Biophysical Journal, Volume *117*

Supplemental Information

Effect of Osmolytes on Conformational Behavior of Intrinsically Disordered Protein α-Synuclein

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SUPPORTING MATERIAL

Effect of osmolytes on conformational behaviour of intrinsically disordered protein α-synuclein

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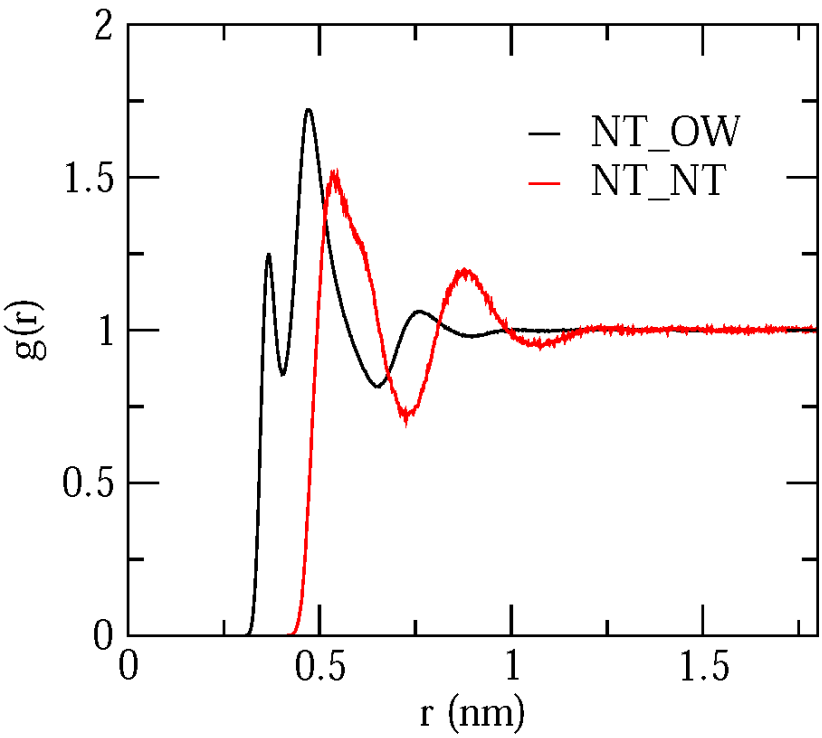
Validation of force field generated from ATB

In order to validate the force field generated from ATB, we have calculated the thermodynamic property (i.e. Density), transport property (self-diffusion coefficient)and structural property (RDF and number of hydrogen bonds) of 3 M TMAO box and compared our result with the Shea force field and United atom (UA) ff (generated from ATB) of TMAO (1). We found that the density of TMAO solution in our case at 3 M TMAO is 980.24 Kg/m^3, for UA model it is 980 Kg/m^3 for solution of concentration up to 4 molal and in case of Shea model it is 990 Kg/m^3 for TMAO solution of molality < 2 mol/Kg **(1).** The ratio of self-diffusion coefficient of water in TMAO water solution to the self-diffusion coefficient of pure water in our case is found to be 0.808, in UA model it is 0.7 and for Shea model it is 0.58 (3.5 m)$\times 10^{-9}$ m^2/s. The number of hydrogen bond per TMAO molecule is 2.13 (3 M TMAO), for UA model it is < 2.5 and for Shea model it is < 3 (~2.8) for ~ 4 mol/Kg solution.

The small variations in the physical constant values could be attributed to the small difference in concentrations. RDF of NT-NT is also calculated and the plot obtained in our case shows similar pattern as shown by UA model (1)**.**

The RDF plot of NT-NT and NT-OW



The thermodynamic properties of UA model shows similarity with the Shea model. However the deviation from the experimental data could be explained in terms of weak solute-water interactions, a consequence of non-optimal assignment of interaction parameter (1)**.**

Table S1: No. of clusters formed and cluster entropy (S = K ln W) in bracket at various RMSD in different conditions. (*Convergence check has been monitored by calculating the number of clusters and cluster entropy during the time interval of 50 ns at various RMSD and it is found that the number of clusters and cluster entropy decreases at the end of the simulation. Since equilibration time for different trajectory is found to be different so we tried to find out a common equilibrated segment from this trajectory as shown in the table below. The number of clusters is 1 for simulation time of 110 ns to 200 ns in all the trajectories except those of urea, which may be because of greater degree of unfolding taking place in urea. It is for this reason that the analysis segment is for the last 90 ns.)*

