Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

SUPPORTING INFORMATION:

Osmolyte Effect on Enzymatic Stability and Reaction Equilibrium of Formate Dehydrogenase

Nicolás F. Gajardo-Parra [a], Harold Akrofi-Mantey [a], Moreno Ascani [a], Esteban Cea-Klapp [b], José Matias Garrido [b], Gabriele Sadowski [a], Christoph Held [a,]*

[a] Laboratory of Thermodynamics, Department of Biochemical and Chemical Engineering, Dortmund University of Technology, Emil-Figge-Str. 70, 44227 Dortmund, Germany

[b] Departamento de Ingeniería Química, Faculty of Engineering, Universidad de Concepción, Concepción, Chile

SUPPORTING FIGURES

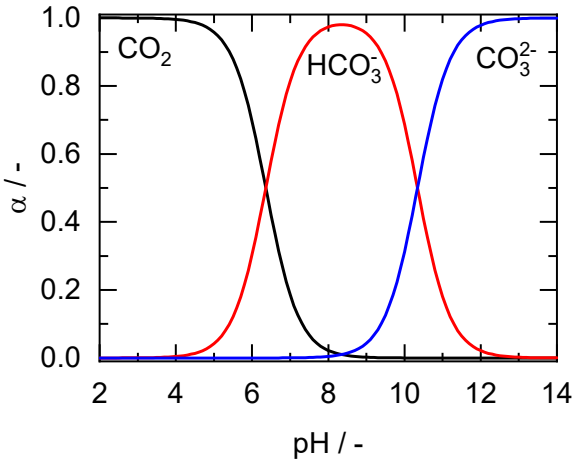


Figure S1: CO_2 species distribution at different pH.

