Supplementary Material (ESI) for Soft Matter

Solvent effects on protein fast dynamics: implications for biopreservation

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Materials and Methods

Sample preparation

Hen-egg white lysozyme (Sigma-Aldrich)[1] was dialyzed in deionized water for 3 days at 4 °C, in order to remove salts and then lyophilized. For the dielectric measurements it was then dissolved in glycerol in the presence of water, and then lyophilized a second time to remove the water. The amount of glycerol in the mixture was measured by weighing the sample before and after lyophilization. No significant amount of water remained in the mixtures (~ 0.05 g per 1 g of the dried glycerol-lysozyme mixture). We present the results as LG as the lysozyme- glycerol system and LW for hydrated lysozyme in H_2O (for dielectric measurements) or in D_2O (for neutron scatterings measurements). For the neutron scattering experiments the dialyzed lysozyme was washed in D_2O

[1] Certain commercial equipment, instruments, or materials (or suppliers, or software, ...) are identified in this paper to foster understanding. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

(Sigma-Aldrich) at pD 7.6 for 36 hours to deuterate all exchangeable hydrogen atoms and then lyophilized again. Heavy water, deuterated glycerol (Sigma-Aldrich), and partially deuterated trehalose were used to reduce the scattering signal from the solvent, and probe primarily the dynamic response from the H-atoms of protein. According to ^{1}H NMR measurements, the level of deuteration of the trehalose was determined as 63 $\pm$ 3 %. The weight ratios of lysozyme to D_20, glycerol and trehalose were 0.5, 1:1 and 1:1, respectively.

Neutron Scattering Measurements

Neutron scattering measurements were conducted, employing the high-flux back scattering spectrometer (HFBS, NG2) [1] and disk-chopper time of flight spectrometer (DCS, NG4) [2] at the NIST Center for Neutron Research, in Maryland, USA. The two spectrometers cover an energy window between 1ueV and 100 meV. Several temperatures within the range 50 K to 370K were measured for dry and wet lysozyme, lysozyme in glycerol, and in trehalose. Additionally pure trehalose was measured at 370 K. The given Q values ranged from 0.25 Å^{-1} to 1.75 Å^{-1} for HFBS and 0.08 Å^{-1} to 1.46 Å^{-1} for DCS.

Elastic scattering scans were also performed on each sample on HFBS (incoming neutron energies is the same as the final analysed energy, such that only elastic events are counted) with an energy resolution of 1ueV at a rate of 0.7K/min across the aforementioned temperature ranges, in order to estimate the mean-squared displacement, $\langle r^2(T)\rangle$. Due to about 40 times higher incoherent scattering cross-section of H-atoms than D-atoms, the obtained neutron scattering spectra mostly reflects the motions of non-exchangeable H-atoms in lysozyme upon being mixed with deuterated solvents. The motions of H-atoms represent internal dynamics of protein because the H-atoms are well distributed over all regions of protein.

We note that, owing to the partial deuteration of the trehalose, about 33% of the scattering signal arises from the non-exchangeable H-atoms of the trehalose in the 1:1 lysozyme-trehalose mixture. So the total scattering function needs to be corrected for spectral contribution of trehalose

to extract the scattering feature of lysozyme (detailed below). The total scattering intensity of all samples was less than 10 % so as to prevent multiple scattering by using sealed aluminium annular cells. A vanadium standard was used to measure the resolution of the spectrometer. All neutron scattering data were normalised to monitor intensity, corrected for background scattering and empty cell scattering. The data was analysed using DAVE program provided by NIST [3]. The neutron scattering spectra were normalized to the mass of lysozyme in each mixture which is proportional to the number of H-atoms. Unless stated otherwise, the Q range used for analysis of the energy-resolved spectra is above 0.5 $Å^{-1}$ to avoid possible multiple-scattering effect on quasielastic scattering.

