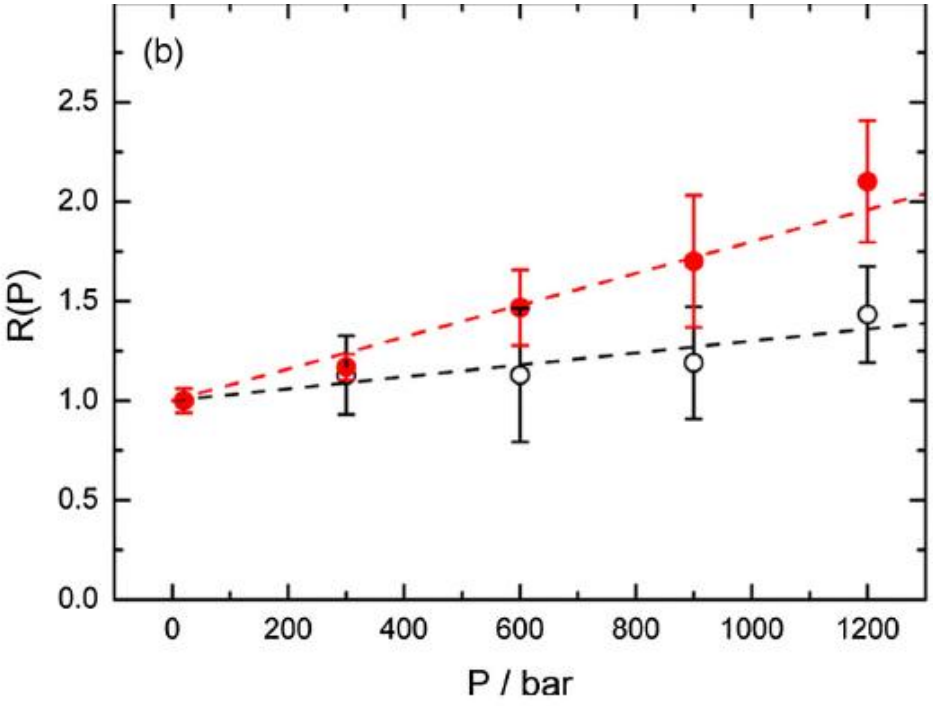
h = 0.19



h = =.42

Testing the FSC hypothesis via the water pressure anomaly





EXPECTED (no FSC)

Exp. data from Prielmeier et al 1988

Simulations from Starr et al 1999

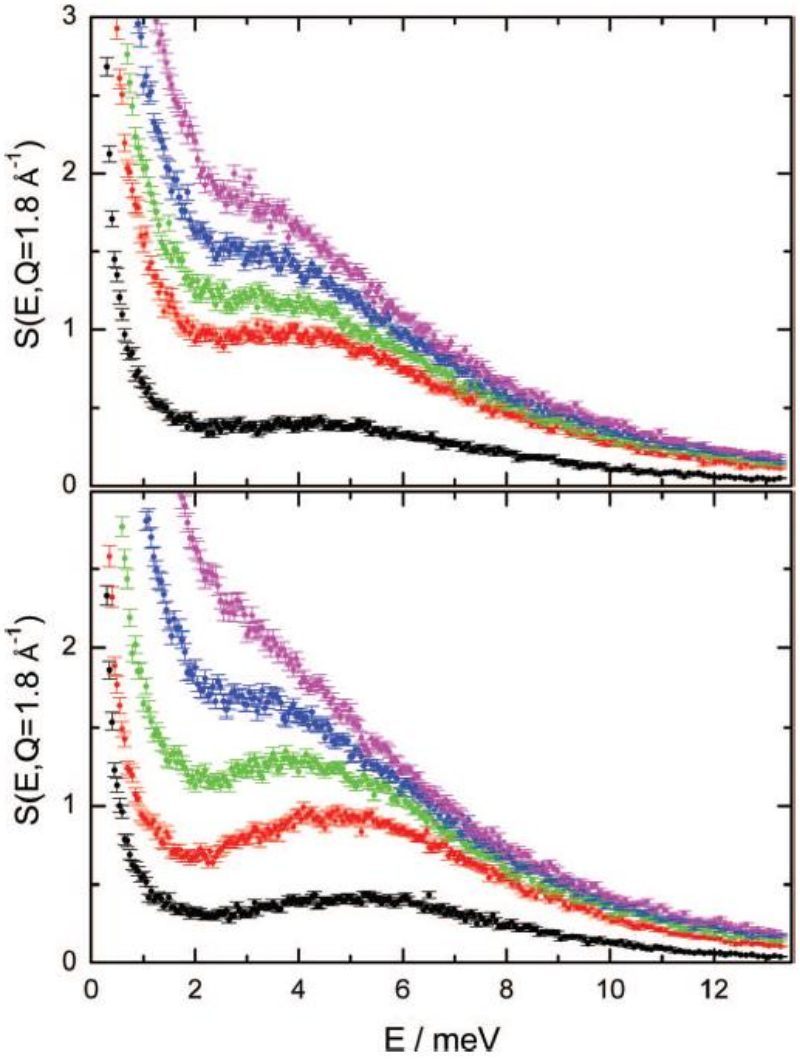
MEASURED

R(P) = MSD(P)/MSD(0)

From the Einstein relation:

$MSD = 6D\tau_{res}$

Testing the FSC hypothesis via the Boson peak



h = 0.19

h = 0.42

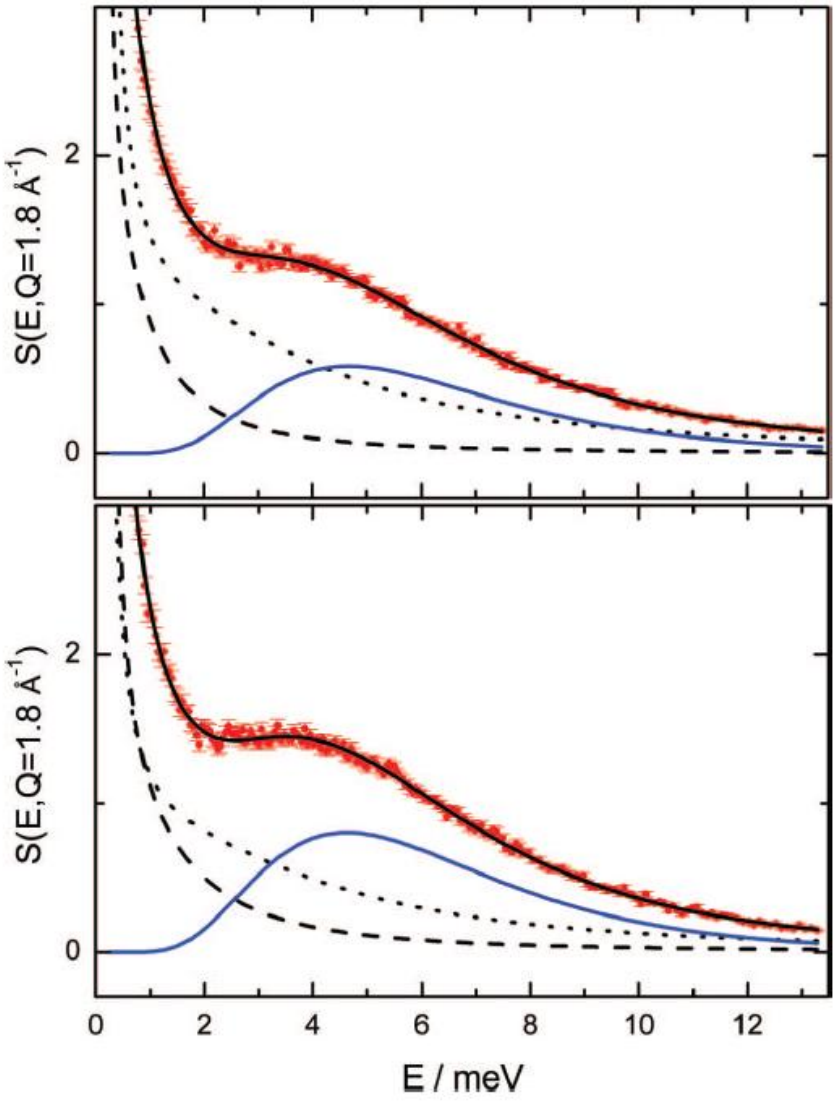
The Boson peak “disappears” at temperatures between 215K (green) and 235K (blue) . However, uncertainties are introduced by the presence of quasi-elastic broadening

