Dynamical Transition of Protein-Hydration Water

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Thin layers of water on biomolecular and other nanostructured surfaces can be supercooled to temperatures not accessible with bulk water. Chen *et al.* [Proc. Natl. Acad. Sci. U.S.A. **103**, 9012 (2006)] suggested that anomalies near 220 K observed by quasielastic neutron scattering can be explained by a hidden critical point of bulk water. Based on more sensitive measurements of water on perdeuterated phycocyanin, using the new neutron backscattering spectrometer SPHERES, and an improved data analysis, we present results that show no sign of such a fragile-to-strong transition. The inflection of the elastic intensity at 220 K has a dynamic origin that is compatible with a calorimetric glass transition at 170 K. The temperature dependence of the relaxation times is highly sensitive to data evaluation; it can be brought into perfect agreement with the results of other techniques, without any anomaly.

In contrast to bulk water, protein-hydration water can be supercooled down to a glass transition at $T_g \simeq 170$ K. Near T_g translational degrees of freedom arrest, which induces discontinuities in the specific heat and the thermal expansion coefficient of the hydration water [1–4]. Because of the dynamic nature of the glass transition, freezing of microscopic degrees of freedom can already be observed far above T_g.

The *protein dynamic transition* is an abrupt onset of atomic displacements on the microscopic length and time scale probed by quasielastic neutron scattering (QENS). First observed 20 years ago in hydrated myoglobin and lysozyme at $T_\Delta \simeq 240$ K [5], it is now known to be a generic property of hydrated proteins, while it is absent in dehydrated systems. It is therefore related to the dynamics of the hydration shell.

In QENS, mean squared dispacements $\langle \delta x^2 \rangle$ are deduced from the elastic scattering intensity $S(q, 0)$. Because of the finite spectrometer resolution (FWHM $2\Delta\omega$), one actually measures $S(q, |\omega| \lesssim \Delta\omega)$. Full spectral measurements show that the anomalous decrease of the central peak is compensated by increasing inelastic wings [5,6]. These effects have been interpreted as precursors of the glass transition. Since QENS probes structural relaxation at $\Delta\omega^{-1} \simeq 100$ ps, while the calorimetric T_g refers to a time scale of 100 s, it is natural that T_Δ is located far above T_g: the protein dynamic transition is the microscopic manifestation of the glass transition in the hydration shell. The time-scale dependence of T_Δ also explains its variation with viscosity [7–9].

Recently, these views have been challenged by the suggestion that there might be a time-scale independent tran- sition. Support came mainly from QENS experiments on the backscattering spectrometer HFBS at NIST. In hydrated lysozyme, DNA and RNA, a kink was found not only in $S(q, |\omega| \lesssim \Delta\omega)$, but also in the α relaxation time $\langle\tau\rangle$ deduced from full spectra $S(q, \omega)$ [10–13]. This change of $\langle\tau\rangle(T)$ from high-T super-Arrhenius to low-T Arrhenius behavior at $T_L \simeq 220$ K has been interpreted as a *fragile-to-strong-transition (FST)* from the high density (HDL) to the less fluid low density phase (LDL) of supercooled water [14]. In this view, a qualitative change in the dynamics occurs when the so-called Widom line is crossed, which extrapolates the phase boundary beyond the conjectured critical point [15,16].

Similar behavior was found for confined water in various nonbiological environments [[17,18] and references therein]; T_L was mostly located between 215 and 228 K. There were, however, two remarkable exceptions: making a substrate hydrophobic lowered T_L by 35 K [19]; using another neutron spectrometer with wider dynamic range lowered T_L by about 10 to 20 K [20]. Critics objected that relaxation times measured by other spectroscopic techniques show no anomaly at T_L [21–23].

In this Letter we will argue that the changing temperature dependence of $\log(\langle\tau\rangle)$ is likely to be an artifact of the data analysis, and that the kink in the elastic intensity also admits a conventional, *dynamic* interpretation. We will refer to the hydration shell of different proteins. If the anomalies observed around T_L were due to an intrinsic property of water, then the choice of the protein should not matter.

