

# Electronic Supplementary Information for "New Insights into the Protein Stabilizing Effects of Trehalose by Comparing with Sucrose"

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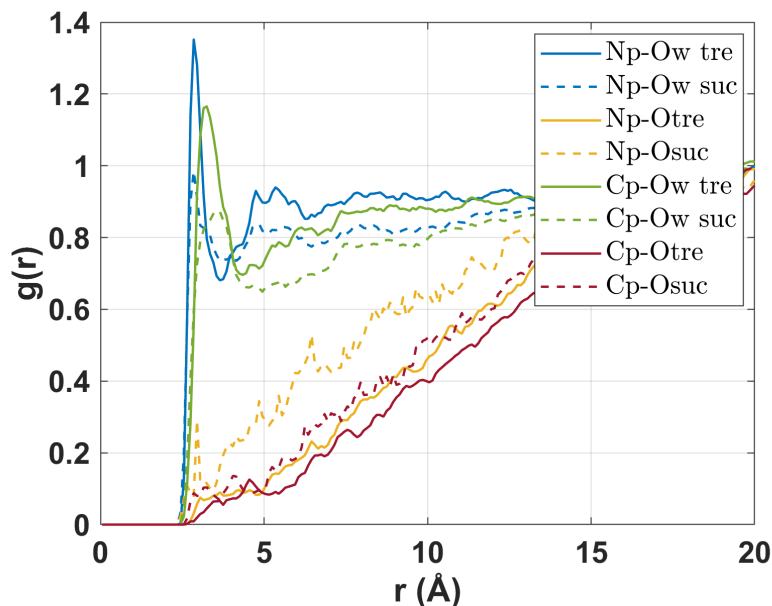
## 1 Structural modeling: Lennard-Jones and coulomb parameters

**Table S1** Lennard-Jones and coulomb parameters for atoms in the EPSR model

Element in protein	$\sigma$ (Å)	$\epsilon$ (kJ/mol)	q(e)
H	0	0	0.0897
O	2.96	0.87864	-0.5
C	3.5	0.27614	0.2
N	3.25	0.71128	-0.76
S	3.55	1.046	-0.47
Fe	2.594	0.0539	2
Element in trehalose	$\sigma$ (Å)	$\epsilon$ (kJ/mol)	q(e)
Ot	3.1	0.71128	-0.5
O1	2.9	0.58576	-0.5
O2	2.9	0.58576	-0.5
O3	3.1	0.71128	-0.5
O4 (for sucrose)	3.1	0.71128	-0.5
Ht	1.7	0.05	0.3005
M	1.7	0.12552	0.0
Ct	3.5	0.27614	0.258
Element in water	$\sigma$ (Å)	$\epsilon$ (kJ/mol)	q(e)
Hw	0	0	0.4238
Ow	3.166	0.65	-0.8476

## 2 Structural modeling: Partial pair correlation functions and coordination numbers

Partial pair correlation functions between nitrogens (Np) or carbons (Cp) atoms on the surface of the protein and oxygen atoms in the water (Ow) or sugar (Otre/Osuc) molecules are displayed in figure S1. Both the trehalose and sucrose systems exhibit a much stronger correlation between Np or Cp and Ow than with Otre/Osuc, however, this difference is larger for the trehalose system, indicating a more pronounced preferential hydration effect. Thus, the correlations between the surface atoms of myoglobin and the sugar oxygens are slightly stronger in the sucrose system, which is also quantitatively shown by the coordination numbers in table S2. The coordination numbers display how many oxygens of the specified molecule type are found within 3.4 Å of any nitrogen or carbon atom on the surface of the protein.



**Figure S1** Partial pair correlation functions between surface protein nitrogens (Np) or carbons (Cp) and water oxygens (Ow) or sugar oxygens (Osuc/Otre). The surface protein atoms were defined as those nitrogen or carbon atoms which are within 2.5 Å of any atom in the solvent. The solid lines represent the trehalose system while the dashed lines represent the sucrose system.

**Table S2** Coordination number ( $N_{X-O}$ ). The values without parenthesis are given for the sucrose system while the values within parenthesis are given for the trehalose system.

