Hydrophobic association and solvation of neopentane in Urea, TMAO and Urea-TMAO solution

Timir Hajari*,†,1, Mayank Dixit*,†,2 and Hari O. S. Yadav[3]

*These authors equally contributed to this work.

Dr. Timir Hajari

[1]Department of chemistry, City College, 102/1, Raja Rammohan Sarani, Kolkata - 700 009, India

E-mail: timir230@gmail.com

Mayank Dixit

[2]Researcher, Graduate School of Engineering, Department of Chemical Engineering Kyoto University-Katsura Nishikyo-ku, Kyoto-shi, Kyoto-fu, 615-8510, Japan

E-mail: mayankdixit.kyoto.iitb@gmail.com and dixit@cheme.kyoto-u.ac.jp

Hari O. S. Yadav

[3]Postdoctoral Fellow, Department of Materials Chemistry, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Aichi, 464-8603, Japan

E-mail: hariyadav.iitd@gmail.com and hari.yadav@chembio.nagoya-u.ac.jp

†Corresponding authors. Email: timir230@gmail.com **and** mayankdixit.kyoto.iitb@gmail.com

ABSTRACT

A detailed knowledge on hydrophobic association and solvation is crucial in understanding the conformational stability of proteins and polymers in osmolyte solutions. Using Molecular Dynamics simulations, we found the hydrophobic association using neopentane molecules is greater in mixed urea-TMAO-water solution in comparison to that in 8 M urea solution, in 4 M TMAO solution and in neat water. The neopentane association in urea solution is greater than that in TMAO solution or neat water. We find the association is even less in TMAO solution than pure water. From free energy calculations, it is revealed that the neopentane sized cavity creation in mixed urea-TMAO-water is most unfavorable and that causes the highest hydrophobic association. The cavity formation in urea solution is either more unfavorable or comparable to that in TMAO solution. Importantly, it is found that the population of neopentane-neopentane contact pair and the free energy contribution for cavity formation step in TMAO solution are very sensitive towards the choice of TMAO force-fields. A careful construction of TMAO force-fields is important for studying hydrophobic association. Interestingly it is observed that the total solute-solvent dispersion interaction energy contribution is always most favorable in mixed urea-TMAO-water. The magnitude of this interaction energy is greater in urea solution relative to TMAO solution for two different force-fields of TMAO, whereas the lowest value is obtained in pure water. It is revealed that the extent of the overall hydrophobic association in osmolyte solutions is mainly governed by the cavity creation step and it nullifies the contribution comes from the solute-solvent interaction contribution.

I. INTRODUCTION

A deep understanding about hydrophobic solvation is very important to gain proper knowledge on the conformational stability of proteins and hydrophobic polymers in aqueous osmolyte solutions. Urea is a common osmolyte that unfolds proteins in aqueous urea solution. On the other hand, TMAO is a protecting osmolyte means it stabilizes the folded form of protein. Interestingly, TMAO prevents the denaturing ability of urea and helps a protein to stay in its folded form in aqueous urea-TMAO solution. Several theoretical and experimental studies have addressed on the destabilizing effect of urea,[1–8] the stabilizing effect of TMAO and the role of TMAO to counteract the denaturing ability of urea.[9–21] It has been shown that the preferential interaction of urea with the protein side-chains as well as protein backbone leads to an accumulation of urea around protein's solvation shell and eventually causes the protein unfolding.[2,3,6] However, the complete molecular level understanding about the role of TMAO in stabilizing protein native folded structure either in aqueous TMAO solution or in water-urea-TMAO mixture is not established yet. Various contradictory theories are proposed to explain the role of TMAO in stabilizing protein native folded structure.[10,11,15,17,20]

The role of hydrophobic effect in protein folding is undeniable. Hence the study of the solvation of small hydrophobic molecules or association behaviors of hydrophobic molecules in osmolyte solutions is very important on this regard. Several simulations work concluded a favourable dispersion interaction between urea and hydrophobic moieties is responsible for a higher population of unfolded conformations of hydrophobic polymers or less positive solvation free energies of hydrophobic molecules in urea-water relative to neat water.[2,22–24] It was also found using model hydrophobic solutes (except methane) that the Hydrophobic solvation is more favourable in urea-water mixture as the dispersion energy contribution is more favourable in aqueous urea solution in comparison to that in pure water.[22,23] For the same reason, association of large hydrophobic molecules is reduced to certain extend in urea-water compared to that in pure water. Contrarily, the hydrophobic association in water-TMAO solution or in water-urea-TMAO is not well understood and several contradictory opinions are present in literature.[11,25–34] The work of Athawale et al.[25] had found that TMAO does not affect the strength of the hydrophobic interactions. Simulations studies also found a greater number of TMAO molecules preferentially bind to the folded form of hydrophobic chain than that of the unfolded form and that leads to a higher population of folded form in TMAO solution.[11,28] Nair and van der Vegt[31] showed by detailed thermodynamic analysis that TMAO stabilizes the collapsed hydrophobic polymer structures at low TMAO concentrations and the unfolded structures are stabilized at higher concentrations. Recent simulation studies from Ganguly et al.[32] have shown that hydrophobic association is very sensitive to the choice of the force fields of TMAO molecule. It was found by Tah and Mondal[33] that the collapsed form of a hydrophobic polymer is destabilized in mixed urea-TMAO solution.

The solvation process for any hydrophobic solute can be imagined as a two step process. Firstly, a cavity is created to accommodate the solute. In the second step, the solute-solvent dispersion interaction is introduced. The cavity formation step is highly unfavorable and it always overcompensate the favorable dispersion interaction contribution. Now the extent of hydrophobic association in various osmolyte solutions is not same because the cavity formation contribution and dispersion contribution are different in various osmolyte solutions. It is really important to study the cavity contributions and the solute-solvent dispersion interaction energy contributions in different osmolyte solutions to get proper understanding about hydrophobic association in osmolyte solutions. In our current manuscript, the hydrophobic solvation and hydrophobic association are studied using neopentane molecules in neat water, in aqueous 8 M urea solution, in aqueous 4 M TMAO solution and in a mixture of urea, TMAO and water solution using molecular dynamics simulations. Hydrophobic association is quantified using pair correlation functions and Kirkwood Buff integral data. The relative density of solvent molecules around neopentane is analyzed using pair correlation functions between neopentane and solvents. Preferential binding between hydrophobic molecule and cosolvent (urea or TMAO) is also estimated. All the analysis mentioned above are also performed using neopentane cavities for the better understanding of hydrophobic association. The two important contributions in hydrophobic solvation namely the free energy required for cavity creation and the solute solvent interaction energies are also computed for a deeper understanding about the solvation process in mixed solvent medium.

II. METHODS AND COMPUTATIONAL DETAILS

A. Molecular dynamics protocol:

We have carried out classical MD simulations using GROMACS package (version 5.1.4)[35] in four different solvent systems: water, aqueous-urea, aqueous-TMAO, and aqueous-urea-TMAO.

We use Partical Mesh Ewald electrostatics[36] with a direct space cutoff of 1.0 nm and a grid spacing of 0.12 nm. For nonbonded van der Waals interactions, a 1.0 nm cutoff is used. Pressure of the system is 1 bar and we have fixed the pressure of systems using the Berendsen barostat.[37] We have maintained the geometry of water, urea and TMAO molecules during the simulations by using the LINCS[38] algorithm. The temperature of the system is 298 K and it was kept constant using the velocity rescaling thermostat.[39]

Two different TMAO force-fields are considered for understanding the force-fields dependencies of hydrophobic association. One model was developed by Garcia and coworkers[16] that is called as

Garcia force-fields in our manuscript. The second one was developed by Netz and coworkers[18] that is named as Netz force-fields. TIP3P[40] and SPC/E[41] water models are considered along with Garcia and Netz force fields for TMAO respectively as this combination was used in the simulation studies of Ganguly et al.[32] Kirkwood buff force field[42] for urea and gromos54a7 force fields[43] for neopentane are used. The force fields used here for neopentane is an united atom model.

The details of the simulation boxes containing neopentane, water, urea and TMAO are given in Table I.

Table I. Details of the systems containing cavity (N_{cavity}), neopentane (N_{neo}), water (N_w), urea (N_u) and TMAO (N_{ta}).

Systems	N_{neo}	N_{cavity}	N_w	N_u	N_{tmao}
I_w	25	0	975	0	0
II_{w-ua}	25	0	10000	2500	0
III_{w-tmao}	25	0	12500	0	1250
$IV_{w-ua-tmao}$	25	0	6000	2500	1250
C_w	0	25	975	0	0
C_{w-ua}	0	25	10000	2500	0
C_{w-tmao}	0	25	12500	0	1250
$C_{w-ua-tmao}$	0	25	6000	2500	1250

System I_w refers to neopentane in water, system II_{w-ua} refers to neopentane in water-urea, system III_{w-tmao} refers to neopentane in water-TMAO, and system $IV_{w-ua-tmao}$ refers to neopentane in water-urea-TMAO mixtures. System C_w refers to cavity in water, system C_{w-ua} refers to cavity in water-urea, system C_{w-tmao} refers to cavity in water-TMAO, and system $C_{w-ua-tmao}$ refers to cavity in water-urea-TMAO mixtures. The numbers of cavity, neopentane, water, urea and TMAO molecules are given by N_{cavity}, N_{neo}, N_w, N_u and N_{tmao} respectively (subscripts referring to neopentane cavity, neopentane, water, urea and TMAO). For cavities the C_6-term in van der Walls equation is set as zero, only C_{12} term is present to represent the excluded volume part. The C_{12} term in cavities is same as the C_{12} term present in neopentane molecules.

Packmol software is used to generate the initial configuration of each system.[44] Initially, MD simulations were performed for 50 ns in NVT ensemble for thermal equilibration of each system at 298K. Subsequently, NPT simulations for 100ns were carried out for equilibration for each system using

velocity rescaling thermostat[39] and Berendsen pressure barostat.[37] Finally we have generated trajectories of 400 ns for each system using NPT MD simulations by using Nosé-Hoover thermostat[45,46] and Parrinello-Rahman barostat.[47]

B. Simulation details of cavity formation free energy calculations

The Thermodynamics Integration (TI)[48] method is employed to estimate the free energy cost to generate a soft cavity in four different solvent mediums. Here, the initial state is where zero solute - solvent interaction is present and in the final state the r^{-12} part of the solute is introduced. To do that the C_6 and C_{12} terms for the van der Walls equation are set as zero for the solute at initial state and the final state only C_{12} is there. For all simulations in this context, the box size is around 4 nm. The numbers of water, urea, TMAO are presented in table II. To perform TI, 16-20 λ points are considered. The leap-frog stochastic dynamics integrator[49] with an inverse friction constant of 0.1 ps is used to maintain the system temperature at 298K. For each λ point, initially 1 ns to 4 ns equilibration runs and 4 ns to 10 ns production runs are performed. We have fixed the pressure of systems 1 bar using the Berendsen barostat[37] for the equilibration and Parrinello-Rahman barostat[47] for the production run. More details regarding the methodology are given in the work of Hajari and van der Vegt.[50]

Table II. The details of the systems for cavitation free energies of neopentane in water, water-urea, water-TMAO and water-urea-TMAO mixtures.

Systems	N_{neo}	N_w	N_u	N_{tmao}
CAV_w	1	2000	0	0
CAV_{w-ua}	1	1250	300	0
CAV_{w-tmao}	1	1250	0	150
$CAV_{w-ua-tmao}$	1	1000	300	150

C. Radial distribution functions (RDFs)

To investigate solvation of neopentane in these systems, we calculate the RDFs between water, urea, TMAO, neopentane and cavity. The RDFs is given by following equation.

$$g_{\alpha\beta}(r) = \frac{\langle \rho_\beta(r) \rangle}{\langle \rho_\beta \rangle_{local}}$$

$$= \frac{1}{\langle \rho_\beta \rangle_{local}} \frac{1}{N_\alpha} \sum_{i \in \alpha}^{N_\alpha} \sum_{j \in \beta}^{N_\beta} \frac{\delta(r_{ij} - r)}{4\pi r^2} \dots\dots\dots\dots\dots(1)$$

Here $\rho_\beta(r)$ is the particle density of type β particles around α particles at a distance r and $\langle \rho_\beta \rangle_{local}$ is the particle density of type β averaged over all spheres around particle α with radius r_c, $N\alpha$ is the total number of α particles and N_β is the total number of β particles.

D. Preferential binding coefficients(γ)

The preferential binding coefficient is defined as[51]

$$\gamma = \left\langle n_c(r) - \frac{N_c - n_c(r)}{N_w - n_w(r)} n_w(r) \right\rangle \dots\dots\dots\dots\dots\dots(2)$$

where $n_c(r)$ represents the number of cosolvents and $n_w(r)$ number of water molecules at distance r from the center of mass of neopentane or cavity, N_c is total number of cosolvents and N_w is the total number of water molecules in the system. The positive value of γ indicates preferential binding of solute with co-solvent while negative value favors solvent i.e. water near the solute.

