Electronic Supplementary Material (ESI) for Molecular Systems Design & Engineering.

Supplementary Information

Kinetics and mechanism of ionic-liquid induced protein unfolding: Application to the model protein HP35

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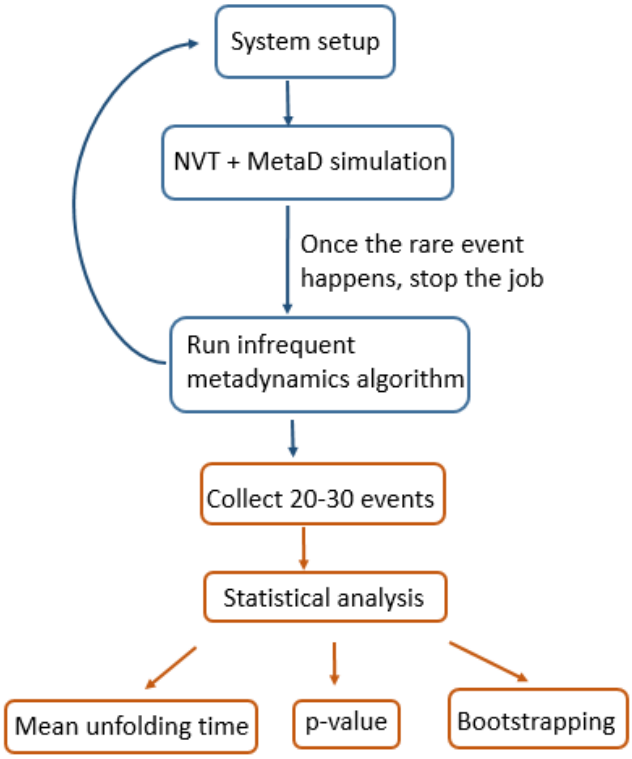

Figure S1: Simulation workflow for a campaign of infrequent MetaD simulations

Description of bootstrapping method for error analysis

For every set of simulations, we performed a sampling procedure by randomly selecting (with replacement) subsamples of 20 unfolding times and analyzing these small sets in the same manner as the original data. If the p-value < 0.05, i.e., the subset did not follow a random Poisson process, we then rejected this subset. The reject rate was calculated from the rejected events out of 2000 iterations of sampling procedure. The same sampling method was also used for sampling mean unfolding time, p-value and other statistics.

Table S1: Further simulation details for each system studied. The PDB entry, IL studied, number of molecules of each type of solvent, temperature, and box dimensions are provided.

Protein	ILs	Cation (#)	Anion (#)	Water (#)	Temperature (K)	Approximate box dimensions (nm)
HP35 (2F4K)	none	0	0	3352	360	4.6 × 5.1 × 4.8
Chignolin (1UAO)	none	0	0	1485	340	3.8 × 3.6 × 3.5
HP35(1YRF)	none	0	0	3179	330	5.1 × 4.9 × 4.1
HP35(1YRF)	[BMIM][Cl]	133	133	5161	330	5.9 × 5.9 × 5.9
HP35(1YRF)	[BMIM][Br]	106	106	5162	330	5.8 × 5.8 × 5.8
HP35(1YRF)	[BMIM][DCA]	113	113	5152	330	5.9 × 6.1 × 5.6
HP35(1YRF)	[BMIM][MTS]	95	95	5276	330	5.8 × 5.8 × 6.0

Table S2: Variation of unfolding time with MetaD Gaussian deposition stride for chignolin unfolding times and KS test p-value (described in main text).