Neutron Scattering Data Analysis

The non-exchangeable H-atoms in the partially deuterated trehalose will add to the scattering of the lysozyme-trehalose sample. This needs to be accounted for in the data analysis. Based on 63 % deuteration of trehalose determined by ^{1}H NMR measurement, we calculate that 33 % of the total incoherent scattering is attributed to H-atoms of trehalose, in sampling 1:1 weight ratio of trehalose to lysozyme (Eq.1).

$$\frac{S_{Trehalose}}{S_{Total}} = \frac{\frac{N_{H-Trehalose}}{M_{Trehalose}}}{\frac{N_{H-Trehalose}}{M_{Trehalose}} + \frac{N_{H-Lysozyme}}{M_{Lysozyme}}} = \frac{\frac{22\times 0.37}{342}}{\frac{22\times 0.37}{342} + \frac{695}{14360}} = 0.33 \qquad (1)$$

where, $N_{H\text{-}Trehalose}$: the number of H-atoms in one trehalose molecule; $N_{H\text{-}Lysozyme}$: the number of H-atoms in one lysozyme molecule; $M_{Trehalose}$: the molecular weight of one trehalose molecule; $M_{Lysozyme}$: the molecular weight of one lysozyme molecule.

Therefore, the data must be corrected to this. The mean-squared displacement of lysozyme in trehalose for example is corrected as :

$$< r^2(T) >_{LT-corrected} = \frac{< r^2(T) >_{LT-measured} - 0.33 < r^2(T) >_{Trehalose}}{0.67} + C\,. \qquad (2)$$

Here, $<r^2(T)>_{Trehalose}$ is mean-squared displacement of pure trehalose and C is a zero-point normalization factor. The final $<r^2(T)>_{LT\text{-}corrected}$ was normalized by the magnitude of $<r^2(T)>$ of dry lysozyme at 5 K , using C. A similar correction procedure has been applied to the frequency-dependent spectral intensities. All the data shown in the main manuscript are corrected accordingly.

$<r^2(T)>$ is calculated using the elastic scattering intensity measured at the HFBS spectrometer in Gaussian approximation:

$$<r^2(T)> = -3Q^{-2} \ln[I_{el}(Q,T)/I_{el}(Q,10K)] \qquad (3)$$

The $I_{el}(Q,T)$ is an elastic incoherent neutron scattering intensity at a particular Q and T. The Q range chosen for the fits is from 0.25 to 1.00 Å^{-1}.

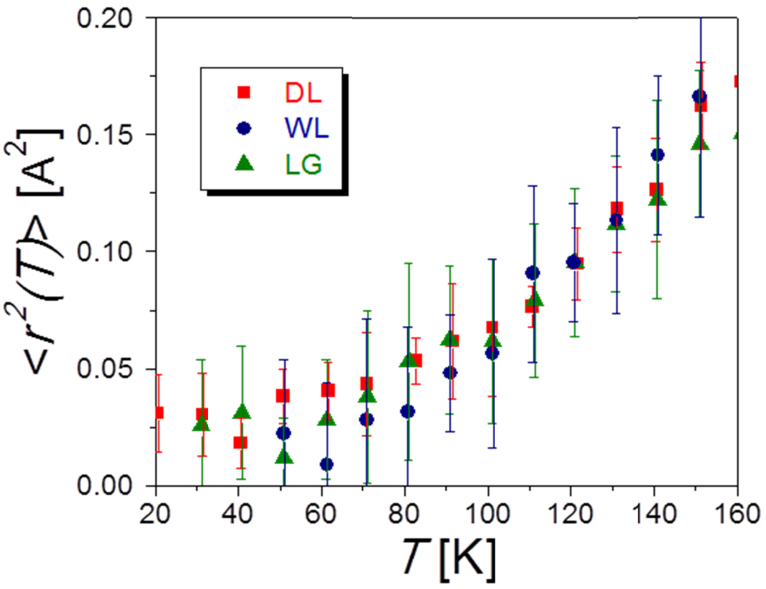

Fig.S1. MSD of dry and hydrated lysozyme (DL and WL) and lysozyme in glycerol (LG) and in trehalose (LT) at low temperatures. Change of the slope at around T=100K is usually ascribed to onset of methyl group rotation on the time scale of the spectrometer (1-2 ns). The values of MSD of all samples in this temperature range remain similar within the error bars which suggests that methyl group dynamics remain a dominant process and are largely unaffected by the type or presence of solvent.

