Electronic Supplementary Information for Structural Adaptations in Bovine Serum Albumin Protein in Archetypal Deep Eutectic Solvent Reline and Its Aqueous Mixtures

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S1 Force-Field Validation

Methodology: Table S1 summarizes the compositions of reline-water mixtures studied. PACKMOL[1] was used to randomly pack the molecules in cubic simulation boxes. The parameters for reline components ($[Ch]^+$, Cl^-, and urea) were taken from CHARMM general force-field[2] as used in our previous studies[3,4] while water molecules were considered as TIP3P model.[5] All the simulation have been carried out for ∼60 ns in NPT ensemble using GROMACS 5.1.1.[6] The temperature was maintained to 303 K using Nosé-Hoover thermostat[7–9] and the pressure was kept constant at 1 bar using Parrinello-Rahman barostat.[10] The equations of motion were integrated at a timestep of 1 fs. Periodic boundary conditions were applied in all the directions. A cutoff distance of 1.2 nm was considered for short-range interactions. The long-range electrostatics were evaluated using PME.[11,12] The last 10 ns trajectory with frame saved at every 100 fs has been utilized to calculate density and X-ray scattering structure function S(q). The X-ray structure functions have been calculated as per the previous literature.[4,13,14]

Table S1: Summary of the system compositions studied.

System	Reline (wt%)	Water (wt%)	$[Ch]^+/Cl^-$ ion pairs	urea	water
rel-1w	93.5	6.5	1000	2000	1000
rel-2w	87.8	12.2	1000	2000	2000
rel-4w	78.3	21.7	1000	2000	4000
rel-10w	59.0	41.0	1000	2000	10000
rel-20w	41.9	58.1	1000	2000	20000
water	0.0	100	-	-	1000

Results and Discussion: Figure S1 illustrates the comparison of bulk densities of reline-water mixtures at varying compositions in presence of TIP3P water with the previously reported SPC/E model.[4] A very good agreement is observed at all the studied concentrations. Figure S2 displays the comparative plots of S(q)s of reline-water mixtures at varying

concentrations. A good overlap can be noticed from the plots in presence of both the water models. However, a slight difference in the S(q)s of pure SPC/E and TIP3P water can be noted with respect to splitting of the principal peak, which is a known fact for TIP3P water model.[5] Apart from this, Figure S2 also conveys that in corroboration with the SPC/E water model, TIP3P water also shows the DES structural transition at and above 41 wt% of water (rel-10w). The molecular arrangement of reline is changed from "water-in-reline" to an aqueous solution of reline components i.e. below 41 wt%, all the S(q)s are similar to that of pure reline but at and above 41 wt% of water the S(q)s resemble pure water structure function. Recently, this structural transition at 41 wt% has also been observed experimentally through 2D IR spectroscopy,[15] which further validates the computational findings.

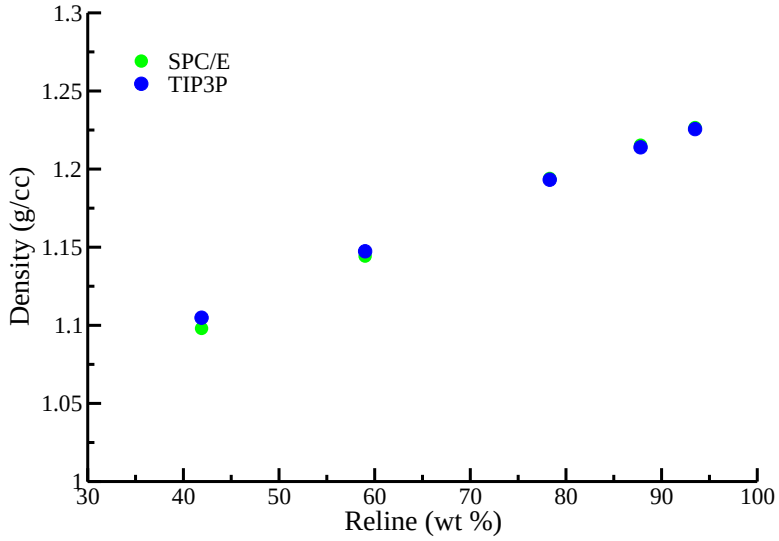

Figure S1: Comparison plots for densities of reline-water mixtures in presence of SPC/E[4] and TIP3P water models. Density data for SPC/E water model has been reprinted with permission from ref.[4] Copyright 2018 American Chemical Society.

