Supplementary Information for the manuscript:

"Role of solvent properties of water in crowding effects induced by macromolecular agents and osmolytes"

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S1.1 Solvatochromic studies

All solutions of polyethylene glycols, osmolytes, and salts were prepared in deionized water. The solvatochromic probes 4-nitroanisole, 4-nitrophenol, and Reichardt's carboxylate-substituted betaine dye were used to measure the dipolarity/polarizability π^*, HBA basicity β, and HBD acidity α of the media in the osmolytes solutions. Aqueous solutions (ca. 10 mM) of each solvatochromic dye were prepared and 5-15 μL of each was added separately to a total volume of 500 μL of the sample solution. A strong base was added to the samples (~5 μL of 1 M NaOH to 500 μL of the sample solution) containing Reichardt's carboxylate-substituted betaine dye to ensure a basic pH. A strong acid (~10 μL of 1 M HCl to 500 μL of the solution) was added to the samples containing 4-nitrophenol in order to eliminate charge-transfer UV/Vis absorption bands of the phenolate anion that were observed in some solutions. The respective blank solutions without dye were prepared separately. The samples were mixed thoroughly in a vortex mixer and the UV/Vis absorption spectra of each solution were acquired. To check the reproducibility, possible aggregation and specific interactions effects, the position of the band maximum in each sample was measured in five separate aliquots. The effects of adding 2 M NaOH on the position of the band maximum of Reichardt's carboxylate-substituted betaine dye in each sample solution and 2 M NaOH on the position of the band maximum of 4-nitrophenol in each sample solution were checked in separate experiments and found to be negligible within the experimental error limits. A UV-VIS microplate reader spectrophotometer SpectraMax Plus384 (Molecular Devices, Sunnyvale, CA, USA) with a bandwidth of 2.0 nm, data interval of 1 nm, and high resolution scan (~0.5 nm/s) was used for acquisition of the UV–Vis molecular absorbance data. The absorption spectra of the probes were determined over the spectral range from 240 to 600 nm in each solution. Pure samples solutions containing no dye (blank) were scanned first to establish a baseline. The wavelength of maximum absorbance in each solution was determined as described previously[S1], using the PeakFit software package (Systat Software Inc., San Jose, CA, USA), and averaged. The standard deviation for the measured maximum absorption wavelength was ≤0.4 nm for all dyes in all solutions examined.

The behavior of the probes (4-nitrophenol, and Reichardt's carboxylated betaine dye) in several solvents (water, n-hexane, methanol) was tested in the presence and absence of HCl (for 4-nitrophenol) and NaOH (for the betaine dye) at different concentrations of the probes, acid or base, and the maximum shifts of the probes were compared to reference values found in the literature and were within the experimental errors in all cases (data not shown).

The results of these solvatochromic studies were used to calculate π^*, β and α as described by Marcus[S2].

Determination of the solvent dipolarity/polarizability π^*. Values of parameter π^* were determined from the wave numbers ($\tilde{\upsilon}_1$ in 1000 cm^{-1} = 1 kK) of the longest-wavelength absorption band of 4-nitroanisole using relationship (1):

$$\pi^* = 0.427 \bullet (34.12 - \tilde{\upsilon}_1) \quad (1)$$

Determination of the solvent hydrogen-bond acceptor (HBA) basicity β. Values of parameter β were determined from the wave numbers ($\tilde{\upsilon}_2$ in 1000 cm^{-1} = 1 kK) of the longest-wavelength absorption band of the 4-nitrophenol using relationship (2):

$$\beta = 0.346 \bullet (35.045 - \tilde{\upsilon}_2) - 0.57 \bullet \pi^* \quad (2)$$

Determination of the solvent hydrogen-bond donor (HBD) acidity α. Values of parameter α were determined from the longest-wavelength Vis absorption band of Reichardt's standard betaine dye no. 30 using relationship (3):

$$\alpha = 0.0649 \bullet E_T(30) - 2.03 - 0.72 \bullet \pi^* \quad (3)$$

The $E_T(30)$ values (in kcal/mol) are based on the negatively solvatochromic standard pyridinium *N*-phenolate betaine dye no. 30 (Reichardt's dye) as probe. They are obtained directly from the wavelength (λ in nm) of the Vis absorption band of the corresponding carboxylate-substituted betaine dye, according to equation (4)[S3]:

$$E_T(30) = (1/0.932) \bullet [(28591/\lambda) - 3.335] \quad (4)$$

S1.2 Experimental and interpolated solvent features of water in aqueous solutions of indicated polymers and osmolytes used in correlations shown in Table 2.

Table S1. Protein/nucleic acid stability (Y) expressed as indicated and experimentally estimated and interpolated solvent dipolarity/polarizability (π^*) and solvent hydrogen bond donor acidity (α) of water in aqueous solutions of indicated individual polymers, osmolytes, and salts or their binary mixtures at concentrations indicated.

