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Dynamical Effects of Trimethylamine *N*-Oxide on Aqueous Solutions of Urea

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Abstract

Trimethylamine *N*-oxide (TMAO) stabilizes protein structures, whereas urea destabilizes proteins, and their opposing effects can be counteracted at a 1:2 ratio of TMAO to urea. To investigate how they affect solution dynamics, molecular dynamics simulations have been carried out for aqueous solutions of TMAO and urea at different concentrations. In the binary solutions, urea mainly slows the diffusion of waters that are hydrogen bonded to it (i.e., hydration water), whereas TMAO dramatically slows the diffusion of both hydration water and bulk water because of long-lived TMAO-water hydrogen bonds. In the ternary solutions, because TMAO decreases the diffusion rate of bulk water, the lifetimes of not only water–water but also urea–water hydrogen bonds increase. In addition, the constant forming and breaking of short lifetime hydrogen bonds between urea and water appears to impart energy into the bulk, whereas the long lifetime hydrogen bonds between TMAO and water slows down the bulk, resulting in the compensating effects on bulk water in the ternary solution. This suggests that the counteracting effects of TMAO on urea denaturation may be both to make longer lived hydrogen bonds to water and to counter the energizing effects of urea on bulk water.

Graphical Abstract

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Supporting Information

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Tables of simulation conditions (Tables S1–S3), more extensive figures of the lifetimes of pairwise hydrogen bonds in the simulations (Figure S1), and $D_W/D_W(0)$ versus $\tau_W(0)/\tau_W$, fitted by power equations (Figure S2) (PDF)

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1. INTRODUCTION

The normal function of proteins relies on a delicate balance between stability and flexibility of proteins, which can be profoundly affected by cosolutes. For instance, trimethylamine *N*-oxide (TMAO) is known to stabilize proteins,¹ whereas urea is a strong denaturant of proteins.² Also, TMAO has been found to counteract the effects of urea on protein denaturation at a 1:2 ratio of TMAO to urea.^{3,4} Interestingly, sharks and other elasmobranchs appear to utilize a 0.6 Osm mixture of urea and TMAO to cope with high saline environments.⁵ Those living near the ocean surface have nearly the same 1:2 ratio mentioned above, which suggests that urea is used because it is readily available as a waste product, whereas TMAO is produced to counteract the negative effects of urea on protein stability.^{3,5} However, some deep sea teleosts, crustaceans, and elasmobranchs have concentrations of TMAO that increase linearly with depth at which the animal lives, leading to the proposition that TMAO may protect against hydrostatic pressure.^{6–8} In the elasmobranchs, the total concentration of urea and TMAO stays at 0.6 Osm but the ratio of TMAO to urea increases.

The mechanisms of how cosolutes affect proteins have been widely studied. Urea is generally believed to strongly interact with the backbone and side chains of proteins, which greatly destabilizes protein structure.^{2,9} Conversely, TMAO molecules appear to be excluded from the surface of proteins,^{3,10,11} which indicates that it stabilizes protein indirectly. Proposed mechanisms include minimization of the protein volume because of preferential exclusion of TMAO¹² or by strengthening water structure.¹³ Evidence indicates that the interactions between TMAO and water are very strong. A TMAO molecule forms hydrogen bonds with ~3 water molecules according to dielectric spectroscopy,¹⁴ Raman spectroscopy,¹⁵ and ab initio molecular dynamics (AIMD) simulations,^{16,17} which have a much longer lifetime than typical hydrogen bonds between polar species based on dielectric spectroscopy¹⁸ and AIMD simulations.^{19–21} Moreover, the counteraction by TMAO of the

denaturing effects of urea is even more debated. Some studies indicate that a direct interaction between TMAO and urea²² or an indirect interaction through a water bridge¹⁴ cancels the effect of urea by removing urea from the protein surface. Most recent research indicates TMAO and urea work in a mostly indirect manner.^{23–25} For instance, TMAO may strengthen the preferential exclusion effect independent of²⁶ or in synergy with²⁷ urea, or it may counter the effects of urea on the water hydrogen bond network.^{28,29}