Number of Clusters

	0-50 ns	**50- 100 ns**	**100-150 ns**	**150-200 ns**
300 K	*92 (4.52)*	*1(0)*	*1(0)*	*1(0)*
350 K	*72(4.276)*	*5(1.61)*	*1(0)*	*1(0)*
400 K	*14(2.64)*	*1(0)*	*1(0)*	*1(0)*
Urea 3 M	*9(2.19)*	*4(1.39)*	*9(2.19)*	*8(2.08)*
Urea 5 M	*19(2.94)*	*10(2.30)*	*11(2.39)*	*4(1.39)*
Urea 8 M	*21(3.04)*	*14(2.64)*	*26(3.26)*	*20(2.99)*
TMAO 3 M	*5(1.61)*	*3(1.09)*	*2(0.693)*	*1(0)*
TMAO 5 M	*1(0)*	*1(0)*	*1(0)*	*1(0)*
TMAO 8 M	*7(1.95)*	*5(1.61)*	*2(0.693)*	*1(0)*

Table S2: Percentage of secondary structure content of α-synuclein in presence of 5 M urea and 5 M TMAO at 300 K, 350 K and 400 K.

Temperature (K)	**Urea 5 M**			**TMAO 5 M**		
	α–Helix	**β–Sheet**	**Coil**	**α–Helix**	**β–Sheet**	**Coil**
300	3	1	76	52	0	24
350	0	19	61	5	1	45
400	0	23	48	8	1	44

Table S3: Fraction of native contacts of Cα atoms of α-Synuclein

Water/Osmolyte	**Average Fraction of native Contact**
300 K	0.745017
350K	0.65617
400K	0.61497
Urea 3 M	0.603184
Urea 5 M	0.60223
Urea 8 M	0.5915
TMAO 3 M	0.642577
TMAO 5 M	0.947012
TMAO 8 M	0.793457

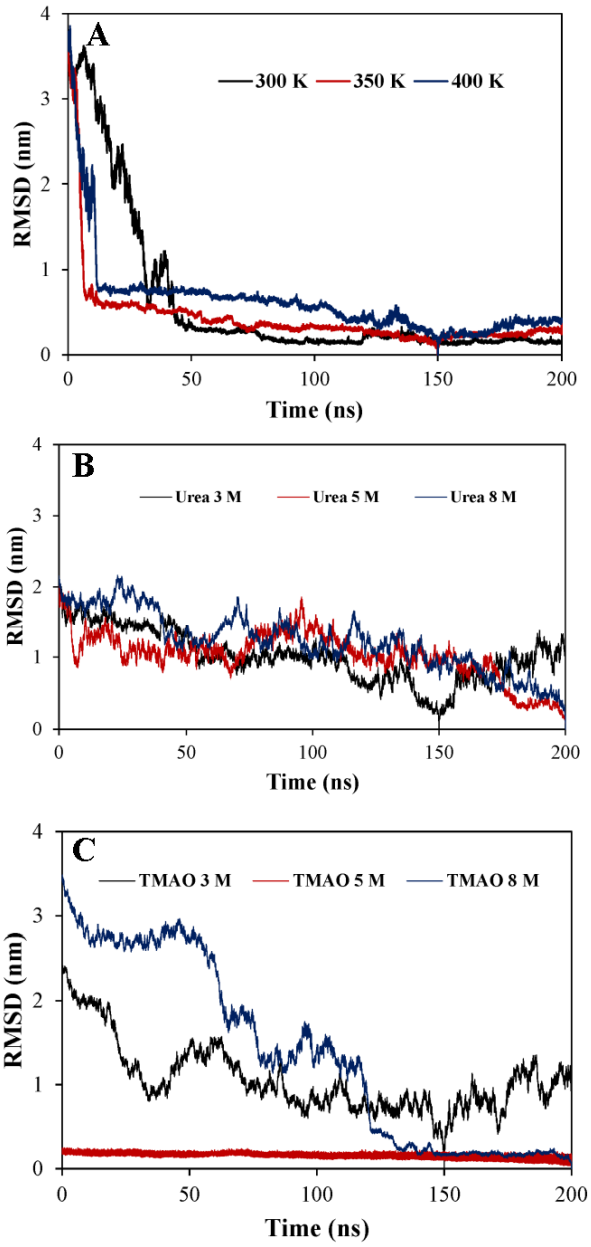


Fig.S1: RMSD of backbone atoms of α-synuclein in water at different temperature and in osmolytes at various concentrations (large fluctuation in RMSD in case of osmolytes is due to large deviation in their conformation from the initial one).