Quantitative analysis

$$S_{\mathrm{exp}}(E, Q = 1.8\,\text{Å}^{-1}) = M(E) + \frac{1}{\pi}\cdot\frac{A_1\gamma_1}{E^2+\gamma_1^2} + \frac{1}{\pi}\cdot\frac{A_2\gamma_2}{E^2+\gamma_2^2} + \frac{A_3}{\sqrt{2\pi}\sigma_{BP}E}\cdot\exp\left[\frac{-(E_{BP}-\ln E)^2}{2\sigma_{BP}^2}\right]$$

h = 0.19

h = 0.42



Quantitative analysis

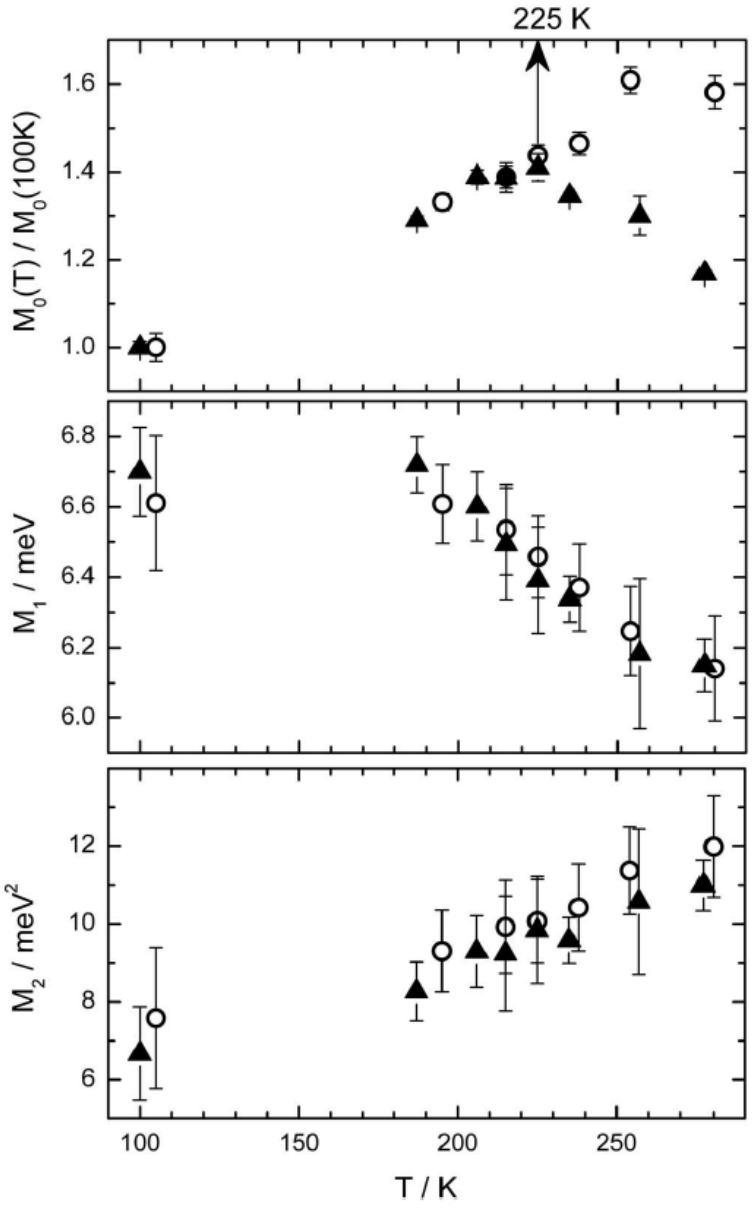
From the lognormal term we obtain the vibrational density of states (VDOS), g(E), according to

$$g(E) \sim \frac{E \cdot \frac{A_3}{\sqrt{2\pi}\sigma_{BP}E} \cdot \exp\left[\frac{-(E_{BP} - \ln E)^2}{2\sigma_{BP}^2}\right]}{n(E,T) \cdot DW(T)}, \quad (2a)$$

$$n(E,T) = \frac{1}{\exp\left(\frac{E}{k_B T}\right) - 1}, \quad (2b)$$

R-VDOS = $g(E)/E^2$

Open circles : h = 0.19
Full triangles : h = 0.42



SUMMARY

-Two anharmonicity onsets are detected in protein dynamics by elastic neutron scattering:

1) MGA ; hydration independent ; its onset temperature depends on energy resolution
2) PDT ; hydration dependent ; its onset temperature does not depend on hydration and energy resolution while the MSD amplitudes do