	$N_{N-O}$	$N_{C-O}$
Protein-Water	158 (203)	194 (287)
Protein-Sugar	6.02 (3.26)	6.48 (2.89)

### 3 Dynamics from QENS: Relative scattering contribution and fit parameters

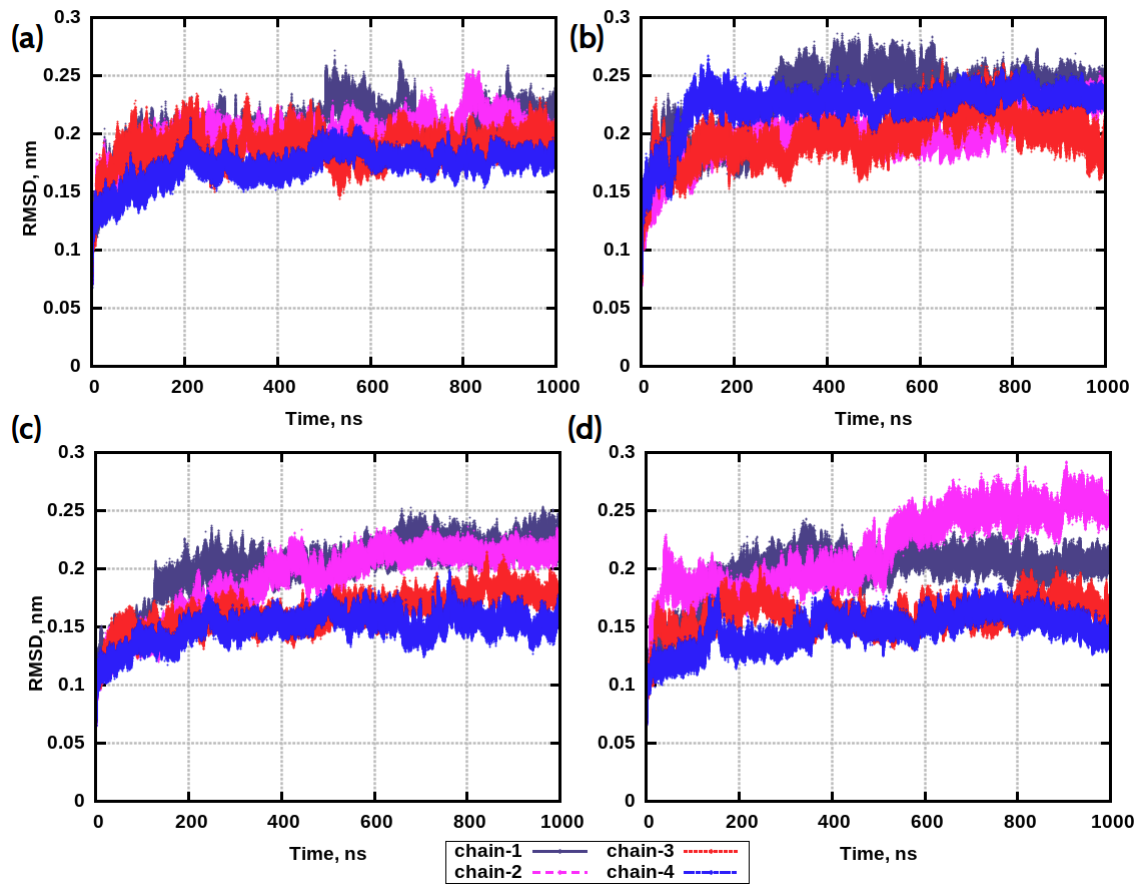
**Table S3** Theoretical relative scattering amplitudes for protein, sugar, and water in the different samples. Values without parenthesis contains only incoherent scattering contribution while the values within parenthesis include both coherent and incoherent scattering contributions.

