Supporting Information

Introduction. In Part 1, we outline steps that lead to an expression for the entropy and size of a cooperative rearranging region based on a generalization of the Adam-Gibbs model (1). In Part 2, we establish bounds on the slope of Debye-Waller factor with respect to temperature. In Part 3, we argue that the negative slope of the Debye-Waller factor may be attributed to the characteristics of the temperature dependence of the excess vibrational entropy of water $S_{\mathrm{exc,vib}}$ for T < 190 K. In Part 4, we address the question of whether the protein-water system at low temperature behaves as a fragile or as a strong liquid.

Part 1. In this part, we derive the equations that give the size of the cooperatively rearranging clusters. We invoke the Adam-Gibbs (AG) idea of a cooperative rearranging region (2). It is defined as that part of a subsystem which is in weak contact with the remaining part of the system, and which is capable of independent rearrangements because of fluctuations of enthalpy (2,3). We assume that the relaxation time for cooperative rearrangements in supercooled water is due to the decrease of its entropy $S(T)$ (4)

$$\tau(T) \approx A \exp - (\Delta\mu s* / k_{\mathrm{B}} T S(T)). \qquad \textbf{[1]}$$

Here $s*$ is the "critical" entropy of the rearranging region, $\Delta\mu$ is the potential energy barrier per molecule, A has units of time and is weakly temperature dependent, and k_{B} is the Boltzmann constant. Observe that Eq. 1 is a generalization of the AG model that includes configurational and vibrational contributions to the entropy of the liquid.

Let us introduce the logarithmic shift factor defined as the logarithm of the ratio of the relaxation time $\tau(T)$ at temperature T and that at reference temperature T_S

$$log \frac{\tau(T)}{\tau(T_S)}. \qquad \textbf{[2]}$$

On substituting Eq. 1 in Eq. 2, the logarithmic shift factor is written in the form of the William, Landel, and Ferry (WLF) equation

$$-log\, a_T = \frac{a_1(T - T_S)}{a_2 + (T - T_S)}, \qquad \textbf{[3]}$$

where the WLF coefficients $a_1(T_S)$ and $a_2(T)$ are found to be

$$a_1(T_s) = \frac{\Delta\mu}{2.303 k_\mathrm{B} T_s F(T_s)}$$

$$a_2(T) = \frac{T_S F(T_S)}{1 + F(T_S) + B(T, T_S, F(T))}. \qquad \textbf{[4]}$$

Here, $F(T) = S(T)/s*$ and $B(T, T_S, F(T))$ is a function of $F(T)$ and the reference temperature (4).

Since $a_2(T)$ is known to be weakly temperature dependent (2, 5) compared with $a_1(T_S)$, we approximate $a_2(T) \approx a_2(T_S)$. This leads to an explicit expression for the temperature dependence of the entropy in glass-forming liquids

$$S(T) - S(T_S) = s * (\frac{T - T_S}{T}). \qquad \textbf{[5]}$$

The Vogel-Fulcher-Tamman (VTF) form accurately describes the temperature dependence of the relaxation time of water as observed in dielectric experiments

$$\tau(T) = \tau_\mathrm{VTF} \exp - (\frac{B}{T - T_\mathrm{o}}), \qquad \textbf{[6]}$$

where B, T_{o}, and τ_{VTF} are empirical parameters. For example, in case of water, existing diffusivity data indicates that T_{o} = 119 K (6). Let us choose the reference temperature as T_o. Because the configurational entropy of water is small for temperatures below 150 K, we assume that configurational entropy vanishes at the VFT T_{o} (5). Then, the critical size of cooperative rearranging region is found to be

$$z^* = \frac{N_A s^*}{S} = \frac{T}{T - T_{\mathrm{o}}} \qquad \textbf{[7a]}$$

The potential barrier for cooperative rearrangements is given by

$$\Delta\mu / k_{\mathrm{B}} \approx C'_1 2.303 T_{\mathrm{g}} (\frac{T_{\mathrm{g}} - T_{\mathrm{o}}}{T_{\mathrm{o}}}), \qquad \textbf{[7b]}$$

where C'_1 is the WLF coefficient with T_{g} as the glass transition temperature.

Part 2. In this part, we establish the details for the bounds on the slope of Debye-Waller factor with respect to temperature.

To explain the characteristics of the Boson peak in glass forming liquids at low temperatures, Sokolov *et al.* (7) suggested expressing the temperature dependence of the relation time of cooperative rearrangements in terms of the mean-squared displacement of the atoms from their equilibrium positions

$$\tau(T) = \tau_u \exp(\frac{u_{\mathrm{o}}^2}{3\langle u^2 \rangle}), \qquad \textbf{[8]}$$

where τ_u and u_{o}^2 are weakly temperature dependent, and $\langle u^2 \rangle$ is the mean-squared displacement of the atoms in supercooled liquid in excess of that of the crystalline solid.