To improve the empirical base, we measured the motion of protein-hydration water with enhanced sensitivity, using

a new neutron spectrometer and a fully deuterated protein.
We chose deuterated C-phycocyanin (D-CPC), a light
harvesting, blue copper protein. Deuteration drastically
reduces scattering from the protein so that we become
more sensitive to weak quasielastic scattering from the
hydration water. CPC has been investigated by neutron
scattering before [24–28]. The sample was purified from
a preparation of Crespi [28,29] with nearly 99% deutera-
tion. The absorbance ratio $A_{620\,nm}/A_{280\,nm} \simeq 7.3$ is above
the analytic-grade value of 5 [30]. The integrity of the
secondary structure was ascertained by CD spectroscopy.
The protein was dialyzed against distilled water, freeze
dried, and rehydrated to $h = 0.3$ g/g [27], the same degree
of hydatrion as in Ref. [10].

    QENS was performed on 500 mg of hydrated D-CPC
with the new backscattering spectrometer SPHERES at
FRM II [31]. It has a resolution (FWHM) of 0.62 μeV
(HFBS 0.85 μeV), high flux, and an outstanding signal-to-
noise ratio of 1000:1. Scattering wave numbers q range
from 0.2 to 1.8 Å^{-1}. We chose about the same energy
range ± 10 μeV as customary at HFBS. Spectra at tem-
peratures T between 100 and 300 K were collected for 6 to
12 h. An empty cell measurement was subtracted and
spectra were normalized to a vanadium standard. The
analysis was performed with the program FRIDA [32].

    Figure 1 displays selected spectra in the relevant tem-
perature range. The logarithmic intensity scale emphasizes
both the broad, asymmetric wing of the instrumental reso-
lution function, unavoidable in a crystal spectrometer, and
the quasielastic scattering. At least two distinct compo-
nents of the scattering function $S(q, \omega)$ can be observed:
(i) An elastic component that has the shape of the instru-
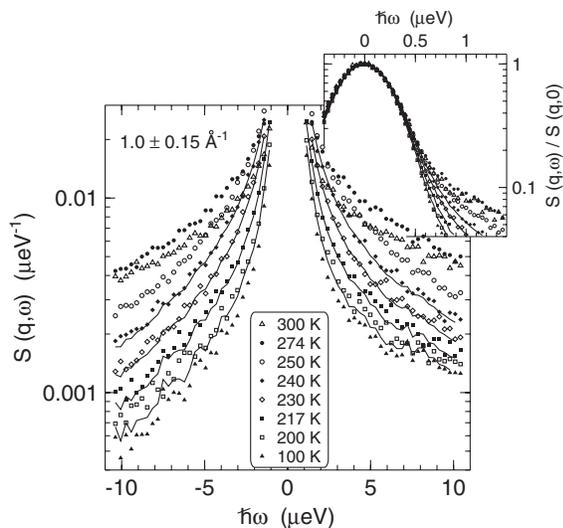mental resolution function $R_q(\omega)$. It is mainly due to the

coherent structure factor of the protein (as evidenced by
strong small-angle scattering for $q \ll 1$ Å^{-1} [27]), as well
as to a protein incoherent term mainly due to the ex-
changed protons (25%). Also immobilized water near
charged groups contributes to this component. (ii) A qua-
sielastic component that appears above about 170 K. It is
mainly due to motion of adsorbed H_2O molecules.

