Protein structural changes characterized by high-pressure, pulsed field gradient diffusion NMR spectroscopy

Venkatraman Ramanujam, T. Reid Alderson, Iva Pritišanac, Jinfa Ying, and Ad Bax

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, 20892, USA

Supporting Information

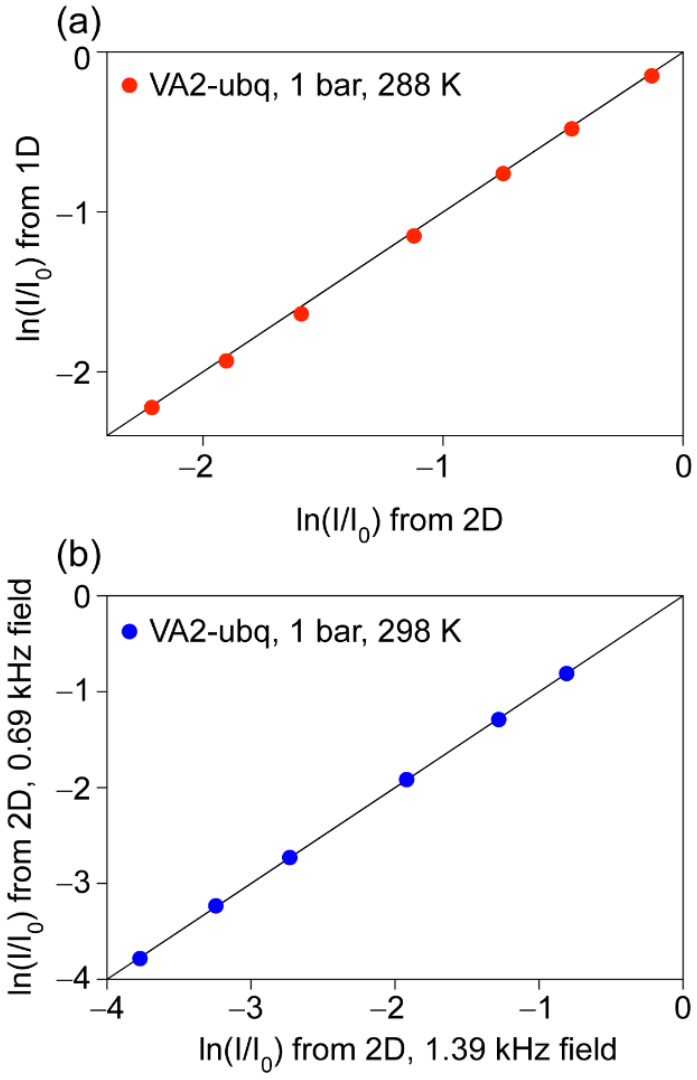

Fig. S1. Comparison of 1D and 2D PFG diffusion results. (**a**) $\ln(I/I_0)$ values determined for VA2-ubiquitin using either a 2D (*x*-axis) or 1D (*y*-axis) PFG NMR diffusion experiment with the diffusion time set to 280 ms and the gradient duration set to 1.5 ms. (**b**) Lowering the ^{15}N decoupling power by a factor of four (1.39 kHz to 0.69 kHz RF field strength; WALTZ-16 decoupling scheme) in the 2D PFG NMR diffusion experiment had no impact on the measured signal attenuation, indicating negligible sample heating from ^{15}N decoupling, and no detectable effect of heating-induced convection on translational diffusion. To determine I/I_0 ratios, the intensities of N = 58 resonances were summed (I) and divided by the summed intensities of these resonances in the plane with the weakest gradient strength (I_0).