Sample	Relative Scattering Contribution ( $A_C^i$ )		
	$A_{Prot}^i$	$A_{Tre}^i$	$A_{Wat}^i$
Mb in H-Sug and D <sub>2</sub> O	0.54 (0.46)	0.40 (0.34)	0.06 (0.19)
Mb in D-Sug and H <sub>2</sub> O	0.23 (0.23)	0.08 (0.09)	0.70 (0.68)
Mb in H-Sug and H <sub>2</sub> O	0.20 (0.20)	0.18 (0.18)	0.62 (0.61)
H-Sug in D <sub>2</sub> O	-	0.88 (0.64)	0.12 (0.36)
H-Suc in H <sub>2</sub> O	-	0.22 (0.23)	0.78 (0.77)
D-Tre in H <sub>2</sub> O	-	0.10 (0.12)	0.90 (0.88)
Mb in D <sub>2</sub> O	0.90 (0.69)	-	0.10 (0.31)
Mb in H <sub>2</sub> O	0.24 (0.25)	-	0.76 (0.75)

**Table S4** Fit parameters and diffusion constants ( $D_x$ ) for the two- and three component systems. Values without parenthesis represent the sucrose systems while the values within parenthesis are the trehalose systems.

Component	$\tau_{res}$ (ps)	$\langle r^2 \rangle^{1/2}$ (Å)	$D_x$ ( $10^{-10} \text{m}^2 \text{s}^{-1}$ )
Water in three-comp	13.16 (17.6)	3.15 (2.74)	12.56 (7.09)
Water in two-comp (sug)	4.20 (4.88)	2.13 (1.89)	18.03 (12.26)
Water in two-comp (mb)	8.84	3.20	18.9
Sugar in three-comp	1.68 (13.83)	0.50 (1.31)	2.49 (2.06)
Sugar in two-comp	14.54 (12.33)	1.46 (1.26)	2.45 (2.16)

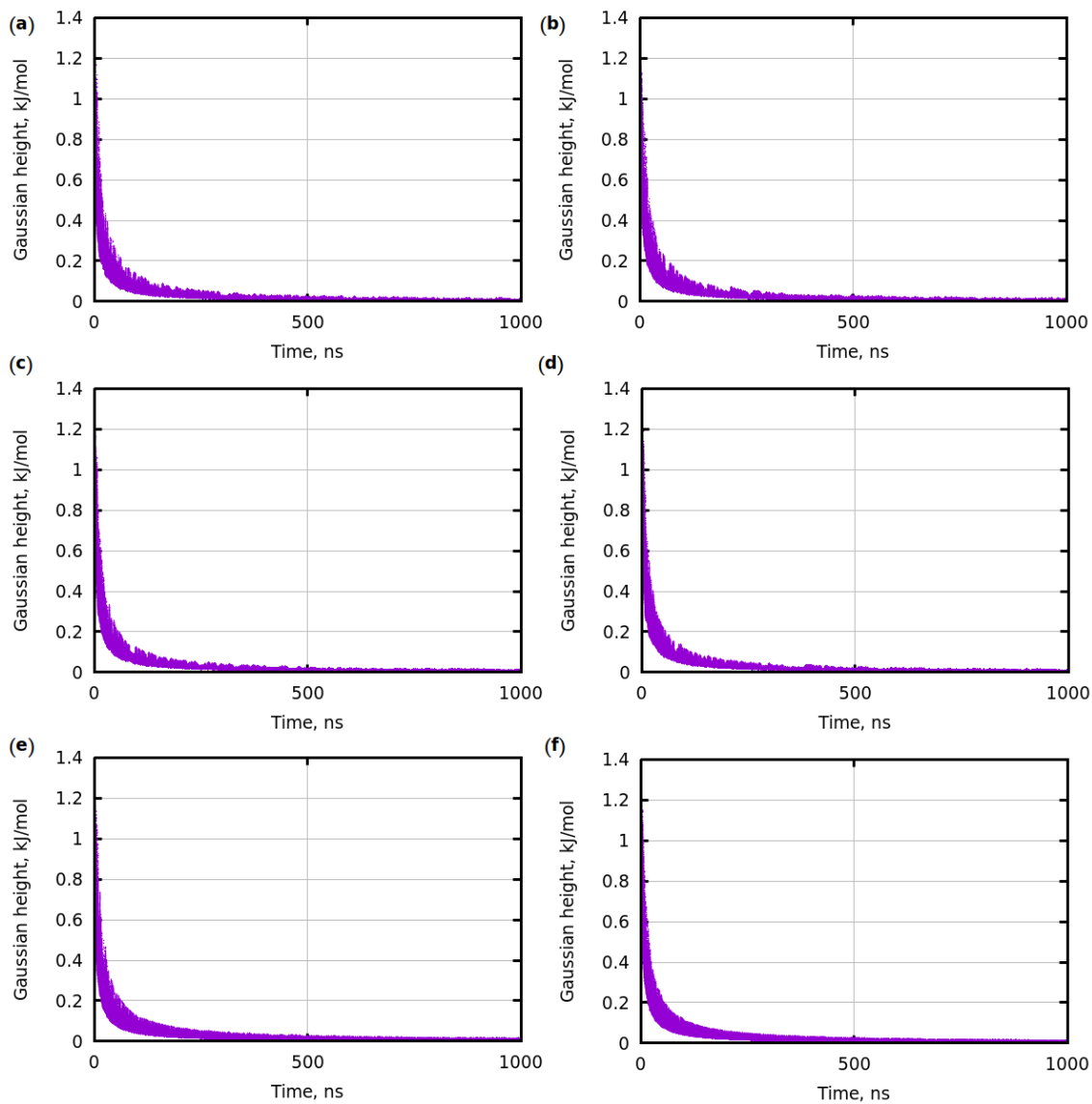
## 4 Classical molecular dynamics: equilibration