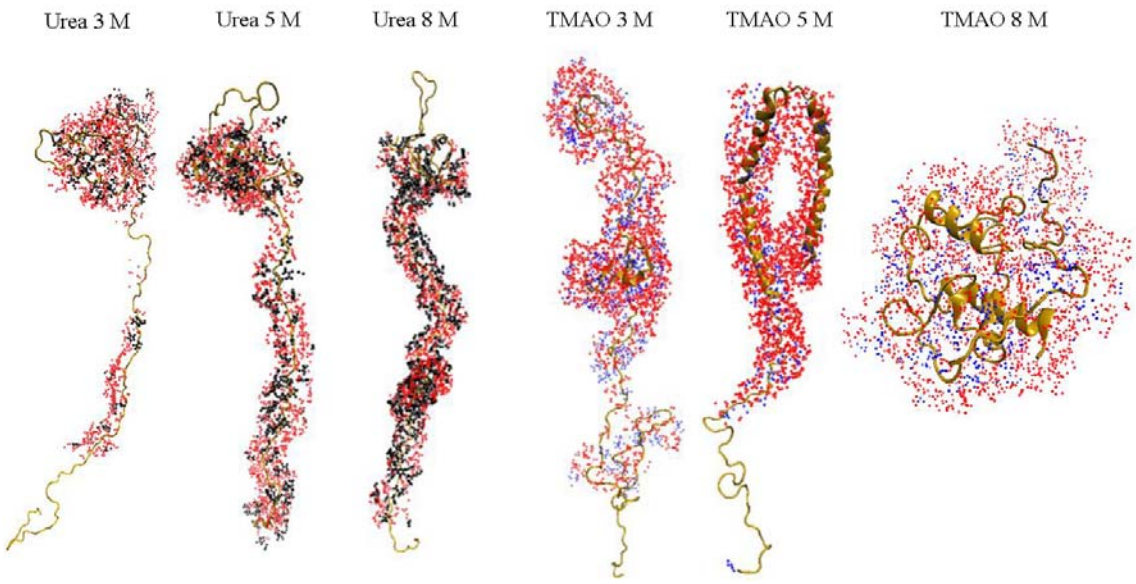


Fig.S2: Protein dehydration for systems containing urea and TMAO within 0.5 nm of α-synuclein surface (black dots are for urea, blue for TMAO and red for water molecules).

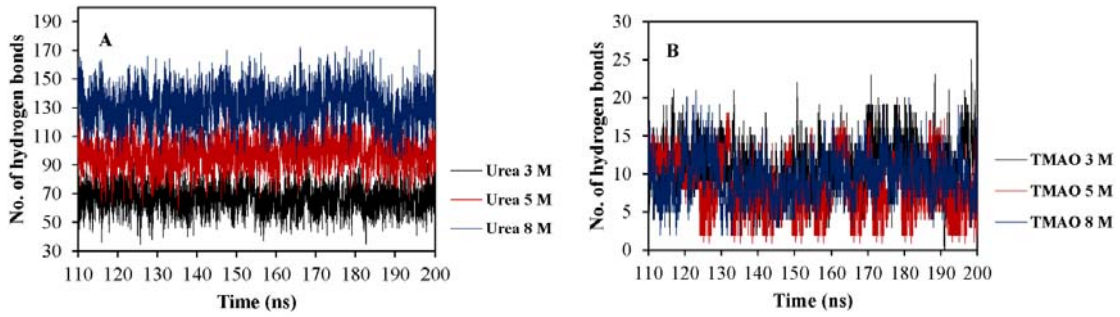


Fig.S3: Number of hydrogen bond formed between side chain of α-Synuclein and osmolytes (A) urea (3 M, 5 M and 8 M) and (B) TMAO (3 M, 5 M and 8 M).