The scattering intensities are collected on both aforementioned spectrometers as a function of energy exchanged between the sample and the neutrons (E=ħω), as the dynamic structure factor, S(Q, E*)*, where ω is the angular frequency [5]. In order to directly compare neutron scattering data to dielectric spectroscopy data, ε”(ν), we prefer to plot the dynamic structure factor as a function of linear frequency ν, remembering that $\omega=2\pi\nu$. We then represent the data as the dynamic susceptibility, $\chi''(\nu)$, which helps understand in more detail the features of energetic distributions of the relaxation modes. The susceptibility relates to S(Q,ν) via the Bose occupation number, $n_B(\nu)=1/[\exp(h\nu/kT)-1]$, such that

$$\chi''_{NS}(Q,\nu) \propto S(Q,\nu)/n_B(\nu). \qquad (4)$$

It has been well known that the data analysis by presenting $\chi''(\nu)$ offers the below-mentioned advantages in characterizing strongly stretched relaxations. At first, relaxation modes are envisioned as distinct peaks. The estimate of characteristic time, τ, is feasible by converting the frequency corresponding to the spectral peak, according to $\nu=(2\pi\tau)^{-1}$; Secondly, the direct comparison with dielectric or mechanical loss is allowed. Thirdly, the frequency dependence of the relaxation modes can be characterized by the power law. Low-frequency and high frequency tail of a relaxation can be described as $\chi''(\nu) \propto \nu^{a}$ and $\chi''(\nu) \propto \nu^{-b}$ ($0 < a$ and $b \leq 1$).. When $a = b = 1$, the relaxation mode is a homogeneous relaxation that can be represented by a single Lorentzian function.

However, it is well known that structural dynamics including segmental relaxation of polymeric molecules appears to be strongly stretched over 2 orders of magnitude. It has been generally accepted that the strongly stretched relaxations can be described well by the Cole-Cole distribution function (Eq. 5).

$$\chi''(\omega) = \chi_0 \frac{(\omega\tau)^{1-\alpha}cos^{\alpha\pi/2}}{1+2(\omega\tau)^{1-\alpha}sin^{\alpha\pi/2}+(\omega\tau)^{2-2\alpha}} \qquad (5)$$

The characteristic frequency (or time, $\nu=(2\pi\tau)^{-1}$) of the slow relaxation for WL was estimated from the analysis of the χ" peak with the Cole-Cole distribution function, assuming that it is frequency-symmetric. The ν value of LG was also assessed by extrapolating the peak maximum from high-frequency tail of the χ" (Figure 4 of the manuscript), assuming that the spectral shape and amplitude are invariable for temperatures and solvents.

Dielectric Spectroscopy Measurements

The dielectric relaxation spectra (DS) were measured in the frequency range of 10^{-2}Hz to 10^{+7}Hz and at temperatures between 150 K to 315 K using a Novocontrol Concept 80 system. Different temperature ranges were selected for each mixture to optimize the accessible observation window for different relaxation processes. Samples were placed in a parallel-plate capacitor and a Teflon spacer. To ensure no water loss or absorption during the measurements an additional external Teflon ring was used. No water losss was confirmed by weighing the samples before and after the measurements. Only hydrated and lysozyme-glycerol systems were investigated.

Real (ε') and imaginary (ε ") parts of the complex dielectric permittivity, ε *= ε '+i ε " were measured and the Kramers-Kronig relationship, $\varepsilon'' \propto -\frac{2}{\pi}\frac{d\varepsilon'}{d\log\nu}$, used to identify the existence of relaxation processes and their characteristic frequency. Mode details analysis was carried out using the WinFIT software from Novocontrol and the spectra were fit by up to three Cole-Cole (CC) distribution functions plus a conductivity tail such that:

$$\varepsilon^{*} = \varepsilon_{\infty} + \sum_{j}\frac{(\Delta\varepsilon)_j}{1+(i\omega\tau_j)^{\alpha_j}} - i\frac{\sigma}{\varepsilon_0\omega^{s}}, j=1,2,3 \qquad (6)$$

where $\omega=2\pi\nu$ is the angular frequency, τ_j is the relaxation time, $\Delta\varepsilon_j$ is the dielectric strength, and αj is the stretching parameter of the j relaxation process, σ represents the amplitude of the

conductivity tail, and s is the exponent describing the tail slope. Details of data analysis are presented in ref [4].

Relaxation times are plotted in the following figure for the dry protein, hydrated and glycerol-protein systems. As mentioned in the text, dielectric spectroscopy finds two relaxations in the latter two systems, both of which are coupled to the solvent relaxations. As we can see there is are no visible changes in the temperature dependence at the dynamical transition temperatures of ~ 200K (for water) and ~ 270 K (for glycerol). In the case of the hydrated system, the slow process of neutron relaxation that is involved with local structural motions can be associated with the so-called 'main' dielectric process. We found even 'slower' process observed in DS.

