Viscosity Dependent Protein Dynamics

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Supplementary Material

A. Determination of Sample Viscosity

In this study, the viscosity of the system was modulated by adding increasing amounts of fructose to a small volume of aqueous protein solutions. To determine the viscosity of a very small volume (~10 μL) of the resulting fructose-protein-water mixture, we developed a spectroscopic method that relates the near infrared (NIR) spectrum of water to the solution viscosity. The NIR spectrum in the ~5000 cm^{-1} region is sensitive to the relative concentrations of both water and fructose and thus has a strong correlation with the solvent viscosity.

Figure S1a shows the NIR absorption spectrum of several fructose-water mixtures of increasing fructose concentration. The peak at ~5150 cm^{-1} has been previously assigned to a water combination band between the intramolecular water stretch and deformation modes(1) and its area is an accurate measure of the water content in the sample. The water combination band at ~5150 cm^{-1} is an attractive candidate for determining the sample water content because it occurs in a relatively clear region of the NIR spectrum and involves only intramolecular water modes, making it relatively insensitive to the detailed nature of the solution composition.

As the sugar concentration increases, there is a decrease in the water combination band at ~5150 cm^{-1} and an increase in the band intensity centered at ~4750 cm^{-1}. The peak at 4750 cm^{-1} has been previously assigned to a combination band involving the sugar OH modes(2, 3). The sugar combination band has some spectral overlap with the water combination band and must be removed to facilitate an accurate analysis of the water content and sample viscosity. The sugar combination band was fit to a Gaussian line shape and the resulting Gaussian component was subtracted from the total NIR spectrum.

The intensity of remaining water peak was integrated between 4700-5400 cm^{-1} and plotted as a function of the measured viscosity in figure 1b. The data is presented on a semi-log plot and the relative water content is normalized to a value of one at 1 cP (no sugar present). The data for all fructose-water and fructose-water-protein solutions was fit to a function of the form

$$\log(\eta) = 8.35\, e^{-\frac{A}{2.35}} \qquad \text{(S1)}$$

where η is the viscosity in centipoise and A is the area under the water overtone band (normalized to a value of one at 1 cP). The solid line is the fit of equation S1 and the dashed lines represent 95% confidence limits of the fit. The fit reproduces the data very accurately for viscosities in the ~10cp-100,000 cP region and can be used to spectroscopically measure the viscosity of solutions with unknown sugar-water compositions. The fit represented by equation S1 is a phenomenological description of

the data with the fewest adjustable parameters over the viscosity range examined in this study.

To verify that addition of protein in concentrations similar to those used for the vibrational echo experiments did not have any significant affect in the NIR water combination band spectral region, we prepared solutions of fructose-water-protein, where the protein was MbCO, HbCO, or horse heart *Cyt. c*. The viscosity of these protein-sugar solutions was measured by spinning-disk rheometric methods. The solutions were analyzed analogously to the fructose-water mixtures and it was determined that the protein does not affect the area under the water combination band at ~5150 cm^{-1} (see figure 1b). The same phenomenological functional form (equation S1) could be used to describe the protein-fructose-water mixtures. The protein contributes relatively little to the NIR spectrum in the 4600-5400cm^{-1} region and the NIR spectrum did not depend on the identity of the protein. Therefore, the area under the water band at 5150 cm^{-1} serves as a quantitative handle on the sample viscosity from 1 to ~100,000 cP.

Spectroscopically determining the viscosity of a solution based on the water content is advantageous when working with very small volumes of material, where standard rheometric methods are not applicable. However, care should be taken when applying the results of figure S1b and equation S1 at temperatures other then 24^{o}C, as the NIR spectrum of water is known to be temperature dependent.(1) The transition dipole moments of water, and thus the area under the band at 5150 cm^{-1} are also sensitive to degree of hydrogen bonding available to H_2O, so the calibration curve presented in this work should be verified if other co-additives are used to affect the sample viscosity. Nonetheless, this is a simple and straightforward approach to measure the viscosity of ~10 μl samples with a very high dynamic range.