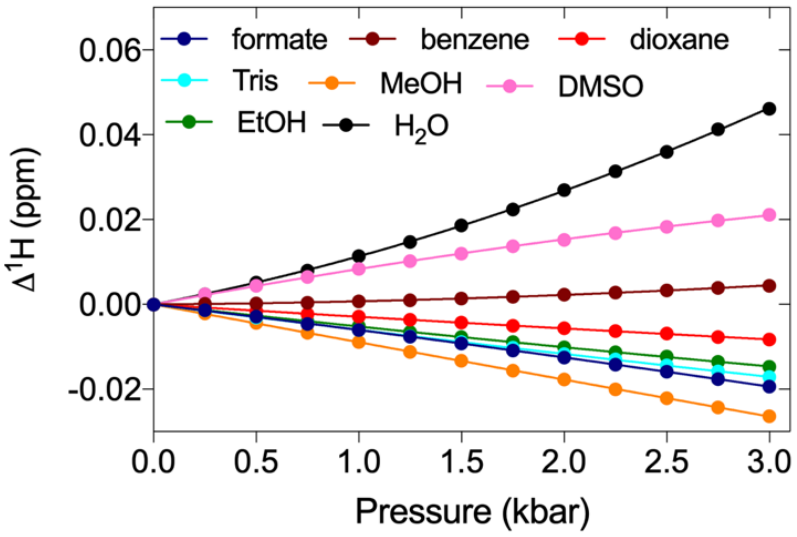

Fig. S2. ^{1}H chemical shift *versus* pressure for water, dioxane, benzene, formate, methylene group of Tris and the methyl groups of methanol, ethanol, and dimethyl sulfoxide (DMSO). Solid lines represent best-fits to a second order polynomial (Table S1). Resonances are referenced to internal DSS, with the probe strongly detuned to avoid radiation damping effects on the apparent chemical shift of water [1]. All data were collected at 700 MHz, 293 K.

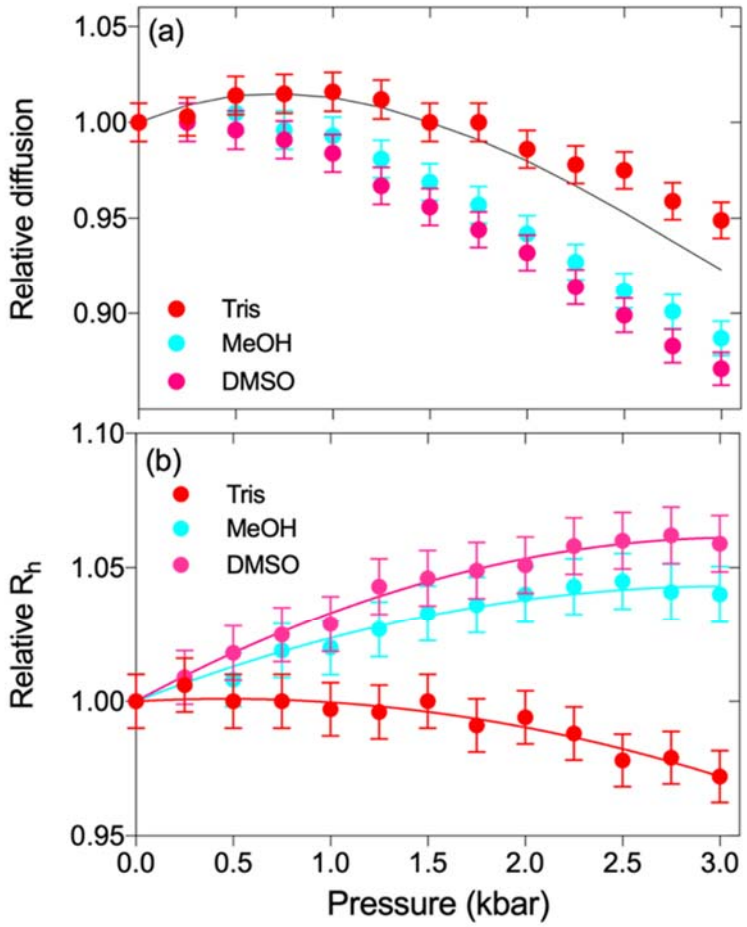

Fig. S3. Pressure-dependence of the translational diffusion and the effective hydration radii for Tris, methanol and DMSO. The solid black line corresponds to the inverse of the water viscosity, normalized at 1 bar. Fits to the I/I_0 intensity ratios are shown in Fig. S4.