τ (ρs)	Unfolding time (μs)	p-value	Events recorded
2	12700 (5700)	0.02 (0.03)	45
5	1330 (474)	0.04 (0.07)	45
10	43.2 (12.0)	0.12 (0.13)	45
14	48.1 (14.1)	0.24 (0.2)	45
20	8.2 (2.9)	0.43 (0.3)	45
50	4.2 (1.0)	0.24 (0.2)	45
60	5.0 (1.6)	0.39 (0.2)	45
100	2.1 (0.5)	0.48 (0.2)	45
120	1.1 (0.3)	0.49 (0.2)	45

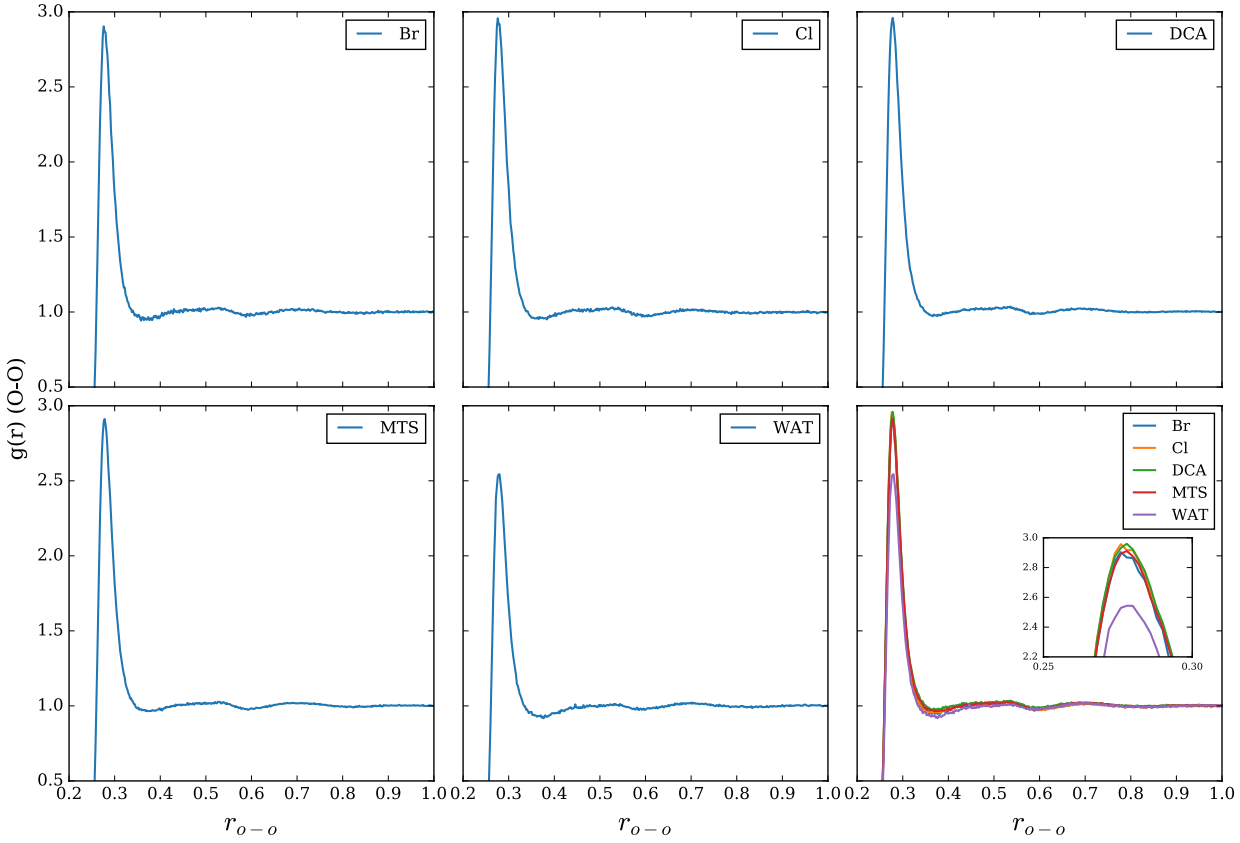

Figure S2: The radial distribution functions (RDFs) of the water (rO-O) in various (20% w/w) ILs/water mixture

Further simulation analyses and details

We also calculated the radial distribution function (RDF) of the protein/IL interactions to study how the anions near the protein behave. The values of the RDFs presented here are averages of three simulations. They support the data shown in Table 4 in the main text. Namely, a clear trend can be seen near 0.5 nm from Cα , $[dca]^-$ anion has highest peak, follow by $[MeOSO_3]^-$, Br^- and Cl^-.

