

Site-specific hydration dynamics of globular proteins and the role of constrained water in solvent exchange with amphiphilic cosolvents

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S1. Sample preparation

Reaction to Form HEWL-RC

The protein labeled complexes (HEWL-RC) and (HuLys-RC) were synthesized according to previously published methods. The proteins, hen egg white lysozyme and human lysozyme, were purchased from Sigma Aldrich (BioUltra, > 98%). No further purification steps were taken prior to performing the reaction. Each reaction was performed by combining the lysozyme protein (HEWL and HuLys) (2 mg/mL) with a 1:1 molar ratio of tricarbonylchloro(glycinato) ruthenium(II) in H₂O or D₂O (Sigma) and stirring at room temperature for 1 hour. The resulting product was then passed through a desalting column (GE Healthcare, PD-10 Disposable Desalting Column) to remove residual tricarbonylchloro(glycinato) ruthenium (II). This reaction was carried out fresh on the day the spectroscopic measurements were made, and no labeled protein was stored to be used at a later date.¹

Tricarbonylchloro(glycinato)ruthenium (II) (CORM-3), which is the labeling molecule, was synthesized from tricarbonyldichlororuthenium (II) dimer (CORM-2, Sigma). In a flask under nitrogen, tricarbonyldichlororuthenium (II) dimer (0.150g) was mixed with glycine (0.040g), followed by the addition of methanol (65 mL) and sodium ethoxide (0.035 g). This reaction was stirred at room temperature for 18 hours. The methanol was then removed under pressure, and the yellow residue was redissolved in THF. Hexane was then used to precipitate the CORM-3 product. The yellow solution was evaporated down to give a pale yellow solid which was stored in closed vials at -20°C.²

S2. FTIR of HEWL-RC in D₂O/TFE

The linear FTIR spectra of HEWL-RC ranging from the amide vibrational modes to the CO vibrational modes in each solvent composition are shown in Figure S1a. Each region is also highlighted to show the consistency of the spectrum in both the amide (b) and CO (c) region upon addition of TFE. It has been well documented, however, that the structure of lysozyme becomes unstable at concentrations of TFE higher than 10%. This suggests that the changes occurring in the protein are not significant enough to cause changes in the FTIR spectrum. Likewise, the preferential solvation of the CO chromophores by TFE, and the subsequent re-solvation by water following the protein partial denaturation, are not evident in the FTIR spectrum. The small changes in the background of the FTIR are the result of differences in the background subtraction, which was taken for each solvent mixture.

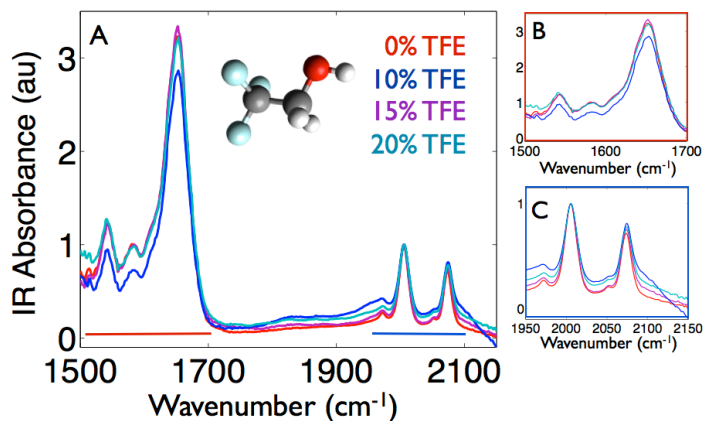


Figure S1. FTIR spectral of HEWL-RC in D₂O/TFE mixtures from 1500-2150 cm⁻¹ (a). The amide vibrational region (b) and the CO (c) vibrational region are highlighted. While there are no significant solvent-effects in either the CO region or the amide region, the ultrafast dynamics reveal that the solvation environment is changing significantly upon the addition of TFE.