E. Kirkwood-Buff integrals

The Kirkwood-Buff Integrals[52] (G_{ij}) between species i and j of binary mixture is defined as

$$G_{ij} = 4\pi \int_0^\infty \left[g_{ij}(r) - 1 \right] r^2 dr \dots\dots\dots\dots\dots(3)$$

Where g_{ij} is the radial distribution function between species i and j. The major contribution in this integral comes from distances r < 1.0 - 1.5 nm.[53] Here we have chosen the upper limit r =1.2 nm for getting the G_{ij} values. A positive value of G_{ij} indicates that the number of particle type j around i

type particle is greater than the number of j type particle present in the bulk where particle type i is absent at the center in the same volume element.

III. RESULTS AND DISCUSSIONS

In this section, we discuss the solvation structures around neopentane and neopentane cavity molecules in water, 8M urea-water, 4M TMAO-water and water-urea-TMAO mixtures for two different force fields of TMAO namely Garcia and Netz force fields. For the analysis of solvation structure of neopentane and cavity, we use radial distribution function and preferential binding coefficients. We also discuss about hydrophobic solvation and it is analyzed in details by computing the solute-solvent interaction energies and the cavity creation free energies. The neopentane association is also quantified using KBI values in osmolyte solutions.

A. Solvation structure of neopentane

Here, we discuss the solvation structure of neopentane in water, water-urea water-TMAO and water-urea-TMAO mixtures for Garcia and Netz models. We present radial distribution function of neopentane, water, urea and TMAO around neopentane in water, water-urea, water-TMAO and water-urea-TMAO mixtures.

A-1. RDFs between neopentane molecules

The RDF between neo-neo is computed in pure water, water-urea, water-TMAO, water-Urea-TMAO using two different force-fields namely Netz and Garcia which is shown in Figure 1. It is clear from the plots the population of neo-neo contact pair (peak) follows the order: water-urea-TMAO> water-Urea> water> water-TMAO for Garcia's TMAO model. The order is as follows: water-Urea-TMAO> water-TMAO> water-urea> water when Netz force field of TMAO is used. The neopentane association at contact pair state is always higher in water-urea-TMAO mixture than other three solutions irrespective of the choice of TMAO's force-fields.

A relatively higher association of neopentane is observed here for TMAO solution with Netz model. By computing potential of mean force (PMF) between two neopentane molecules, Su et al.[54] also showed a higher association at contact distance in TMAO solution relative to that in pure water using Netz model. A similar kind of observation was pointed out by Ganguly et al.[32] and they explained the fact as the TMAO is more hydrophilic in nature in Netz force field. Possibly for this reason, we can observe second highest contact pair population in water-TMAO for Netz force fields. Contrarily, the lowest population is noticed in water-TMAO when Garcia force field is employed for TMAO. This is possible because the TMAO is less hydrophilic in nature. So, in TMAO solution the neo-neo association at this state is very sensitive and quite dependent on the force-fields.

On the other hand, the population of neo-neo solvent mediated pair shows different trends: urea-water ~ urea-water-TMAO> water> water-TMAO. Lee and van der Vegt[55] showed that urea sits in between

neopentane molecules hence the population of solvent mediated pair is enhanced in urea-water compared to water. For the same reason the populations in water-urea-TMAO and in water-urea are comparable. For both force field of TMAO, it is observed that the population of solvent mediate pair is lowest in water-TMAO mixture.
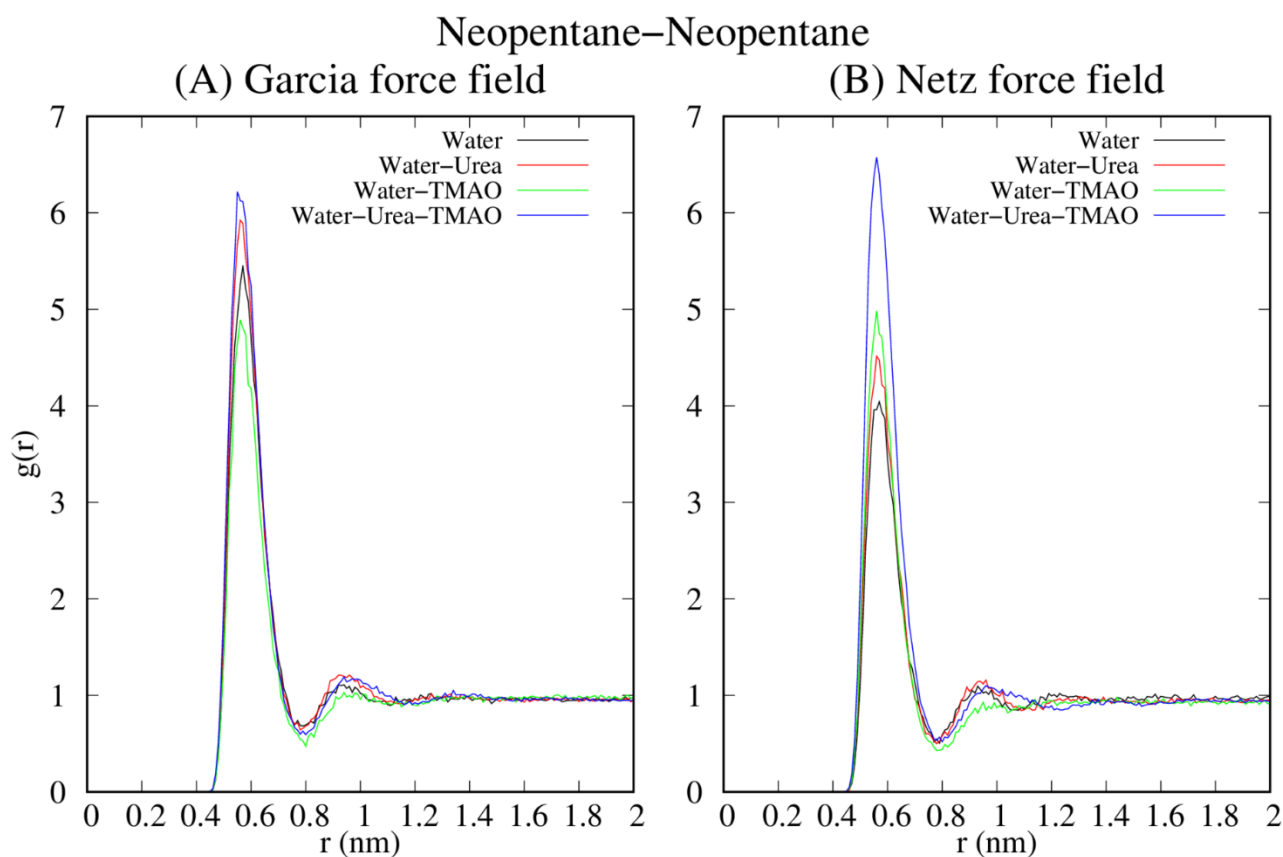

Figure 1. Radial distribution functions between neopentane-neopentane in water, water-urea, water-TMAO and water-urea-TMAO mixtures for Garcia (A) and Netz (B) force fields.

A-2 Structure of water around neopentane

The water structure around neopentane molecules is quantified by computing the pair correlation (g(r)). Figure 2 represents g(r) between the center of masses of neopentane and water. When Garcia's model of TMAO employed, the water density in the first solvation shell of neopentane follows the following order water-urea-TMAO> water-urea> water-TMAO > water. A completely different order is found when Netz's TMAO force field is used. The order is water-urea>water-TMAO~water> water-urea-TMAO.

In both TMAO force fields, the water density in the 1st solvation shell of neopentane is quite similar for pure water and water-TMAO solutions. A significantly high population of water molecules around hydrophobe is always found in water-urea solution compare to that in pure water or water-TMAO. In this context, it is important to mention that a strong force field dependency is identified for water-

urea-TMAO mixture. In the case of Garcia's TMAO force field, the peak height is highest and for Netz's TMAO force field, the peak height is lowest in comparison to that in pure water, water-TMAO and water-urea mixtures.

So, upon the addition of urea to the water-TMAO solution or the addition of TMAO in water-urea, the water density around neopentane's 1st solvation shell is either slightly increased or decreased which depends on the nature of the TMAO's force field.
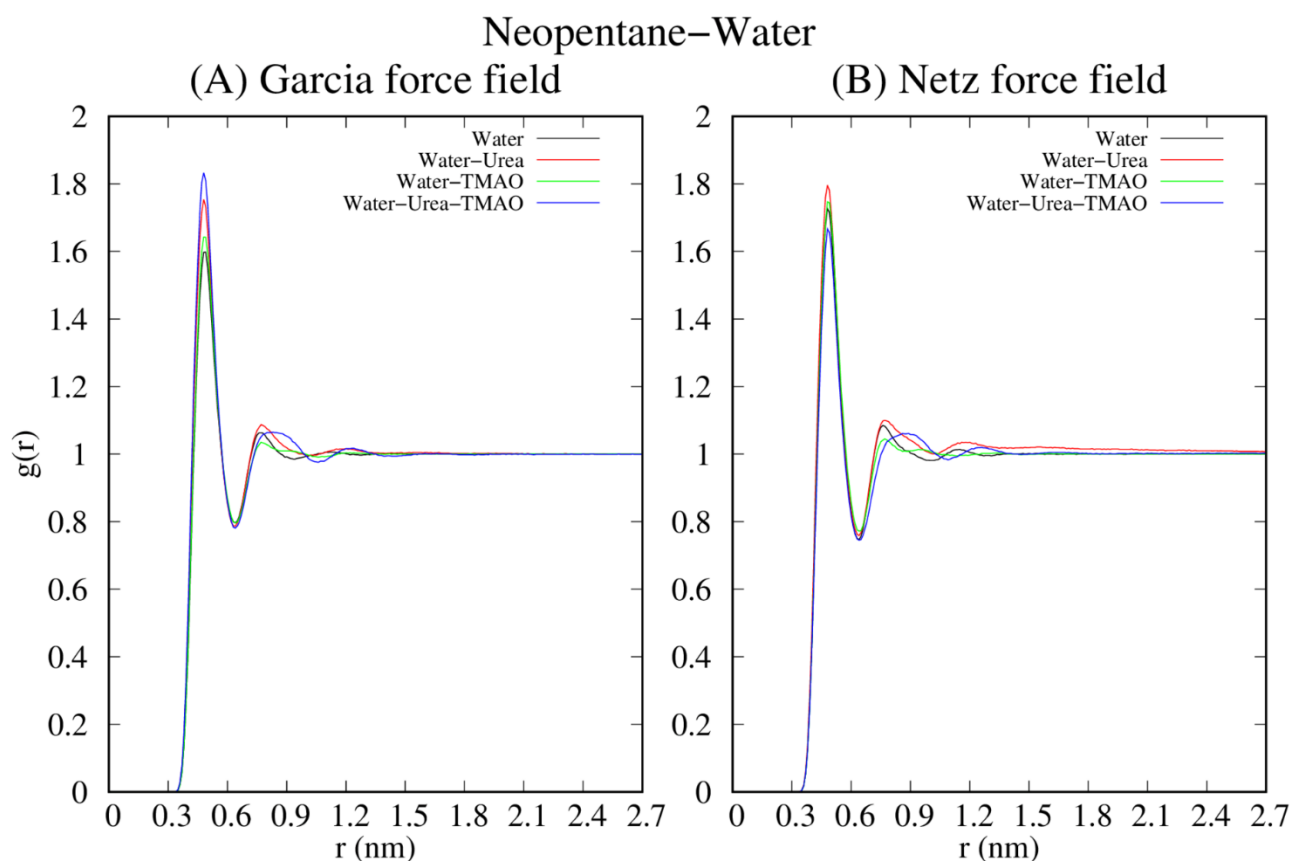

Figure 2. Radial distribution functions between neopentane-water in water, water-urea, water-TMAO and water-urea-TMAO mixtures for Garcia (A) and Netz (B) force fields.

A-3. Structure of cosolvent around neopentane

Figure 3 represents the g(r) between neopentane and urea molecule in water-urea solution and in water-urea-TMAO solution. Independent of the choice of the force-fields of TMAO, a slightly higher urea density in the 1st solvation shell of neopentane is observed in water-urea-TMAO solution in comparison to that in water-urea solution. This increment is more when Netz's TMAO force field is used.