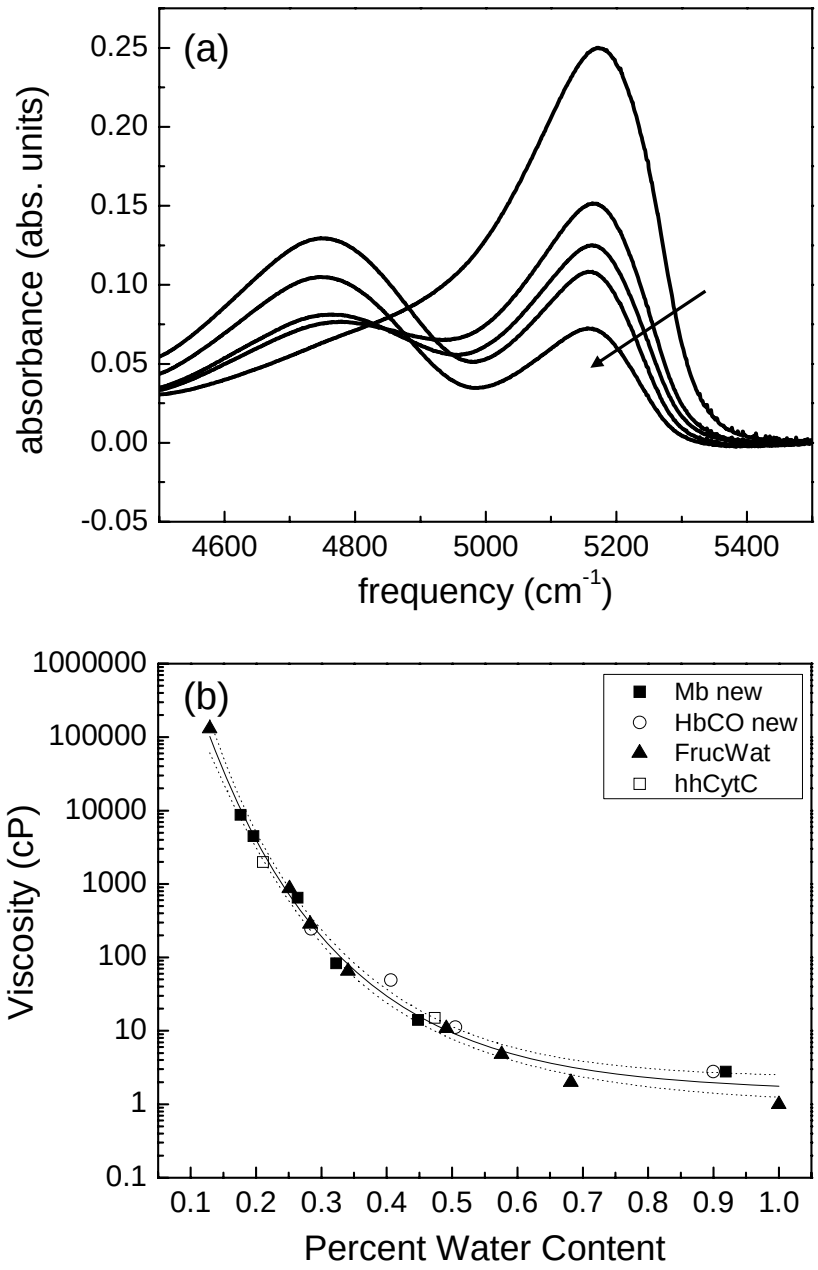

Figure S1. (a) The NIR absorption spectrum of fructose-water solutions between ~1 – 100,000 cP. The NIR combination band of water is centered at ~5150 cm^{-1} and that of the sugar OH modes is centered at ~4750 cm^{-1}. The arrow indicates direction of increasing viscosity. (b) A calibration curve was constructed to relate the solution viscosity to the relative water composition, as measured by the area under the NIR peak of the water combination band. The data is presented on a semi-log scale. The area under the NIR water band was normalized to one at 1 cP. Several proteins were added to the fructose-water mixtures to determine that the NIR band was water was insensitive to the presence or detailed nature of the protein. Filled squares, MbCO; open squares, M61A; open circles, HbCO; filled triangles, fructose-water only. The solid curve is an exponential fit to the data and dotted lined indicate the 95% confidence limits of the curve. See the text for additional model details.

