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Supplementary Information

Determination of osmolyte geometry

Calculations were performed with GAMESS version R1 released in 2020[1]. Ground state geometries were optimized with water implicit solvent using second-order Møller-Plesset (MP2) perturbation theory[2] and the 6-311+G(d,p) basis set[3,4]. The C-PCM solvation model[5] with an iterative solver was used with the solute's quantum mechanical density (SMD)[6] for C-PCM electrostatics. These calculations were performed using a direct SCF calculation[7] for the restricted Hartree-Fock type wavefunction, and utilizing Pulay's DIIS interpolation[8] and Davidson damping of the Fock matrix[9]. For the optimized geometries, the largest component of the gradient was less than 10^{-5} hartree/bohr.

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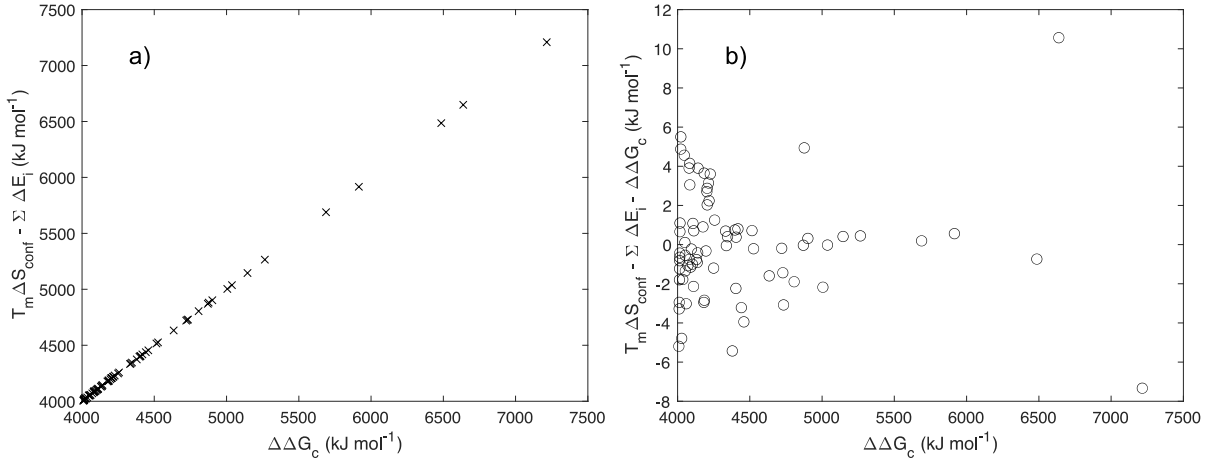

Fig. S1 Parameter predictions or $\Delta\Delta G_c$ for CRP with a spheroidal D-state. In a) the parameter-predicted right-hand side of eqn (4) is plotted against the SPT calculated $\Delta\Delta G_c$ values. In b) the difference between the predicted and calculated values is plotted on the y-axis. Each plot includes 76 points in total from 13 different osmolytes. The predictions utilize the osmolyte identity, molarity and the experimental T_m.