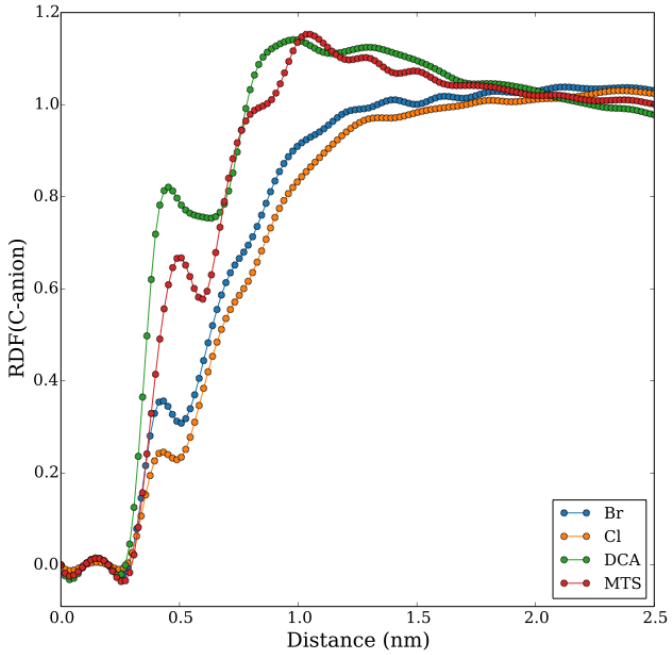

Figure S3: The RDF of the Cα-anion between HP35 and different ILs solutions. The distance calculated is that between the Cα atom and the anion center of mass.

Three trials of 100 ns metadynamics simulation were performed to study the affinity of PHE and the anions we used in this study. We biased the distance between the center of mass of the ring of an isolated neutral PHE (capped with acetate and NME) and one anion. The isolated system (vacuum with no periodic boundary conditions) carried a net charge of -1. Cutoffs of 2.0 nm were used for the LJ and electrostatic potentials. The system temperature was 330 K to coincide with the unfolding simulations. We selected a value of 0.02 nm for the width of the Gaussian 'hills' in the MetaD bias potential. We used well-tempered metadynamics with a biasfactor (γ) of 9 and initial Gaussian height of 2.0 kJ/mol. Energy wall is applied at distance 2.0 nm, the force constant of the wall is 1000 kJ/nm^2

Figure 5 in the main document presents the free energy profiles as the average value of three trials. Convergence was assessed for a single simulation by the tracking minimum basin time-independent free energy value through whole simulation as suggested by Tiwary and Parrinello. Figure S5 shows our simulations are converged after ~ 50 ns, suggesting the three independent trials provide an excellent assessment of the binding free-energy between the anion and PHE.

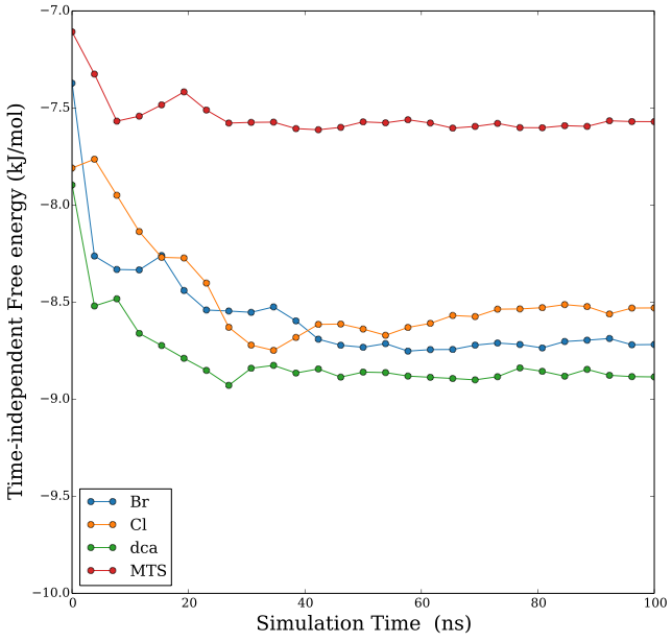

Figure S4: Convergence test of the PHE/anion binding simulations described above and in the main text.