**Figure S2** Root mean square deviation for single protein chains in simulated systems. (a) With sucrose and counter ions (b) With sucrose, counter ions and ions for pH (c) With trehalose and counter ions (d) With trehalose, counter ions and ions for pH.

## 5 Free energy calculations: Gaussian heights

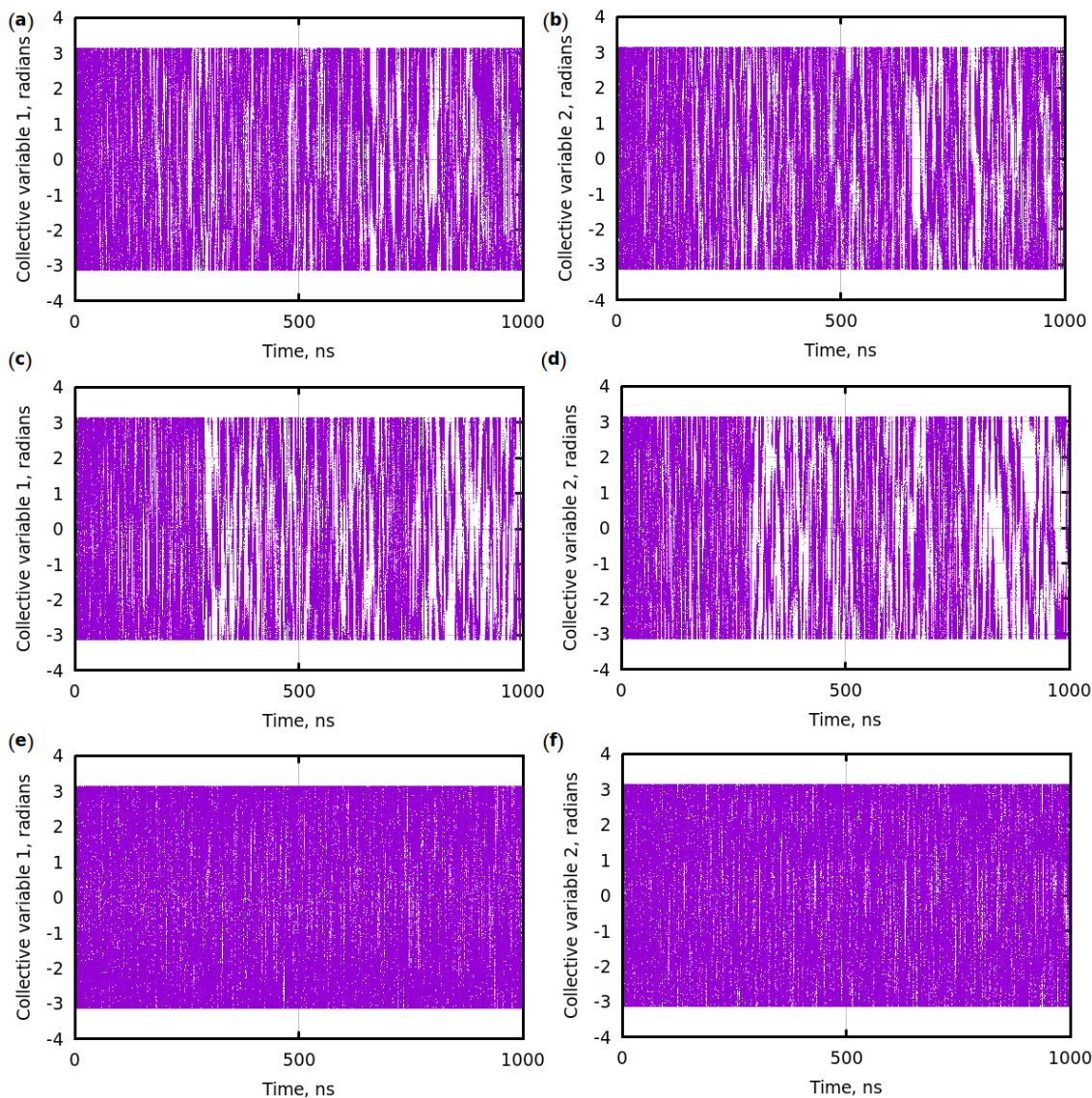
This part demonstrates the evolution of Gaussian heights over the simulation time. The importance of figure S3 is that it shows that despite that there were 2 CVs, the simulation time of  $1 \mu\text{s}$  was enough in order to observe the convergence of simulations, i.e. Gaussian heights asymptotically reach the value  $0 \text{ kJ/mol}$ .<sup>1,2</sup>