The RDFs between neopentane and TMAO is shown in Figure 4. Again, a marginally higher TMAO density is found in water-urea-TMAO solution than that in water-TMAO solution. Hence upon

addition of TMAO in water-urea solution a bit more accumulation of urea around neopentane is found. Similarly, an addition of urea in water-TMAO mixture, an enhanced accumulation of TMAO is found near hydrophobes.
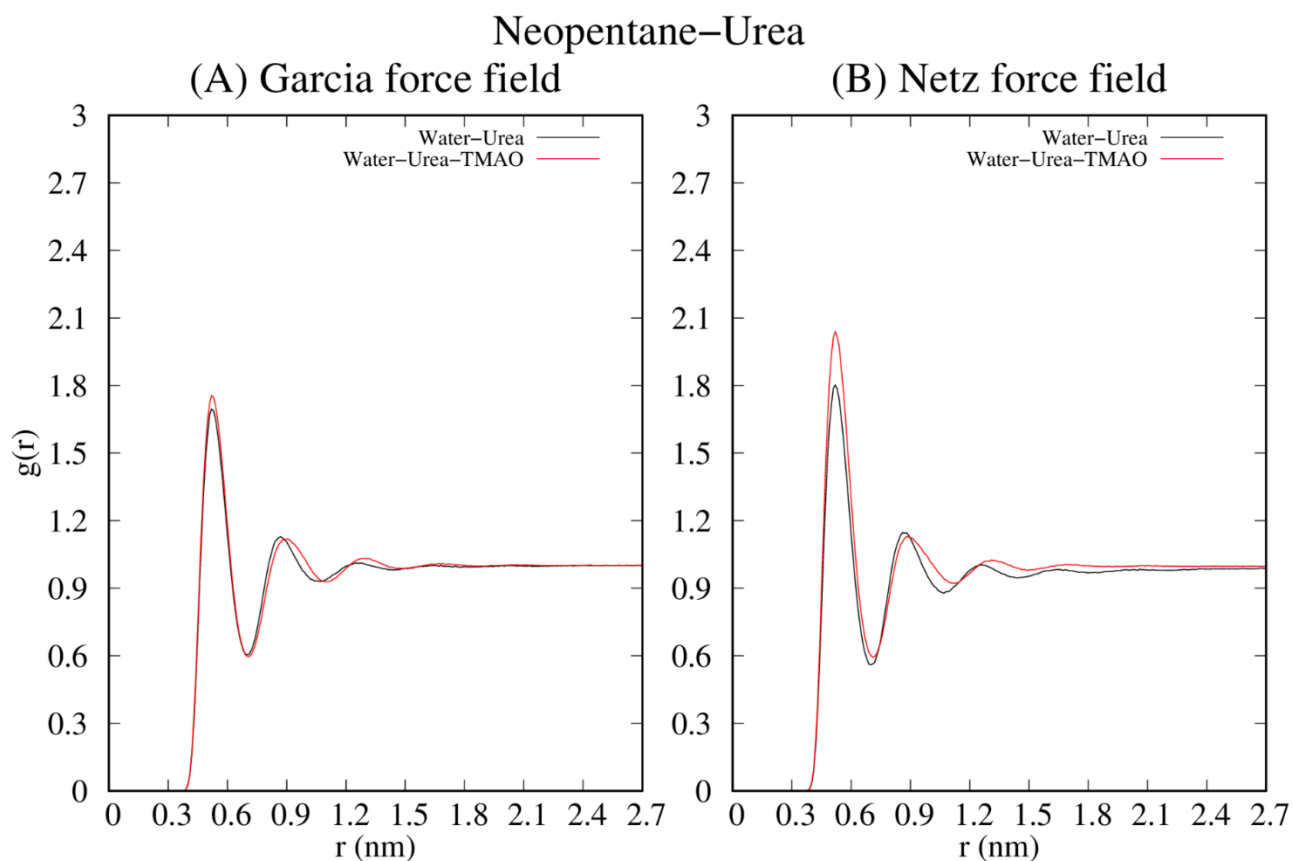

Figure 3. Radial distribution function between neopentane and urea in water-urea and water-urea-TMAO mixtures for Garcia (A) and Netz (B) force fields.
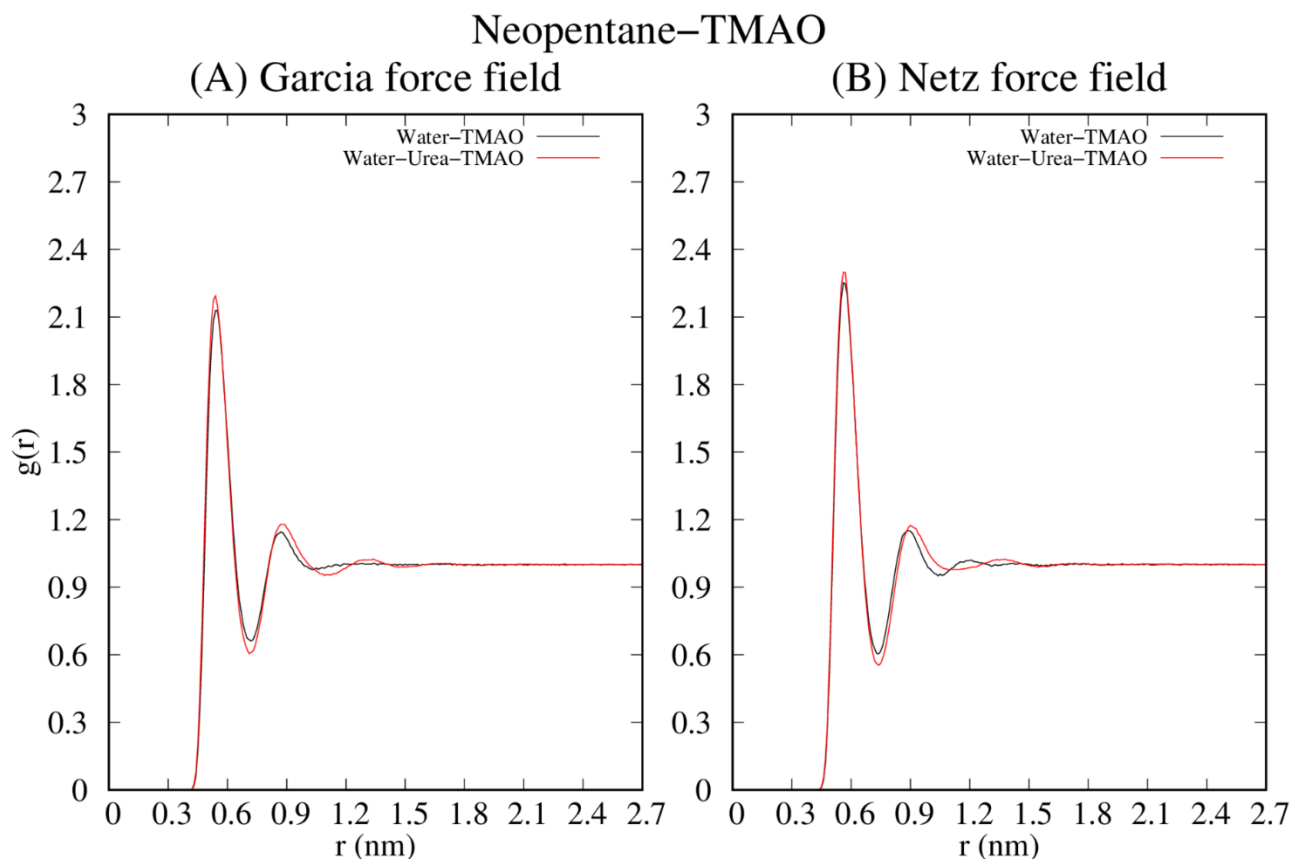
Figure 4. Radial distribution function between neopentane-TMAO in water-TMAO and water-urea-TMAO mixtures for Garcia (A) and Netz (B) force fields.

A-4. Preferential binding coefficient (γ)

If a cosolvent prefers to interact with a solute, it will replace the water molecules from the surroundings of the solute. So, a positive value of Γ indicates a pronounced accumulation of cosolvent around the solute whereas a negative γ value indicates that a preferential exclusion of cosolvent from the surroundings of the solute. However, the effect of cosolvent is negligible when $|\gamma| < 1$.[56]

In our current discussion the solute is neopentane and the cosolvent is either urea or TMAO. Around the neopentane molecule, the preferential binding coefficient for urea and for TMAO in water, water-urea, water-TMAO and water-urea-TMAO mixtures are computed using Eq. 2 and the data is shown in Table III. It can be seen that the γ values for urea are always negative. Using this finding, it can be concluded that urea is preferentially excluded to neopentane in water-urea mixture or in water-urea-TMAO mixture. From this data, we can see $|\gamma| < 1$ for TMAO in water-TMAO or in water-urea-TMAO mixture, no matter which force fields are used for TMAO. Hence, TMAO does not provide any significant change in water binding to neopentane. A positive (>1) value of γ for TMAO around hydrophobic polymer is always observed by Mondal et al. both in collapsed and extended form of the polymer.[11,28] The number of interacting sites are more for the polymer molecule in comparison to that

in small molecule like neopentane that may be one possible reason. The interaction parameters (σ, ε) on each site of the hydrophobe is also equally important.

Table III. Preferential binding coefficients of neopentane with urea and TMAO cosolvents.

| Mixtures | Preferential binding coefficients (γ) | | | |
| | GARCIA | | NETZ | |
	Urea	TMAO	Urea	TMAO
water-urea	-2.01±0.07		-2.91±0.38	
water-TMAO		0.18±0.017		-0.05±0.04
water-urea-TMAO	-1.56±0.24	-0.12±0.03	-0.46±0.32	-0.11±0.05

B. Solvation structure of neopentane cavity in water, urea and tmao mixtures

Here, we discuss the association between cavity molecules and solvation structures of cavity in water, urea and TMAO mixtures for Garcia and Netz force fields. In this section, we present radial distribution function of cavity, water, urea and TMAO around cavity in water, water-urea, water-TMAO and water-urea-TMAO mixtures.

B-1. RDFs between neopentane cavity molecules

We have shown the radial distribution functions between cavity in four mixtures for Garcia and Netz force fields in Figure 5. The cavity-cavity contact pair (CP) and solvent mediated pair (SMP) are observed in each mixture. The intensity of SMP is very weak than that of CP. The values of peak intensity of CP are very high in four mixtures for both force fields. The cavity molecules clustered together because of the repulsion between cavity and solvent molecules.

