Kuttner et al., Supporting material

Materials and Methods

Viscosity measurements. Macroscopic solution viscosity was measured using a Cannon- Fenske Routine Viscometer 150/I750 at 25 °C in a water bath. Some of the viscosity measurements were done with a rotational viscometer (Haake Roto visco 1, Thermo Electron). Measured values of viscosity for glycerol were in good agreement with CRC published data. The viscosity of glycerol solutions was also assessed from their refractive index, measured using a Fisher Tabletop Refractometer (Fisher Scientific).

Fluorescence correlation spectroscopy. Our fluorescence correlation spectrometer was constructed based on an inverted microscope equipped with a water-immersion objective (UplanApo 60x NA 1.2, Olympus). The sample temperature was maintained at 25(±0.2) °C using a custom-made feedback-looped electrical heater. The sample was illuminated by a 488nm Ar+ ion laser (35 LAP 431, Melles Griot, USA), focused through the objective. Laser power was kept 13 μW to maintain the Gaussian shape of the sampling volume. Fluorescence collected through the objective was filtered by a the dichroic mirror (500 DCLP, Chroma) and a long-pass interference filter (HQ 500 LP, Chroma), before being focused on a 50 μm pinhole to filter out-of-focus light. Fluorescent photons were detected with a single photon counting avalanche photodiode (EG&G SPCM15), and their arrival times at the detector were registered using a National Instruments counting card and stored on the computer. Data analysis was described in the main text.

Some recent experiments suggested that in polymer solutions and within cells an appropriate description of translational dynamics should involve the notion of anomalous diffusion, where the mean-squared displacement is not linear with time but rather proportional to a certain power of the time, t^{α} (see e.g. 1). However, in our experiment we found that Equation 2 of the main text gave a satisfactory fit in all solutions measured, including high-viscosity polymer solutions (see also reference 2 for a similar conclusion). Indeed, attempts to explicitly include anomalous diffusion in the fit showed no significant deviation from normal diffusion, with $\alpha \sim 1$.

Association rate measurements. The measurements were carried out under second order kinetic conditions, with equal concentrations (0.5 μM) of both proteins, on a

stopped-flow fluorescence spectrometer (Applied PhotoPhysics). Tryptophan fluorescence was used to follow the progress of the association reaction, with excitation wavelength of 280 nm and emission detected at >320 nm. The data were fitted to the following equation that describes the association reaction for equal concentrations of the two associating proteins [3]:

$$\frac{1}{A_0 - C} - \frac{1}{A_0} = k_a t \qquad (1)$$

where A_0 is the initial concentration of each of the proteins, C is the product concentration k_a is the association rate constant (in M^{-1} sec^{-1}). This equation is derived under the assumption that the reaction of A+B$\rightarrow$AB is irreversible. While this is never true, at conditions where $k_a >> k_{off}$ (where k_{off} is the dissociation rate) this assumption is valid and will not alter significantly the measured apparent k_a. The standard error of mean for k_a determined at second order conditions was ±12.5%.

Modeling translational and rotational diffusion with the Brinkman theory

A consistent treatment of both translation and rotation in polymer solutions is provided by the theory of diffusion in a Brinkman fluid [4,5]. The important parameter in the theory is the hydrodynamic screening length of the medium, commonly equated to the correlation length of the polymer solution, $\xi = R_g (c_p / c_p^*)^{-\beta}$, where c_p is the polymer concentration, c_p^* the overlap concentration and $\beta = 3/4$ in a good solvent. The relative translational correlation time, $\bar{\tau}_D$, and rotational correlation time, $\bar{\theta}$, are written in terms of ξ and R, the hydrodynamic radius of the protein [5]:

$$\bar{\tau}_D = 1 + R/\xi + 1/9(R/\xi)^2, \qquad (2)$$

$$\bar{\theta} = \frac{1 + R/\xi + 1/3(R/\xi)^2}{1 + R/\xi}, \qquad (3)$$

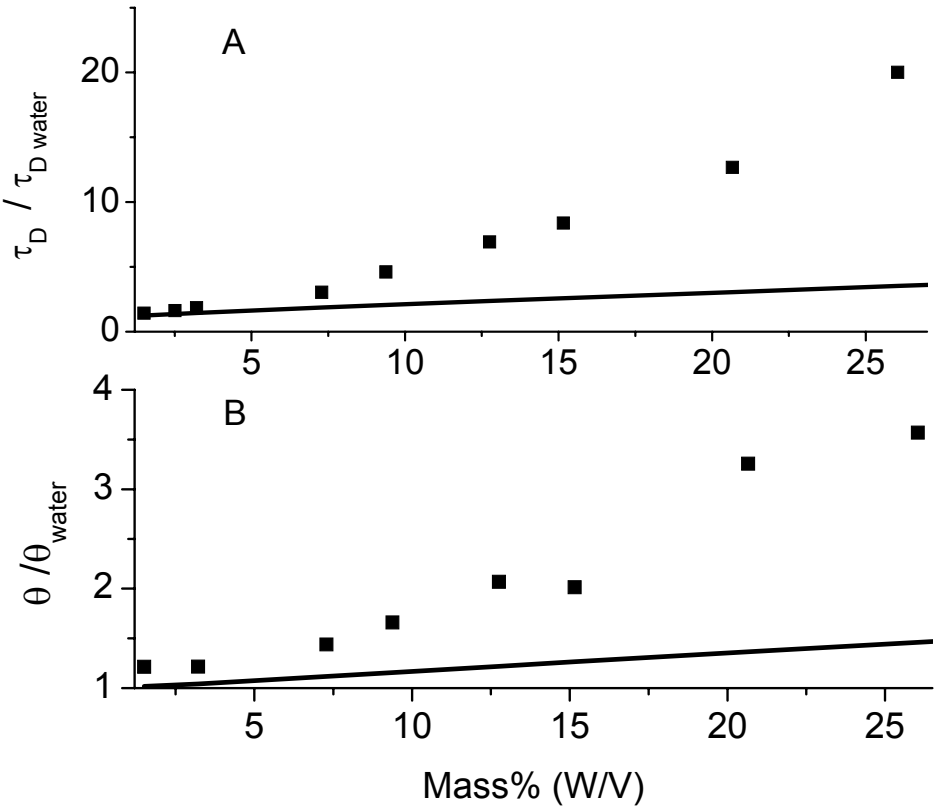

Figure 1: Brinkman theory prediction of the dependence of the translational correlation times (black line in A) and rotational correlation times (black line in B) on mass % of the polymer in PEG 8000 solutions, compared to the experimental results (black squares, same as in Figures 2 and 3 in the main text).

Figure 1 compares these functions to the experimental translational (A) and rotational (B) correlation times. Clearly, the Brinkman fluid theory underestimates the experimental data in both cases.

Dependence of energies of attraction and repulsion on concentration of additives. In the main text (Figure 5) we used the difference between the DLA and the measured results for the relative association times to calculate the energies involved in non-specific induced attractive and repulsive interactions between the proteins. For completeness, we give here the functional forms of the fits given in Figure 5. It will be interesting to see how universal is the behavior measured here with the pair TEM-BLIP.

Energy of attraction in PEG 8000 solutions, in kT: U=-0.03M, with M the concentration of the polymer in mass % (w/v).

Energy of repulsion in glycerol solutions, in kT: $U=0.02M+0.0003M^2$ with M the concentration of glycerol in mass % (w/v).

References

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