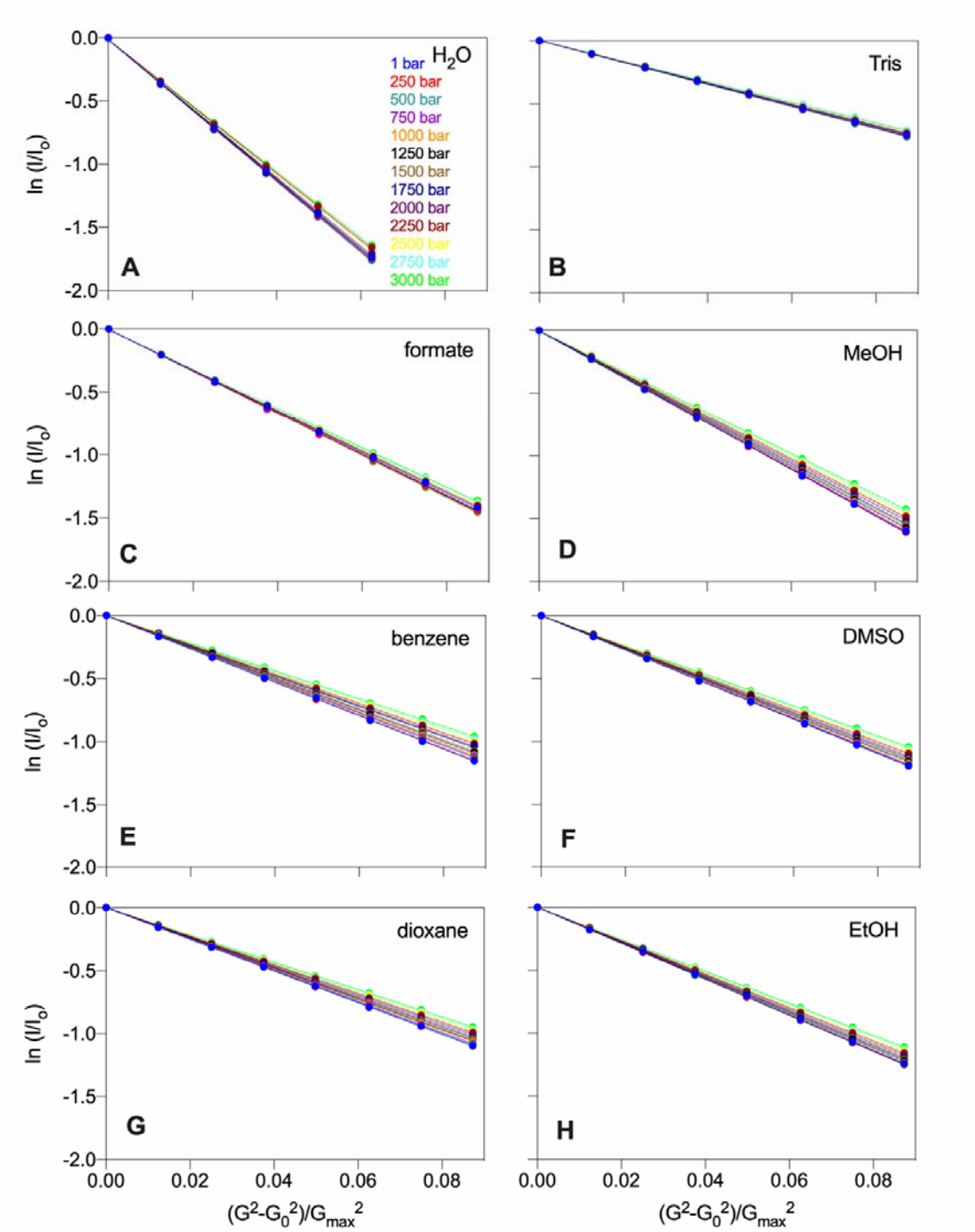

Fig. S4. PFG NMR data used to determine the solution viscosity and pressure-induced changes to the hydration radii of tracer molecules. **(A-H)** Natural logarithm of the intensity ratio (I/I_0) and best-fit lines to $y = \exp(-Ax)$. Note that the x-axis depicts the gradient strength squared, where G_0 is the weakest gradient (2.2 G/cm) and G_{max} = 33.4 G/cm. Panels A-H show data measured at different pressures for eight different molecules, including H_2O, Tris, formate, methanol, benzene, DMSO, dioxane, and ethanol. I