The order of population of cavity-cavity contact minima: w-ua-ta>w-ua~w-ta> w for Garcia force field. In the case of Netz force field, the order is w-ua-ta~w-ta> w-ua> w. The peak intensity of contact minima is lowest in water in comparison to other mixtures. Therefore, the cavity creation is easier in water than w-ua, w-ta and w-ua-ta mixtures. Similar trend is observed using Netz model. We disclosed that cavity creation in urea-water mixture is difficult than in water. Similar results were previously reported.[22–24]
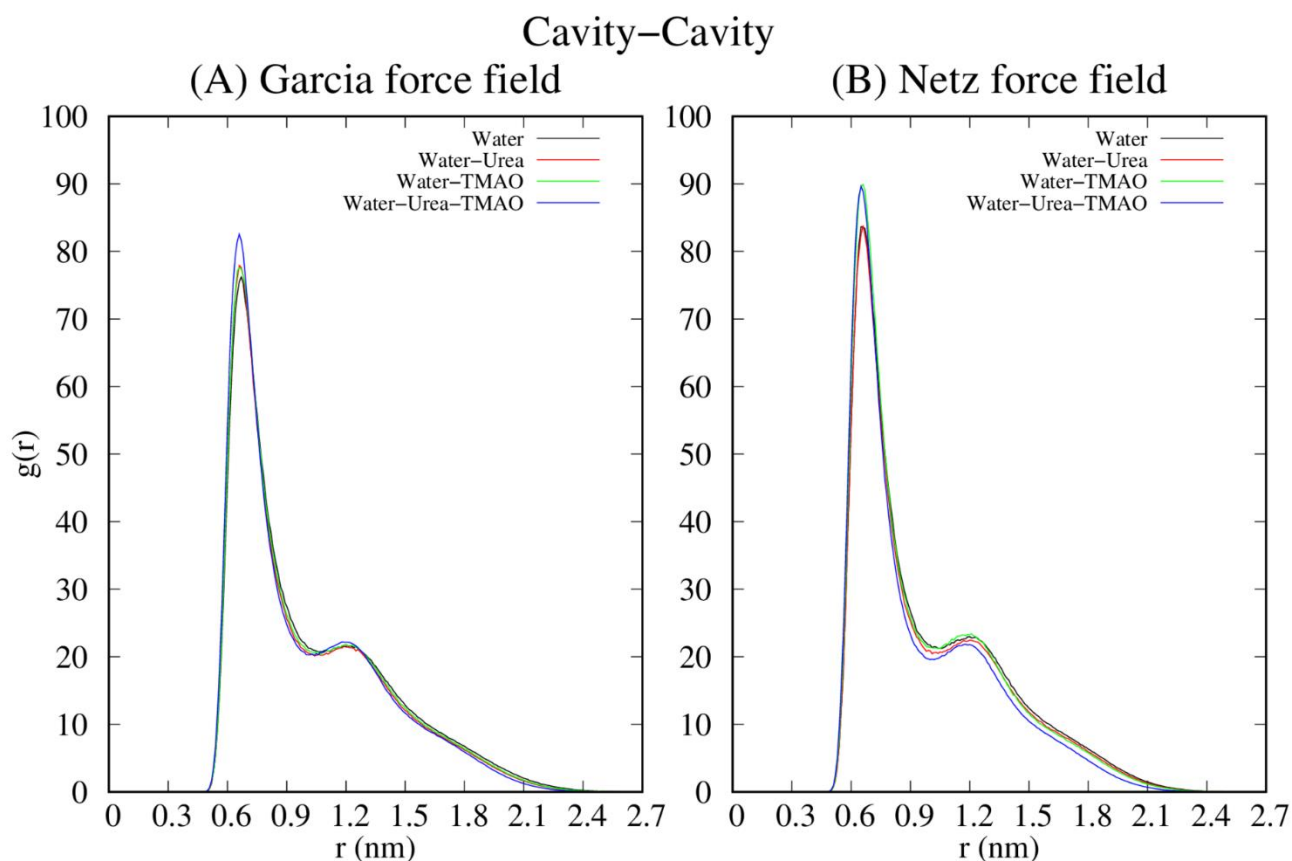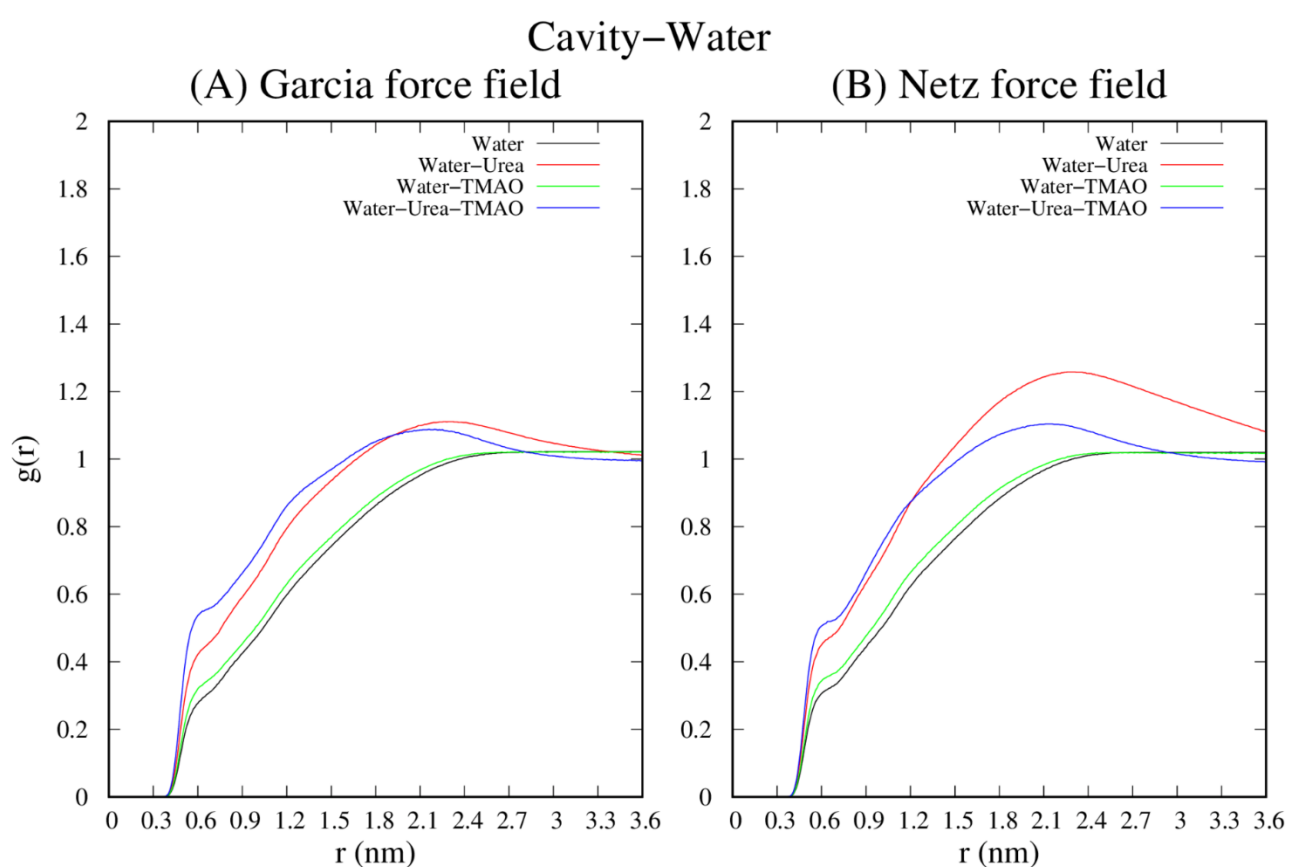
Figure 5. Radial distribution functions between cavity-cavity in water (w), water-urea (w-ua), water-TMAO (w-ta) and water-urea-TMAO (w-ua-ta) mixtures for Garcia (A) and Netz (B) force fields. The peak intensity of contact pair is highest in water-urea-TMAO mixtures for both force fields which suggest that cavity creation is most difficult in water-urea-TMAO mixtures. For Netz model, the population of CP in water-TMAO mixture is comparable to water-urea-TMAO mixture and bit higher than water and water-urea mixtures. Therefore, cavity creation is more difficult in water-TMAO mixture as TMAO is more hydrophilic in nature. Contrarily, the peak intensity of contact pair in water-TMAO mixture is similar to water-urea mixture and lower than water-urea-TMAO mixture for Gacia model of TMAO. Thus, cavity creation is easier in water-TMAO in comparison to water-urea-TMAO mixture possibly because Garcia TMAO is not so hydrophilic. So the cavity association in TMAO solution is very much dependent on the choice of the force-fields of TMAO.

B-2. Structure of water around neopentane cavity

We have estimated the radial distribution functions (g(r)) of water molecules around cavity which is presented in Figure 6. For g(r) calculations, we considered center of mass of cavity and water molecules. We observed that the density of water in the first coordination shell of cavity follows the following trend: water-urea-TMAO> water-urea> water-TMAO > water for Garcia's force field.

Similar trend is obtained for Netz model. Independent of the choice of the TMAO force field, it can be noticed that the cavity-water RDFs are quite similar in neat water and TMAO-water.

On the other hand, the RDFs are similar in nature in urea-water and urea-TMAO solution. A relatively high water density around cavity molecules causes the solvation of cavities more unfavorable in urea water or urea TMAO water relative to TMAO-water or pure water. Upon addition of urea in water-TMAO mixture, the water density around cavities is significantly enhanced in water-urea-TMAO mixture for both force-fields. On other hand, on addition of TMAO in water-urea mixture, the water density in the solvation shells of cavity molecules is significantly increased for Garcia's model of TMAO while it is slightly enhanced for Netz model.

Figure 6. Radial distribution functions between cavity-water in water (w), water-urea (w-ua), water-TMAO (w-ta) and water-urea-TMAO (w-ua-ta) mixtures for Garcia (A) and Netz (B) force fields.

B-3. Structure of cosolvent around neopentane cavity

We have estimated the RDFs between cavity and urea molecules in water-urea and water-urea-TMAO mixtures which is shown in Figure 7. The density of urea in the solvation shell of cavity is higher in water-urea-TMAO mixture in comparison to water-urea mixture for Garcia force field although the considerable increment is observed for Netz force field. In the case of Netz force field, a sharp peak is found which indicates more ordered structure of urea around cavity.

The computed g(r) between cavity and TMAO molecules is exhibited in Figure 8. For both force fields, the density of TMAO in the coordination shell of cavity is larger in water-urea-TMAO solution than that of water-TMAO mixture. The first RDFs peak of TMAO is significantly larger and sharper in water-urea-TMAO solution for Netz model. Hence, the structure of TMAO around cavity is more ordered and density of TMAO in the coordination shell of cavity is more in water-urea-TMAO solution in comparison to water-TMAO mixture.
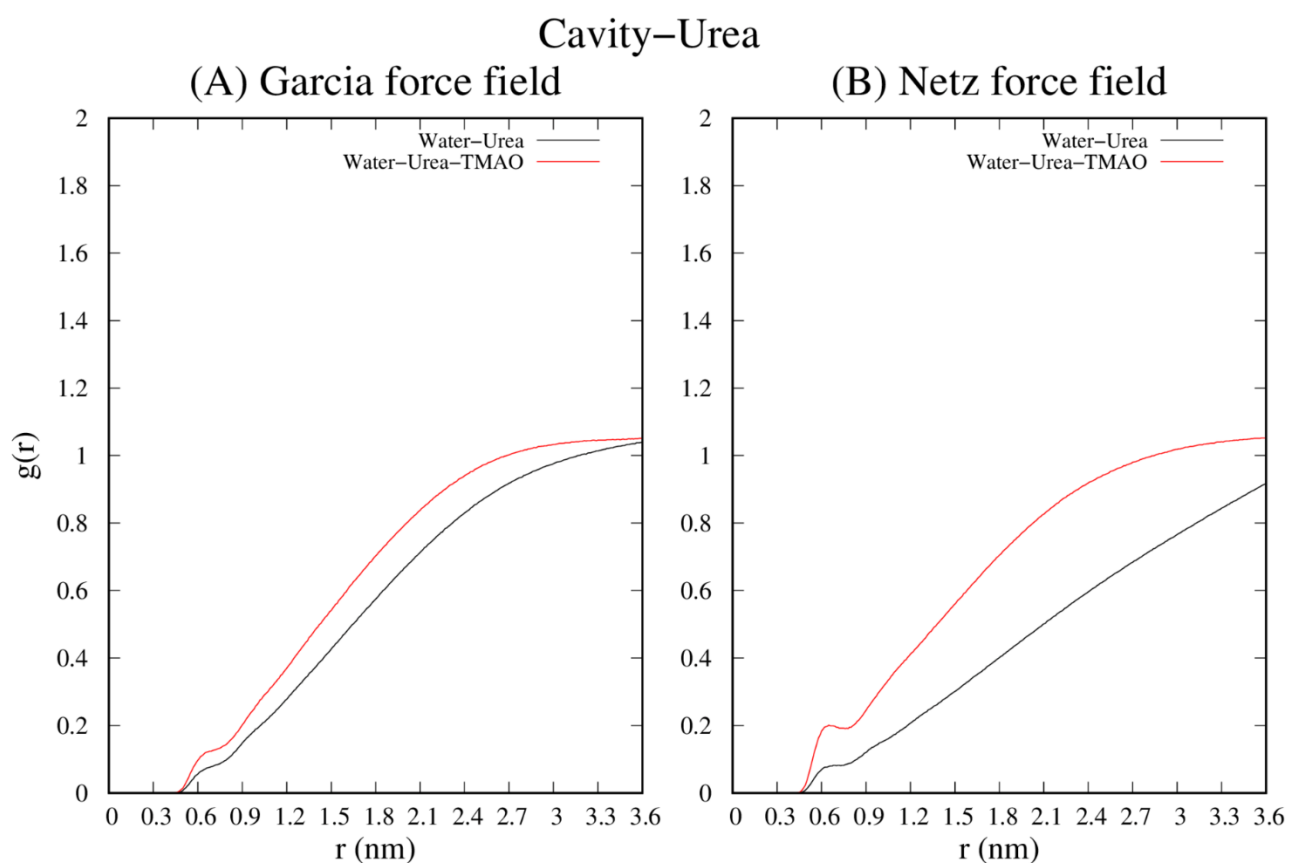

Figure 7. Radial distribution function between cavity-urea in water-urea (w-ua) and water-urea-TMAO (w-ua-ta) mixtures for Garcia (A) and Netz (B) force fields.
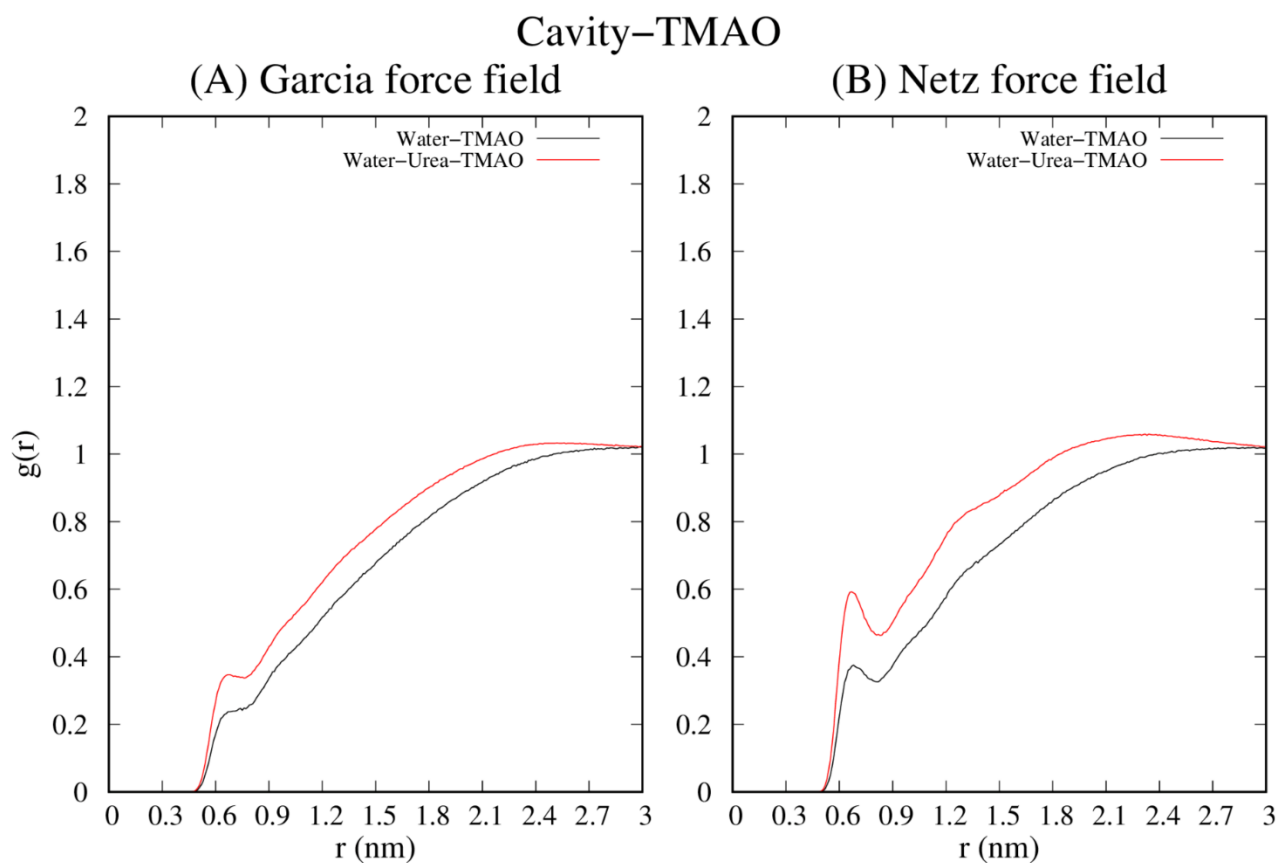
Figure 8. Radial distribution function between cavity- and TMAO molecules in water-TMAO (w-ta) and water-urea-TMAO (w-ua-ta) mixtures for Garcia (A) and Netz (B) force fields.