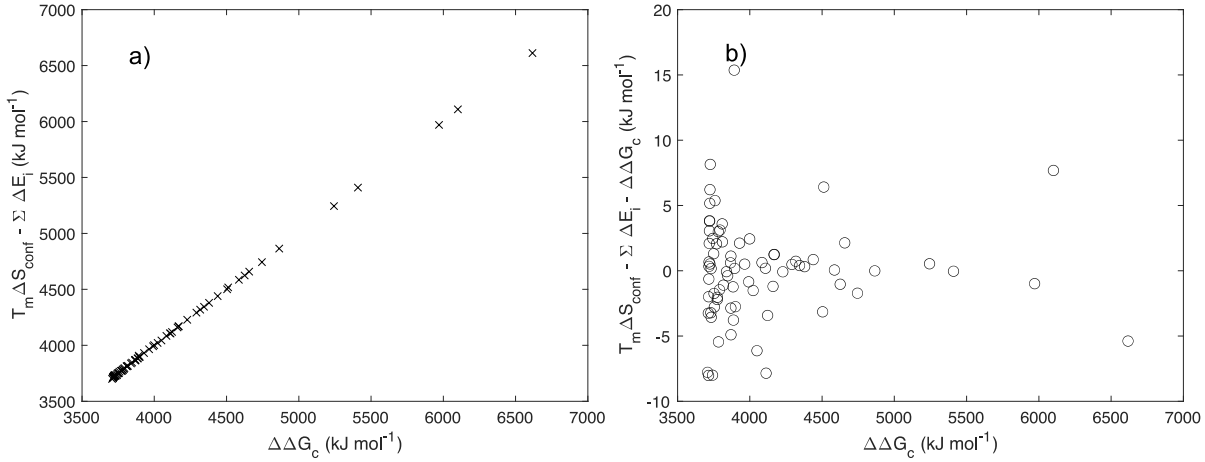

Fig. S2 Parameter predictions or $\Delta\Delta G_c$ for TNFα with a spherocylindrical D-state. In a) the parameter-predicted right-hand side of eqn (4) is plotted against the SPT calculated $\Delta\Delta G_c$ values. In b) the difference between the predicted and calculated values is plotted on the y-axis. Each plot includes 76 points in total from 13 different osmolytes. The predictions utilize the osmolyte identity, molarity and the experimental T_m.

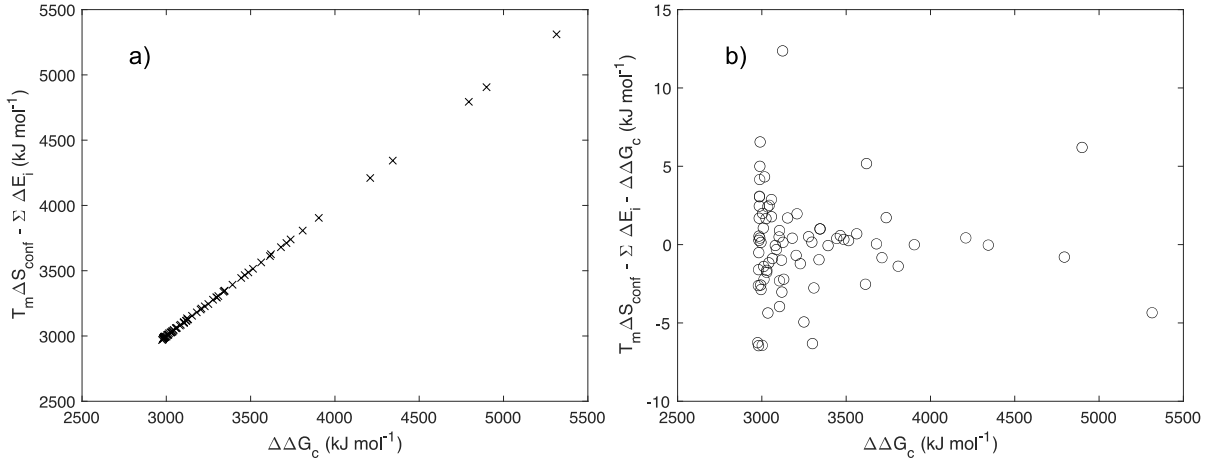

Fig. S3 Parameter predictions or $\Delta\Delta G_c$ for TNFα with a spheroidal D-state. In a) the parameter-predicted right-hand side of eqn (4) is plotted against the SPT calculated $\Delta\Delta G_c$ values. In b) the difference between the predicted and calculated values is plotted on the y-axis. Each plot includes 76 points in total from 13 different osmolytes. The predictions utilize the osmolyte identity, molarity and the experimental T_m.