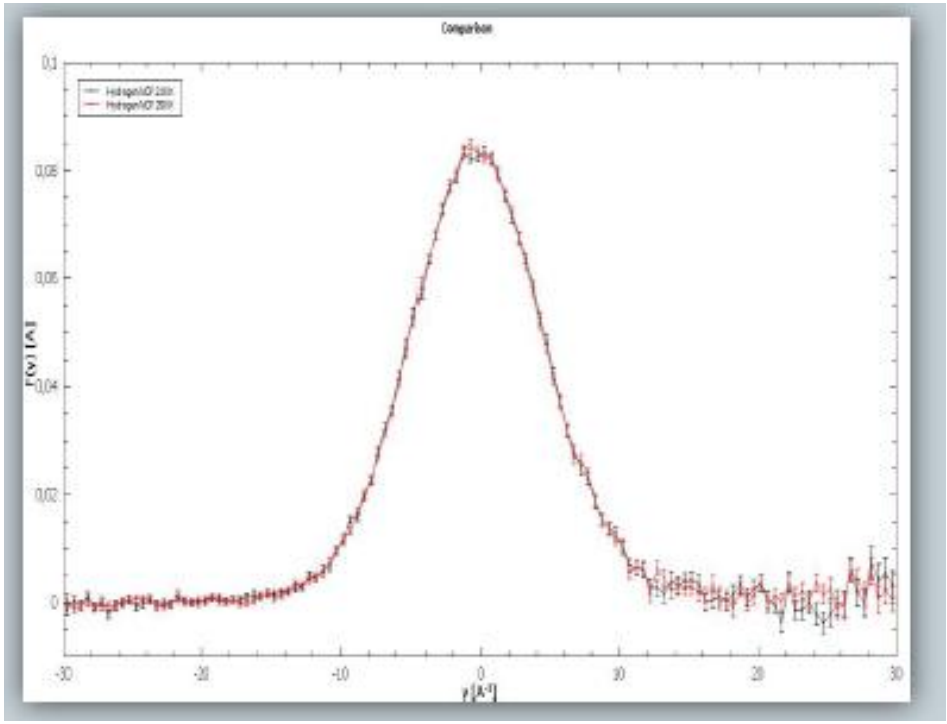
-The PDT is not a mere resolution effect but reflects a real physical effect clearly related to hydration water

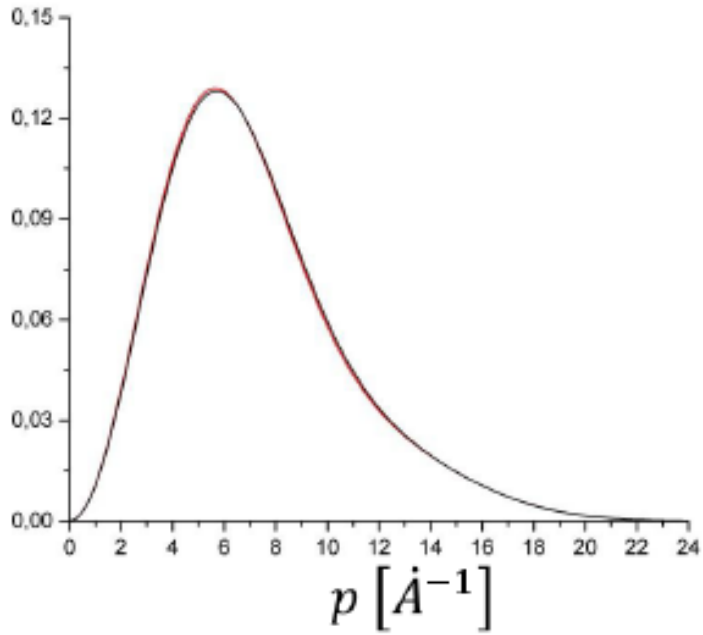
-Possible connection with the LDL->HDL crossover in hydration water at about 220 K

-Experimental evidence on the FSG crosssover in water confined in silica hydrogel and mimicking protein hydration water obtained through the pressure anomaly and the boson peak

A Deep Inelastic Neutron Scattering Study on water confined in a silica hydrogel: preliminary results

V. De Michele
G. Romanelli
A. Cupane



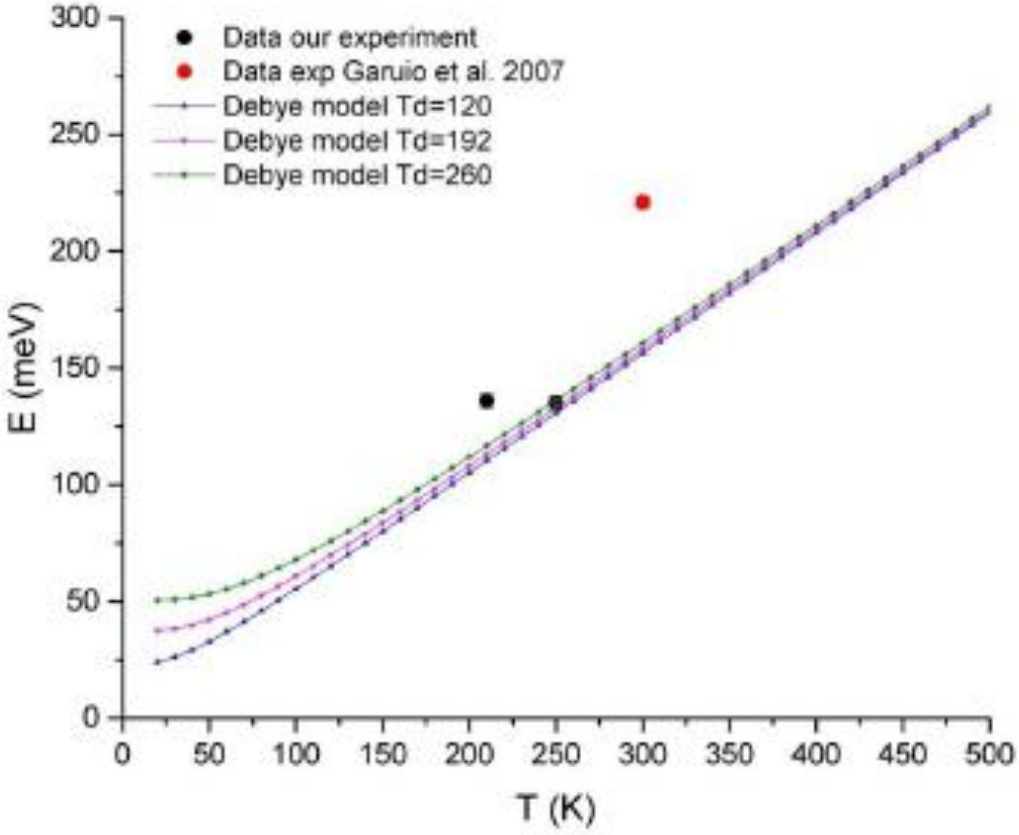


Proton NCP at 250 K (red) and at 210 K (black)

Proton momenum distribution

$< E_K > = 135 \pm 2$ mev T = 250 K

$< E_K > = 136 \pm 2$ mev T = 210 K



The fact that $< E_K >$ (210K) = $< E_K >$ (250K) is an unexpected and puzzling result. Indeed, a decrease of $< E_K >$ with decreasing T would be expected. However, more experimental data are needed before speculating

THE JOURNAL OF PHYSICAL CHEMISTRY Letters

Letter

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Neutron Scattering Reveals Enhanced Protein Dynamics in Concanavalin A Amyloid Fibrils