Generally, the effects of cosolutes on aqueous solutions are studied in terms of water structure, thermodynamics, and hydrogen bonding. However, dynamic properties such as the diffusion coefficient, which is inversely proportional to the viscosity, can also be affected by cosolutes. These readily measurable properties are indicative of underlying changes in water structure that can influence protein structure and dynamics. For instance, the diffusion coefficient appears to be indicative of the strength of the hydrogen bond network.³⁰ In previous work,³¹ the diffusion coefficients of water in aqueous solutions of ions, TMAO, and urea were examined in terms of hydration water, which is defined as water in the first hydration shell of a solute, and bulk water, which is the rest of the water, in molecular dynamics simulations. For molecular solutes, only water around hydrogen bond donors or acceptors in the solute was counted. A simple model was used in which the average water diffusion coefficient D_w was assumed to be equal to the sum of diffusion rate of bulk water D_{bulk} times the fraction of water in the bulk and the diffusion rates of any solute D_s times the fraction of water in hydrating that solute. For ions, D_{bulk} and D_s can be estimated as the diffusion coefficients of pure water and of the solute estimated at infinite dilution, respectively; in other words, D_w linearly decreases with increasing molar ratio of solute to water. Thus, D_w in aqueous solutions of ions appears mainly affected by hydration water because more water molecules strongly act with an ion and not by changes in bulk water diffusion. However, for TMAO and urea, the number of hydrating waters is fewer per solute, so D_w is mainly determined by bulk water. In addition, D_{bulk} was much slower than that for pure water at high TMAO concentration, so the decrease was nonlinear and experimental studies of TMAO solutions show an exponentially decaying relationship between D_w and the molar ratio³² because the number of waters affected by a TMAO molecule appears to extend beyond the first hydration shell at low concentrations. In addition, the concentration dependence of the diffusion of the solute $D_s(c)$ was considered to be a good measure of the concentration dependence of the bulk water diffusion $D_{\text{bulk}}(c)$.³¹ These results suggest that TMAO could be affecting the hydrogen bonding between urea and water by decreasing the mobility of bulk water.

Classical molecular dynamics simulations can provide useful information about these problems because AIMD simulations are still limited in the size and timescale of the simulations. A number of force fields for TMAO have been proposed, including the Kast model,³³ the Netz model,³⁴ the Shea model,¹¹ and the Hölzl model,³⁵ whereas the OPLS³⁶ force field and the Kirkwood–Buff derived model³⁷ are often used for urea. However, the choice of force fields appears to critically affect the balance of the TMAO–water, urea–water, and TMAO–urea interactions.^{24,38} Moreover, a very recent study combining AIMD simulations, polarization-resolved femtosecond infrared pump-probe spectroscopy and nuclear magnetic resonance spectroscopy indicates that the large mismatch between the strong TMAO–water hydrogen bond and the weak urea–water hydrogen bond disfavors a

direct hydrogen bond between TMAO and urea, whereas MD simulations using the Kast force field for TMAO and the OPLS force field for urea are unable to predict the mismatch and instead predict a stable direct hydrogen-bonded interaction.²¹ However, a major drawback in most MD simulations of these systems is that they use three-point SPC/E³⁹ or TIP3P⁴⁰ water models, which have been shown to be less accurate, so that their conclusions are less convincing.

In this study, aqueous solutions of TMAO, urea, and a 1:2 ratio of TMAO and urea at different concentrations are investigated by molecular dynamics simulations. Because our future studies will be of how TMAO and urea alter the effects of temperature and pressure on proteins using the CHARMM36 force field,^{41,42} this force field was used here for TMAO and urea for consistency with future studies. In addition, while a variety of improved water models have been suggested,⁴³ the four-point TIP4P–Ew model⁴⁴ was used here for water because it has been utilized with biomolecular force fields and is able to reproduce the temperature and pressure dependence of many liquid-state properties. The agreement of the MD simulations using these force fields with AIMD and experiment is demonstrated first. Next, the number and lifetimes of hydrogen bonds between all possible pairs, including between water and TMAO or urea, and between TMAO and urea, were calculated. In addition, the diffusion coefficients of water and the cosolutes were calculated and correlated with the hydrogen bond lifetimes. The results demonstrate that TMAO can greatly retard the diffusion of bulk water, not just water in its hydration shell, whereas urea mostly affects water in its hydration shell. The reasons for this difference are explored by investigating the dynamics of water that is hydrogen bonded to urea versus TMAO. The consequences appear to be that the slowed diffusion of bulk water in the ternary solutions increases the lifetimes of urea–water hydrogen bonds.

2. METHODS

The molecular dynamics simulations were performed using the molecular mechanics package CHARMM version 41a2⁴⁵ with the CHARMM36 all-atom force field^{41,42} for TMAO and urea, and the TIP4P–Ew model for water.⁴⁴ The nonbonded interactions were handled as follows. Pair and image lists were generated out to 12 Å and updated heuristically. The van der Waals interactions were turned off using the CHARMM switching function from 8 to 10 Å. The particle-mesh Ewald summation method⁴⁶ with a β -spline coefficient of 6, a kappa value of 0.34 Å⁻¹, 100 *k*-vectors in each Cartesian direction, and a real space truncation at 10 Å, was used to account for long-range electrostatic interactions.