is the peak intensity recorded in a given spectrum and I_0 is the peak intensity at the weakest gradient strength.

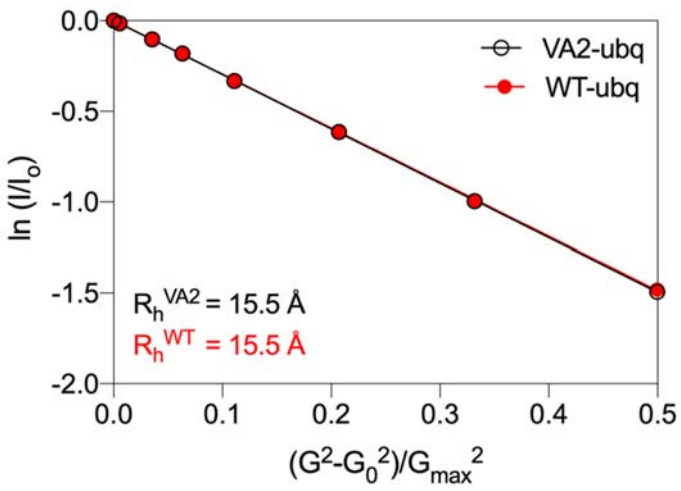

Fig. S5 | Comparison of the signal intensity attenuation resulting from diffusion of VA2- (black) and WT-ubiquitin (red) for measurements carried out at 1 bar, 1.3 mM protein, pH 6.5, 288 K. The R_h values obtained from comparison to dioxane are listed in the lower left corner. 1D BPP-LED measurements were recorded at 288 K and 1 bar. G is the applied gradient strength, G_0 = 2.2 G/cm; G_{max} = 33.4 G/cm.