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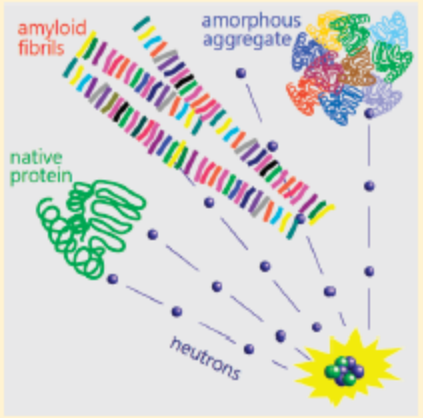
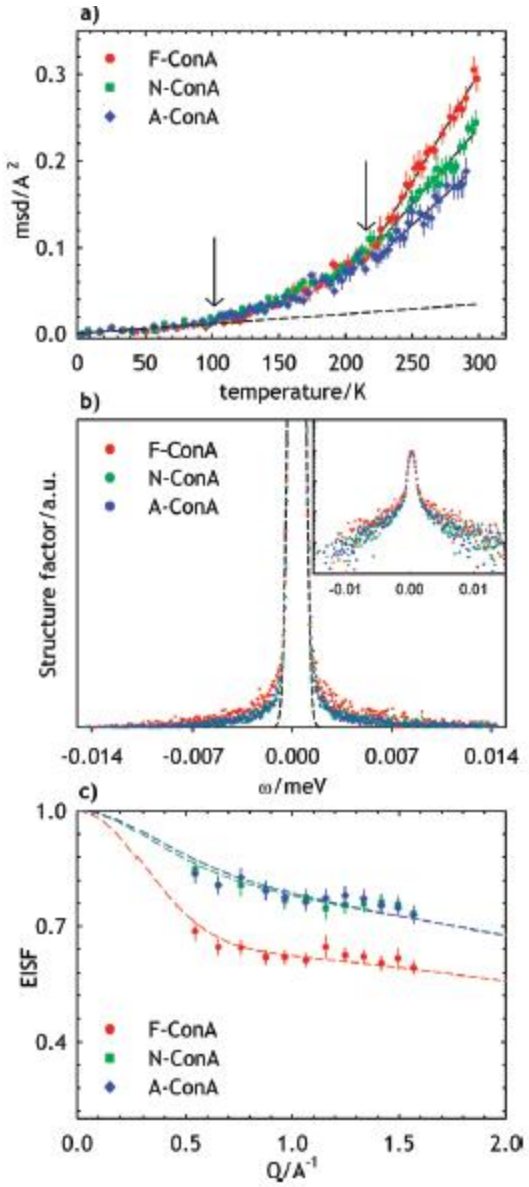
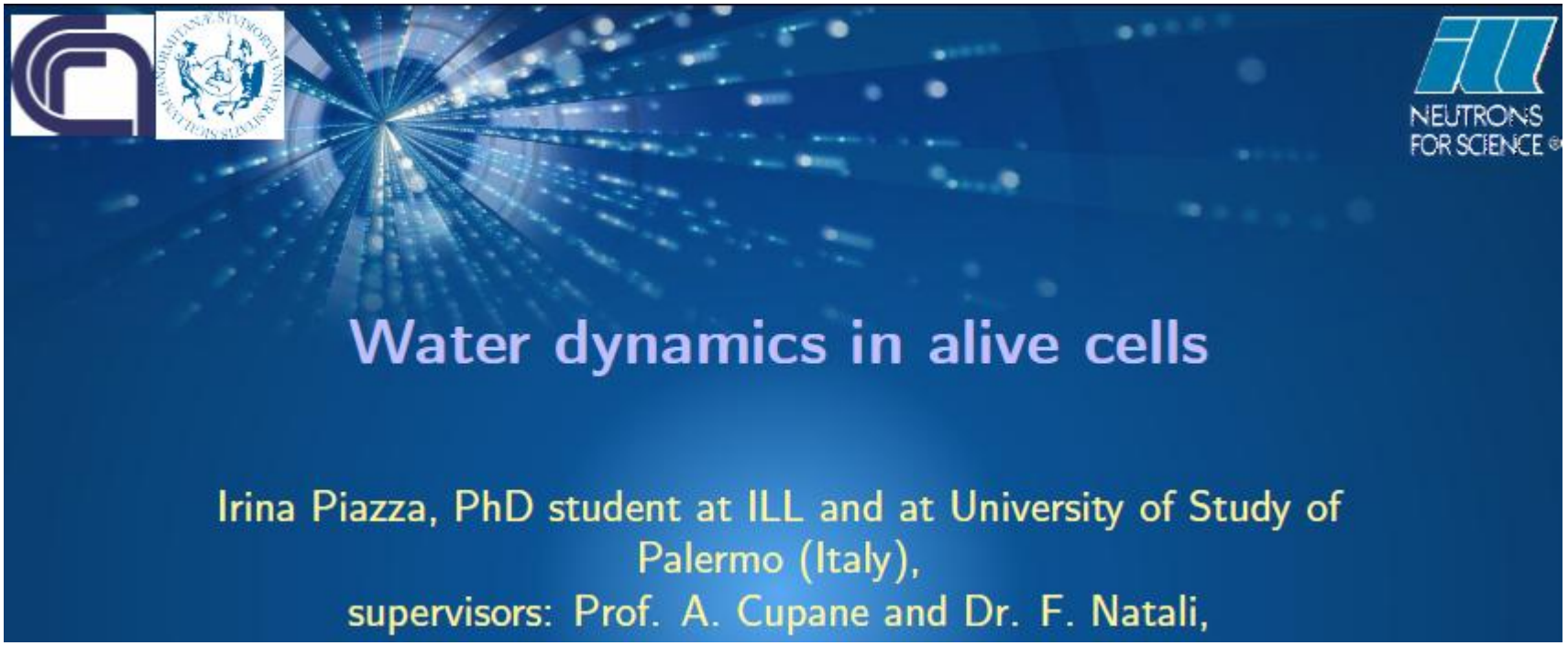
Supporting Information

ABSTRACT: Protein aggregation is one of the most challenging topics in life sciences, and it is implicated in several human pathologies. The nature and the role of toxic species is highly debated, with amyloid fibrils being among the most relevant species for their peculiar structural and functional properties. Protein dynamics and in particular the ability to fluctuate through a large number of conformational substates are closely related to protein function. This Letter focuses on amyloid fibril dynamics, and, to our knowledge, it is the first neutron scattering study on a protein (Concanavalin A) isolated in its fibril state. Our results reveal enhanced atomic fluctuations in amyloid fibrils and indicate that the protein is "softer" in the fibril state with respect to the native and amorphous aggregate states. We discuss this finding in terms of a structural interpretation and suggest that the paradigm ordered structure ↔ lower flexibility can be questioned when considering the local fast side-chain protein dynamics.