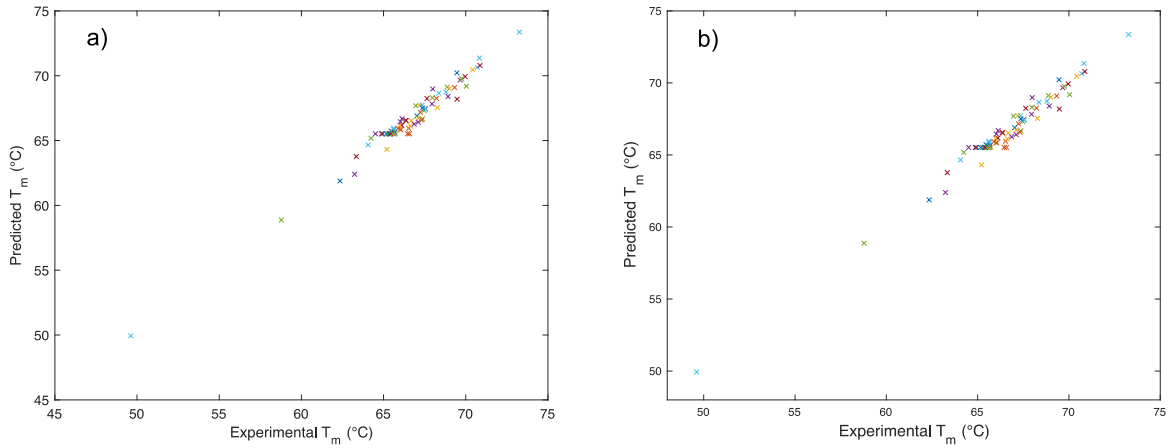

Fig. S4 T_m predictions (y-axis) for CRP for a D-state that is a) spherocylindrical and b) spheroidal as compared to the experimental T_m values.

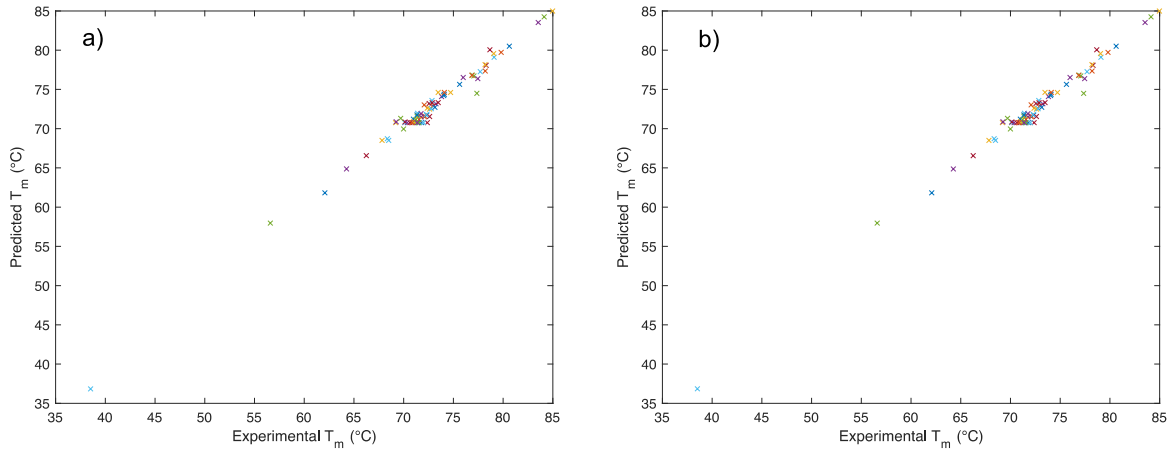

Fig. S5 T_m predictions (y-axis) for TNFα for a D-state that is a) spherocylindrical and b) spheroidal as compared to the experimental T_m values.