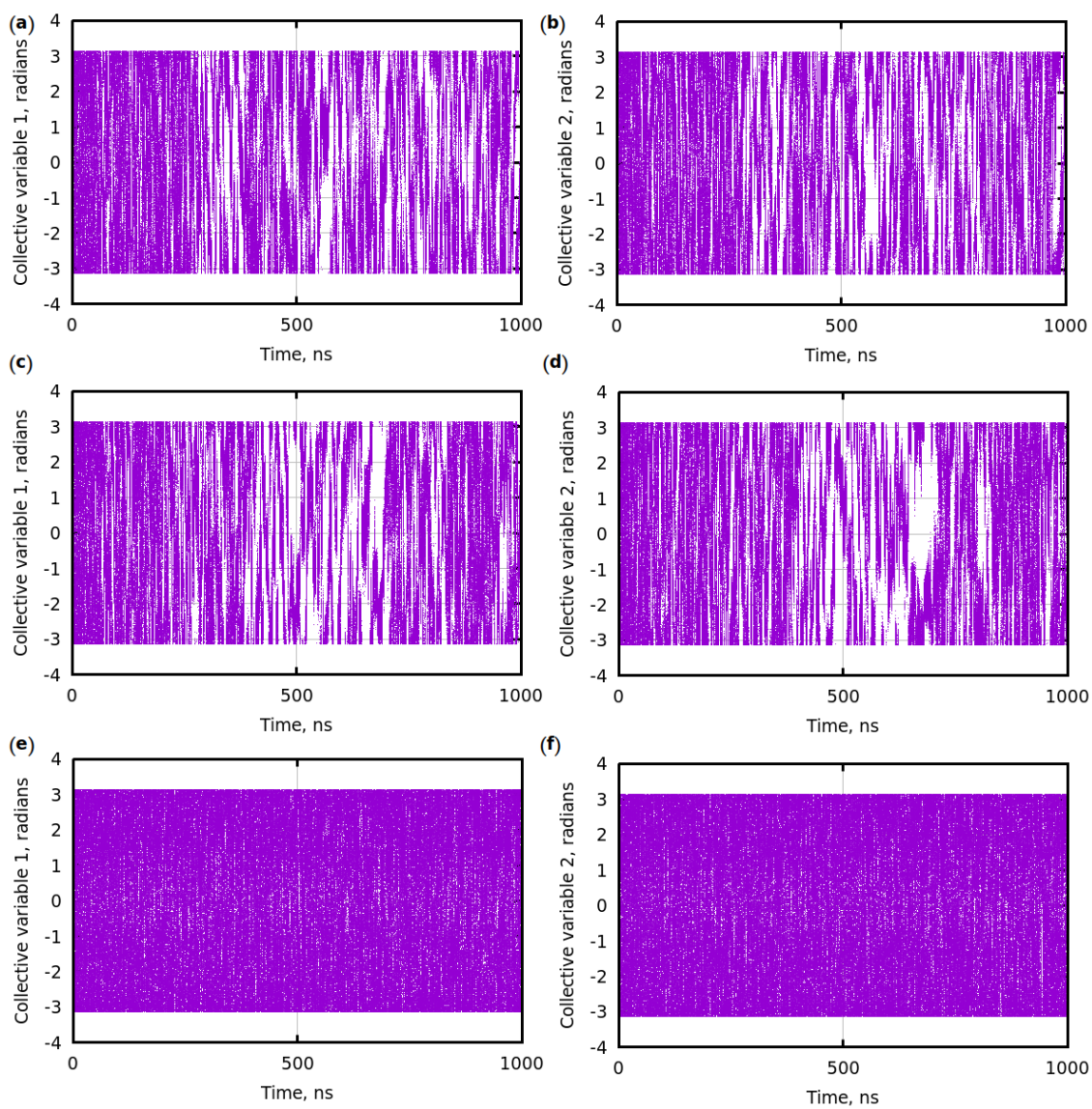
**Figure S3** Profiles with evolution of Gaussian heights over the simulation time. (a) System with myoglobin and sucrose including ions for experimental pH. (b) System with myoglobin and sucrose with counter ions. (c) System with myoglobin and trehalose including ions for experimental pH. (d) System with myoglobin and trehalose with counter ions. (e) System with single molecule of sucrose. (f) System with single molecule of trehalose.