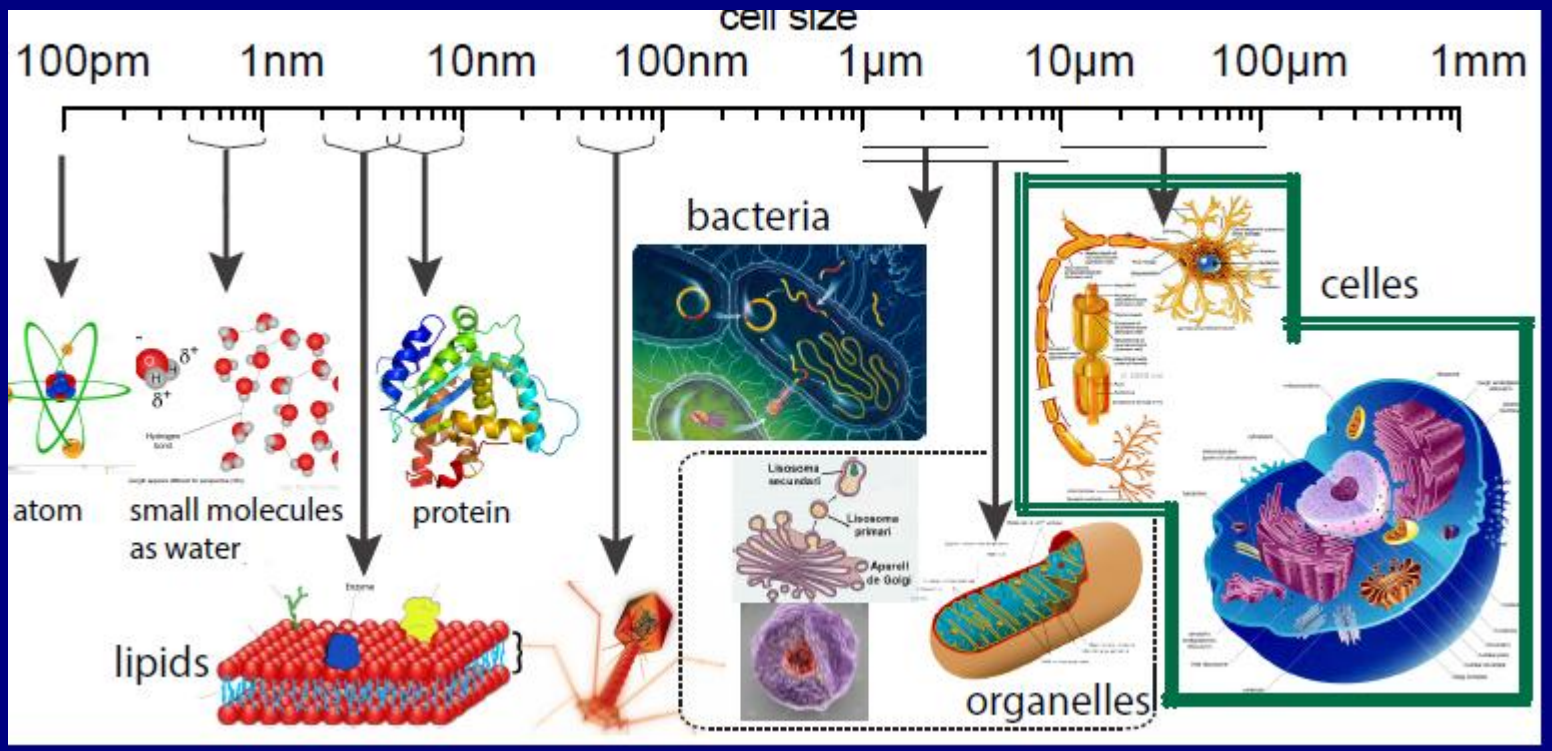


SECTION: Biophysical Chemistry and Biomolecules

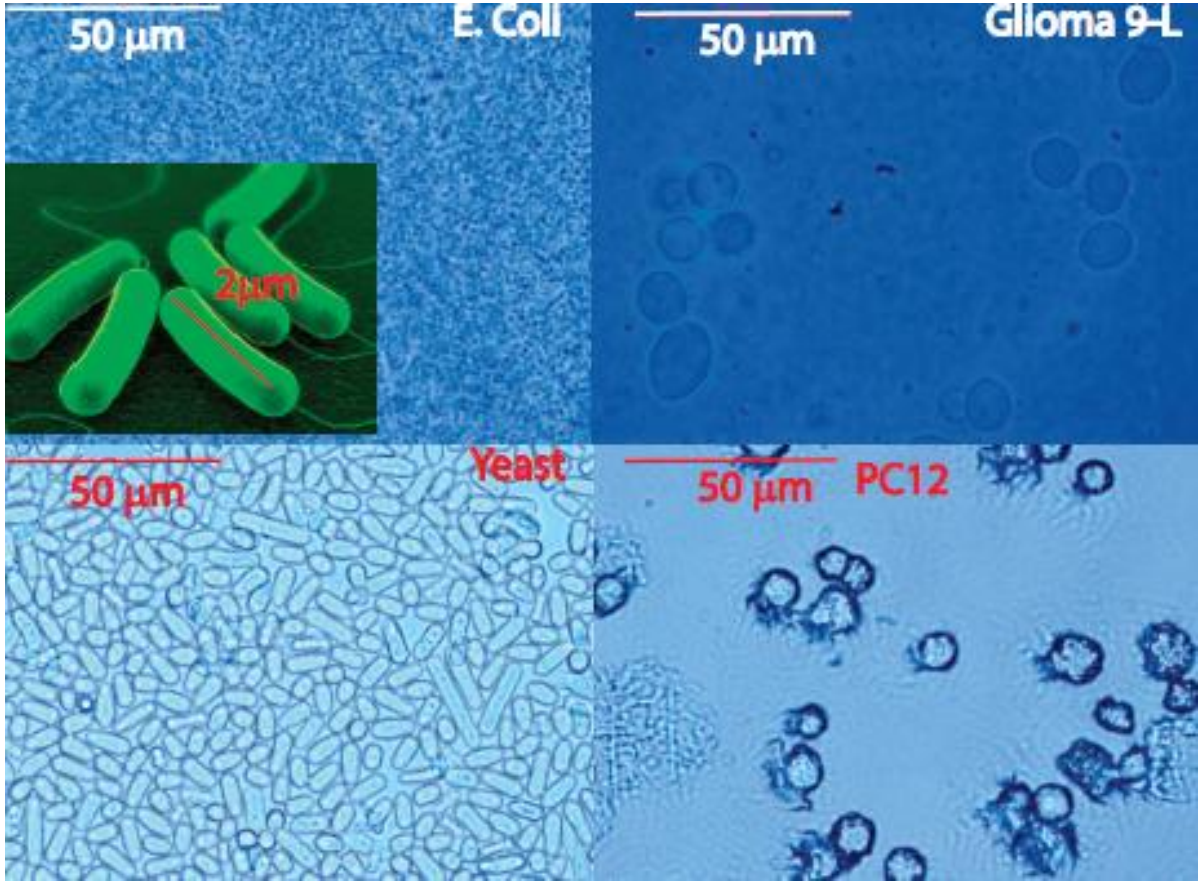

a)
F-ConA
N-ConA
A-ConA
msd/A²
temperature/K
b)
Structure factor/a.u.
ω/meV
c)
EISF
Q/A⁻¹



Aim : to investigate water dynamics in biological systems more complex than protein powders