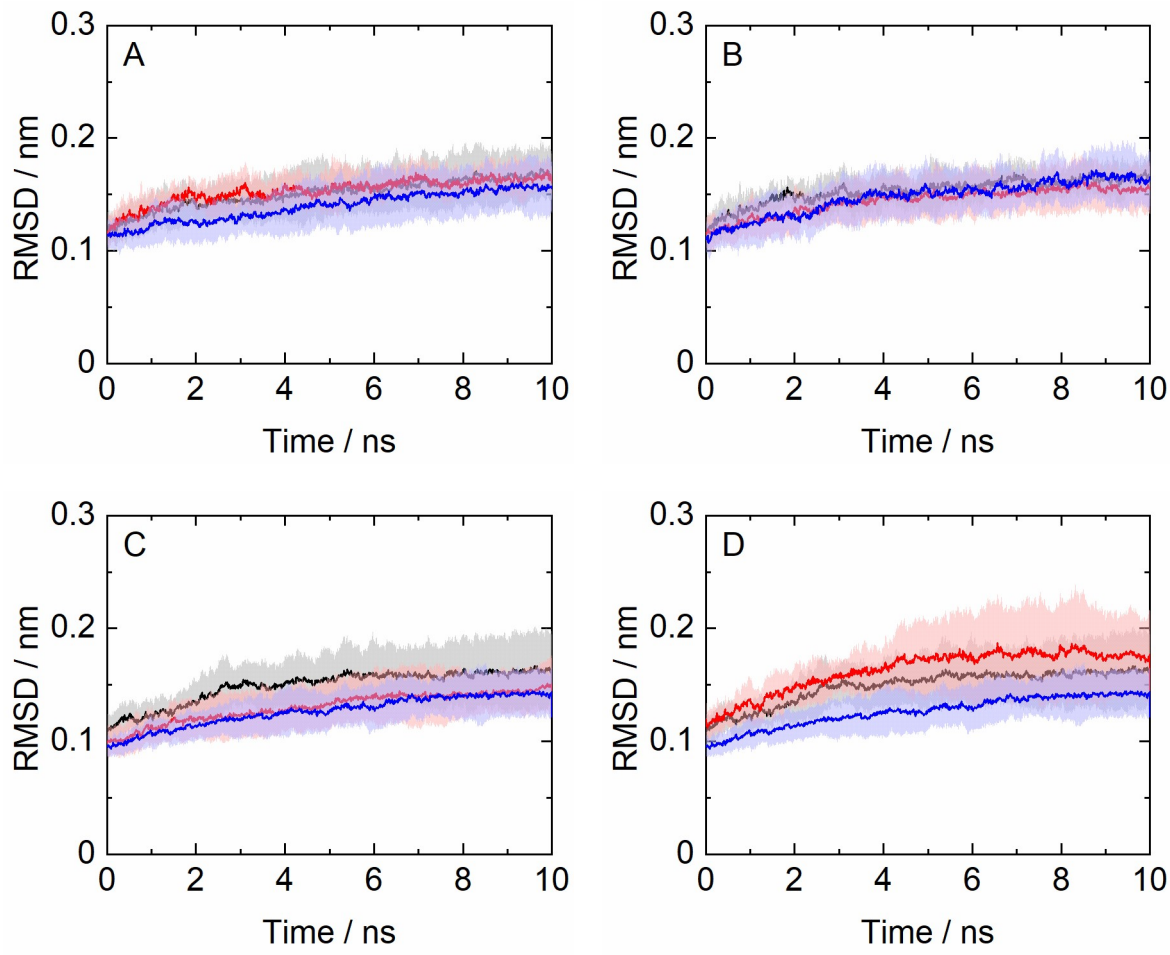


Figure S2: Protein backbone root-mean-square deviation (RMSD) for (A) betaine, (B) glycerol, (C) trehalose and (D) TMAO. Lines represent diferent concentrations: 0.5 mol/kg (black), 1 mol/kg (red) and 1.5 mol/kg (blue). Standard errors computed from 20 independent runs are shown as shaded regions.

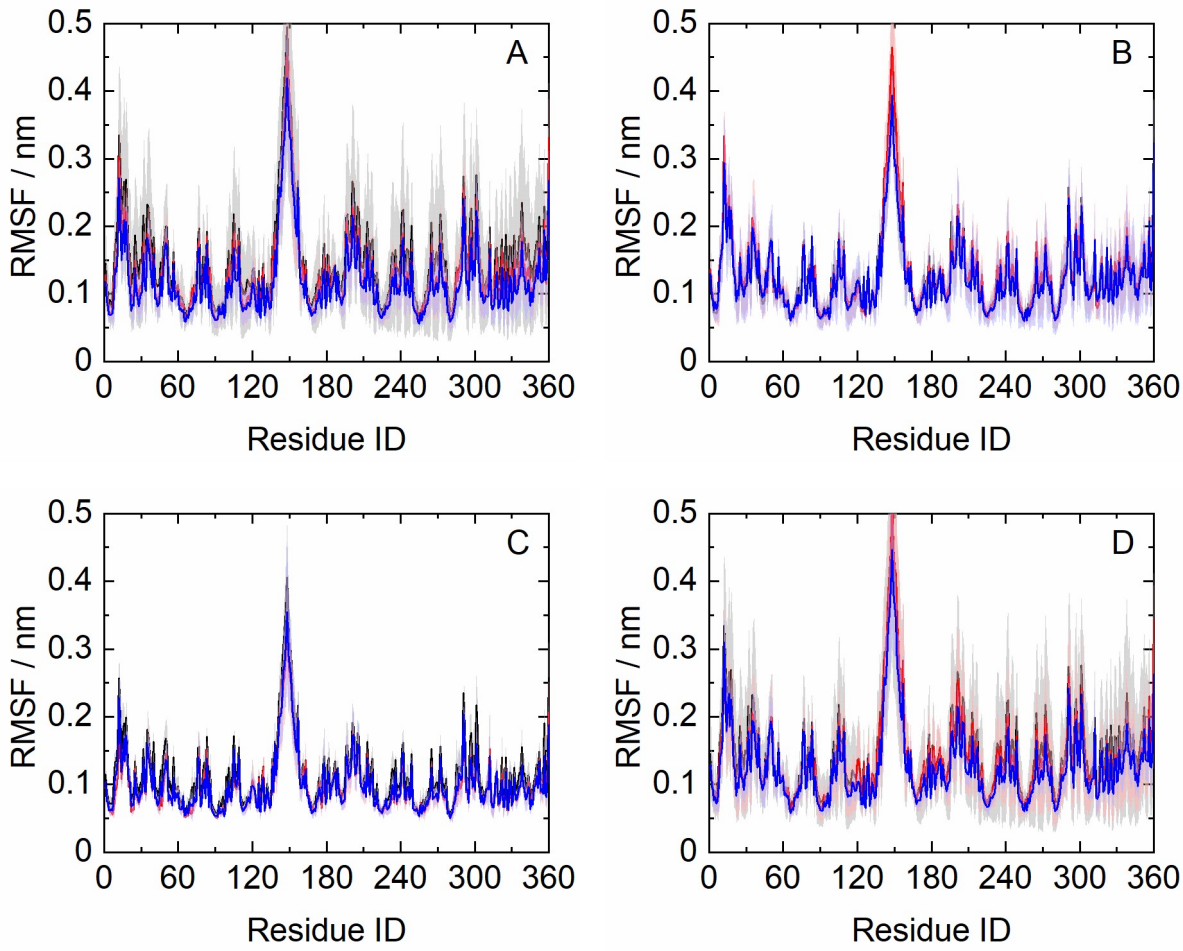


Figure S3: Protein residues root-mean-square fluctuation (RMSF) for (A) betaine, (B) glycerol, (C) trehalose and (D) TMAO. Lines represent diferent concentrations: 0.5 mol/kg (black), 1 mol/kg (red) and 1.5 mol/kg (blue). Standard errors computed from 20 independent runs are shown as shaded regions.