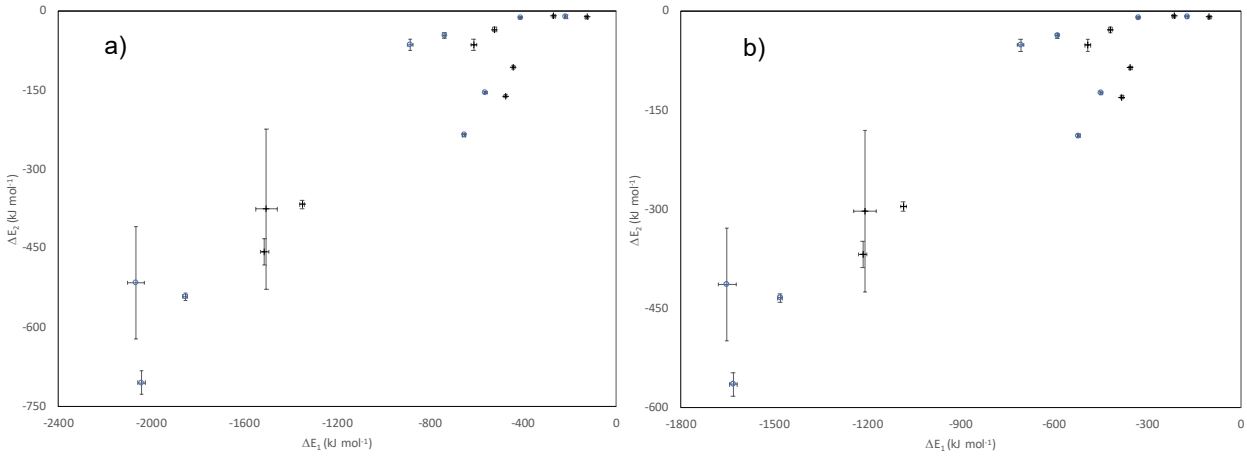

Fig. S6 The correlation between the ΔE_1 and ΔE_2 parameters for each protein. The open circles correspond to CRP, and the crosses to TNFα. Plot a) is for a spherocylindrical D-state, while plot b) for a spheroidal D-state.

Table S1 Experimental T_m values for CRP[1] and those predicted using the fitted parameters for a) a spherocylindrical D-state and b) a spheroidal D-state, along with the solution density equations from Ref 17. The standard deviation of the experimental temperature data and the estimated error in the predicted values are included in parentheses. Uncertainty less than 0.1°C is omitted. The star indicates a data value that was not included in Ref. 1.

Lactose	M	0	0.05	0.1	0.2	0.344			
	T_m (°C)[1]	65.3	65.7	66.1(0.1)	67.0(0.1)	68.2			
	T_m (°C)[a]	65.5(0.1)	65.9(0.3)	66.2(0.6)	66.9(1.3)	68.3(2.8)			
	T_m (°C)[b]	65.5(0.1)	65.8(0.3)	66.2(0.6)	66.9(1.3)	68.3(2.8)			
Trehalose	M	0	0.1	0.25	0.375	0.5	0.67		
	T_m (°C)[1]	66.5(0.1)	67.1(0.1)	68.0(0.1)	68.0	68.8(0.1)	70.0(0.1)		
	T_m (°C)[a]	65.5(0.1)	66.7(0.3)	67.8(0.6)	68.3(1.0)	68.7(1.5)	69.9(2.2)		
	T_m (°C)[b]	65.5(0.1)	66.7(0.3)	67.8(0.6)	68.3(1.0)	68.7(1.5)	69.9(2.2)		
Sucrose	M	0	0.1	0.25	0.5	1	1.125		
	T_m (°C)[1]	65.7(0.1)	66.1(0.1)	67.0(0.2)	67.4(0.1)	69.5(0.6)	69.5(0.4)		
	T_m (°C)[a]	65.5(0.1)	66.7(0.2)	67.7(0.4)	67.7(0.8)	68.2(1.7)	70.2(1.8)		
	T_m (°C)[b]	65.5(0.1)	66.7(0.2)	67.7(0.4)	67.7(0.8)	68.2(1.7)	70.2(1.8)		
Fructose	M	0	0.1	0.238	0.475	0.95	1.5		
	T_m (°C)[1]	65.6(0.3)	66.5(0.1)	66.7(0.2)	67.5(0.2)	68.9(0.1)	70.7(0.1)		
	T_m (°C)[a]	65.5(0.1)	65.9(0.2)	66.5(0.3)	67.5(0.6)	69.1(1.3)	70.6(2.4)		
	T_m (°C)[b]	65.5(0.1)	65.9(0.2)	66.5(0.3)	67.5(0.6)	69.1(1.3)	70.6(2.4)		
Glucose	M	0	0.1	0.475	0.95	1.1			
	T_m (°C)[1]	65.4(0.2)	65.6(0.1)	67.2(0.2)	69.0(0.2)	69.7(0.2)			
	T_m (°C)[a]	65.5(0.1)	65.9(0.2)	67.2(1.1)	69.0(2.6)	69.7(3.2)			
	T_m (°C)[b]	65.5(0.1)	65.9(0.2)	67.2(1.1)	69.0(2.6)	69.7(3.2)			
Hydroxyectoine	M	0	0.1	0.25	0.5	1	2		
	T_m (°C)[1]	65.2(0.1)	66.4	67.2(0.3)	68.0(0.1)	70.0(0.2)	73.3(0.2)		
	T_m (°C)[a]	65.5(0.1)	66.5(0.2)	67.7(0.3)	69.0(0.5)	69.2(1.0)	73.4(2.1)		
	T_m (°C)[b]	65.5(0.1)	66.5(0.2)	67.7(0.3)	69.0(0.5)	69.2(1.0)	73.4(2.1)		
Ectoine	M	0	0.1	0.2	0.3	0.5	1	2	
	T_m (°C)[1]	65.4	67.3	68.3(0.1)	68.9(0.1)	69.8*	70.8(0.1)*	70.9*	
	T_m (°C)[a]	65.5(0.1)	66.6(0.2)	67.5(0.2)	68.4(0.3)	69.8(0.4)	71.4(0.9)	70.8(2.3)	
	T_m (°C)[b]	65.5(0.1)	66.6(0.2)	67.5(0.2)	68.4(0.3)	69.8(0.4)	71.4(0.9)	70.8(2.3)	
L-Alanine	M	0	0.1	0.2	0.3	0.4	0.5	0.75	1
	T_m (°C)[1]	64.9(0.1)	65.5(0.1)	65.9(0.2)	66.0(0.2)	67.1(0.2)	67.4(0.3)	67.5(0.4)*	67.6(0.3)*
	T_m (°C)[a]	65.5(0.1)	65.7(0.1)	65.9(0.2)	66.2(0.2)	66.4(0.2)	66.7(0.3)	67.4(0.4)	68.2(0.5)
	T_m (°C)[b]	65.5(0.1)	65.7(0.1)	65.9(0.2)	66.2(0.2)	66.4(0.2)	66.7(0.3)	67.4(0.4)	68.2(0.5)