B. Viscosity Dependent Frequency Correlation Functions

The frequency-frequency correlation functions (FFCF) for all four proteins were obtained by fitting the spectrally dispersed vibrational echo decays as a function of wavelength and T_w at each viscosity. A bi-exponential FFCF of the form displayed below was necessary to simultaneously reproduce the full dynamic T_w dependence and the linear absorption spectrum, as described in section IIC of the manuscript. The results of the fits are presented in the tables below:

Table S1: H64V FFCF Parameters

Viscosity (cP)	Δ_0 (cm-1)	T_2^* (ps)[a]	Δ_2 (cm-1)	τ_2 (ps)	T_c^R (ps)
2.8	2.8		2.3	3.3	8.3
41	3.4		1.8	5.9	11.4
670	3.4	—[b]	1.4	6.0	18.8
6,400	3.7		1.2	6.2	24.2
28,400	4.0		0.92	9. 7	51.0
∞ (film)	4.6	12.4	0.56	11	

Table S2: M61A FFCF Parameters

Viscosity (cP)	Δ_0 (cm-1)	T_2^* (ps)[a]	Δ_2 (cm-1)	τ_2 (ps)	T_c^R (ps)
3	4.7		3.6	2.3	3.7
10.1	5.2		3.1	2.5	4.1
660	5.1	—[b]	2.3	3.7	6.3
1,400	5.0		1.6	3.9	9.7
13,400	4.7		1.4	5.1	10.0
∞ (film)	4.6	11.6	0.87	6.5	

Table S3: HbCO Main Band FFCF Parameters

Viscosity (cP)	Δ_0 (cm-1)	T_2^* (ps)[a]	Δ_2 (cm-1)	τ_2 (ps)	T_c^R (ps)
2.8	2.5		2.5	3.2	8.0
13	2.8		2.1	4.3	9.8
120	3.1	—[b]	1.8	4.3	11.5
800	3.1		1.6	3.7	12.8
4,100	3.5		1.2	3.3	17.6
20,000	3.5		1.1	4.5	20.6
∞ (film)	3.7	9.65	0.24	6.8	

Table S4: MbCO A_1 Band FFCF Parameters[c]

viscosity (cP)	Δ_0 (cm-1)	T_2^* (ps)[a]	Δ_2 (cm-1)	τ_2 (ps)	T_c^R (ps)
2.8	2.7		4.0	3.6	4.0
13	2.7		3.6	4.2	4.9
130	4.1	—[b]	2.3	4.6	9.0
2,200	4.1		1.6	3.3	15
3,900	3.8		2.0	4.2	16
130,000	4.2		1.0	4.4	28
∞ (film)	4.4	11.0	0.57	4.8	

[a] Δ_1 and τ_1 can not be determined independently because this term of the FFCF is motionally narrowed. Instead, we report a pure dephasing time, T_2^*. See Section IIC of the manuscript for further information.

[b] The motionally narrowed FFCF component was independent of solvent viscosity and was set to be the same as that of the protein-glass.

[c] Strong spectral overlap between the A_1 and A_3 states of MbCO precluded an accurate determination of the A_3 FFCF and increased the uncertainty in the FFCF parameters for the A_1 state.