S3. Two-Dimensional Infrared Spectroscopy

Mid-IR pulses centered at 2000 cm⁻¹ are generated using a dual stage optical parametric amplification (OPA) and difference frequency generation (DFG) setup pumped by a regeneratively amplified Ti:Sapphire laser. The mid-IR pulses are split into fields E_1 , E_2 , E_3 , and E_{LO} , with respective wavevectors k_1 , k_2 , k_3 and k_{LO} (100 fs, 150 cm⁻¹ band-

width, 400 nJ/pulse) where the first three pulses are focused onto a sample cell in a boxcar geometry to generate the third-order nonlinear signal, and the fourth pulse is used for heterodyne detection of the signal. In our experiments we implement chirped pulse upconversion, where the emitted mid-IR signal and reference local oscillator are mixed in a sum-frequency crystal (5% MgO doped LiNbO₃) with a highly chirped pulse centered at 800 nm and fwhm = 160 ps, to allow detection in the visible spectral region using a 0.5 m grating spectrograph equipped with a silicon CCD camera. The spectrometer provides the detection frequency, and this spectrum is collected as a function of the time delay between the first two pulses, τ_1 . Fourier transformation with respect to τ_1 provides the excitation frequency axis in the 2D-IR spectrum. Population dynamics, such as vibrational relaxation, are obtained by stepping the waiting time (t_2) between the second and third pulses.

S4. Experimental fits to lifetime data

Table S1 shows the fits to the experimental vibrational lifetime data. The data were consistently fit with a single exponential decay, with the exception of the HEWL-RC complex in 10% TFE, which has a significantly longer lifetime. The single exponential decay was used for systems where the relaxation was dominated by water-assisted vibrational relaxation, where the observed lifetime were around or less than 5 ps. In all cases, the vibrational relaxation is competing with intramolecular vibrational redistribution (IVR), which occurs on sub-5 ps timescales. Because of the coincidence of the IVR timescale and the vibrational relaxation timescale being on the same order for systems dominated by water-assisted vibrational relaxation, single exponential functions with a constant offset were found to give the most accurate fits.

$$\varphi(t) = A_1 \exp\left(-\frac{t}{\tau_1}\right) + y_0 \quad (1)$$

Here, τ is the relaxation time constant and y_0 is a constant offset of the function. This functional form, with the constant offset, is often used to account for a slight heating induced bleach that results in the decay not going fully to 0 and is found to accurately represent the relaxation data.³

For the HEWL-RC in 10% TFE system, however, the vibrational lifetime is found to increase significantly due to a dehydrated solvation environment that is comprised of alcohol solvent and the protein. Due to a well defined separation of the IVR decay time and the much slower vibrational relaxation, it was found that a bi-exponential decay was needed to fit the data.

$$\varphi(t) = A_1 \exp\left(-\frac{t}{\tau_1}\right) + A_2 \exp\left(-\frac{t}{\tau_2}\right) + y_0 \quad (2)$$

Here, τ_1 describes the slow vibrational relaxation (on the order or 30 ps) and τ_2 describes the fast IVR component, where the vibrational energy redistributed to the asymmetric mode of the vibrational label. While both of these relaxation mechanisms are present in the other data, the slow vibrational relaxation of HEWL-RC in 10% TFE allows them to be fit separately.

Table S1. Exponential fits to the vibrational lifetimes

HEWL-RC	TFE v/v %	y_0 (ps)	A_1	τ_1 (ps)	A_2	τ_2 (ps)
	0	0.09 ± 0.01	0.92 ± 0.03	3.87 ± 0.29	----	----
	10	0.20 ± 0.05	0.64 ± 0.09	32.3 ± 4.2	0.35 ± 0.04	1.05 ± 0.42
	15	0.18 ± 0.02	0.85 ± 0.05	3.99 ± 0.60	----	----
	20	0.17 ± 0.02	0.84 ± 0.03	4.41 ± 0.48	----	----

HuLys-RC	TFE v/v%	γ_0 (ps)	A_1	τ (ps)	A_2	τ_2 (ps)
	0	0.11 ± 0.02	0.84 ± 0.03	4.59 ± 0.32	-----	-----
	10	0.06 ± 0.01	0.82 ± 0.02	5.32 ± 0.16	-----	-----
	15	0.08 ± 0.01	0.84 ± 0.04	5.63 ± 0.24	-----	-----
	20	0.08 ± 0.02	0.89 ± 0.05	5.12 ± 0.38	-----	-----

References

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