Glycine	M	0	0.1	0.25	0.5	1	1.5
	T_m (°C)[1]	65.0(0.1)	65.5(0.5)	66.0(0.3)	66.9(0.2)	67.5(0.1)	68.4
	T_m (°C)[a]	65.5(0.1)	65.6(0.1)	65.9(0.2)	66.2(0.2)	67.3(0.4)	68.7(0.5)
	T_m (°C)[b]	65.5(0.1)	65.6(0.1)	65.8(0.2)	66.3(0.2)	67.3(0.4)	68.7(0.5)
Guanidine HCl	M	0	0.1	0.25	0.5	1	
	T_m (°C)[1]	66.6	65.2(0.1)	63.2(0.4)	58.8(0.1)	49.6(0.1)	
	T_m (°C)[a]	65.5(0.1)	64.3(0.1)	62.4(0.2)	58.9(0.4)	49.9(0.9)	
	T_m (°C)[b]	65.5(0.1)	64.3(0.1)	62.4(0.2)	58.9(0.4)	49.9(0.9)	
TMG	M	0	0.1	0.25	0.5	1	2
	T_m (°C)[1]	65.4(0.1)	65.7(0.2)	66.0(0.1)	66.6(0.1)	66.0(0.3)*	65.6(0.1)*
	T_m (°C)[a]	65.5(0.1)	65.7(0.2)	65.8(0.3)	66.1(0.5)	66.4(1.1)	65.5(3.0)
	T_m (°C)[b]	65.5(0.1)	65.7(0.2)	65.8(0.3)	66.1(0.5)	66.4(1.1)	65.5(3.0)
Urea	M	0	0.1	0.25	0.5	1	
	T_m (°C)[1]	64.5(0.2)	64.2(0.1)	64.1(0.1)	63.3(0.1)	62.3(0.1)	
	T_m (°C)[a]	65.5(0.1)	65.2(0.1)	64.7(0.2)	63.8(0.3)	61.9(0.6)	
	T_m (°C)[b]	65.5(0.1)	65.2(0.1)	64.7(0.2)	63.8(0.3)	61.9(0.6)	
TMAO	M	0.1	0.25	0.5	1	2	
	T_m (°C)[1]	65.6	66.4	67.4(0.1)	69.3(0.1)	70.4(0.1)*	
	T_m (°C)[a]	65.5(0.2)	66.6(0.3)	67.5(0.5)	69.1(1.1)	70.5(2.9)	
	T_m (°C)[b]	65.5(0.2)	66.6(0.3)	67.5(0.5)	69.1(1.1)	70.5(2.9)	

Table S2 Experimental T_m values for TNFα[1] and those predicted using the fitted parameters for a) a spherocylindrical D-state and b) a spheroidal D-state, along with the solution density equations from Ref. 17. . The standard deviation of the experimental temperature data and the estimated error in the predicted values are included in parentheses. Uncertainty less than 0.1°C is omitted. The star indicates a data value that was not included in Ref. 1.