    To start with a model-free data characterization, we
examined the T dependence of the integrated inelastic
intensity for different ω intervals, preferentially on the
$\omega < 0$ side where the resolution wing is weaker. Between
180 and 230 K, the inelastic intensity begins to rise
strongly. There is no sharp kink; the temperature of
maximum inflection depends on the ω range and on q
[Fig. 2(a)]. This is a first indication for a dynamic phe-
nomenon rather than a hidden phase transition: the q and T
dependence is compatible with a smooth onset of transla-
tional diffusion. The elastic intensity in compensation
shows a steplike decrease at 220 K in parallel with data
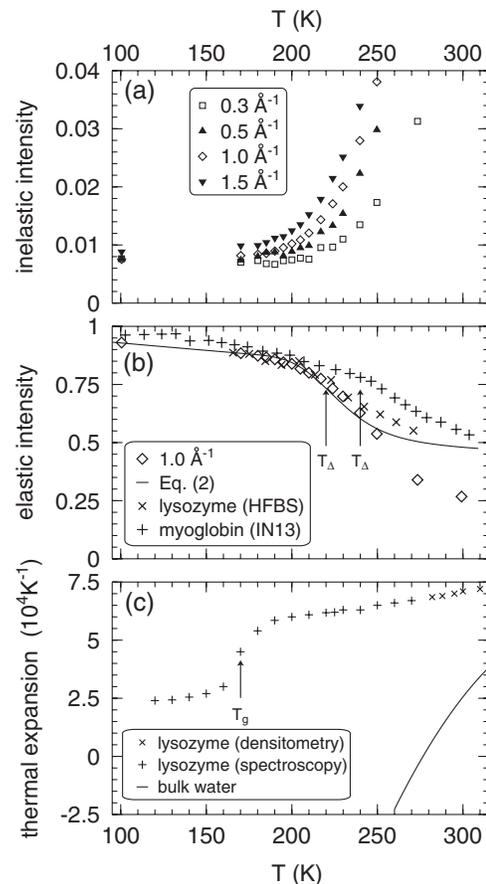obtained with lysozyme. This suggests a common transi-

FIG. 1.   Neutron scattering spectra of H_2O on D-CPC at $q =$ 1.0 $\pm$ 0.15 Å^{-1}. Note the truncated logarithmic scale. Solid lines are fits with (1), convoluted with the instrumental resolution (sample data at 100 K).—Inset: central peak, normalized to the value at $\omega = 0$.

FIG. 2.   (a) Scattering intensity integrated over $\hbar\omega < -3$ μeV. (b) Elastic scattering intensity $S(q, 0)$ and result of (2), compared to literature data [5,10]. (c) Thermal expansion coefficient α_P of water in hydrated lysozyme powder ($+$, spectroscopic measure-ment [3]), of hydration water in lysozyme solution ($\times$, densi-tometry, [36]), of bulk water (line).

tion temperature of both hydrated proteins [Fig. 2(b)]. In
the following, we concentrate on $q = 1.0 \pm 0.15$ Å^{-1}.

To analyze the spectra, we use the same model as
Chen *et al.* [10], consisting of an elastic line and a
Kohlrausch-Williams-Watts function (KWW, Fourier
transform of a stretched exponential [33]):

$$S_{\text{th}}(q, \omega) = f_q\left\{a_1\delta(\omega) + a_2\int_{-\infty}^{\infty}\frac{dt}{2\pi}e^{i\omega t - (|t|/\tau)^\beta}\right\}. \quad (1)$$

The Debye-Waller factor $f_q = \exp(-\langle\delta x^2\rangle q^2)$ accounts
for phonon scattering outside our energy window. The
mean squared displacement $\langle\delta x^2\rangle$ is assumed to be linear
in T; from 100 and 170 K: $\partial\langle\delta x^2\rangle/\partial T = 7 \times 10^{-4}$ Å^2/K.
β was fixed to 0.5 as in the analysis of Chen *et al.*

Equation (1) can fit the data sets for $T \leq 240$ K, hereby
covering the temperature range of the proposed transition.
At higher T, the data start to deviate from Eq. (1): addi-
tional quasielastic scattering, likely due to relaxational or
fast anharmonic motion of the protein, is not included in
this bimodal model.

In a first attempt to extract the relaxation times, we have
used a standard fitting procedure for the data evaluation.
Fitting the quasielastic wings for $T < 240$ K, we are in the
regime $\omega\tau \gg 1$, where the KWW function goes in first
order with $(\omega\tau)^{-1-\beta}$. In such a power-law regime, the fit
parameters amplitude and time-scale τ are degenerate.
Therefore, the 240 K data were used to determine the
relative contribution of the water $a_2/(a_1 + a_2)$ to 0.42
which was then used as input for the other temperatures.