B-4. Preferential binding coefficient (γ) of cavity with cosolvents

In this section, we present the preferential biding coefficients of cavity with urea and TMAO in water-urea, water-TMAO and water-urea-TMAO mixtures. The values of preferential binding coefficients of cavity are provided in Table IV. Urea molecules are always preferentially excluded from the solvation shells of neopentane cavities. The highly negative value of γ for urea indicates an excessive depletion urea from the solvation shells of cavity. Interestingly, TMAO molecules is also preferentially excluded from the solvation shells of cavity, however the magnitude of depletion is less compared to the urea depletion around cavity. In contrast to urea, it can be noticed that TMAO is preferentially excluded more in water-urea-TMAO solution.

Table IV Preferential binding coefficients of cavity with urea and TMAO cosolvents.

Mixtures	Preferential binding coefficients (γ)			
	GARCIA		NETZ	
	Urea	TMAO	Urea	TMAO
water-urea	-16.57±0.08		-19.97±0.118	
water-TMAO		-2.17±0.03		-1.56±0.04
water-urea-TMAO	-17.28±0.18	-4.51±0.04	-14.90±0.43	-2.30±0.08

C. The cavitation free energies of neopentane in water, urea and tmao mixtures

A single neopentane sized molecular cavity is grown in w,w-ua, w-ta and w-ua-ta mixtures. At initial state, the C_6 and C_{12} terms for the van der Walls equation are set as zero for the solute and the final state only C_{12} is there. The free energy change to grow the cavity is estimated using thermodynamic Integration method, is called ΔG_{cavity}. The simulation details are discussed in the methods and computational details section. A higher positive value of the ΔG_{cavity} is indicating a more unfavorable is the cavity creation. The cavitation free energies of neopentane in mixed solvents are provided in Table V.

For Garcia TMAO ff, the order is ΔG_{cavity} (w)<ΔG_{cavity} (w-ta)<ΔG_{cavity} (w-ua)<ΔG_{cavity} (w-ua-ta) whereas the order is following for NetzΔG_{cavity} (w)<ΔG_{cavity} (w-ta)~ ΔG_{cavity} (w-ua)<ΔG_{cavity} (w-ua-ta).

Table V. The cavitation free energies of neopentane in water, water-urea, water-TMAO and water-urea-TMAO mixtures. The errors in the cavitation free energies are within 2 kJ/mol.

Mixtures	Cavitation free energies (ΔG_{cavity})/(kJ/mol)	
	Garcia	Netz
CAV_w	73	78
CAV_{w-ua}	83.1	87.8
CAV_{w-tmao}	78	87.5
$CAV_{w-ua-tmao}$	87	93.4

Independent of the force field employed, the cavity generation is toughest in urea-TMAO solution and is easiest in pure water. Depending on the chosen model for TMAO molecule, the cavity formation in urea solution is more difficult or equally difficult relative to that in TMAO solution.

We have discussed cavity-cavity association behavior using cavity-cavity RDFs in the Fig. 5. Now this ΔG_{cavity} data makes it clear why the association behavior is in that way. Independent of all force-fields, it was found the highest cavity-cavity association in w-ua-ta mixture. The reason is that the cavity creation is highly unfavorable as the ΔG_{cavity} holds higher values. On the other hand, it is also found a high cav-cav aggregation in w-ta mixture for Netz model. It is because the cavity formation is difficult in TMAO (Netz) ($\Delta G_{cavity} \sim 87$ kJ/mol) and is compare to the value in urea solution.

D. Solute-solvent interaction energy

Attractive dispersion interaction plays a vital role in hydrophobic solvation or hydrophobic association. The neopentane-water, neopentane-urea, neopentane-TMAO and total interaction energies are estimated and the data are presented in Figure 9 and Figure 10. Here the numbers of neopentane, urea, water and TMAO molecules are same as in the Table I.
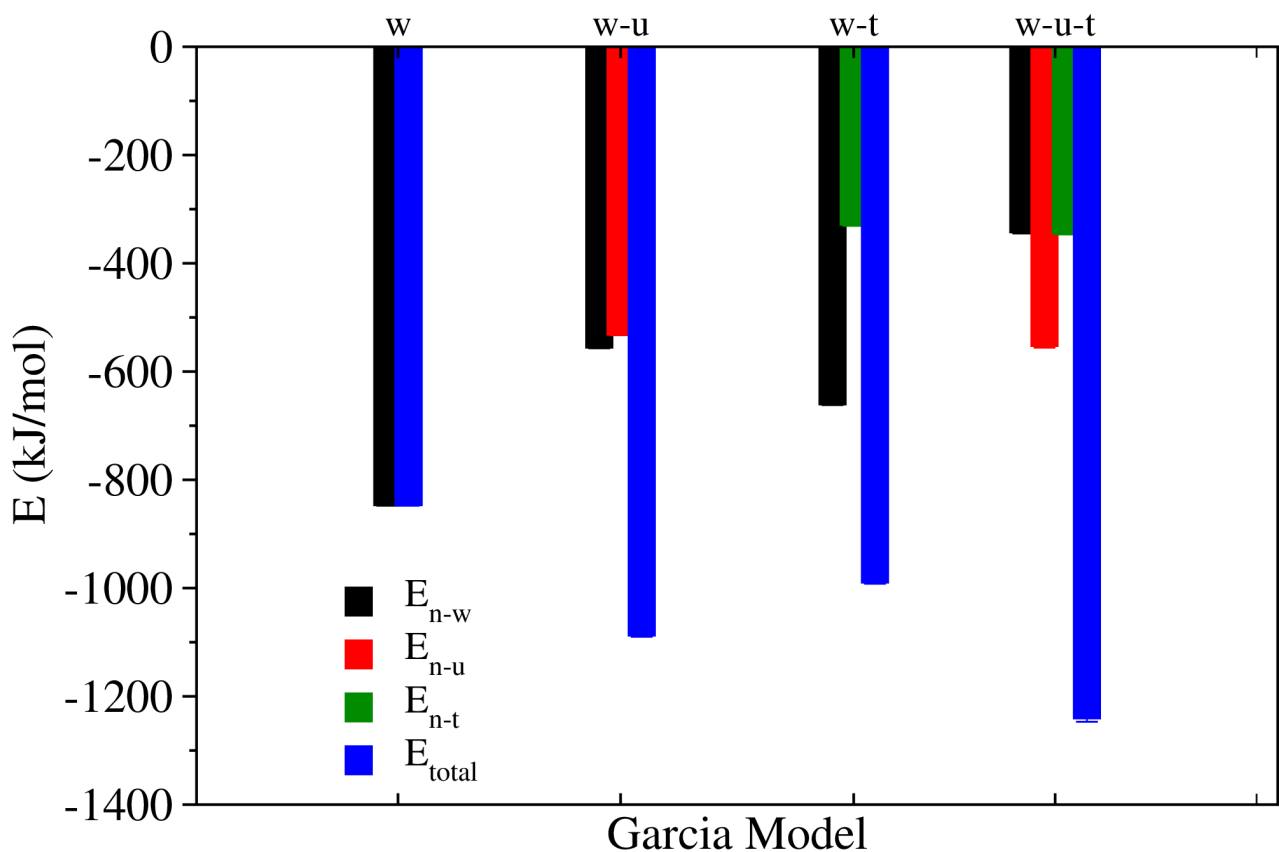

Figure 9. The interaction energies of neopentane with water, urea and TMAO in w, w-ua, w-ta and w-ua-ta mixtures for Garcia model.
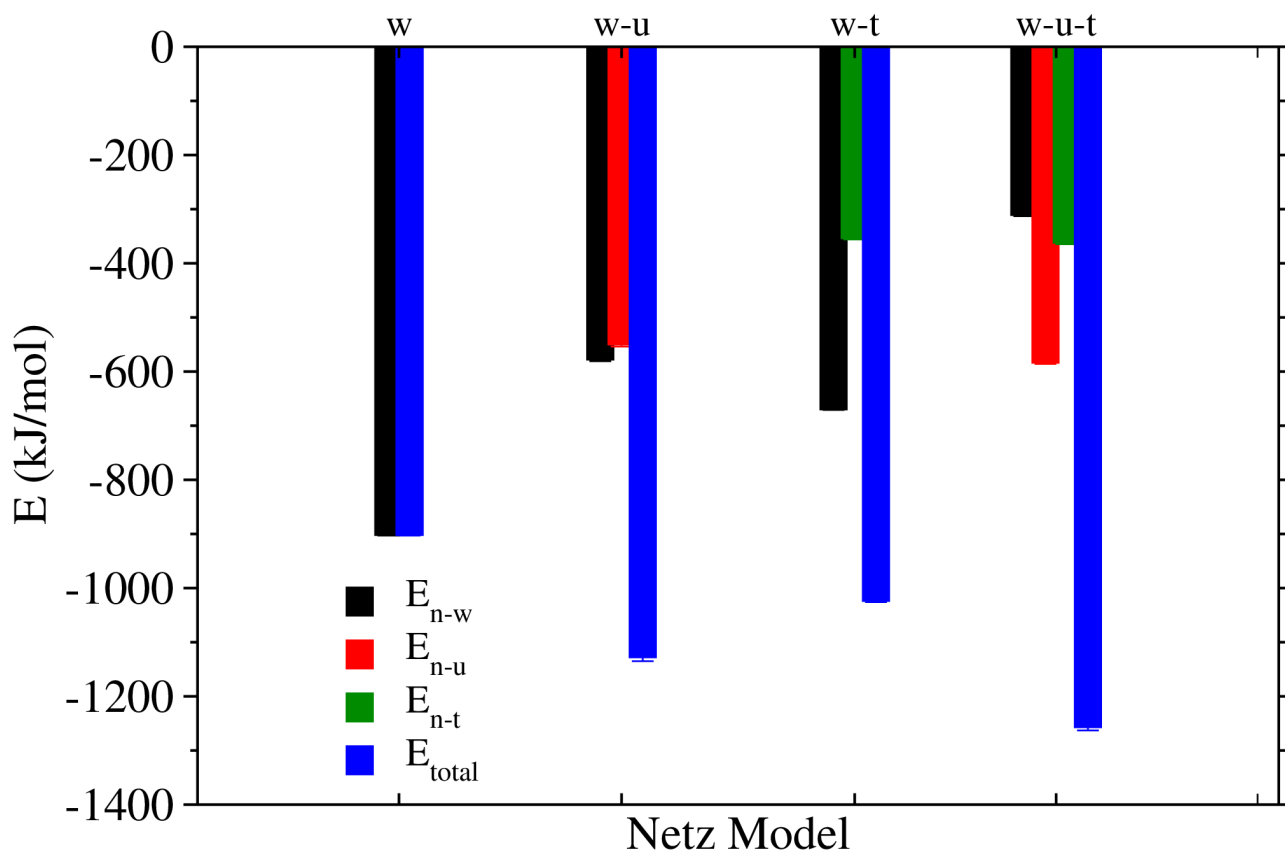
Figure 10. The interaction energies of neopentane with water, urea and TMAO in w, w-ua, w-ta and w-ua-ta mixtures for Netz model.

It is important to mention that the neopentane force field that is used, has zero partial charges on each atom. The interaction energies showed in Figures 9 and 10 are only dispersion interaction energies and the raw data is provided in Table S-III (Table S-III is given in supplementary material). The total solute-solvent interaction energies follow the order as for the two different force fields of TMAO E(w)> E(w-ta)> E(w-ua) > E(w-ua-ta). It is well established that urea stabilizes hydrophobes via favorable dispersion interactions[22-24] and that is why the more negative solute-solvent interaction energy is observed in urea-water than that in pure water.

The interaction energy is more negative in water-TMAO mixture compared to pure water. These results indicate hydrophobic solvation gets a more favorable contribution in urea-water or water-TMAO mixtures in comparison to pure water. Interestingly, it can be seen that the most favored dispersion interaction (solute-solvent) is obtained in water-urea-TMAO mixture. If we look the individual solute-solvent contribution, it is clear that E(neo-urea) is more negative than E(neo-TMAO) in different mixtures. This is because the number of urea present in the system is twice to the number of TMAO. Interestingly, it can be seen that the most favored dispersion interaction (solute-

solvent) is obtained in water-urea-TMAO mixture. Contrarily, the overall association that is most in urea-TMAO solution. We already found (Table V) the unfavorable cavity formation free energy contribution in this solution is so high that overcompensate the effect of the favorable solute-solvent dispersion interaction contribution. It leads to this excessive neopentane-neopentane aggregation in urea-TMAO-water mixture relative to other three solutions. Again, the total solute-solvent energy is more negative in urea solution than that in TMAO solution still the hydrophobic association is more in aqueous urea solution that is because of this energy-entropy cancellation effect. So it is revealed that the extent of hydrophobic association in aqueous osmolyte solutions is mainly governed by the cavity formation step.