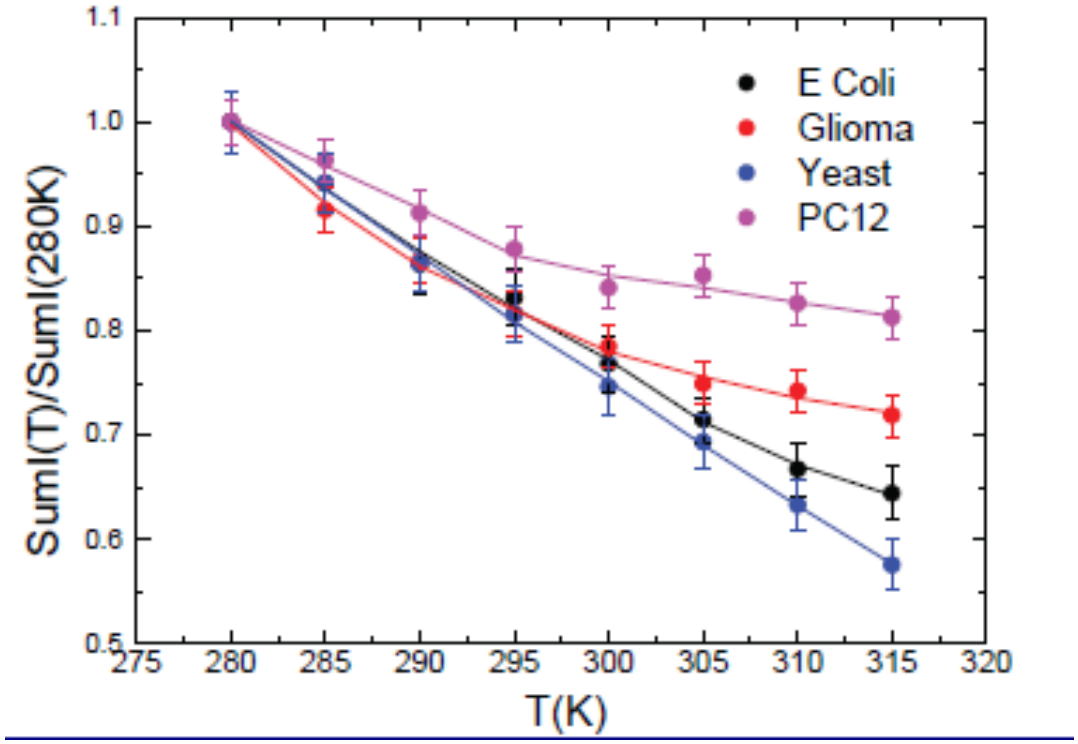


Neutron scattering technique uses neutron beam as a probe to investigate macromolecules and water molecules dynamics.



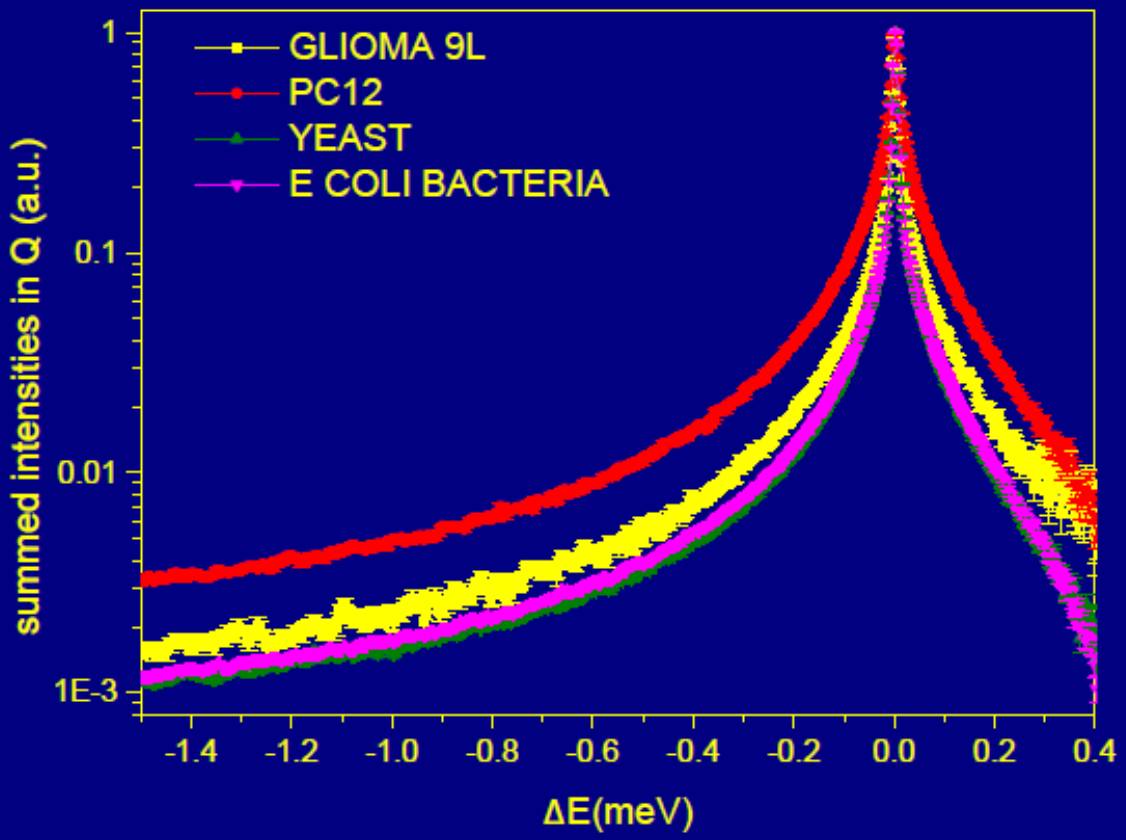
Samples:
Cell pellets of
E. Coli ; 38% H_2O
Yeast ; 52% H_2O
PC12 ; 34% H_2O
Glioma 9-L ; 39% H_2O