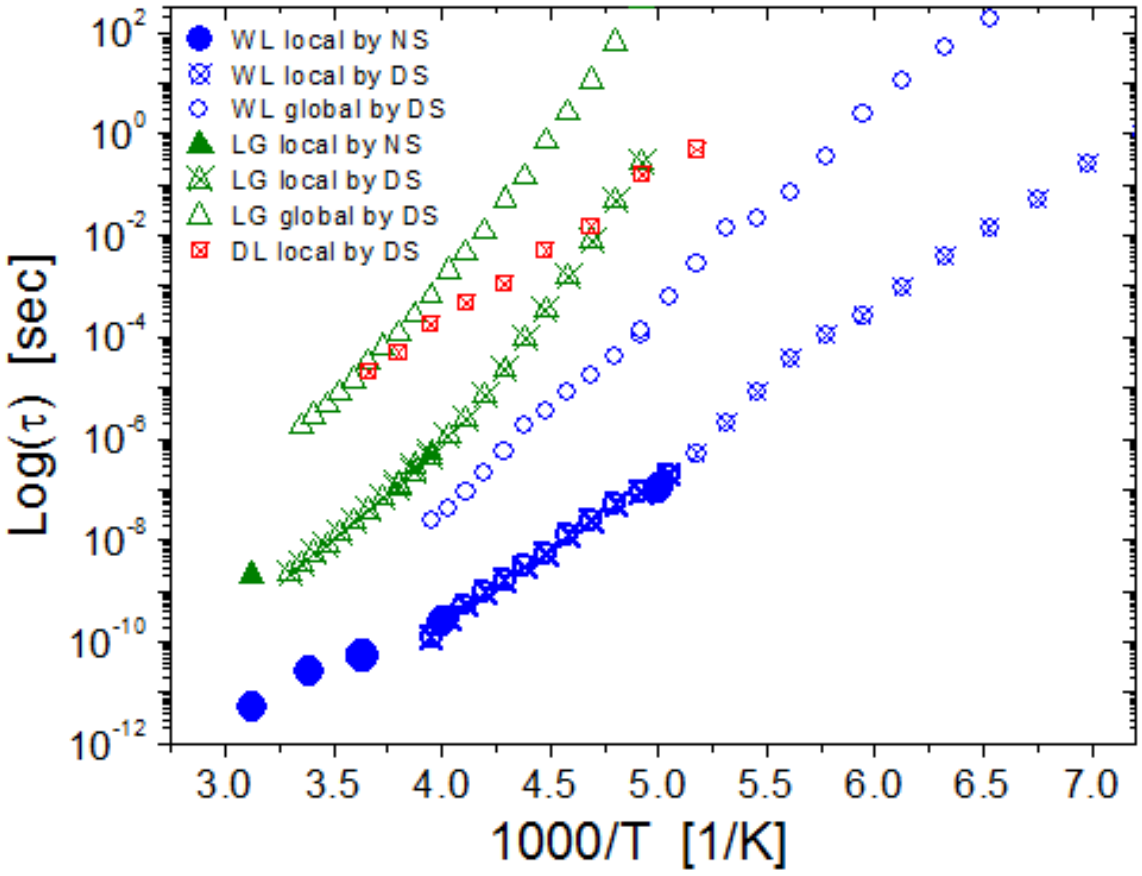

Fig.S2. Temperature dependence of the characteristic relaxation times. Structural relaxation in hydrated lysozyme estimated from neutron scattering measurements. Local motion: slow process obtained from NS and main mode from DS. Global motion: slower mode obtained from DS.

References

1. A. Meyer, R. M. Dimeo, P. M .Gehring, and D. A. Neumann, *Rev Sci Instrum,* 2003, **74**, 2759;
2. J.R.D. Copley and J.C. Cook, *Chem. Phys*., 2003, **292**, 477;
3. R.T. Azuah, L.R. Kneller, Y. Qiu, P.L.W. Tregenna-Piggott, C.M. Brown, J.R.D. Copley, and R.M. Dimeo, *J. Res. Natl. Inst. Stan. Technol.,* 2009, **114**, 341;
4. S Khodadadi, S Pawlus and AP Sokolov , *J Phys Chem B*, 2009, **112**, 14273
5. G. L Squires, *Introduction to thermal neutron scattering,* Cambridge University Press (1978)