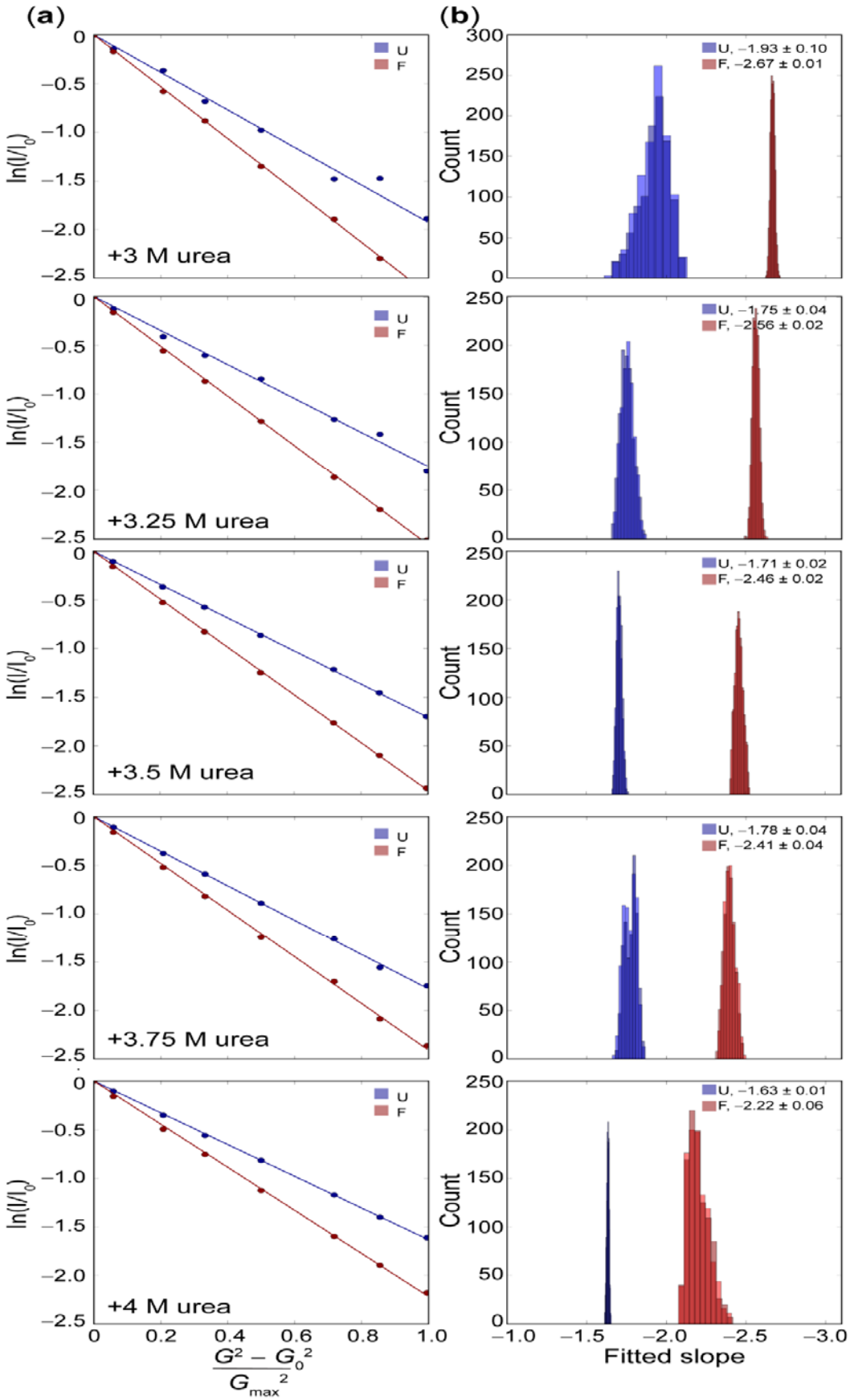
(a)
(b)
U
F
+3 M urea
+3.25 M urea
+3.5 M urea
+3.75 M urea
+4 M urea
ln(I/I0)
Count
U, −1.93 ± 0.10
F, −2.67 ± 0.01
U, −1.75 ± 0.04
F, −2.56 ± 0.02
U, −1.71 ± 0.02
F, −2.46 ± 0.02
U, −1.78 ± 0.04
F, −2.41 ± 0.04
U, −1.63 ± 0.01
F, −2.22 ± 0.06
G² − G0² / Gmax²
Fitted slope