Lactose	M	0	0.0125	0.0625	0.125	0.25	0.3125
	T_m (°C)[1]	70.1(0.2)	70.1	71.0(0.3)	72.1(0.8)	72.4(0.1)	73.3(0.3)
	T_m (°C)[a]	70.8(0.2)	70.9(0.2)	71.2(0.6)	71.6(1.3)	72.6(3.1)	73.2(4.3)
	T_m (°C)[b]	70.8(0.2)	70.9(0.2)	71.2(0.6)	71.6(1.3)	72.6(3.1)	73.2(4.3)
Trehalose	M	0	0.1	0.25	0.375	0.5	0.67
	T_m (°C)[1]	70.4(0.6)	71.3(0.1)	72.8(0.4)	73.5(0.3)	74.1(0.2)	76.9(0.7)
	T_m (°C)[a]	70.8(0.2)	71.6(0.4)	72.5(0.9)	73.3(1.4)	74.4(2.0)	76.8(2.9)
	T_m (°C)[b]	70.8(0.2)	71.6(0.4)	72.5(0.9)	73.3(1.4)	74.4(2.0)	76.8(2.9)
Sucrose	M	0	0.1	0.25	0.5	1	1.125
	T_m (°C)[1]	71.2(0.3)	71.4(0.2)	72.6(0.1)	74.0(0.3)	78.2(0.1)	79.1(0.1)
	T_m (°C)[a]	70.8(0.2)	72.0(0.3)	73.2(0.6)	74.2(1.1)	77.3(2.2)	79.6(2.3)
	T_m (°C)[b]	70.8(0.2)	72.0(0.3)	73.2(0.6)	74.2(1.1)	77.3(2.2)	79.6(2.3)
Fructose	M	0	0.1	0.238	0.475	0.95	1.5
	T_m (°C)[1]	71.8(0.1)	72.1(0.2)	72.6(0.1)	73.8(0.1)	76.9(0.5)	79.1(0.6)
	T_m (°C)[a]	70.8(0.2)	71.5(0.2)	72.5(0.4)	74.1(0.8)	76.8(1.7)	79.1(3.2)
	T_m (°C)[b]	70.8(0.2)	71.5(0.2)	72.5(0.4)	74.1(0.8)	76.8(1.7)	79.1(3.2)
Glucose	M	0	0.1	0.238	0.475	0.95	1.1
	T_m (°C)[1]	72.4(0.3)	72.3(0.2)	73.0(0.5)	74.7(0.3)	76.0(0.2)	77.1(0.9)
	T_m (°C)[a]	70.8(0.2)	71.7(0.3)	72.9(0.6)	74.6(1.3)	76.5(3.0)	76.7(3.8)
	T_m (°C)[b]	70.8(0.2)	71.7(0.3)	72.9(0.6)	74.6(1.3)	76.5(3.0)	76.7(3.8)
Hydroxyectoine	M	0	0.1	0.25	0.5	1	2
	T_m (°C)[1]	70.6(0.3)	71.4(0.3)	72.1(0.9)	73.5(0.4)	77.5(0.3)	84.1(0.9)
	T_m (°C)[a]	70.8(0.2)	71.8(0.2)	73.0(0.4)	74.6(0.7)	76.4(1.3)	84.2(2.5)
	T_m (°C)[b]	70.8(0.2)	71.8(0.2)	73.0(0.4)	74.6(0.7)	76.4(1.3)	84.2(2.5)
Ectoine	M	0	0.1	0.25	0.5	1	2*
	T_m (°C)[1]	71.5(0.3)	71.6(0.4)	71.5(0.2)	71.7(0.3)	71.2(0.6)	68.5(0.2)
	T_m (°C)[a]	70.8(0.2)	71.2(0.2)	71.6(0.4)	71.9(0.7)	71.1(1.5)	68.5(3.9)
	T_m (°C)[b]	70.8(0.2)	71.2(0.2)	71.6(0.4)	71.9(0.7)	71.1(1.5)	68.5(3.9)
L-Alanine	M	0	0.0154	0.0769	0.384	0.662*	0.94*
	T_m (°C)[1]	70.9(0.5)	71.0(0.7)	71.8(0.2)	77.4(0.3)	77.7(0.3)	78.7(0.2)
	T_m (°C)[a]	70.8(0.2)	70.9(0.2)	71.5(0.2)	74.5(0.3)	77.3(0.5)	80.0(0.7)
	T_m (°C)[b]	70.8(0.2)	70.9(0.2)	71.5(0.2)	74.5(0.3)	77.3(0.5)	80.0(0.7)