However, this approach neglects a severe limitation of
the standard fitting procedure for narrow lines: When fit-
ting the central peak, the convolution of S_{th} with the
experimental resolution $R_q(\omega)$ becomes nontrivial for $T \lesssim$
220 K where the peak in the KWW function becomes so
sharp (compared to the experimental energy binning) that
the straightforward calculation $S_{\text{th}}(q, \omega) \otimes R_q(\omega)$ as a
Riemann sum yields a wrong amplitude a_1. Because of
the mentioned degeneracy of amplitude and time scale, this
results in a distorted $\langle\tau\rangle$. We have therefore repeated the fits
with an improved fitting procedure, neglecting the errone-
ous a_1 and fixing directly a_2 at 0.42.

Figure 3 shows an Arrhenius plot of the mean relaxation
time $\langle\tau\rangle = \int dt \exp(-t/\tau)^\beta = \beta^{-1}\Gamma(\beta^{-1})\tau$ resulting
from both fitting procedures. As can be seen, the tempera-
ture dependence of the relaxation times obtained from the
standard fitting procedure resembles the results of Chen
et al. This trend is however not observed any more in the
results of the improved fitting procedure which agree well
with the structural (α-)relaxation times of water on other
proteins taken from literature (also shown). There is a good
agreement with high-T myoglobin data measured by
QENS over a wider energy range than covered here [8]
and for $T \lesssim 220$ K with both, NMR [34] and dielectric
spectroscopy [4] which show a smooth increase without
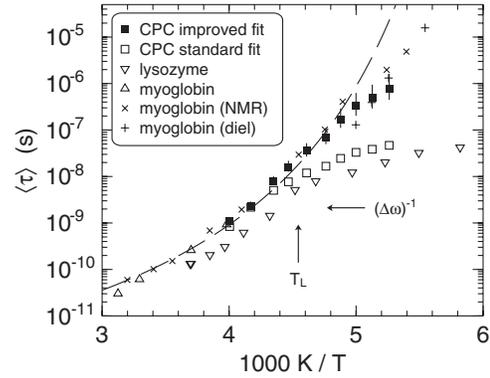inflection at 220 K. The curvature of $\langle\tau\rangle$ in the Arrhenius

FIG. 3. Average relaxation times $\langle\tau\rangle$ versus reciprocal tem-
perature for hydration water on different proteins: (■):
c-phycocyanin with improved fitting procedure and (□): with
a standard fitting procedure. Error bars were estimated by vary-
ing β between 0.45 and 0.55. (▽): lysozyme, QENS [10]. (△):
myoglobin, QENS with wide ω range [8]. (×): myoglobin,
NMR [34]. (+): myoglobin, dielectric loss [4], vertical arrow:
conjectured T_L of the FST, horizontal arrow: instrumental reso-
lution $(\Delta\omega)^{-1}$. Dashed line: Vogel-Fulcher fit to the data above
200 K with fixed $T_g = 170$ K, yielding $\langle\tau\rangle_0 = 6.5 \times 10^{-13}$ s,
$T_H = 745$ K, and $T_0 = 147$ K.

plot indicates fragile behavior according to a Vogel-
Fulcher relation, $\langle\tau\rangle = \langle\tau\rangle_0 \exp[T_H/(T - T_0)]$.

For low T, our $\langle\tau\rangle$ exceeds the resolution $\Delta\omega^{-1} \simeq 2$ ns
by some orders of magnitude. Still, for $|\hbar\omega| \gtrsim 2$ μeV
there are clear quasielastic wings that allow to determine
τ within the chosen model with fixed parameters a_2 and β.
Therefore, this analysis can be used to probe the spectral
behavior for the predicted FST discontinuity.