C. Representative Quality of Nonlinear Response Function Fits to the Data

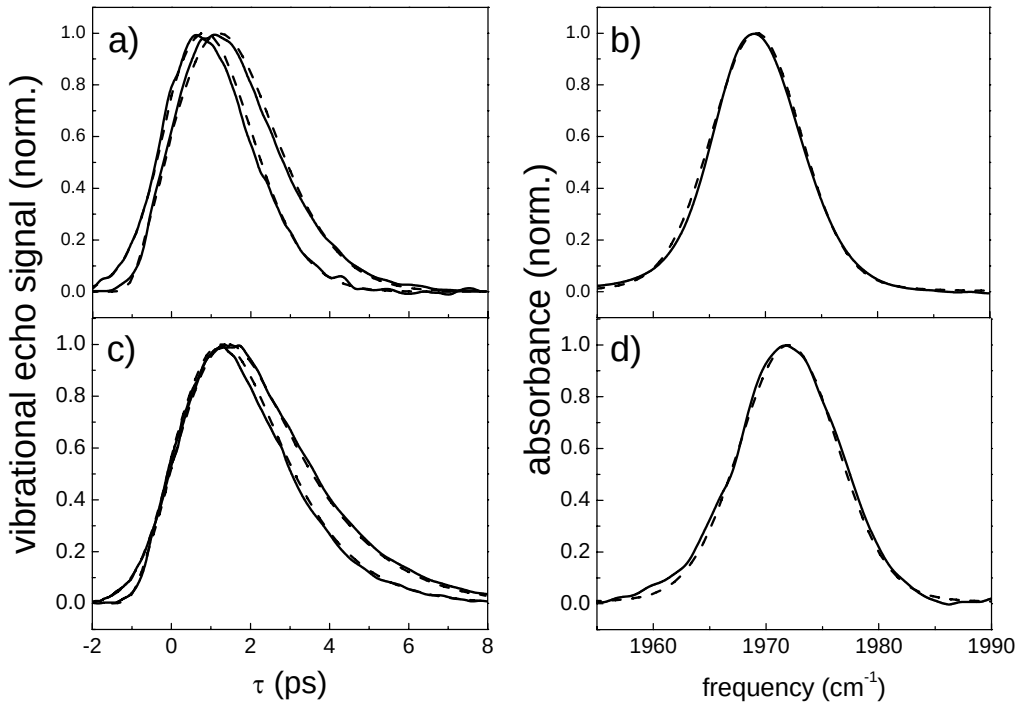

Figure S2. Representative quality of fits for H64V vibrational echo and FT-IR data as a function of viscosity. At each viscosity, a family of vibrational echo decays at different T_ws, as well as the FT-IR spectrum, were fit simultaneously to a single FFCF of the form described in equation 1 in the paper. Panels (a) and (c) present the vibrational echo data and corresponding fit for T_w=0.5 ps and 16 ps for a low viscosity (41 cP) and high viscosity (20,000 cP) samples, respectively. The T_w=0.5 ps data has a larger peak shift and a slower vibrational echo decay. Panels (b) and (d) present the FT-IR data and corresponding fits for H64V at the low and high viscosity, respectively. The data is shown in solid line, and fits are dashed curves. The same excellent quality of fit was achieved at all T_ws and viscosities for all proteins in this study.

References

1. Bertie, J., and Z. Lan. 1996. Infrared intensities of liquids .20. The intensity of the OH stretching band of liquid water revisited, and the best current values of the optical constants of H2O(1) at 25 degrees C between 15,000 and 1 cm(-1). Applied Spectroscopy 50:1047-1057.
2. Jung, B., M. J. McShane, S. Rastegar, and G. L. Cote. 1998. Effects of temperature on the near-infrared spectroscopic measurement of glucose. Proc. of SPIE 3253:41-47.
3. Hazen, K. H., M. A. Arnold, and G. W. Small. 1994. Temperature-insensitive near-infrared spectroscopic measurement of glucose in aqueous solution. Applied Spectroscopy 48:477-483.