Eq. 8 can be derived by considering the hopping dynamics in configurational space together with an estimate of the average activated barriers between free energy minima in the glassy state (8).

On differentiating Eq. 8 with respect to temperature and making use of Eq. 1, we obtain an explicit expression for the slope of the mean-squared displacement in terms of experimental measurable quantities

$$\frac{d\langle u^2\rangle}{dT} = \frac{\langle r_o^2\rangle/3}{(\ln(A/\tau_u)+\frac{C}{TS})^2}(\frac{C}{T^2S})(1+\frac{\Delta C_P}{S}), \qquad \textbf{[9]}$$

where $C = \frac{\Delta\mu}{R} N_A s*$. The quantity in brackets in Eq. 9 is proportional to the fragility index m (1); observe that the slope is positive. One anticipates that Eq. 9 is valid for temperatures approximately larger than 150 K because the assumed validity of the Adam-Gibbs and VFT equation for dielectric relaxation time is in this range.

At lower temperatures, the surface of the protein induces substantial perturbations on the structure of water. We account for the perturbations by introducing an effective force constant k (9)

$$k(T,\Gamma) = \frac{k_B T}{\langle(\Delta E(T,\Gamma)^2\rangle}. \qquad \textbf{[10]}$$

Here, Γ is an appropriate order parameter that describes the reaction coordinate in configuration space. The temperature dependence of the relaxation time is

$$\tau(T) = \tau_k \exp(\frac{k_o}{k}). \qquad \textbf{[11]}$$

We choose the reference system such that k_{o} given by Eq. 11 is at $T = T_{\mathrm{g}}$, and τ_k is weakly dependent on temperature. From Eqs. 8 and 11 we obtain

$$\frac{d\left\langle u^2\right\rangle}{dT} = -\frac{\left\langle r_{\mathrm{o}}^2\right\rangle/3}{(\ln(\tau_k/\tau_u)+\dfrac{k_{\mathrm{o}}}{k})^2}\left(\frac{C_{\mathrm{p}}(T)V(T)F(T)}{C_{\mathrm{p}}(T_{\mathrm{g}})V(T_{\mathrm{g}})F(T_{\mathrm{g}})T_{\mathrm{g}}}\right)\left(1+T\Delta\alpha+\frac{T}{C_{\mathrm{P}}}\frac{dC_{\mathrm{P}}}{dT}+\frac{T}{F}\frac{dF}{dT}\right) \qquad \textbf{[12]}$$

where $F(T) = \left\langle(\Delta\Gamma)^2\right\rangle$ is the mean-squared fluctuation of the order parameter, $\Delta\alpha$ is the change in thermal expansion between the liquid and the glass, and we have assumed that $\left\langle(\Delta E(T,\Gamma)^2\right\rangle \approx \left\langle(\Delta E(T)^2\right\rangle\left\langle(\Delta\Gamma)^2\right\rangle$. The second term in brackets is positive. In the last term in brackets, the contributions from the first three quantities are positive, whereas the temperature variation of F may be negative. If this term is small in comparison with the remaining terms in the brackets, then there is an overall positive contribution. Under these circumstances, the slope of the Debye-Waller factor is negative.

In the temperature range of interest, configuration freezing of entropy is expected, and the excess entropy satisfies the inequality $S_2 < S(T) < S_1$. Continuity of excess free energy at 153 and at 236 K requires S_1 to be less than 2.0 J / (mol • K) (5,10). S_2 is the excess residual entropy and is approximately 0.4 J/(mol • K) (5,10); it is defined as the residual entropy of glassy water minus the residual entropy of hexagonal ice. The quantity $(1+\dfrac{\Delta C_{\mathrm{P}}}{S})$ is related to the fragility index m/m_{min}, where m_{min} is the minimum fragility, which is usually taken to be around 16 (11). Enthalpy measurements indicate a value for m around 39 for water (12). The excess configurational entropy at 150 K is approximated by $S_{\mathrm{exc}}(150\mathrm{K})$ - this is assessed to be 3.8 J/(mol • K) (5). Because

$z*(150\text{K})$ = 4.3, one obtains an estimate of $N_A s* = z*S_{\text{conf}}$ = 18.48 J/(mol • K). The barrier to cooperative rearrangements and the constant C is found to be $\Delta\mu/R \approx 447$ K and 8,269 J/mol, respectively.