Whereas our first analysis, using the standard fitting
procedure, reproduces the results from hydrated lysozyme
[10]—the basis for the postulated FST at 220 K—we can
rule out any discontinuity at this temperature using the
improved fitting procedure. We suspect therefore that
also the various results backing the FST are affected by
the problems of the standard fitting procedure: In the
power-law regime $\omega\tau \gg 1$, fitted $\langle\tau\rangle$ are meaningful
only if the amplitude a_2 has been fixed at a physically
reasonable value. In the FST reports, the amplitude, if
mentioned at all, is just called a *scaling constant* [17].
The fitted $\langle\tau\rangle(T)$ are therefore possibly distorted by an
uncontrolled drift of $a_2(T)$. This could explain why the
crossover temperature T_L varies with pressure, while the
crossover time appears constant [13].

Below approximately 200 K, the relaxation times ex-
tracted from our improved technique deviate slightly from
the Vogel-Fulcher relation. Being orders of magnitude
above the instrumental resolution, a detailed discussion
of this effect seems inadequate. If it were real, one could
attribute it to the onset of the glass transition which is
known to be about 30 K broad [4,35]. The observed process
would then no longer be the isotropic α relaxation [34].

In contrast to the kink in $\langle\tau\rangle$, the kink in the observed
elastic scattering intensity $S(q, |\omega| \lesssim \Delta\omega)$ is an undisput-

able experimental fact. Figure 2(b) shows that observations at SPHERES (D-CPC) and HFBS (lysozyme [10]) almost coincide below 240 K. The difference at higher T can be explained by our use of deuterated CPC, which reduces the relative contribution of the protein.

The expected elastic scattering is

$$S_{\exp}(q, 0) = \int d\omega S_{\text{th}}(q, \omega) R_q(-\omega)$$
$$= f_q \left\{ a_1 R_q(0) + (1 - a_1) \int_0^\infty \frac{dt}{\pi} e^{-(t/\tau)^\beta} \tilde{R}_q(t) \right\}$$
(2)

with $\tilde{R}_q(t) = \int d\omega \cos(\omega t) R_q(\omega)$. Indeed, the low-$T$ data are perfectly reproduced by Eq. (2) using the values from the Vogel-Fulcher relation for $\langle \tau \rangle$. This confirms the long established explanation of the protein dynamic transition as a time-scale dependent glass transition: When τ^{-1} exceeds the instrumental resolution $\Delta\omega$, then the central part of the KWW spectrum starts to broaden so that $S(q, |\omega| \lesssim \Delta\omega)$ falls below f_q.

This is further corroborated by elastic scattering ($q = 1.4 \text{ Å}^{-1}$) from hydrated myoglobin on the thermal neutron backscattering spectrometer IN13 [5]. Its resolution width $2\hbar\Delta\omega = 8 \mu\text{eV}$ is an order of magnitude larger than that of HFBS or SPHERES, and the kink in $S(q, |\omega| \lesssim \Delta\omega)$ is observed accordingly at a higher temperature, namely, the often reported $T_\Delta \simeq 240$ K.

Previous QENS experiments were motivated by the hope that hydration layers would provide an opportunity to study water in a deeply supercooled state that is not accessible in the bulk. However, thin hydration layers seem to be qualitatively different from bulk water [21]. This view is also supported by literature data of the thermal expansion coefficient α_P of lysozyme hydration water [Fig. 2(c)]. In concentrated solution, α_P is nearly T independent and much larger than the bulk value [36]. In hydrated powder, α_P is estimated from the O-H stretching frequency of water, which varies with the average H bond length. A striking step is found near 170 K, induced by the softening of the O-H–O hydrogen bond network [3]. No anomaly is observed at $T_L \simeq 220$ K.

All observations agree, however, with a glass transition of the hydration shell and a dynamic onset temperature T_Δ, which varies with the probe frequency [Fig. 2(b)]. The different values of α_P for bulk and hydration water could indicate that the protein acts as a patch-breaker [37], suppressing critical fluctuations present in bulk water.

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