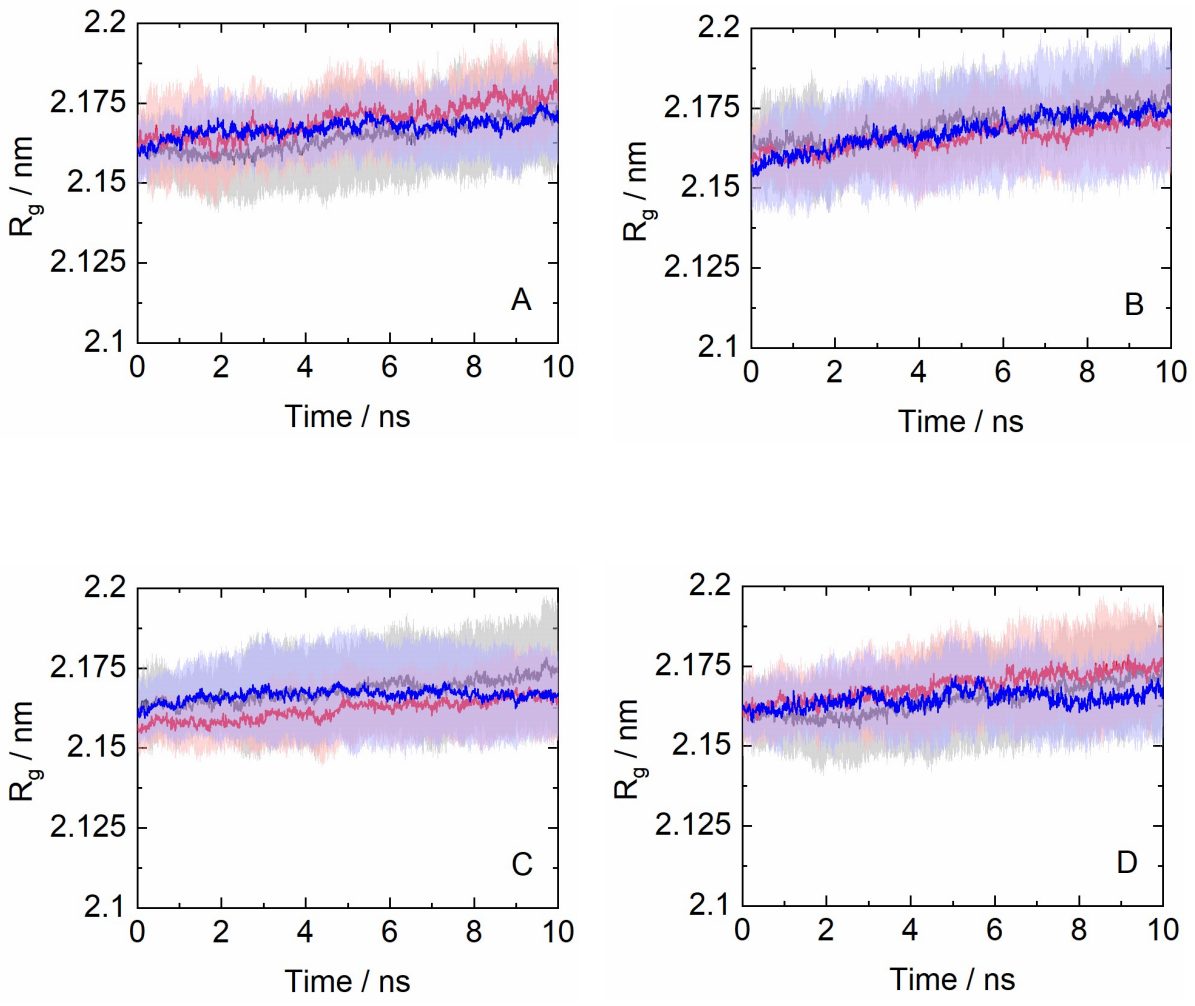


Figure S4: Protein radius of gyration (R_g) for (A) betaine, (B) glycerol, (C) trehalose and (D) TMAO. Lines represent diferent concentrations: 0.5 mol/kg (black), 1 mol/kg (red) and 1.5 mol/kg (blue). Standard errors computed from 20 independent runs are shown as shaded regions.