It is important to point out that the neo-urea interaction energy contribution is nearly same in urea-water and in water-urea-TMAO mixture. Similarly, the neo-TMAO interaction energy is almost same as in water-TMAO and water-urea-TMAO mixture. Neo-urea and Neo-TMAO interaction have provided an additive contribution in water-urea-TMAO mixture.

E. Association of neopentane

To quantify the overall neopentane-neopentane association, we have estimated the Kirkwood Buff integral between neopentane molecules using Eq. 3 in these four different types of solutions. The data are shown in Table VI.

Table VI. The Kirkwood Buff integral between neopentane molecules in water, water-urea, water-TMAO and water-urea-TMAO mixtures.

S.No	Kirkwood−Buff integral	
	Garcia	Netz
water	1.56±0.06	0.47±0.30
water-urea	1.79±0.27	0.73±0.20
water-TMAO	0.83±0.25	0.32±0.27
water-urea-TMAO	2.02±0.23	1.83±0.59

A more positive value of KBI indicates a more neo-neo association. It is clear from the data table the neo-neo association follows the order as: water-urea-TMAO ~ water-Urea> water> water-TMAO for Garcia's TMAO force field. The order is as followed water-urea-TMAO > water-Urea> water ~ water-TMAO. Hence it can be concluded that the hydrophobic association is highest in water-urea-TMAO relative to other three solutions for both the two force-fields of TMAO. On the contrary, it is found by Tah et al.[33] that the population of the extended conformers of a model hydrophobic polymer is enhanced in water-urea-TMAO solution than other three types of solutions. So, the hydrophobic

association behaviors in osmolyte solutions are different and it depends on the choice of the molecules to study hydrophobic association. If small molecules like neopentane are considered the result is completely different than the result obtained using a hydrophobic polymer. We can see the lowest neo-neo aggregation behaviors in TMAO-water mixture using two different force-fields of TMAO. Interestingly, the neopentane association in urea-water mixture is higher than pure water or water-TMAO mixture. It can be noted that the KBI value in TMAO solution is closer to the KBI value in urea solution when Netz's model is employed, whereas the KBI value in urea solution is significantly high for Garcia model. The cavity creation penalty is the key factor to govern the overall association. For Garcia force-fields of TMAO, the highest positive ΔG_{cavity} is in urea-TMAO solution and the second highest value is obtained for urea-water. The ΔG_{cavity} value in urea solution is higher than that in TMAO solution for Garcia model. Hence the association is significantly high in urea solution relative to TMAO solution and the neo-neo association is following that order when the TMAO model is the Garcia's one. The ΔG_{cavity} value is highest in urea-TMAO solution for Netz model and as a result of that the neopentane association is the most here. We already found the ΔG_{cavity} values are comparable in aqueous urea solution and aqueous TMAO solution and this is why the difference in KBI values for these two solutions is relatively less here and the association in urea solution is slightly higher than that in TMAO solution for Netz model.

IV. CONCLUSIONS

A detailed understanding about hydrophobic solvation in mixed solvent medium is extremely useful to explain various chemical or biological processes. In our current study, an attempt is made to shed light on hydrophobic solvation and association in water-urea, water-TMAO and water-urea-TMAO solutions by using the help of the radial distribution functions, preferential binding coefficients, free energy required for cavity formation and the hydrophobe-solvent or cosolvent interaction energies data.

The population of neopentane-neopentane contact pair is highest in water-urea-TMAO mixture for two different TMAO force fields. Contrarily, it is revealed that the population of contact pair of neopentane in water-TMAO solutions is highly sensitive to the choice of the TMAO force fields. The probability of getting neopentane contact pair is second highest when the more hydrophilic TMAO force field (Netz model) is used. On the other hand, the population is lowest if the TMAO force field is a relatively less hydrophilic force field namely Garcia model. Interestingly, it is found that the population of neopentane-neopentane solvent mediated pair is always lowest for water-TMAO solution. A relatively greater population of this state is observed for both water-urea and water-urea-TMAO. The lesser population of solvent mediated pair in water-TMAO eventually leads a lesser overall neo-neo association in water-TMAO in comparison to that in pure water, water-urea and in water-urea-TMAO mixtures.

For a better understanding of hydrophobic solvation in mixed solvent medium, the free energy penalty to generate a soft cavity of neopentane is estimated. Independent of the choice of the force fields, the ΔG_{cavity} is the most unfavourable in water-urea-TMAO mixture. Again, it is found that the ΔG_{cavity} value is very sensitive towards the choice of the force fields. For Netz force fields, the ΔG_{cavity} for water-TMAO is almost same as the ΔG_{cavity} for water-urea. Contrarily, a greater ΔG_{cavity} for water-urea is found in comparison to that in water-TMAO for Garcia's TMAO model. Free-energetically it is relatively less unfavorable to create a soft cavity in pure water than that in mixed solvent medium. The analysis of solute (neopentane)- solvent interaction energies reveals the following order: $|E(w)| < |E(water\text{-}TMAO)| < |E(water\text{-}urea)| < |E(water\text{-}urea\text{-}TMAO)|$. The hydrophobic solvation gets the most favourable contribution from solute-solvent interaction energy part in case of water-urea-TMAO solution still the hydrophobic association is highest here. The total solute-solvent interaction energy is more favorable in urea solution than that in TMAO solution still the hydrophobic association is more in aqueous urea solution. It can be seen that the ΔG_{cavity} provides the most unfavorable contribution in hydrophobic solvation in water-urea-TMAO mixtures. The ΔG_{cavity} in aqueous urea solution is more positive for Garcia model and the association is significantly higher in aqueous urea solution than TMAO solution. The ΔG_{cavity} values are almost same in urea solution and aqueous TMAO solution if Netz model of TMAO is considered. The neopentane aggregation is marginally higher in urea solution than that in TMAO solution. The unfavorable ΔG_{cavity} contribution always tries to compensate the attractive interaction energy contribution. The overall solvation process is governed by the resultant of these two opposite effects. It can be concluded that the unfavorable free energy contribution due to cavity creation in the osmolyte solutions is the key factor to govern the overall association. It is also important to point out that the choice of force fields of TMAO is extremely crucial. If the chosen force fields are able to reproduce properly these contributions then only the study of hydrophobic solvation is worthwhile.

SUPPLEMENTRY MATERIAL

The Figure S1 and Tables S-I to S-III are provided in supplementary material. The radial distribution functions between cavity and different sites of urea molecule in water-urea mixture and RDFs between cavity and different sites of TMAO are given in Figure S1. The running coordination number of water, urea and TMAO molecules around neopentane and cavity are provided in Tables S1 and S2 respectively. The raw data of solute-solvent interaction energy is given in Table S3.

AUTHOR CONTRIBUTIONS

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

DATA AVAILABILITY

The data that support the findings of this research are available on request from the Corresponding authors.

REFERENCES

[1] J.M. Scholtz, D. Barrick, E.J. York, J.M. Stewart, and R.L. Baldwin, Proc. Natl. Acad. Sci. U. S. A. **92**, 185 (1995).

[2] A.W. and, D.G. Covell, and D. Thirumalai*, J. Am. Chem. Soc. **120**, 427 (1998).

[3] L. Hua, R. Zhou, D. Thirumalai, and B.J. Berne, Proc. Natl. Acad. Sci. **105**, 16928 (2008).

[4] M. Auton, L.M.F. Holthauzen, and D.W. Bolen, Proc. Natl. Acad. Sci. **104**, 15317 (2007).

[5] B.J. Bennion and V. Daggett, Proc. Natl. Acad. Sci. **100**, 5142 (2003).

[6] M.C.S. and and H. Grubmüller*, J. Am. Chem. Soc. **129**, 16126 (2007).

[7] B. Moeser and D. Horinek, J. Phys. Chem. B **118**, 107 (2013).

[8] D.R. Canchi, D. Paschek, and A.E. García, J. Am. Chem. Soc. **132**, 2338 (2010).

[9] † Qin Zou, ‡ Brian J. Bennion, †,‡ and Valerie Daggett, and † Kenneth P. Murphy*, J. Am. Chem. Soc. **124**, 1192 (2002).

[10] B.J. Bennion and V. Daggett, Proc. Natl. Acad. Sci. **101**, 6433 (2004).

[11] J. Mondal, D. Halverson, I.T.S. Li, G. Stirnemann, G.C. Walker, and B.J. Berne, Proc. Natl. Acad. Sci. U. S. A. **112**, 9270 (2015).

[12] P. Ganguly, P. Boserman, N.F.A. Van Der Vegt, and J.E. Shea, J. Am. Chem. Soc. **140**, 483 (2018).

[13] P. Ganguly, J. Polák, N.F.A. Van Der Vegt, J. Heyda, and J.E. Shea, J. Phys. Chem. B **124**, 6181 (2020).

[14] H. Wei, Y. Fan, and Y.Q. Gao, J. Phys. Chem. B **114**, 557 (2009).

[15] C. SS, R. G, S. JE, and T. D, J. Phys. Chem. B **115**, 13401 (2011).

[16] D.R. Canchi, P. Jayasimha, D.C. Rau, G.I. Makhatadze, and A.E. Garcia, J. Phys. Chem. B (2012).

[17] D.R. Canchi and A.E. García, Http://Dx.Doi.Org/10.1146/Annurev-Physchem-040412-110156 **64**, 273 (2013).

[18] E. Schneck, D. Horinek, and R.R. Netz, J. Phys. Chem. B **117**, 8310 (2013).

[19] J. Ma, I.M. Pazos, and F. Gai, Proc. Natl. Acad. Sci. **111**, 8476 (2014).

[20] P. Ganguly, T. Hajari, J.-E. Shea, and N.F.A. van der Vegt, J. Phys. Chem. Lett. **6**, 581 (2015).

[21] Y.-T. Liao, A.C. Manson, M.R. DeLyser, W.G. Noid, and P.S. Cremer, Proc. Natl. Acad. Sci. **114**, 2479 (2017).

[22] M. Ikeguchi, S. Nakamura, and K. Shimizu, J. Am. Chem. Soc. **123**, 677 (2001).

[23] D. Trzesniak, N.F.A. van der Vegt, and W.F. van Gunsteren, Phys. Chem. Chem. Phys. **6**, 697 (2004).

[24] R. Zangi, R. Zhou, and B.J. Berne, J. Am. Chem. Soc. **131**, 1535 (2009).

[25] M. V. Athawale, J.S. Dordick, and S. Garde, Biophys. J. **89**, 858 (2005).

[26] M. Mukherjee and J. Mondal, J. Phys. Chem. B **123**, 8697 (2019).

[27] P. S and P. GN, J. Phys. Chem. B **111**, 7932 (2007).

[28] J. Mondal, G. Stirnemann, and B.J. Berne, J. Phys. Chem. B **117**, 8723 (2013).

[29] F. Rodríguez-Ropero, P. Rötzscher, and N.F.A. van der Vegt, J. Phys. Chem. B **120**, 8757 (2016).

[30] N.F.A. Van Der Vegt and D. Nayar, J. Phys. Chem. B **121**, 9986 (2017).

[31] N. D and van der V. NFA, J. Phys. Chem. B **122**, 3587 (2018).

[32] P. Ganguly, N.F.A. Van Der Vegt, and J.E. Shea, J. Phys. Chem. Lett. **7**, 3052 (2016).

[33] I. Tah and J. Mondal, J. Phys. Chem. B **120**, 10969 (2016).

[34] M. M and M. J, J. Phys. Chem. B **122**, 6922 (2018).

[35] M.J. Abraham, T. Murtola, R. Schulz, S. Páll, J.C. Smith, B. Hess, and E. Lindah, SoftwareX **1**–**2**, 19 (2015).

[36] U. Essmann, L. Perea, and M.L. Berkowitz, J. Chem. Phys. (1995).

[37] H.J.C. Berendsen, J.P.M. Postma, W.F. Van Gunsteren, A. Dinola, and J.R. Haak, J. Chem. Phys. (1984).

[38] B. Hess, H. Bekker, H.J.C. Berendsen, and J.G.E.M. Fraaije, J. Comput. Chem. (1997).

[39] G. Bussi, D. Donadio, and M. Parrinello, J. Chem. Phys. (2007).

[40] W.L. Jorgensen, J. Chandrasekhar, J.D. Madura, R.W. Impey, and M.L. Klein, J. Chem. Phys. (1983).

[41] H.J.C. Berendsen, J.R. Grigera, and T.P. Straatsma, J. Phys. Chem. (1987).

[42] S. Weerasinghe and P.E. Smith, J. Chem. Phys. **118**, 10663 (2003).

[43] N. Schmid, A.P. Eichenberger, A. Choutko, S. Riniker, M. Winger, A.E. Mark, and W.F. Van Gunsteren, Eur. Biophys. J. **40**, 843 (2011).

[44] L. Martinez, R. Andrade, E.G. Birgin, and J.M. Martínez, J. Comput. Chem. **30**, 2157 (2009).

[45] W.G. Hoover, Phys. Rev. A **31**, 1695 (1985).

[46] S. Nosé, J. Chem. Phys. **81**, 511 (1984).