Glycine	M	0	0.01825	0.0913	0.456	1.14	1.5
	T_m (°C)[1]	69.2(0.1)	69.3(0.1)	69.7(0.6)	72.8(0.2)	78.3(0.4)	80.6(0.6)
	T_m (°C)[a]	70.8(0.2)	70.9(0.2)	71.3(0.2)	73.6(0.3)	78.1(0.5)	80.5(0.7)
	T_m (°C)[b]	70.8(0.2)	70.9(0.2)	71.3(0.2)	73.6(0.3)	78.1(0.5)	80.5(0.7)
Guanidine HCl	M	0	0.1	0.25	0.5	1	
	T_m (°C)[1]	70.8(0.3)	67.8(0.3)	64.3(0.9)	56.6(0.4)	38.5(0.6)	
	T_m (°C)[a]	70.8(0.2)	68.5(0.2)	64.9(0.3)	58.0(0.5)	36.8(2.1)	
	T_m (°C)[b]	70.8(0.2)	68.5(0.2)	64.9(0.3)	58.0(0.5)	36.8(2.1)	
TMG	M	0	0.1	0.25	0.5	1	2
	T_m (°C)[1]	72.0(0.2)	72.6(0.7)	73.1(1.0)	74.1(0.9)	78.2(0.4)	83.5(0.8)
	T_m (°C)[a]	70.8(0.2)	71.6(0.2)	72.7(0.4)	74.6(0.6)	78.2(1.3)	83.5(3.4)
	T_m (°C)[b]	70.8(0.2)	71.6(0.2)	72.7(0.4)	74.6(0.6)	78.2(1.3)	83.5(3.4)
Urea	M	0	0.1	0.25	0.5	1	
	T_m (°C)[1]	71.4(0.7)	70.0(0.3)	68.4(0.1)	66.2(0.2)	62.1(0.1)	
	T_m (°C)[a]	70.8(0.2)	70.0(0.2)	68.7(0.3)	66.6(0.5)	61.8(1.0)	
	T_m (°C)[b]	70.8(0.2)	70.0(0.2)	68.7(0.3)	66.6(0.5)	61.8(1.0)	
TMAO	M	0	0.1	0.25	0.5	1	2
	T_m (°C)[1]	71.5(0.4)	72.3(0.8)	72.9(0.3)	75.6(0.4)	79.8(0.5)	85.0(0.5)
	T_m (°C)[a]	70.8(0.2)	71.8(0.2)	73.3(0.4)	75.6(0.7)	79.7(1.4)	85.0(3.6)
	T_m (°C)[b]	70.8(0.2)	71.8(0.2)	73.3(0.4)	75.6(0.7)	79.7(1.4)	85.0(3.6)

Table S3 Ratios of parameter values, TNFα/CRP, for each osmolyte in the pairwise study. The ratio for ΔS_{conf}, which is independent of osmolyte is 73%.

	ΔE_1	ΔE_2
Lactose	73 ± 3%	73 ± 33%
Trehalose	74 ± 1%	65 ± 4%
Sucrose	73 ± 1%	68 ± 2%
Glucose	69 ± 2%	100 ± 24%
Fructose	71 ± 1%	75 ± 13%
Hydroxyectoine	73 ± 1%	69 ± 1%
Ectoine	79 ± 1%	69 ± 2%
Guanidine HCl	84 ± 1%	n/a
L-Alanine	65 ± 1%	n/a
Glycine	68 ± 1%	n/a
TMG	65 ± 2%	76 ± 26%
Urea	81 ± 2%	n/a
TMAO	58 ± 3%	104 ± 39%

Table S4 Ratios of WASA changes upon denaturation based on the overall protein, the backbone and the sidechain as determined from the values in Ref. 1.

	TNFα / CRP	$\Delta WASA_{CRP}$ Å^2
Overall	75%	15318
Backbone	70%	3777
Sidechain	77%	11541
Polar	79%	4636
Nonpolar	74%	10682