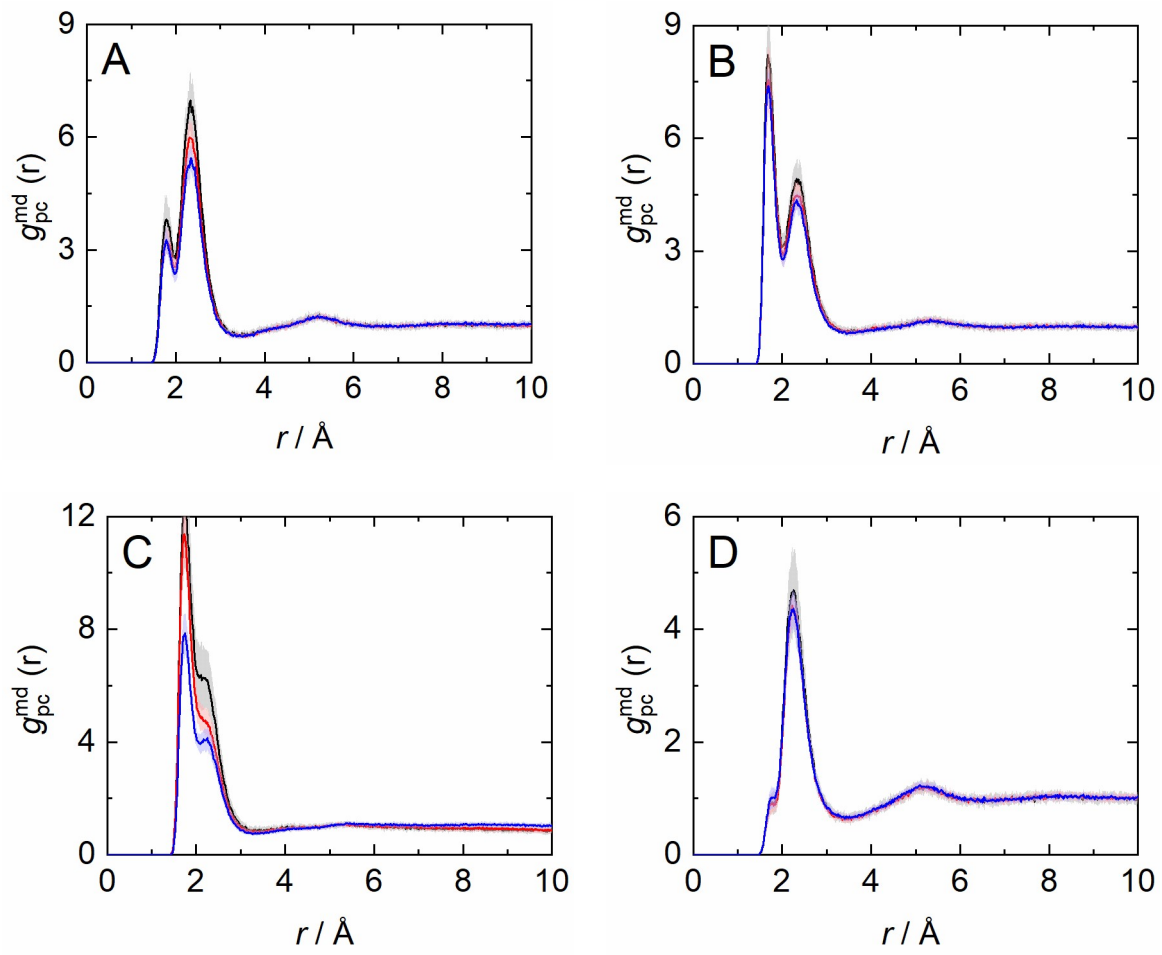


Figure S5: Minimun-distance distributions functions for protein – osmolyte interactions (g_{pc}^{md}) for (A) betaine, (B) glycerol, (C) trehalose and (D) TMAO. Lines represent diferent concentrations: 0.5 mol/kg (black), 1 mol/kg (red) and 1.5 mol/kg (blue). Standard errors computed from 20 independent runs are shown as shaded regions.

