Supporting Information

Specific Buffer Effects on the Intermolecular Interactions Among Protein Molecules at Physiological pH

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Chemicals

Bovine serum albumin (BSA, ≥ 98 %), lysozyme from chicken egg (LYZ, ≥ 98 %), sodium phosphate dibasic (≥ 99 %), citric acid monohydrate (≥ 99 %), deuterium oxide (99.9 % D), sodium hydroxide (≥ 97.0 %), hydrochloric acid (37 %) were purchased from Sigma-Aldrich (Milan, Italy). Sodium dihydrogen phosphate (≥ 98 %) was purchased from Thermo Fisher Scientific. Tris-HCl (≥ 99.8 %) was purchased from Bio-Rad Laboratories, Inc.

NMR measurements

Samples for NMR experiments were prepared using D_2O. NMR experiments were carried out using a Bruker Ascend^{TM} 600 at 14.0954 T. ^{1}H, ^{23}Na, ^{31}P and ^{35}Cl NMR measurements were performed at the operating frequencies of 600.13, 158.746, 242.938 and 58.8 MHz, respectively. ^{1}H NMR self-diffusion coefficients were determined through the PGSE acquisition sequence and DOSY toolbox to obtain the self-diffusion coefficients *D* from the usual equation,[1]

$$I = I_0 e^{-D\gamma^2 g^2 \delta^2 (\Delta - \delta/3)} \quad (1)$$

where *I* is the integral of the peak at each field gradient intensity, I_o is the signal intensity in the absence of field gradient, γ is the magnetogyric ratio, g, δ, and Δ are the magnitude, the duration and the separation of the field gradient pulses, respectively.

Spin-lattice (R_1) and spin-spin (R_2) relaxation rates for ^{23}Na and ^{31}P nuclei were performed using the inversion recovery and CPMG spin-echo usual sequences $R_1 = 1/T_1$ (Eq. 2) and $R_2 = 1/T_2$ (Eq. 3) values were obtained using the following equations,[2]

$$M_z = M_0 \left(1 - 2e^{-\frac{\tau}{T_1}}\right) \quad (2)$$

$$M_z = M_0 e^{-\frac{\tau}{T_2}} \quad (3)$$

where M_z is the signal intensity at each delay τ, and M_o is the signal intensity when the delay τ is long enough to allow for full recovery of the signal intensity. All experiments were run at the controlled temperature of 298 K, and reported values are the average of three different measurements for each experiment.

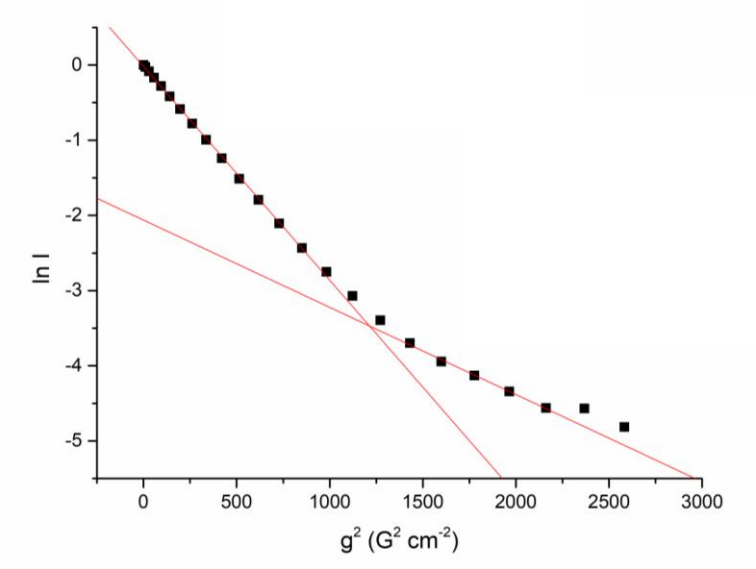

Figure S1. Citrate anion in the presence of BSA: ^{1}H NMR PGSE intensity decay (ln I) vs gradient strength ($g^2 = \gamma^2 g^2 \delta^2 (\Delta - \delta/3)$) to display the two slopes that bring to calculate the two D values 3.78 and 1.54 × 10^{-10} m^2 s^{-1} for citrate anion.