Ref	Protein/ Nucleic acid	Property	Buffer	Crowder	Data	π^*	α
S5	RNase A	ΔG_D	0.05 M cacodylic, acid buffer, containing 0.1 M KCl	Buffer	10.3	1.096	1.237
				0.25 M TMAO	10.7	1.096	1.226
				0.50 M TMAO	10.8	1.096	1.213
				0.75 M TMAO	11.2	1.096	1.201
				1.00 M TMAO	11.8	1.096	1.19
				0.25 M Sarcosine	11.2	1.107	1.215
				0.50 M Sarcosine	11.7	1.112	1.201
				0.75 M Sarcosine	12.2	1.116	1.186
				1.00 M Sarcosine	12.5	1.12	1.179
				0.25 M Sorbitol	10.8	1.103	1.231
				0.50 M Sorbitol	11	1.111	1.225
				0.75 M Sorbitol	11.4	1.121	1.221
				0.50 M Sorbitol	11.9	1.132	1.21
				0.25 M Betaine	10.8	1.104	1.225
				0.50 M Betaine	11.2	1.103	1.211
				0.75 M Betaine	11.7	1.101	1.199
				1.00 M Betaine	12.1	1.1	1.185
S6	Ubiquitin	T_m	Aq. solution pH 2.0	12 % w/v Dextran-40	328.47 ±0.27	1.131	1.156
				23.5 % w/v Dextran-40	334.38 ±0.006	1.152	1.088
				35 % w/v Dextran-40	340.5 ±0.2	1.17	1.028
				44.5 % w/v Dextran-40	345.98 ±0.23	1.184	0.985
				12 w/v Glucose	329.07 ±0.04	1.123	1.193
				23.5 % w/v Glucose	334.3 ±0.16	1.145	1.157
				35 % w/v Glucose	340.18 ±0.13	1.164	1.126
				44.5 % w/v Glucose	345.23 ±0.09	1.18	1.102
				10.5 % w/v PEG-20 000	321.43 ±0.09	1.109	1.08
				21.5 % w/v PEG-20 000	320.53 ±0.16	1.115	0.952
				21.5 % w/v PEG-20 000	320.53 ±0.16	1.115	0.952
S7	Chymotrypsin inhibitor 2	ΔG	0.05 M Sodium, acetate pH 5.4	Buffer	4.9 ±0.1	1.096	1.237
				10 % w/v Ficoll-70	5.4 ±0.1	1.128	1.105
				30 % w/v Ficoll-70	5.8 ±0.2	1.159	0.988
				20 % w/v Sucrose	5.4 ±0.1	1.137	1.176
S8	Alkaline phosphatase	ΔG_{N-U}	0.1 M Tris-HCl, pH 8.0, Guanidinim, chloride	Buffer	61.27 ±3.6	1.096	1.237
				10 % w/v PEG-4000	106.98 ±7.7	1.109	1.06
				10 % w/v Dextran-70	89.21 ±7.81	1.1	1.172
				10 % w/v Ficoll-70	54.11 ±1.56	1.128	1.105
S9	G-actin	T_m	0.002 M Tris-HCL , pH 8.2, small amounts CaCl2, DTT, ATP	Buffer	49.4 ±0.3	1.096	1.237
				1 M Urea	47.4 ±0.3	1.138	1.184
				2 M Urea	43.6 ±0.2	1.175	1.139
				1 M TMAO	53.4 ±0.3	1.096	1.19
				2 M TMAO	57.5 ±0.3	1.096	1.137
				1 M TMAO/2 M Urea	49 ±0.2	1.154	1.112
				5 % w/v Ficoll-70	50.5 ±0.4	1.116	1.155
				10 % w/v Ficoll-70	53.3 ±0.5	1.128	1.105
				20 % w/v Ficoll-70	59.5 ±2.7	1.144	1.049
S10	α/β-Tubulin	k_{app}	0.26 M glycerol + 0.08 M PIPES pH 6.8, 37 ^oC	Buffer	9.43 ±0.23	1.096	1.237
				0.10 M TMAO	11 ±0.29	1.096	1.233
				0.25 M TMAO	12.81 ±0.365	1.096	1.226
				0.50 M TMAO	15.33 ±0.48	1.096	1.213

Table S1. Protein/nucleic acid stability (Y) expressed as indicated and experimentally estimated and interpolated solvent dipolarity/polarizability (π^*) and solvent hydrogen bond donor acidity (α) of water in aqueous solutions of indicated individual polymers, osmolytes, and salts or their binary mixtures at concentrations indicated (continue).