Part 3. In this section, we show that the negative slope of the DW factor can be attributed to the temperature dependence of the excess vibrational entropy of water $S_{\text{exc,vib}}$ for $T < 190$ K.

The excess vibrational entropy of water is defined as

$$S_{\text{exc,vib}}(T) = S_{\text{vib,water}}(T) - S_{\text{vib,ice}}(T), \qquad \textbf{[13]}$$

where, $S_{\text{vib,water}}(T) = S_{\text{water}}(T) - S_{\text{conf,water}}(T)$. The total entropy of supercooled water is defined as $S_{\text{water}}(T) = S_{\text{ice}}(T) - S_{\text{exc}}(T)$. The excess entropy $S_{\text{exc}}(T)$ at temperature T is that of supercooled water above that of hexagonal ice. This quantity is evaluated along the same lines as Johari, namely, by choosing the C_{p} path for which the excess entropy at 150 K is 0.47 J/(mol • K) (5). Thus, this approach is valid for temperatures $T > 150$ K. The entropy of hexagonal ice $S_{\text{ice}}(T)$ is calculated from knowledge of the residual entropy of hexagonal ice, which is 3.37 J/mol and the integral of $C_{\text{p,ice}} d\ln T$ (13).

The configuration entropy of water is obtained from Eq. 5

$$S_{\text{conf,water}}(T) = N_A s*(\frac{T - T_{\text{o}}}{T}) \qquad \textbf{[14]}$$

As shown earlier, $z*(150K)$ = 4.3 whereas $N_A s* = z*S_{\text{conf}}$ = 18.48 J/(mol • K). In agreement with Johari, the excess vibrational entropy of water over ice as a function of temperature becomes negative around 190 K (5).

Part 4. In this section, we address the question of whether the protein-water system at low temperature behaves as a fragile or as a strong liquid. By a strong liquid we mean a glass-forming liquid that resists change of structure, and for which the temperature dependence of relaxation time is Arrhenius (14). On the other hand, the structure of fragile liquids is assumed to fluctuate over a wide range of length scales, and for which the temperature dependence of relaxation is strongly non-Arrhenius (14). Based on these definitions, it is reasonable to conclude that, because there is a distinct change of slope in the mean-squared displacement around 180 K, it would imply that protein-water system behaves like fragile glass formers.

Does the above picture hold for the protein-water system near its apparent glass transition temperature T_g =136 K? To address this question, we take into account the literature data for the self-diffusion coefficient D of water for temperatures larger than 245 K, together with recent measurements by Smith and Kay in the temperature range 150-157 K (15). The extended diffusion data is fitted to the Vogel-Fulcher-Tamman (VFT) form $D(T) = D_o \exp-(\frac{B}{T - T_o})$. The parameters of the VFT fit are B = 864 ± 100, T_o = 119 ±3 K, and D_o = 3.06 x 10^{-3} cm^2/s (6). The relaxation time as obtained by dielectric experiments is deduced from the Stokes-Einstein relation $\tau(T) = l^2/6D$, where l is the distance that a water molecule hops; it is approximately 0.33 nm from geometrical considerations (5,16).

At or near T_g, VFT data predict, in agreement with conventional line of thought, an apparent Arrhenius temperature dependence of relaxation time; this result is so because the configurational entropy is almost constant (17). Thus, it appears that water behaves as a strong liquid near T_g. However, a plot of the relaxation time vs. inverse temperature. shows that the relaxation time data for $T < 200$ K appears to be Arrhenius. A signature of Arrhenius behavior, according to the classification scheme of strong and fragile liquids (14), is that a plot of $log\,\tau(T)$ versus $1/T$ is a straight line whose intercept should be 10^{-14} s; this is not the case (5). Further, based on the diffusivity data, the relaxation time near the glass transition temperature $T_g = 136$ K is not 100 s. The fragility index based on enthalpy and dielectric measurements also differs (12). This result underscores the fact that, in glass-forming liquids, the relaxation time as measured by calorimetric, shear, dielectric, or x-ray and neutron scattering techniques may differ (3, 5, 12, 14). To put it differently, protein-water system exhibits fragile or strong characteristics depending on the physical property that is measured and the technique used to probe these degrees of freedom (14).

The argument presented above is for bulk water. But, water in crambin is sequestered. The only relaxation time data for sequestered water come from measurement in various size pores of poly(2-hydroxy-ethyl methacrylate) (5, 18). In this case, one finds the relaxation time to be larger than that for bulk water, reinforcing the idea that supercooled water may not necessarily be strong near the glass transition (5, 18).

References

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