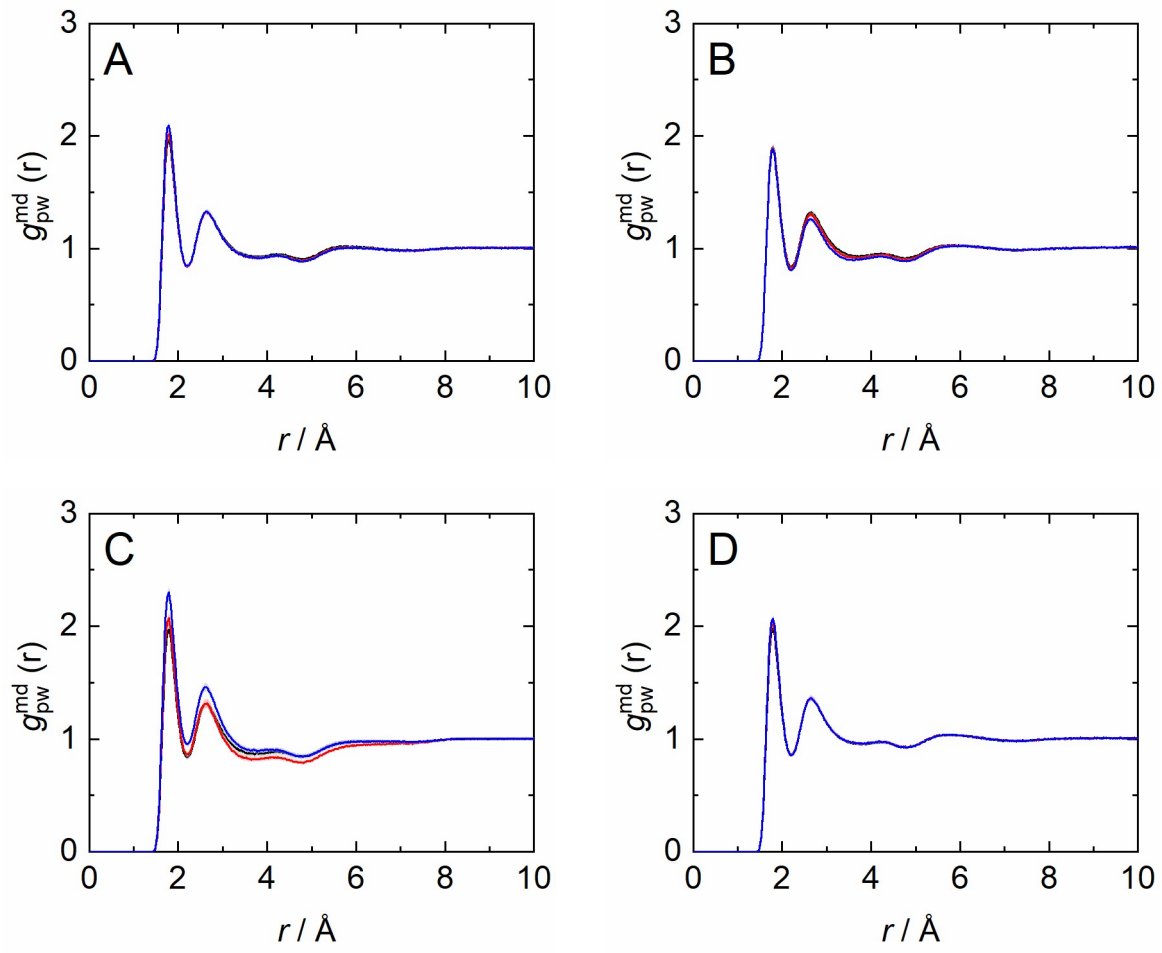


Figure S6: Minimun-distance distributions functions for protein – water interactions (g_{pw}^{md}) under osmolyte influence: (A) betaine, (B) glycerol, (C) trehalose and (D) TMAO. Lines represent diferent concentrations: 0.5 mol/kg (black), 1 mol/kg (red) and 1.5 mol/kg (blue). Standard errors computed from 20 independent runs are shown as shaded regions.