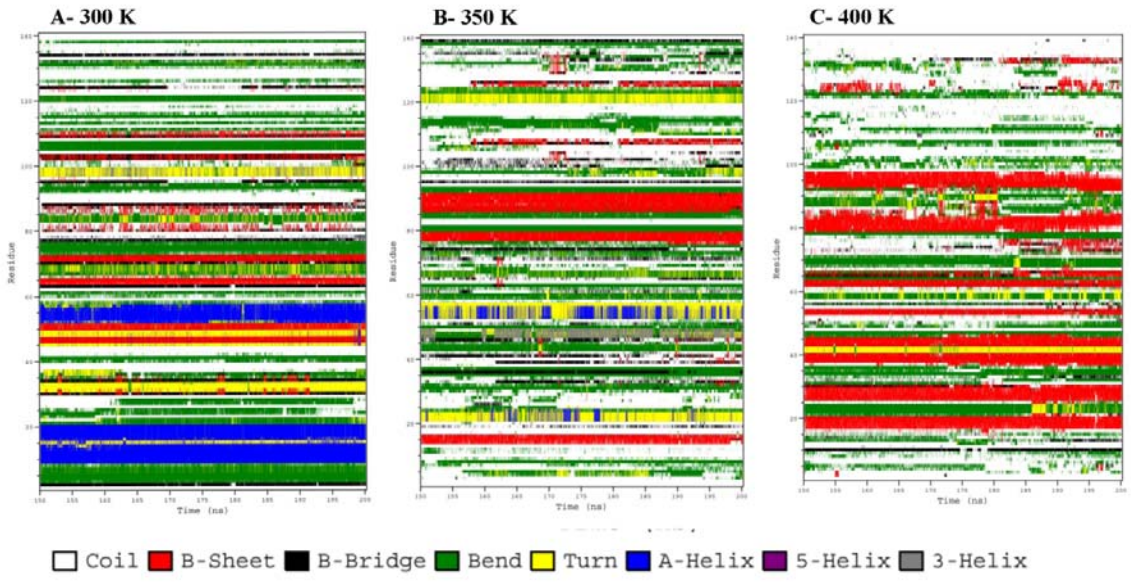


Fig.S4: Secondary Structure plot of α-Synuclein in water at (A) 300 K, (B) 350 K and (C) 400 K.

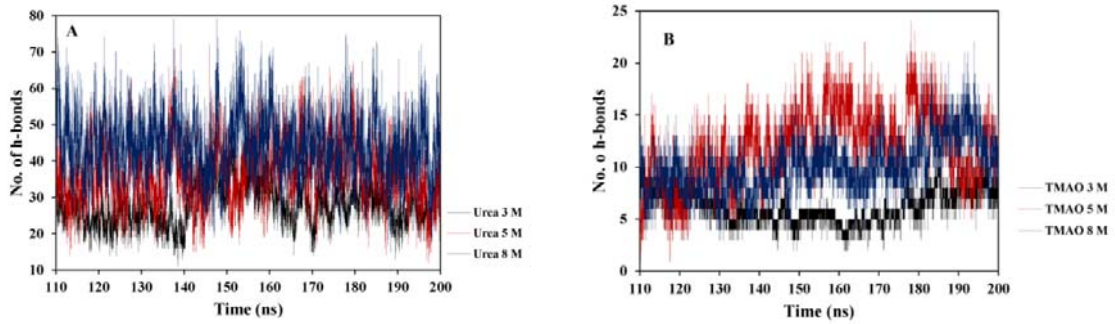


Fig.S5: Number of hydrogen bond formed between water and osmolytes in the first hydration shell (A) Urea (3 M, 5 M and 8 M), (B) TMAO (3 M, 5 M and 8 M)

Supporting References

1. Markthaler, D., J. Zeman, J. Baz, J. Smiatek, and N. Hansen. 2017. Validation of Trimethylamine-N-oxide (TMAO) Force Fields Based on Thermophysical Properties of Aqueous TMAO Solutions. *The Journal of Physical Chemistry B* 121(47):10674-10688.