Ref	Protein/ Nucleic acid	Property	Buffer	Crowder	Data	π^*	α
S10	α/β-Tubulin	k_{app}	0.26 M glycerol + 0.08 M PIPES pH 6.8, 37 °C	1.00 M TMAO	15.73 ±0.49	1.096	1.19
				1.50 M TMAO	18.47 ±0.63	1.096	1.163
				0.10 M Urea	6.46 ±0.13	1.104	1.227
				0.25 M Urea	5.01 ±0.09	1.112	1.22
				0.50M Urea	3.08 ±0.04	1.122	1.205
				10 % w/v Sucrose	9.11 ±0.22	1.116	1.209
				15 % w/v Sucrose	6.12 ±0.12	1.126	1.193
				20 % w/v Sucrose	5.92 ±0.11	1.137	1.176
				10 % w/v Ficoll-70	22.3 ±1.39	1.128	1.105
S11	dsDNA	T_m	0.1 M KCl + 0.01 M K_2HPO_4 pH 7.0	Buffer	66.4	1.096	1.237
				5 %wt. Urea	64.9	1.135	1.193
				10 %wt. Urea	61.7	1.153	1.17
				15 %wt. Urea	59.5	1.172	1.142
				20 %wt. Urea	58	1.19	1.116
				5 %wt. TMAO	67.4	1.096	1.205
				10 %wt. TMAO	69	1.096	1.172
				15 %wt. TMAO	69	1.096	1.137
	gqDNA	T_m	0.1 M KCl + 0.01 M K_2HPO_4 pH 7.0	Buffer	66.5	1.096	1.237
				5 %wt. Urea	63.1	1.135	1.193
				10 %wt. Urea	60.8	1.153	1.17
				15 %wt. Urea	58.2	1.172	1.142
				20 %wt. Urea	55.7	1.19	1.116
				5 %wt. TMAO	71.6	1.096	1.205
				10 %wt. TMAO	74.1	1.096	1.172
				15 %wt. TMAO	77.1	1.096	1.137
S12	RNA	T_m	Dulbecco's, phosphate-buffered saline, pH 7.4	Buffer	47.4 ±0.6	1.096	1.237
				100 g/L Sucrose	44.8 ±0.8	1.115	1.211
				200 g/L Sucrose	42 ±1	1.134	1.18
				300 g/L Sucrose	39.7 ±1.3	1.153	1.149
				100 g/L Ficoll-70	47.1 ±1.2	1.128	1.105
				200 g/L Ficoll-70	46.7 ±1.7	1.144	1.049
				300 g/L Ficoll-70	46.2 ±0.7	1.159	0.988
				300 g/L PEG-6000	50 ±2.6	1.114	0.875
				300 g/L PEG-20 000	51.3 ±0.6	1.12	0.874
S13	RNA	$C^{MgCl2}{}_m$	0.02 M Tris-HCl, pH 7.5	5 % w/v PEG-1000	0.82 ±0.17	1.102	1.162
				10 % w/v PEG-1000	0.62 ±0.12	1.107	1.085
				15 % w/v PEG-1000	0.39 ±0.04	1.109	1.038
				18 % w/v PEG-1000	0.36 ±0.04	1.1	1.004
				20 % w/v PEG-1000	0.21 ±0.02	1.11	0.987
S14	RNA	$C^{MgCl2}{}_m$	0.02 M Tris-HCl, pH 7.5	Buffer	0.65 ±0.05	1.096	1.237
				5 %w/v PEG-1000	0.49 ±0.03	1.102	1.162
				8 % w/v PEG-1000	0.44 ±0.02	1.104	1.123
				12 % w/v PEG-1000	0.38 ±0.02	1.107	1.073
				14 % w/v PEG-1000	0.37 ±0.01	1.108	1.049
				18 % w/v PEG-1000	0.31 ±0.01	1.11	1.004
				1 % w/v PEG-8000	0.47 ±0.02	1.098	1.215
				2 % w/v PEG-8000	0.46 ±0.02	1.1	1.2
				4 % w/v PEG-8000	0.39 ±0.02	1.102	1.171
				6 % w/v PEG-8000	0.39 ±0.02	1.103	1.142
				10 % w/v PEG-8000	0.37 ±0.03	1.106	1.088
				14 % w/v PEG-8000	0.32 ±0.01	1.108	1.037

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Ref	Protein/ Nucleic acid	Property	Buffer	Crowder	Data	π^*	α
S14	RNA	C^{NaCl}_m	0.02 M Tris-HCl, pH 7.5	Buffer	112 ±9	1.096	1.237
				5 % w/v PEG-1000	94 ±5	1.102	1.162
				18 % w/v PEG-1000	42 ±3	1.11	1.004
				2 % w/v PEG-8000	102 ±12	1.1	1.2
				4 % w/v PEG-8000	96 ±16	1.102	1.171
				8 % w/v PEG-8000	77 ±5	1.105	1.115
				12 % w/v PEG-8000	63 ±4	1.107	1.062
				16 % w/v PEG-8000	40 ±7	1.109	1.013
S15	wt-$tRNA^{Phe}$	T_m	0.01 M Na-cacodylate, pH 7.0, 0.14 M KCl	Buffer	53.5	1.096	1.237
				20 % w/v PEG-4000	59	1.112	0.963
				20 % w/v PEG-8000	60	1.11	0.974
				20 % w/v Dextran-70	53.5	1.128	1.105
				20 % w/v Ficoll-70	56.5	1.144	1.049
				2 M Proline	44.5	1.138	1.254
				2 M TMAO	56	1.096	1.137
				2 M Betaine	53	1.096	1.126
S16	RNA	T_m	0.015 M Tris-HCl, pH 7.4	0.025 M KCl	316 ±0.1	1.106	1.23
				0.15 M KCl	323.4 ±0.6	1.114	1.225
				0.30 M KCl	327.2 ±0.2	1.123	1.222

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