Dynamic light scattering measurements

A stock solution of 10 mg/mL concentration of lysozyme was prepared in 10 mM sodium phosphate, sodium citrate and Tris-HCl buffers at pH 7.15 ± 0.05. By dilution, 8 lysozyme solutions were prepared in the concentration range 1 – 9 mg/mL. A 10 mM phosphate buffer stock solution with a concentration of BSA 20 mg/mL at pH 7.15 ± 0.05 as initially prepared. Then 5 solutions at concentrations, 2 mg/mL, 5 mg/mL, 8 mg/mL, 10 mg/mL and 15 mg/mL, were prepared by dilution. The same preparation procedure was used for the 20 mM, 50 mM and 100 mM buffer concentration solutions. The whole series was repeated in citrate and tris pads. Table 2.1 shows the weighs made for the preparations of the 20 mg/mL stock solutions. All the prepared solutions were analyzed by dynamic light scattering (DLS) by putting about 1 mL of the sample solutions in polystyrene cuvettes (SARSTEDT D-51588). and analyzing in a ZetaSizer Nano ZSP instrument (Zetasizer software Version 7.03) at T = 298.0 K. Measurements were carried out at least in triplicate to confirm the reproducibility of the obtained data.

Table S1: Values of diffusion coefficient (D_0), hydrodynamic radius (R_H) and interaction parameter k_D) for Lysozyme obtained by linear fitting apparent diffusion coefficients (D_{app}) *vs* protein concentration. Experiments were carried out at 10 mM buffer concentration, pH 7. 15 and 298 K. Errors are on the last digit and reported within brackets.

Buffer	**R_H [a] (nm)**	**D_0 [b] × 10^{10} (m^2 s^{-1})**	**k_D [b] (cm^3 mg^{-1})**
Tris HCl	1.81(2)	1.35(2)	0.048(3)
Sodium phosphate	1.854(3)	1.321(2)	0.0013(2)
Sodium citrate	1.84(1)	1.33(1)	-0.013(1)

[a] $R_H = k_BT/(6\pi\eta D_0)$. [b] $D_{app} = D_0 (1+k_DC_{protein})$

Table S2: Values of diffusion coefficient (D_0), hydrodynamic radius (R_H) and interaction parameter k_D) for BSA obtained by linear fitting apparent diffusion coefficients (D_{app}) *vs* protein concentration. Experiments were carried out for different buffer concentrations (10-100 mM) at pH 7. 15 and 298 K. Errors are on the last digit and reported within brackets.

Buffer	**Concentration (mM)**	**I [a] (mM)**	**κ^{-1} [b] (nm)**	**R_H (nm)**	**D_0 × 10^{11} (m^2 s^{-1})**	**k_D (cm^3 mg^{-1})**
Tris-HCl	10	8.91	3.22	3.78(6)	5.8(1)	0.027(1)
Tris-HCl	20	17.81	2.28	3.79(1)	5.79(2)	0.0150(1)
Tris-HCl	50	44.52	1.44	3.84(3)	5.58(4)	0.0068(6)
Tris-HCl	100	89.05	1.02	3.85(3)	5.57(4)	0.0050(6)
Sodium phosphate	10	23.63	1.98	3.84(2)	5.62(2)	0.0172(4)
Sodium phosphate	20	47.25	1.40	3.89(3)	5.54(4)	0.0118(6)
Sodium phosphate	50	118.13	0.89	3.80(1)	5.52(2)	0.0055(3)
Sodium phosphate	100	236.27	0.63	3.81(1)	5.51(1)	0.0026(2)
Sodium citrate	10	55.47	1.29	3.86(2)	5.62(3)	0.0095(5)
Sodium citrate	20	110.94	0.91	3.90(4)	5.55(5)	0.0064(8)
Sodium citrate	50	277.35	0.58	3.83(1)	5.56(1)	0.0027(2)
Sodium citrate	100	554.71	0.41	4.01(1)	5.31(1)	0.0012(2)

[a] ionic strength $\left(I = \frac{1}{2}\sum_i c_i z_i^2\right)$; [b] *Debye length* $\left(\boldsymbol{\kappa^{-1}} = \sqrt{\frac{k_B T \varepsilon_0 \varepsilon_r}{N_A e^2 1000 \times 2I}}\right)$

Table S3. Correlation coefficients (R^2) of [a] k_D *vs* I (Figure 3B) and [b] k_D *vs* κ^{-1} (Figure 3C) plots.

Buffer	**R^2 [a]**	**R^2 [b]**
Tris-HCl	0.698	0.982
Sodium phosphate	0.842	0.996
Sodium citrate	0.836	0.995

References

(1) Stilbs, P. Fourier Pulsed-Gradient Studies of Molecular Diffusion. *Prog. NMR Spectrosc.* **1987**, *19*, 1–45.

(2) Becker, E. D. *Relaxation, in 'High Resolution NMR*; Academic Press, 2000.