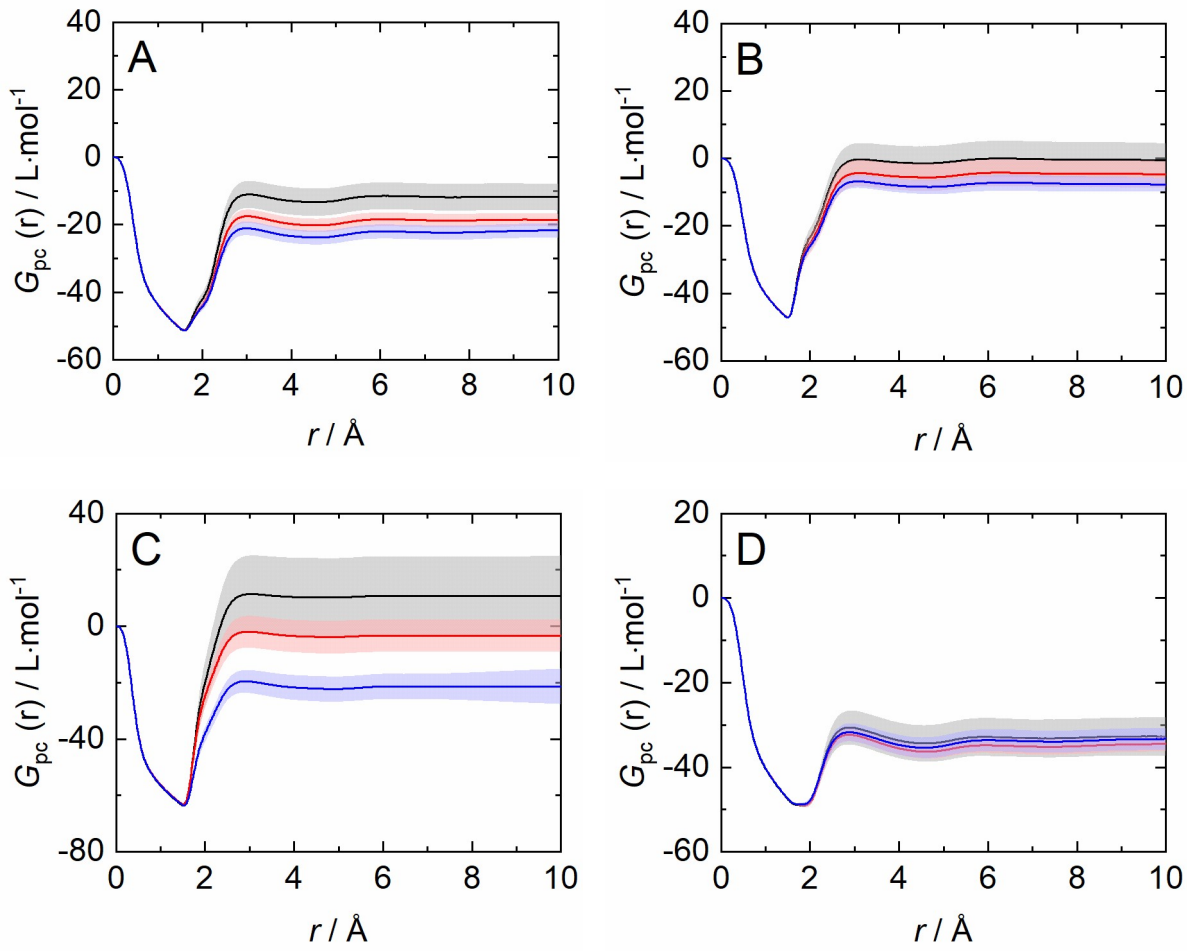
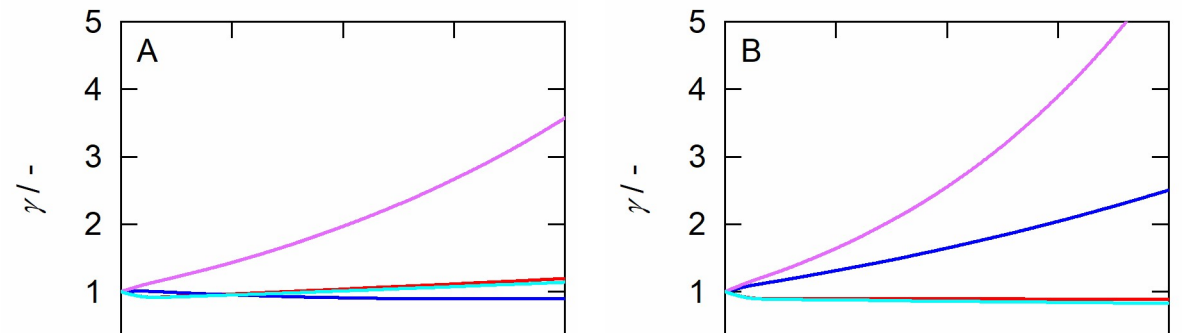


Figure S7: Kirkwood-buff integral profile for protein – osmolyte interactions (G_{pc}). (A) betaine, (B) glycerol, (C) trehalose and (D) TMAO. Lines represent diferent concentrations: 0.5 mol/kg (black), 1 mol/kg (red) and 1.5 mol/kg (blue). Standard errors computed from 20 independent runs are shown as shaded regions.