Fig. S6. 2D PFG NMR translational diffusion measurements of 1.3 mm VA2-ubiquitin in the presence of increasing urea concentrations. Interleaved 2D PFG HSQC NMR experiments were used to simultaneously measure the translational diffusion of the unfolded and folded states in the same sample (Fig. 5, main text). (**a**) Signal intensity, $\ln(I/I_0)$, for both the folded (red) and unfolded (blue) states, as a function of $(G^2-G_0^2)/G_{max}^2$ where I_0 is the intensity in the 2D spectrum with the weakest gradient, G is the encoding gradient strength, G_0 = 2.2 G/cm; G_{max} = 33.4 G/cm. Resonances from each state with signal-to-noise ratios > 10 were selected and pooled, and fit with a single exponential function to the summed intensities at each gradient strength. The solid lines represent best linear fits. (**b**) A jackknife procedure was employed to estimate the errors associated with the fitted slopes in panel (**a**). For each state, resonances were randomly split into two groups, with the intensities from the two groups then separately summed and fit to a straight line, as in panel (**a**). This procedure was repeated 1000 times to generate 2 × 1000 best-fit lines for both the folded and unfolded states. The error was derived by computing the root-mean-square difference between the two sets of best-fit lines and dividing by two. The results are plotted in a histogram format with the two groups depicted for each state in darker and lighter shades of blue (unfolded) and red (folded). Count (y-axis) refers to the number of instances of a given fitted slope (x-axis). The value of the slope derived from fitting all summed intensities is indicated in the upper right. The relative population of unfolded and folded state is depicted in Fig. 5a, main text.

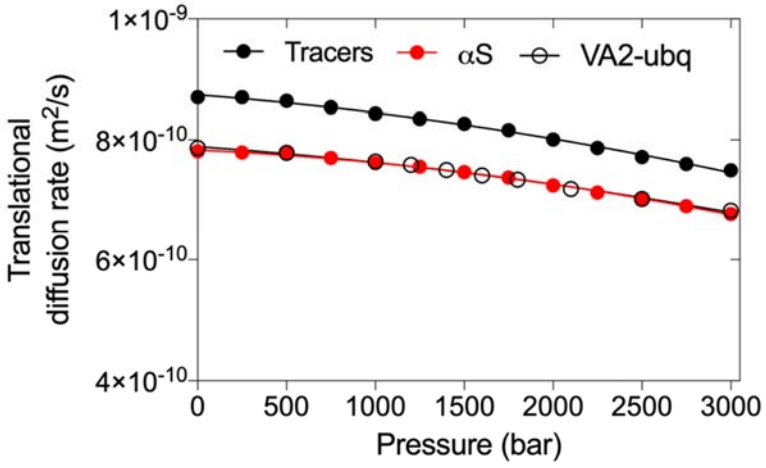

Fig. S7. Translational diffusion rate of dioxane in the samples of tracers (black; 293K), 1.3 mM VA2-ubiquitin (open grey circle; 288K), 0.24 mM α-synuclein (red, 288K).

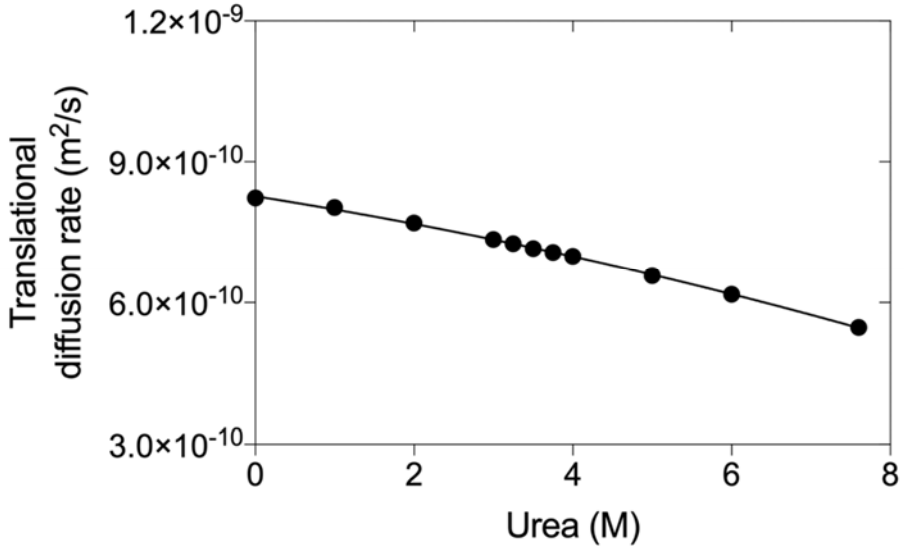

Fig. S8. Translational diffusion rate of dioxane at 288 K as a function of urea concentration, measured on samples used for Fig. 5a, main text.