Preliminary result from ENS

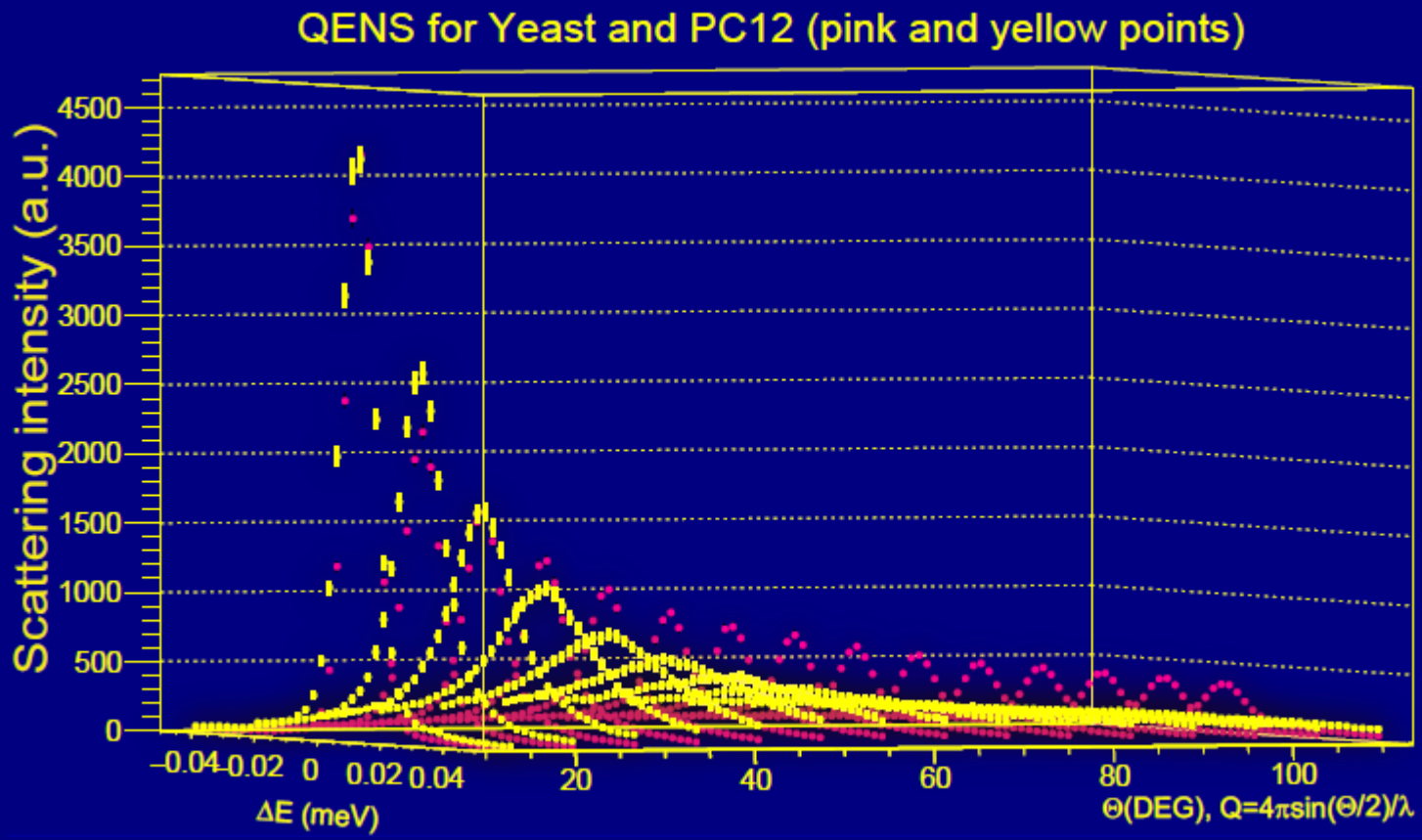


The summed elastic intensities decay slower for cancer cells

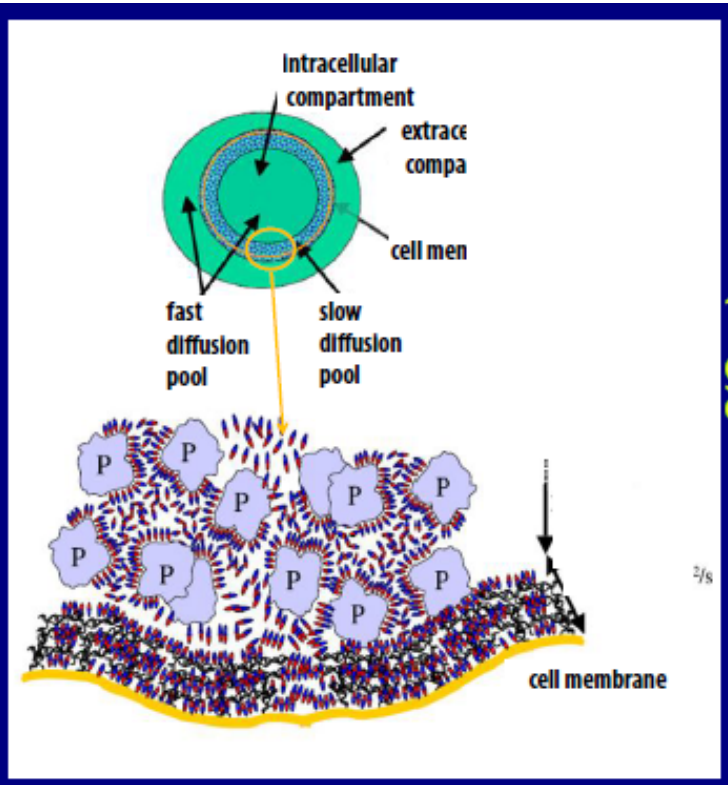
QENS measurement on alive cells

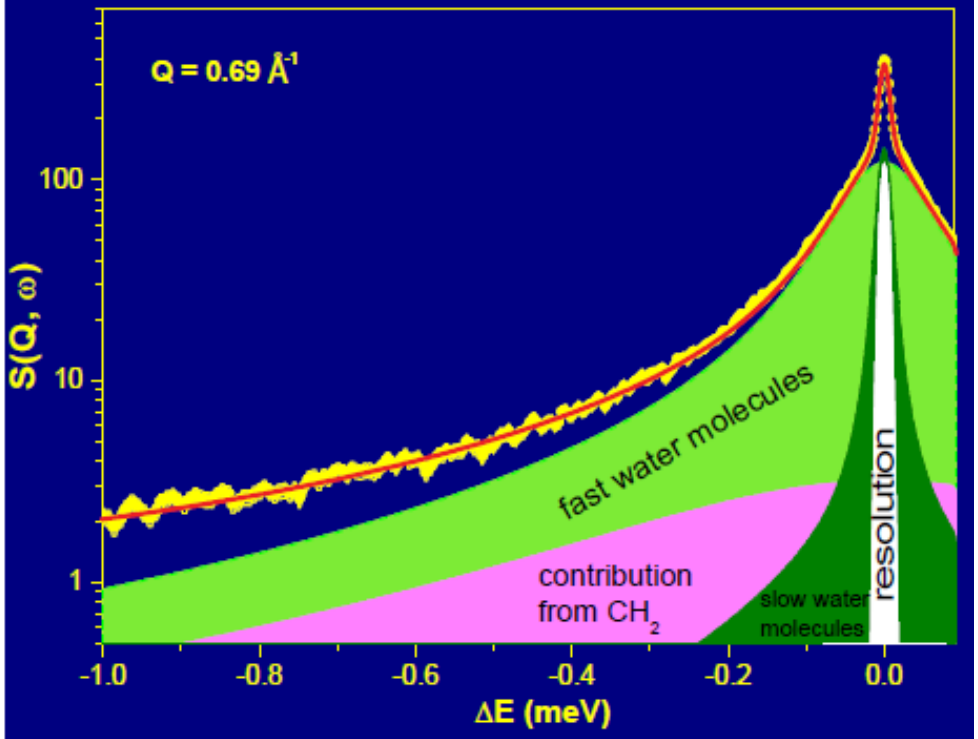


QENS on alive cells



A model to describe complex systems





$$S(Q,\omega) \propto R(Q,\omega) \otimes [f\,\delta(\omega) + p_{fast} S_{fast}(Q,\omega, D^T_{fast}, \tau^T_{fast}, D^R_{fast}) + p_{slow} S_{slow}(Q,\omega, D^T_{slow}, \tau^T_{slow}, D^R_{slow}) + p_{CH_2} S^R_{CH_2}(Q,\omega) + bk]$$

$$S_{fast}(Q,\omega, D^T_{fast}, \tau^T_{fast}, D^R_{fast}) = S^T_{fast}(Q,\omega) \otimes S^R_{fast}(Q,\omega)$$