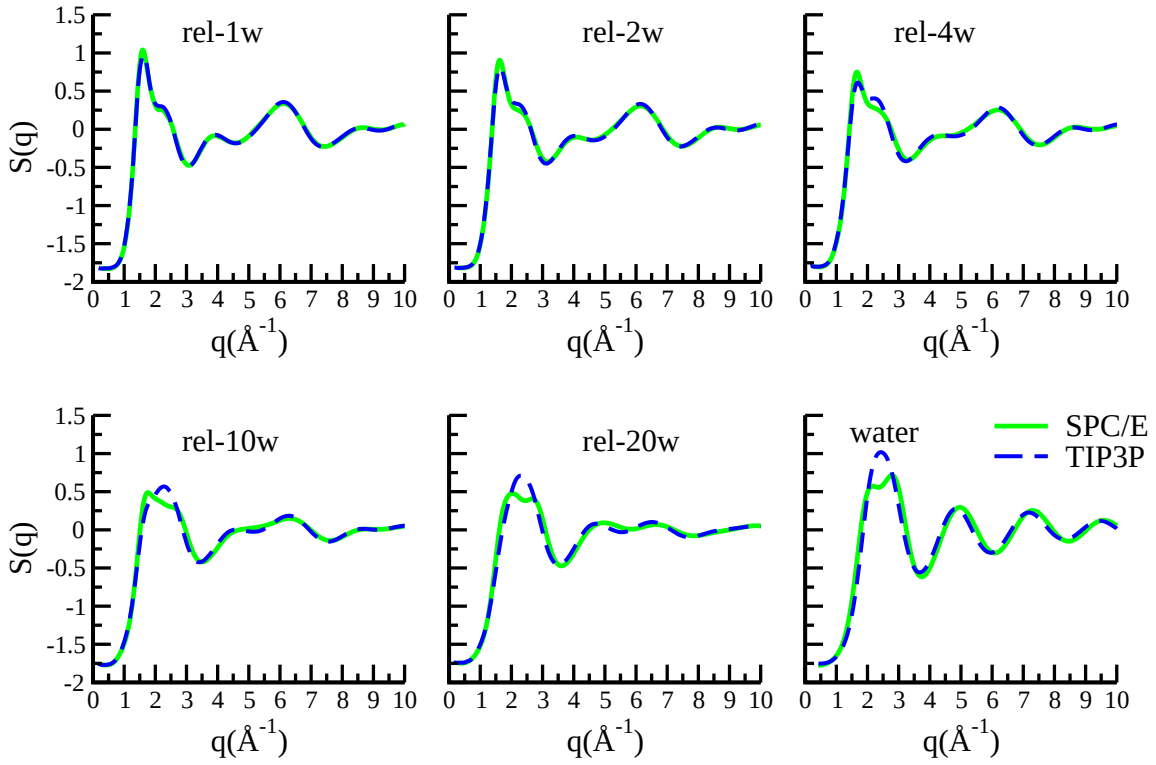

Figure S2: Comparison of simulated X-ray structure functions for reline-water mixtures using SPC/E[4] and TIP3P water model. X-ray scattering data for SPC/E water model has been reprinted with permission from ref.[4] Copyright 2018 American Chemical Society.

Table S2: Summary of all the systems investigated for reversibility of structural modulations.*

System	Denatured BSA conformation taken from	water
R50	50/50 reline/water	50706
R75	75/25 reline/water	60072
R100	pure reline	36576

*Each system was neutralized using 17 K^+ ions.