Table S1. ^{1}H chemical shifts of sodium formate, benzene, water, dioxane, methanol (MeOH), DMSO, and ethanol (EtOH) as a function of hydrostatic pressure at 293 K, pH 6.5. Methylene 1H shifts are listed for Tris and methyl protons for MeOH, EtOH and DMSO. Chemical shifts are relative to the methyl resonance of internal DSS.

P (bar)	Formate (ppm)	Benzene (ppm)	Water (ppm)	Dioxane (ppm)	Tris (ppm)	MeOH (ppm)	DMSO (ppm)	EtOH (ppm)
1	8.4402	7.4298	4.8328	3.7484	3.7206	3.3481	2.7167	1.1713
250	8.4388	7.4299	4.8352	3.7476	3.7192	3.346	2.7191	1.1700
500	8.4373	7.4300	4.8379	3.7468	3.7175	3.3437	2.7211	1.1685
750	8.4356	7.4302	4.8408	3.7461	3.7161	3.3414	2.7232	1.1673
1000	8.4342	7.4305	4.8442	3.7455	3.7146	3.3392	2.7251	1.1662
1250	8.4326	7.4308	4.8475	3.7448	3.7131	3.3369	2.7269	1.1648
1500	8.4310	7.4312	4.8514	3.7441	3.7117	3.3348	2.7287	1.1636
1750	8.4293	7.4316	4.8552	3.7434	3.7104	3.3325	2.7304	1.1624
2000	8.4277	7.4321	4.8598	3.7428	3.7089	3.3304	2.7319	1.1612
2250	8.4260	7.4326	4.8642	3.7421	3.7076	3.3281	2.7335	1.1600
2500	8.4243	7.4331	4.8688	3.7415	3.7062	3.326	2.735	1.1590
2750	8.4226	7.4337	4.8741	3.7407	3.7048	3.3238	2.7365	1.1578
3000	8.4208	7.4342	4.8789	3.7401	3.7035	3.3217	2.7378	1.1566

Table S2. Translational diffusion rates of αS and folded WT-ubiquitin at various pressures, measured at 288 K, pH 6.4.

P (bar)	0.1 mM αS (10^{-11} m^2/s)	0.2 mM αS (10^{-11} m^2/s)	0.5 mM WT-ubiquitin (10^{-11} m^2/s)
1	5.71 ± 0.02	5.51 ± 0.02	11.17 ± 0.04
250	5.80 ± 0.01	5.58 ± 0.02	11.45 ± 0.04
500	5.83 ± 0.01	5.62 ± 0.02	11.58 ± 0.03
750	5.84 ± 0.02	5.62 ± 0.02	11.58 ± 0.04
1000	5.85 ± 0.01	5.65 ± 0.02	11.55 ± 0.03
1250	5.84 ± 0.01	5.61 ± 0.02	11.55 ± 0.04
1500	5.80 ± 0.01	5.60 ± 0.02	11.42 ± 0.03
1750	5.78 ± 0.01	5.58 ± 0.01	11.38 ± 0.03
2000	5.74 ± 0.02	5.52 ± 0.02	11.29 ± 0.04
2250	5.73 ± 0.01	5.50 ± 0.02	11.20 ± 0.03
2500	5.70 ± 0.01	5.46 ± 0.01	10.96 ± 0.04
2750	5.64 ± 0.01	5.41 ± 0.02	10.83 ± 0.04
3000	5.59 ± 0.01	5.38 ± 0.02	10.65 ± 0.04

Reference

1. Torchia, D.A., Slight mistuning of a cryogenic probe significantly perturbs the water H-1 precession frequency. J. Biomol. NMR. 45 (2009) 241-244.