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Supporting Material

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Supplemental information

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Attractive Protein-Polymer Interactions Markedly Alter the Effect of Macromolecular Crowding on Protein Association Equilibria

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Running Title: Temperature dependent crowding

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Supplemental Data

TABLE S1
Best-fit values ± 2 standard errors of estimate (95% confidence limits) of $\log_{10} K_a$ and $\ln \Gamma$.

T (°C)	Additive	Additive concentration (g/l)	$\log_{10} K_a$	$\ln \Gamma$
37.0	none		5.0 ± 0.1	
	Dextran 70	200	6.1 ± 0.2	2.5 ± 0.7
	Ficoll 70	200	6.65 ± 0.15	3.8 ± 0.6
	PEG 2000	200	6.4 ± 0.1	3.2 ± 0.5
30.0	none		4.95 ± 0.10	
	Dextran 70	200	5.85 ± 0.20	2.1 ± 0.7
	Ficoll 2000	200	6.25 ± 0.10	3.00 ± 0.45
	PEG 2000	200	6.05 ± 0.10	2.55 ± 0.45
25.0	none		4.9 ± 0.2	
			4.70 ± 0.05	
	glycerol	100	4.8 ± 0.3	-0.25 ± 1.15
		200	4.7 ± 0.4	-0.45 ± 1.40
		300	4.6 ± 0.5	-0.7 ± 1.6
	Dextran 70	100	5.55 ± 0.25	1.5 ± 1.0
		200	5.65 ± 0.25	1.70 ± 1.05
		300	6.1 ± 0.2	2.75 ± 0.90
	Ficoll 70	100	5.3 ± 0.2	0.45 ± 0.80
		200	5.8 ± 0.3	2.30 ± 1.15
		200	5.95 ± 0.05	2.65 ± 0.25
		300	6.0 ± 0.2	2.5 ± 0.9
	PEG 2000	100	5.3 ± 0.2	0.9 ± 0.9
		200	5.75 ± 0.15	1.95 ± 0.80
		300	5.80 ± 0.15	2.05 ± 0.80
15.0	none		4.60 ± 0.15	
	Dextran 70	200	4.9 ± 0.5	0.7 ± 1.5
	Ficoll 70	200	5.0 ± 0.5	0.9 ± 1.5
	PEG 2000	200	4.8 ± 0.3	0.70 ± 1.05
8.0	none		3.95 ± 0.20	
	Dextran 70	200	4.00 ± 0.15	0.05 ± 0.80
	Ficoll 70	200	3.95 ± 0.20	0.0 ± 0.9
	PEG 2000	200	3.85 ± 0.20	-0.2 ± 0.9

Thermodynamic description of the effect of nonideality on binding of a ligand to a protein bearing *n* equivalent binding sites

Let protein A have *n* equivalent and noninteracting binding sites for protein B. We denote the equilibrium association constant for binding a molecule of B to a molecule of AB_{i-1} by K_i. Due to the change in number of available binding sites as A becomes progressively saturated with B,

$$K_i = \frac{(i-1)!(n-i+1)!}{i!(n-i)!} K_{i,site} \tag{S1}$$

where $K_{i,site}$ denotes the equilibrium constant for binding of B to an individual site on A. Since we have specified that these sites are equivalent and non-interacting, it follows that in the ideal limit

$$K^0_{i,site} = K^0_{site} \tag{S2}$$

However, in the general case (text reference 7)

$$K_{i,site} = \frac{\gamma_B \gamma_{AB_{i-1}}}{\gamma_{AB_i}} K^0_{i,site} = \frac{\gamma_B \gamma_{AB_{i-1}}}{\gamma_{AB_i}} K^0_{site} \tag{S3}$$

It follows from equations (S1) and (S3) that one can define a distinct nonideality factor for each sequential binding step

$$\Gamma_i = \frac{K_i}{K^0_i} = \frac{\gamma_B \gamma_{AB_{i-1}}}{\gamma_{AB_i}} \tag{S4}$$

In principle, nonspecific interactions between polymeric crowder and the trace proteins could have a differential effect upon the values of successive binding constants, and hence upon the shape and position of the overall binding isotherm. To obtain a numerical estimate of the magnitude of this effect we calculated the radius and surface area of spheres with masses equal to that of catalase with 0 to 4 molecules of bound SOD, and partial specific volume 0.73 cm^3/g. Using these values together with Equations 11 and 13 in the text, we estimate that the ratio $\gamma_{AB_{i-1}} / \gamma_{AB_i}$, and hence the value of Γ_i, varies by no more than about 10% as *i* increases from 1 to 4. To a good approximation, for the system catalase + SOD, we may set $\Gamma_i \approx \Gamma$, and the predicted binding isotherm should closely resemble a single site binding isotherm under highly nonideal as well as ideal conditions. This prediction is borne out by our fluorescence titrations carried out in highly nonideal solution, all of which are fit with excellent precision by calculated single site isotherms (text Equations 2 and 3).