## 6 Free energy calculations: collective variables

This section shows the evolution of 2 CVs over the simulation time. Here the accent is on demonstrations of the good sampling over values of dihedrals. Additionally, one can see in (e) and (f) of figures S4 and S5 that the rotational motion is faster for single sugar molecules in water than for sugar molecules in systems with myoglobin, see ((a)-(d) of figures S4 and S5). This is evident from the more white regions in the interval  $[-\pi; \pi]$  for the systems containing myoglobin.



**Figure S4** Profiles with evolution of collective variables (CVs, dihedrals) over the simulation time for systems with sucrose. (a) CV1 ( $\varphi$ ) in the system with myoglobin and counter ions. (b) CV2 ( $\psi$ ) in the system with myoglobin and counter ions. (c) CV1 ( $\varphi$ ) in the system with myoglobin and ions for experimental pH. (d) CV2 ( $\psi$ ) in the system with myoglobin and ions for experimental pH. (e) CV1 ( $\varphi$ ) in the system with a single molecule. (f) CV2 ( $\psi$ ) in the system with a single molecule.

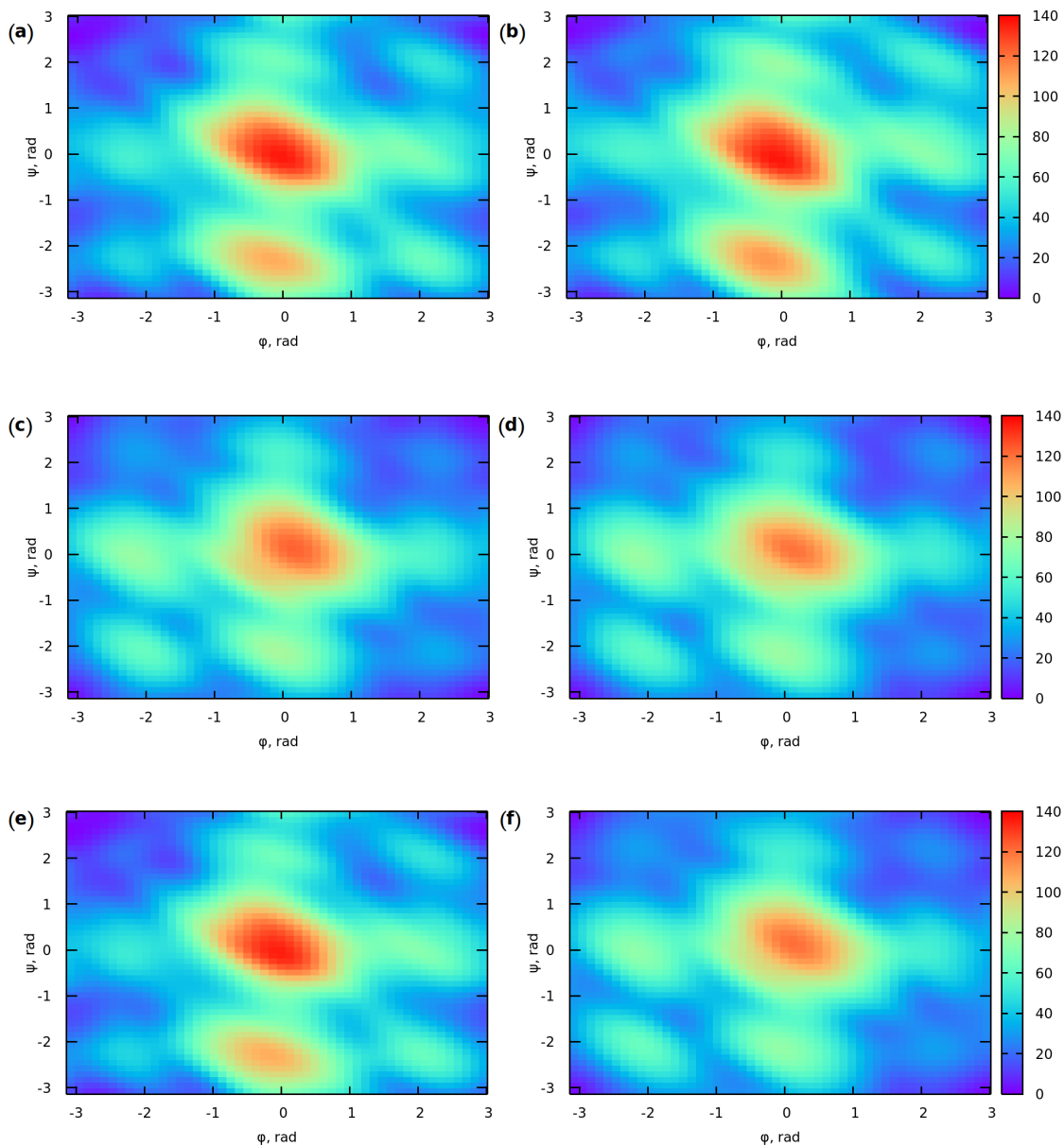