Translational motion will be described with jump diffusion model diffusion:

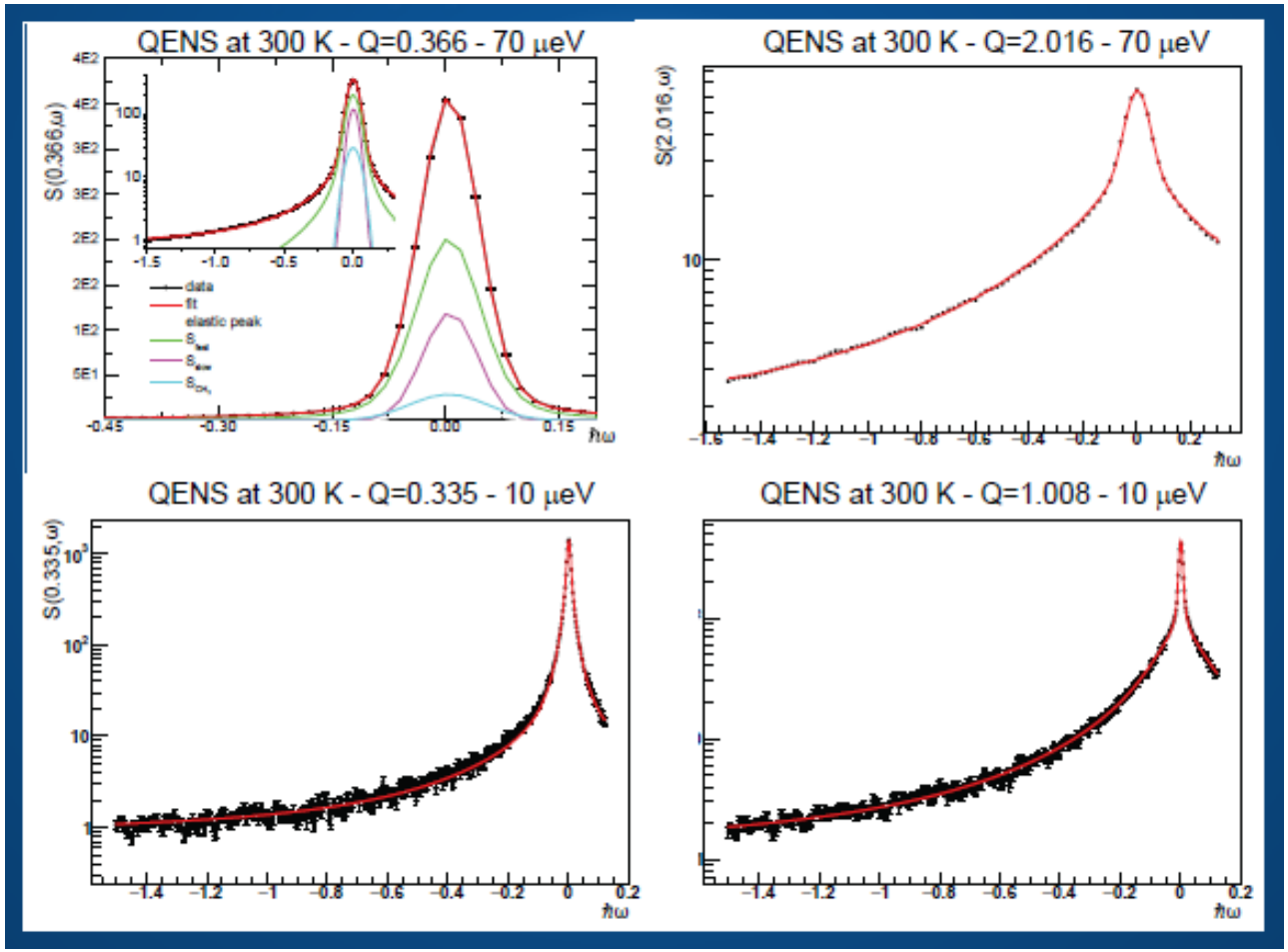
$$S^T_{fast}(Q,\omega) = \frac{\Gamma_{fast}}{\omega^2 + \Gamma^2_{fast}}, \qquad \Gamma_{fast} \propto \frac{D^T_{fast} Q^2}{1 + \tau_{fast} D^T_{fast} Q^2}$$

Rotational motion will be described with continuous rotational diffusion on a circle where the radius a=0.98 Å (bond length for O-H):

$$S^R_{fast}(Q,\omega) = j_0^2(Qa)\delta(\omega) + \sum_{t=1}^{3} j_t^2(Qa) \frac{t(t+1)D_R}{1 + (t(t+1)D_R)^2}$$

Simultaneous fitting of spectra measured at all Q values and at 2 different instrumental resolutions

A typical fit



Some results

$$S(Q,\omega) \propto R(Q,\omega) \otimes [f\delta(\omega) + p_{fast} S_{fast}(Q,\omega, D^T_{fast}, \tau^T_{fast}, D^R_{fast}) + p_{slow} S_{slow}(Q,\omega, D^T_{slow}, \tau^T_{slow}, D^R_{slow}) + p_{CH_2} S^R_{CH_2}(Q,\omega) + bk]$$

Natali model - MpFit- 26 spectra - 5 and 10 Å

	Yeast in PSB		G9L in PBS		E. Coli 300 K	
	value	error	value	error	value	error
f %	8.23	0.08	6.52	0.11	8.37	0.05
P_1 %	65.00	0.10	81.0	0.2	65.80	0.07
D_1[cm^2/s]	2.033E-05	7E-08	2.16E-05	1.2E-07	2.002E-05	5E-08
τ_1[ps]	1.66	0.02	0.90	0.03	0.883	0.012
r[A]	0.98	-	0.98	-	0.98	-
D_{1r}[1/s]	1.98E-05	na	1.98E-05	na	1.98E-05	na
P_2 %	17.72	0.13	6.5	0.2	14.40	0.08
D_2[cm^2/s]	2.17E-06	3E-08	2.74E-06	1.7E-07	2.95E-06	4E-08
τ_2[ps]	33.9	1.1	60	4	30.0	0.9
r[A]	0.98	-	0.98	-	0.98	-
D_{2r}[1/s]	4.3E-06	na	9.88E-06	na	9.88E-06	na
Γ[meV]	0.273	0.003	0.118	0.002	0.1702	0.0007
P_3 %	9.0	0.3	6.0	0.6	11.4	0.2
χ^2_{rid}	0.87		0.53		1.72	

G9-L tumoral cells show a higher water mobility with respect to normal cells