Table S3: Average percentage of BSA secondary structures for 350 ns reversibility runs calculated using DSSP algorithm. Last 100 ns trajectories were used for the computation of average values.

System	α-helix	3_{10}-helix	coil	turn	bend
R50	60.1	3.0	18.3	8.2	10.3
R75	46.3	3.2	23.5	14.1	12.4
R100	52.8	8.3	18.9	14.0	10.8

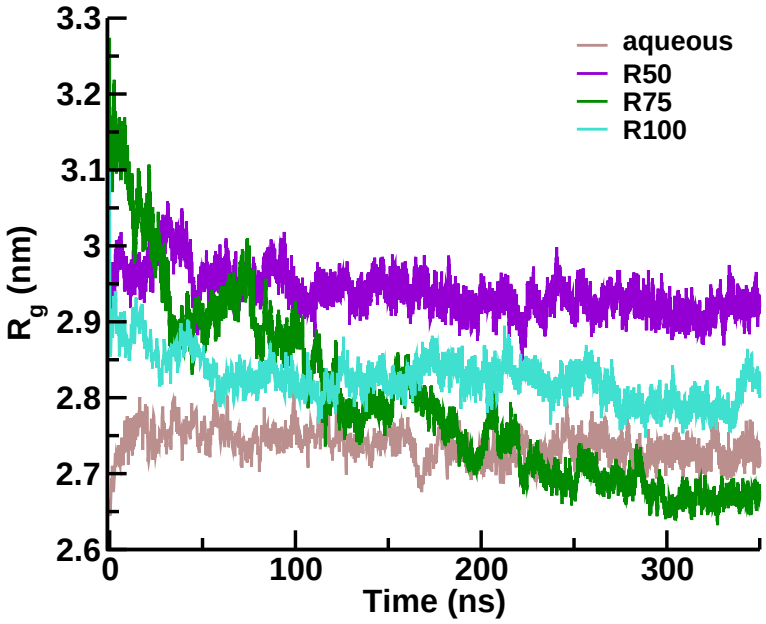

Figure S3: Time-dependent variations in radius of gyration (R_g) on aqueous re-equilibration of denatured protein conformations. Aqueous system data are also plotted for the comparison.

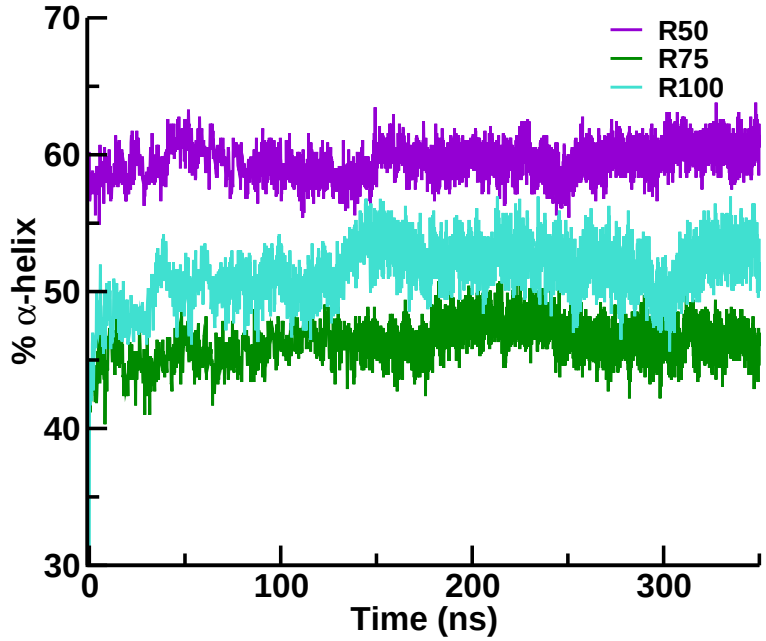

Figure S4: Time evolution of percentage α-helical content for re-equilibration runs of denatured protein systems.

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