**Figure S5** Profiles with evolution of collective variables (CVs, dihedrals) over the simulation time for systems with trehalose. (a) CV1 ( $\varphi$ ) in the system with myoglobin and counter ions. (b) CV2 ( $\psi$ ) in the system with myoglobin and counter ions. (c) CV1 ( $\varphi$ ) in the system with myoglobin and ions for experimental pH. (d) CV2 ( $\psi$ ) in the system with myoglobin and ions for experimental pH. (e) CV1 ( $\varphi$ ) in the system with a single molecule. (f) CV2 ( $\psi$ ) in the system with a single molecule.



## 7 Free energy calculations: final potential of mean force (PMF) profiles

This section demonstrates final PMFs, which were obtained after 1  $\mu$ s of simulation. These PMFs were integrated into PMFs for every dihedral as well as for rotational free energies, which are shown in the main paper.



**Figure S6** Final profiles for PMF. Color-map represents values for potential of mean force in kJ/mol. (a) System with myoglobin, sucrose and counter ions. (b) System with myoglobin, sucrose and ions for experimental pH. (c) System with myoglobin, trehalose and counter ions. (d) System with myoglobin, trehalose and ions for experimental pH. (e) Single molecules of sucrose. (f) Single molecule of trehalose.

### Notes and references

- [1] J. F. Dama, M. Parrinello and G. A. Voth, *Phys. Rev. Lett.*, 2014, **112**, 240602.
- [2] A. Barducci, G. Bussi and M. Parrinello, *Phys. Rev. Lett.*, 2008, **100**, 020603.