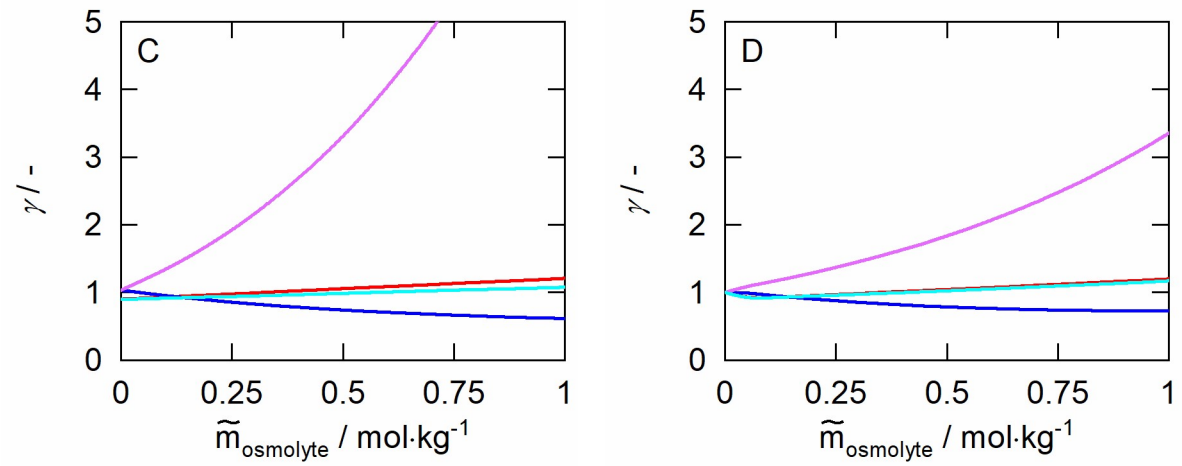


Figure S8: Reaction components activity coefficients at infinite dilution in osmolyte aqueous solutions at 25 °C as function of the osmolyte concentration. (A) betaine, (B) glycerol, (C) trehalose and (D) TMAO. Lines represent different concentrations components: Formate (red), NAD^+ (blue), NADH (magenta) and HCO_3^- (cyan).

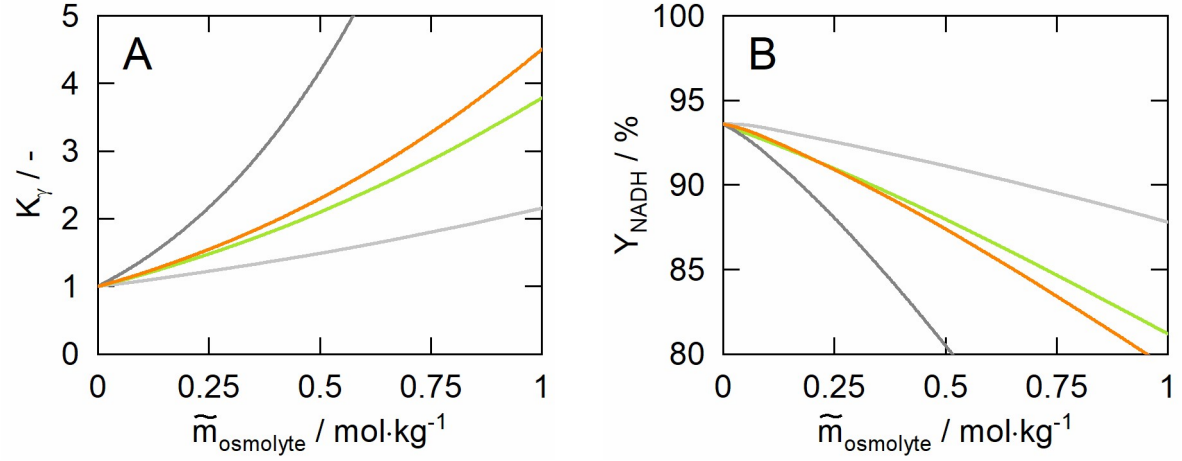


Figure S9: ePC-SAFT calculated K_γ (A) and Yield (B) in osmolyte aqueous solutions at 25 °C as function of the osmolyte concentration. TMAO (orange), betaine (green), glycerol (light gray), and trehalose (dark gray).

SUPPORTING TABLES

Table S1. Enzymatic activity tests at 25 °C.