[47] M. Parrinello and A. Rahman, J. Appl. Phys. **52**, 7182 (1981).

[48] J.G. Kirkwood, J. Chem. Phys. **3**, 300 (1935).

[49] W.F. Van Gunsteren and H.J.C. Berendsen, Mol. Simul. **1**, 173 (1988).

[50] T. Hajari and N.F.A. Van Der Vegt, J. Chem. Phys. **142**, (2015).

[51] M.T. Record, Jr, and C.F. Anderson, Biophys. J. **68**, 786 (1995).

[52] J.G. Kirkwood and F.P. Buff, J. Chem. Phys. **19**, 774 (1951).

[53] P. Ganguly and N.F.A. van der Vegt, J. Chem. Theory Comput. **9**, 1347 (2013).

[54] Z. Su, G. Ravindhran, and C.L. Dias, J. Phys. Chem. B **122**, 5557 (2018).

[55] M.-E.L. and and N.F.A. van der Vegt*, J. Am. Chem. Soc. **128**, 4948 (2006).

[56] R. Chitra and P.E. Smith, J. Phys. Chem. B **105**, 11513 (2001).

Figures:

Figure-1

Figure-2

Neopentane−Water

(A) Garcia force field
(B) Netz force field

Figure-3

Neopentane−Urea

(A) Garcia force field   (B) Netz force field

Figure-4

Neopentane–TMAO

Figure-5

Cavity−Cavity

(A) Garcia force field   (B) Netz force field

Figure-6

Cavity−Water
(A) Garcia force field   (B) Netz force field

Figure-7

Cavity–Urea

(A) Garcia force field   (B) Netz force field
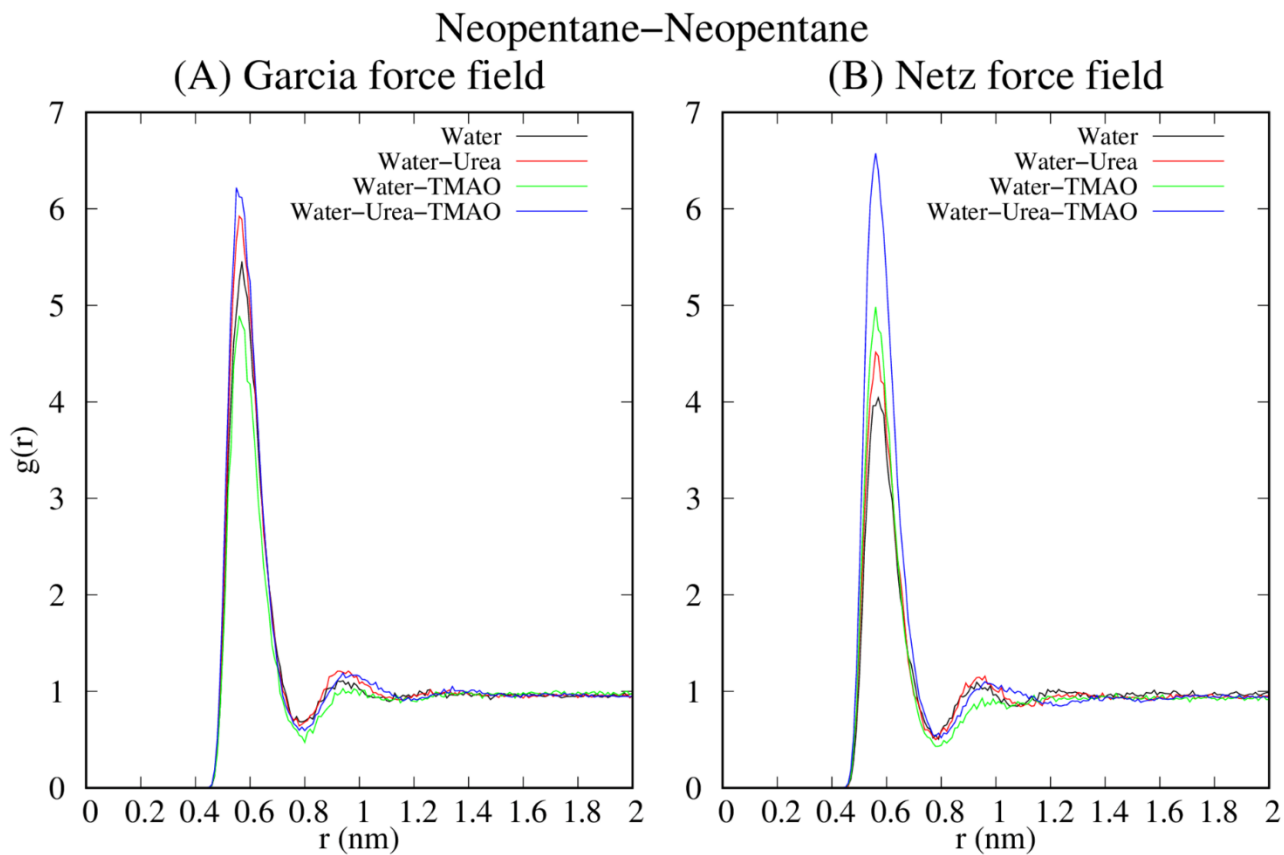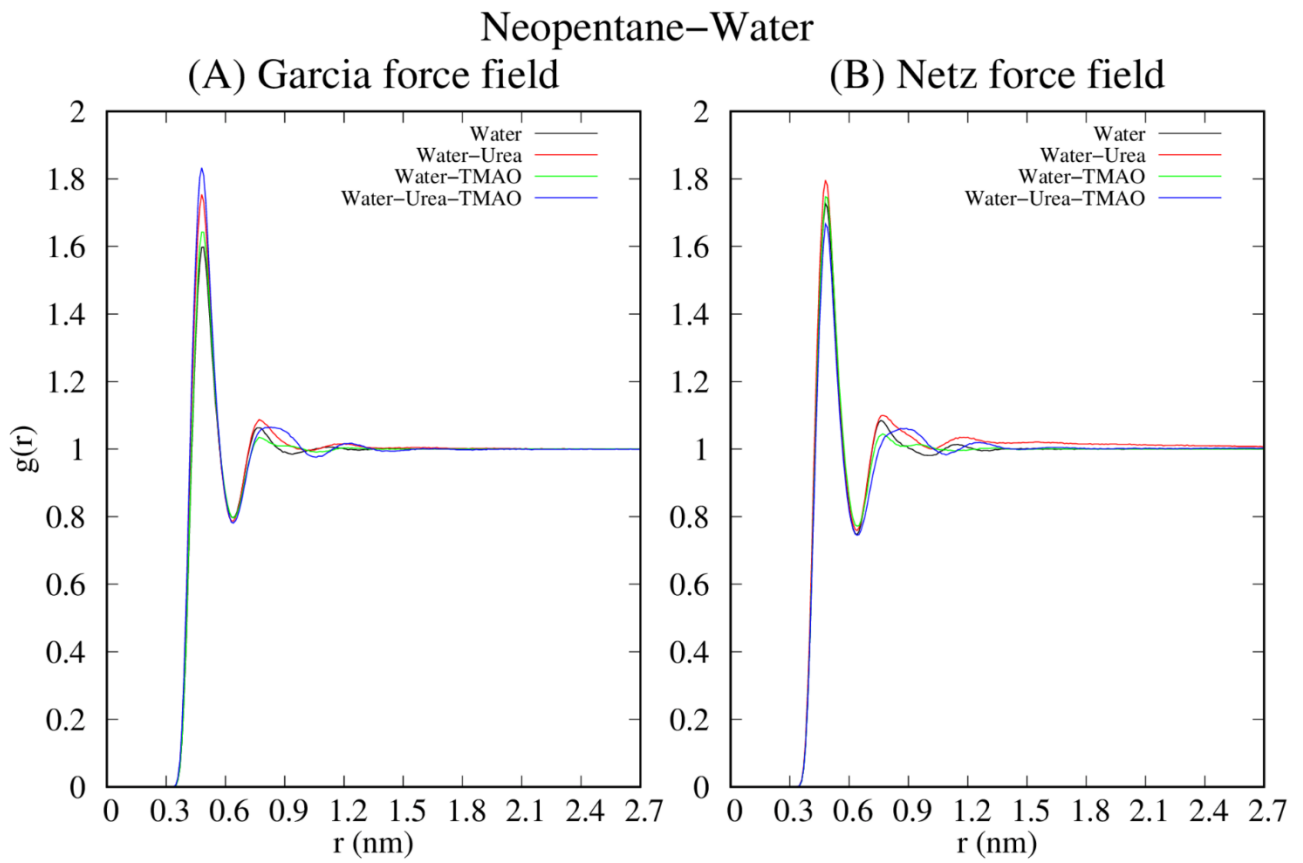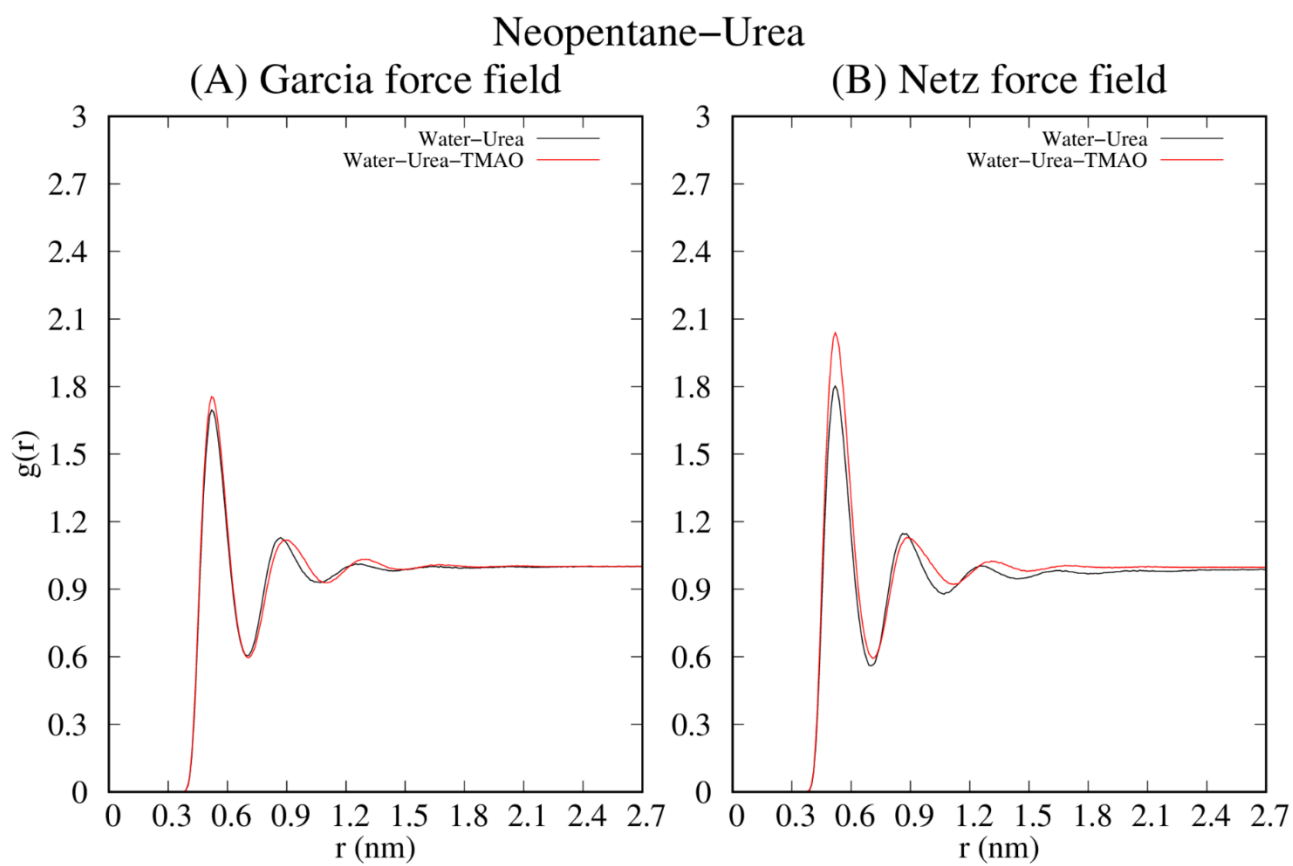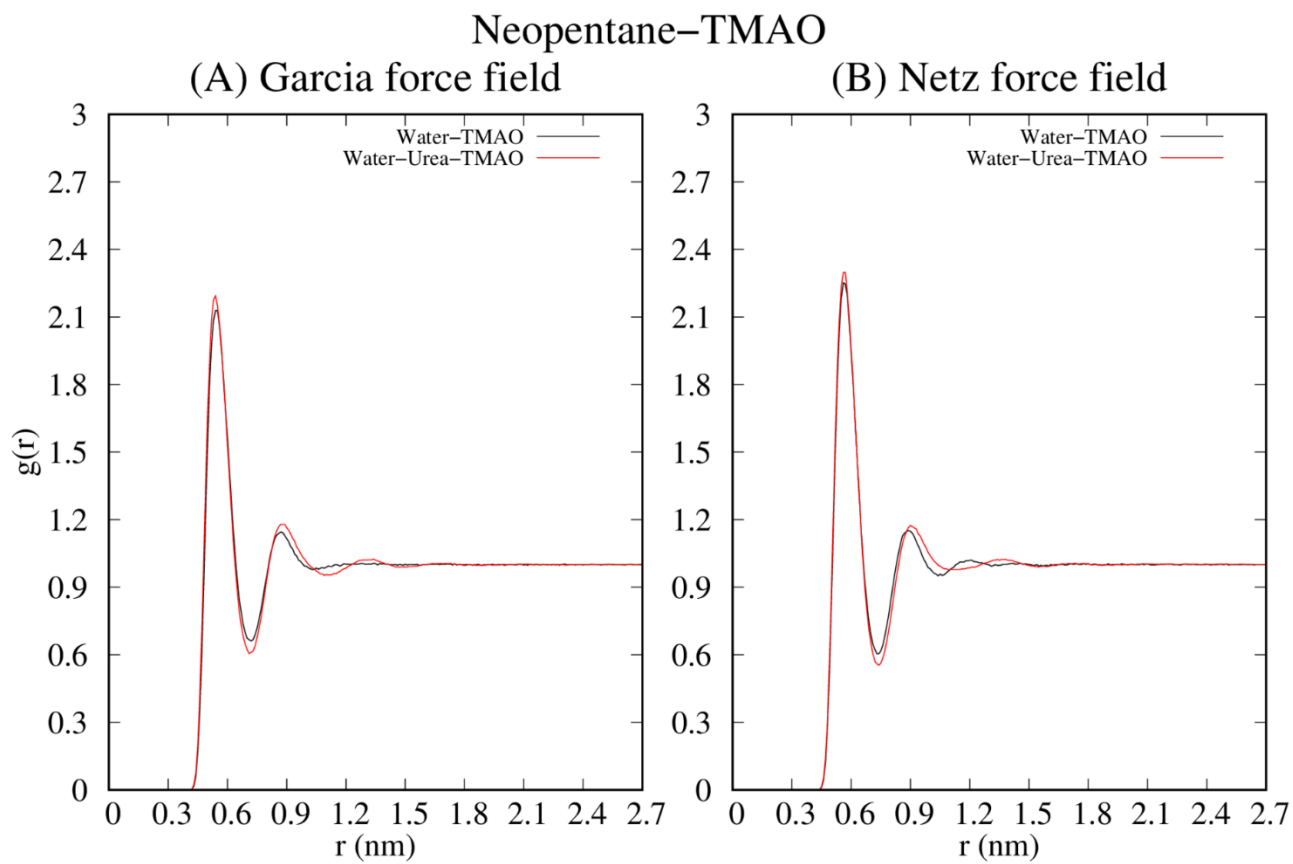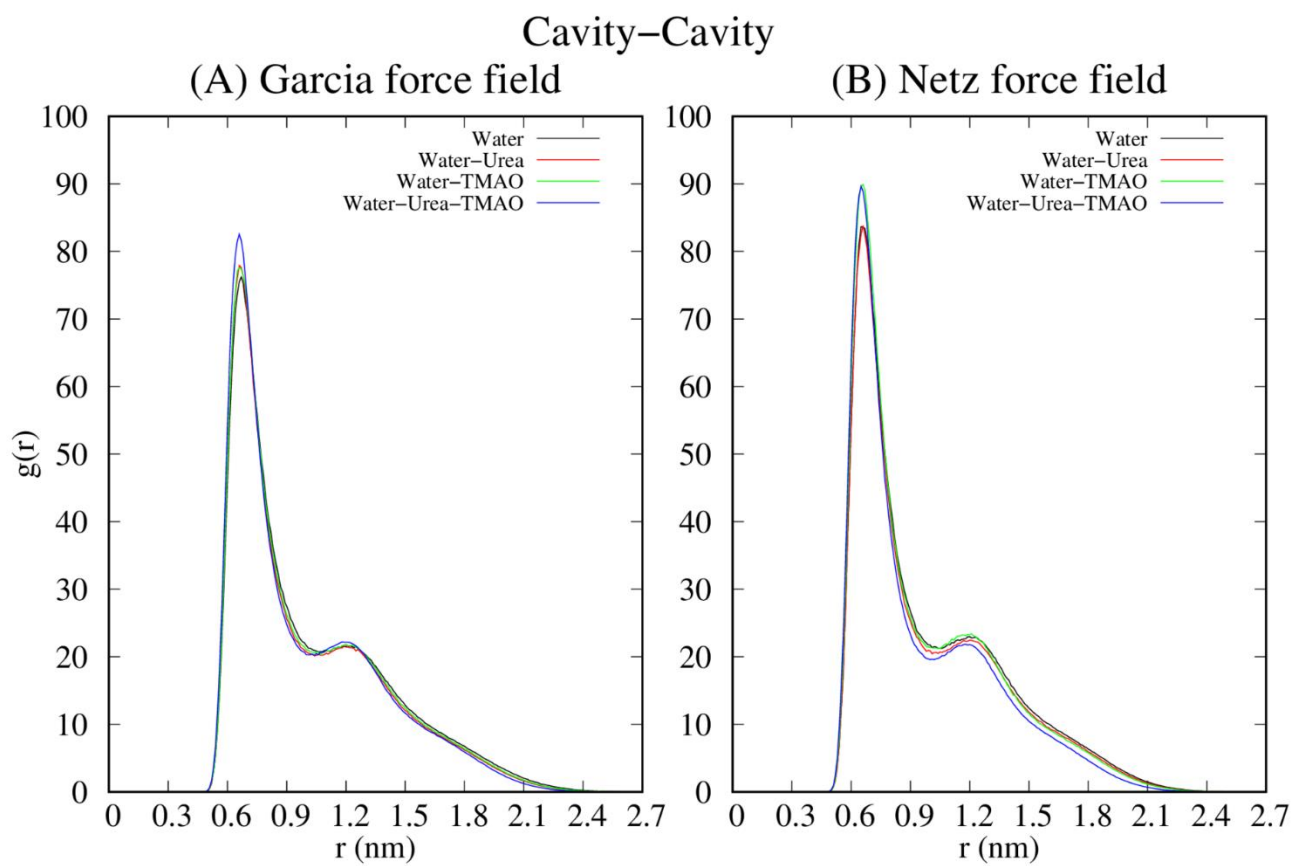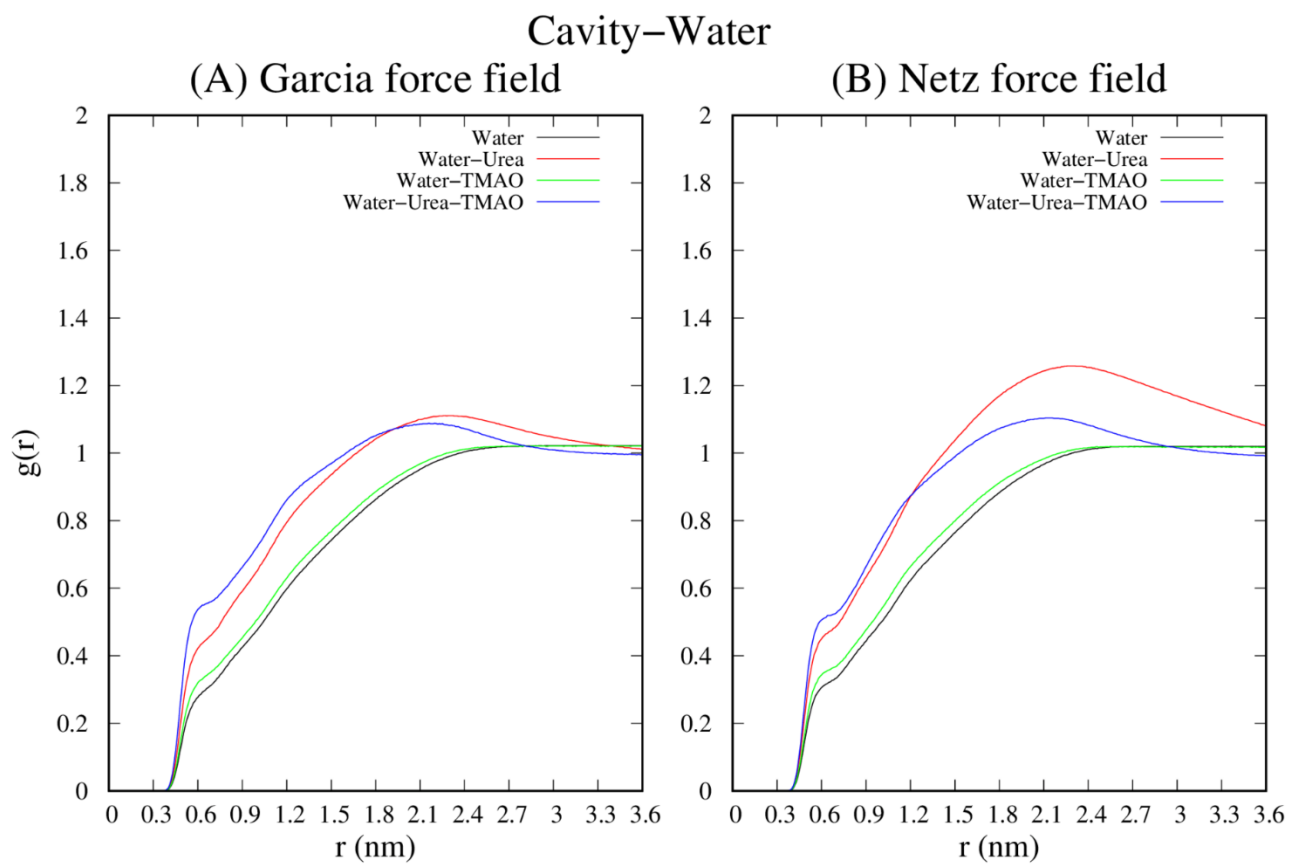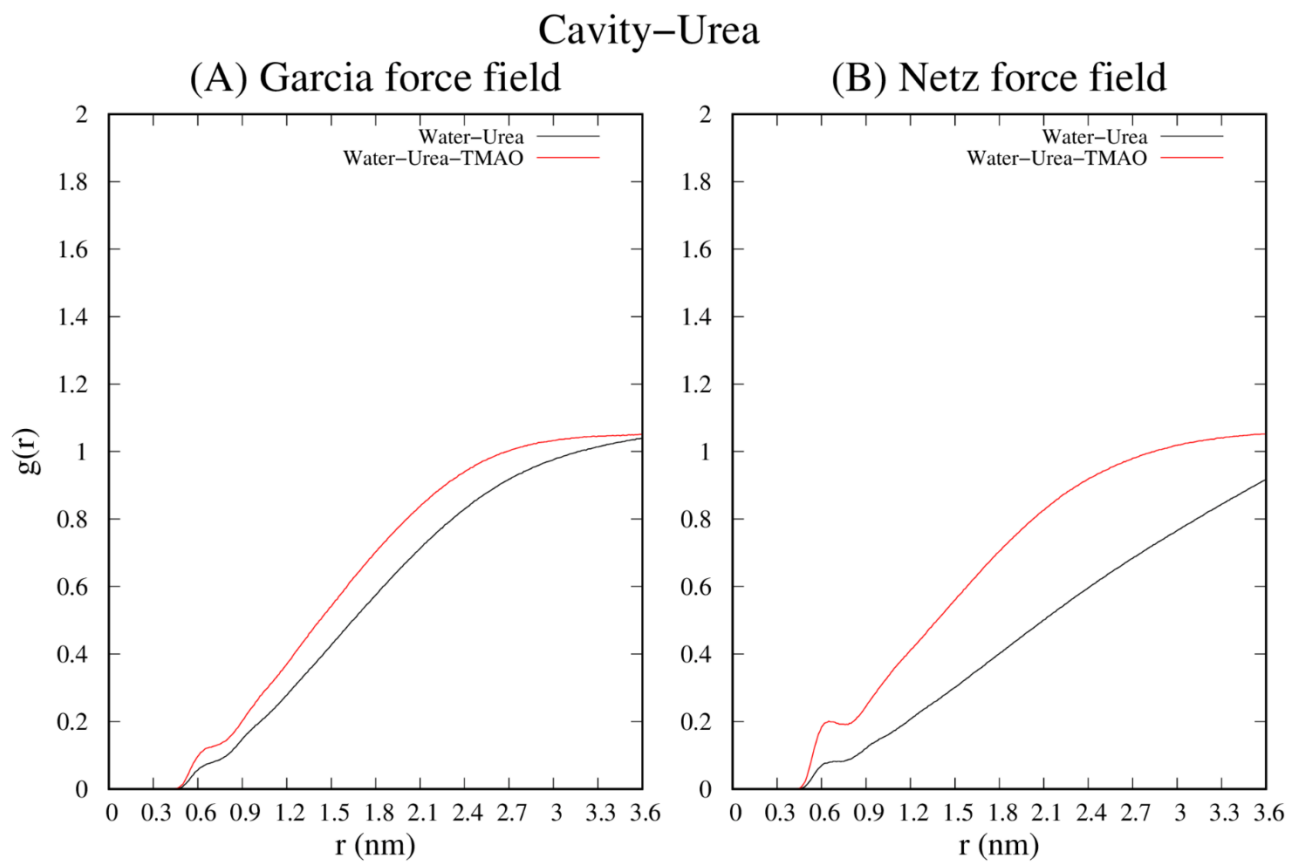
Figure-8

Figure-9

Figure-10