Osmolyte	$\tilde{m}_{osmolyte}$ **[mol/kg]**	$\tilde{m}_{NAD^+}^{t=0}$ **[mmol/kg]**	$\tilde{m}_{For^-}^{t=0}$ **[mol/kg]**	**Relativy activity**
None	0	0.1	100	1
Betaine	0.25	0.1	100	1.029 ± 0.03
Betaine	0.5	0.1	100	1.052 ± 0.05
Betaine	1	0.1	100	0.907 ± 0.04
Glycerol	0.25	0.1	100	1.033 ± 0.02
Glycerol	0.5	0.1	100	1.062 ± 0.03
Glycerol	1	0.1	100	1.027 ± 0.05
Trimethylamine N-oxide	0.25	0.1	100	1.049 ± 0.03
Trimethylamine N-oxide	0.5	0.1	100	1.122 ± 0.04
Trimethylamine N-oxide	1	0.1	100	1.030 ± 0.06
Trehalose	0.25	0.1	100	1.014 ± 0.04
Trehalose	0.5	0.1	100	1.107 ± 0.06
Trehalose	1	0.1	100	0.939 ± 0.03

Table S2. Thermal stability assays.

Osmolyte	$\tilde{m}_{osmolyte}$ **[mol/kg]**	T_{on-set} **[°C]**	T_m **[°C]**
None	0	50.5	61.0
Betaine	0.25	51.2	61.5
Betaine	0.5	51.8	61.7
Betaine	1	52.4	62.6
Glycerol	0.25	51.0	61.3
Glycerol	0.5	51.0	61.4
Glycerol	1	51.8	62.0
Trimethylamine N-oxide	0.25	50.7	61.4
Trimethylamine N-oxide	0.5	52.0	62.0
Trimethylamine N-oxide	1	52.8	62.9
Trehalose	0.25	51.6	61.9
Trehalose	0.5	52.5	62.8
Trehalose	1	54.0	64.2

Table S3. Reaction equilibrium assays at 25 °C.

Osmolyte	$\tilde{m}_{osmolyte}$ **[mol/kg]**	$\tilde{m}_{NAD^+}^{t=0}$ **[mmol/kg]**	$\tilde{m}_{For^-}^{t=0}$ **[mol/kg]**	Y_c **[%]**	**pH**
None	0	0.1	100	91.65 ± 0.33	8.36
Betaine	0.5	0.1	100	89.57 ± 0.62	8.42
Glycerol	0.5	0.1	100	90.20 ± 1.04	8.31
Trimethylamine N-oxide	0.5	0.1	100	91.39 ± 0.48	8.27
Trehalose	0.5	0.1	100	86.51 ± 1.06	8.32

Table S4. The number of molecules in each box.

System	$\tilde{m}_{osmolyte}$ [mol/kg]	*N° water*	*N° co-solvent*
Pure water	-	18000	-
Glycerol 0.5	0.5	18000	162
Glycerol 1.0	1.0	18000	324
Glycerol 1.5	1.5	18000	486
Trehalose 0.5	0.5	18000	162
Trehalose 1.0	1.0	18000	324
Trehalose 1.5	1.5	18000	486
TMAO 0.5	0.5	18000	162
TMAO 1.0	1.0	18000	324
TMAO 1.5	1.5	18000	486
Betaine 0.5	0.5	18000	162
Betaine 1.0	1.0	18000	324
Betaine 1.5	1.5	18000	486

Table S5. ePC-SAFT Parameters used in this work.

Component	m_i^{seg}	σ_i	u_i/k_B	$N_{i,assoc}$	ε^{AiBi}/k_B	k^{AiBi}	z

		[Å]	[K]		[K]		
Water	1.205	*	353.945	1:1	2425.7	0.045099	0
Betaine	8.466	2.547	266.59	1:1	2541.62	0.0384	0
Glycerol	2.007	3.815	430.82	1:1	4633.5	0.0019	0
Trehalose	13.692	2.856	319.85	8:8	5000	0.1	0
Trimethylamine N-oxide	8.928	2.248	245.44	1:1	0	0.0451	0
NAD^+	25.088	2.271	299.04	8:8	3557.3	0.001	-1
NADH	27.395	2.756	380.52	8:8	3711.9	0.001	-2
CO_2	2.077	2.785	169.21	-	-	-	0
For^-	1	3.308	190	-	-	-	-1
HCO_3^-	1	2.929	70	-	-	-	-1
CO_3^{2-}	1	2.442	249.26	-	-	-	-2

* For water, a temperature-dependent segment diameter σ_i=2.7927+10.11· exp(-0.01775·T) - 1.417· exp(0.01146·T) was used.

Table S6. PC-SAFT binary interaction parameters k_{ij} used in this work

Binary System	k_{ij}
Water - Betaine	-0.0922
Water - Glycerol	-0.005
Water - Trehalose	0.0274 + 0.000219(T-298.15 K)
Water - TMAO	-0.1489
Water - NAD+	-0.074
Water - NADH	-0.056
Water - CO_3^{2-}	0.25
Water - CO_2	-0.0021 + 0.000453(T-298.15 K)
Water – For^-	0.25