The simulations were carried out as follows (detailed information can be found in Tables S1–S3). The initial structures of TMAO and urea were generated from the CHARMM internal coordinate table. Different numbers of TMAO, urea, or TMAO + urea (in a 1:2 ratio) molecules were placed and rotated randomly in an equilibrated cubic box of water with side length ~40 Å to render different concentrations. Water molecules were removed if their oxygen was less than 2.5 Å from any heavy atom of a solute molecule. The system was subjected to initial energy minimization (200 steps of the steepest descent method). During energy minimization, heavy atoms of water and cosolute molecules were harmonically restrained to their initial position with a spring constant of 2 kcal/(mol·Å²). This constraint

was removed afterwards. The molecular dynamics simulations employed the leapfrog Verlet algorithm, using a 1 fs integration time step. After heating from 0 to 300 K in 30 ps, the system was scaled every 0.2 ps for 170 ps in the *NPT* ensemble using the Langevin piston method.⁴⁷ The system was further equilibrated unperturbed for 5 ns, and followed by 25 to 40 ns of production run at 300 K also in the *NPT* ensemble. In addition, a 20 ns simulation of pure water was performed to obtain corresponding pure liquid properties. Coordinates were saved every 1 ps for analysis.

The analysis of dynamics and hydrogen bonding was calculated as follows. The diffusion coefficients were calculated with a correction term for system size dependence.⁴⁸ As in previous work, experimental viscosities were used for the correction.⁴⁹ They were calculated for every 5 ns interval to determine error bars. The criterion for a hydrogen bond is a distance cutoff of 2.4 Å between the hydrogen atom and an acceptor, with no angle cutoff.⁵⁰ If an angle cutoff of 135° is added, the average number of hydrogen bond between TMAO and water decreases by about 1.5%, and its lifetime decreases by about 13%. However, the dependence on the concentration is not affected and other criteria such as heavy atom distance or hydrogen bond energies should not affect our results beyond the differences due to lack of angle criteria. The lifetime of a hydrogen bond is calculated by integrating the autocorrelations of a quantity specifying formed versus broken hydrogen bonds.⁵¹ In addition, the average hydrogen bond lifetime for a molecule type i is defined as

$$\langle \tau_i \rangle = \frac{\sum_j \langle \tau_{i-j} \rangle \langle N_{i-j} \rangle N_j}{\sum_j \langle N_{i-j} \rangle N_j} \quad (1)$$

in which $\langle \tau_{i-j} \rangle$ is the average lifetime of a hydrogen bond between molecule type i and molecule type j , N_j is the number of molecule type j , and N_{i-j} is the average number of molecule type i hydrogen bonded to a molecule j .

As mentioned in the Introduction, fewer water molecules act as hydrogen bond donors or acceptors with polar solutes in aqueous solution and changes in D_w are mainly in bulk water diffusion; in other words, $D_w(c) \approx D_{\text{bulk}}(c)$ where c is the concentration of solute. Using a Stokes–Einstein model ($D = k_B T / 6\pi\eta r$) in which the diffusion of either water or solute in a solution is inversely proportional to its radius and the viscosity of the solution and the hydration remains constant, the decrease in the diffusion of water or a solute should be the same so that

$$D_w(c)/D_w(0) \approx D_{\text{bulk}}(c)/D_{\text{bulk}}(0) \approx D_s(c)/D_s(0) \quad (2)$$

where $D_{\text{bulk}}(0) = D_w(0)$. In addition, because our previous simulations suggest the dependence of the diffusion of water in TMAO aqueous solutions is nonlinear, and experiment suggests an exponentially decaying dependence on the molar ratio of solute to solvent,³² the diffusion of water in aqueous solutions of solute is expressed as

$$D_w(x_t, x_u, x_w) = D_w(0) \cdot \exp(-(\alpha_t x_t + \alpha_u x_u)/x_w) \quad (3)$$

where $D_w(0)$ is the pure water diffusion coefficient and x_i is the mole fraction of i , where “t” is for TMAO, “u” for urea, or “w” for water. The parameters α_i are obtained by fitting the binary solutions and these values are used for the ternary solution.

3. RESULTS AND DISCUSSION

Overall, the simulations appear to be qualitatively reasonable compared with experimental results. The densities from the simulations (Figure 1a,b) agree well with experiment for the urea solutions⁵² (Figure 1b) but somewhat overestimate experiment for the TMAO solutions¹³ at high TMAO concentrations (Figure 1a), as seen in other simulations with a variety of force fields,^{19,33} although a newer force field slightly underestimates it.³⁵ The diffusion coefficients for both cosolutes are somewhat overestimated in the simulations compared to the experiment^{32,53} (Figure 2a,b), which is seen in other simulations as well.^{11,32} The overestimate is expected here because the diffusion coefficient of pure TIP4P-Ew water is slightly faster than real water (2.8 vs 2.35×10^{-9} m²/s, respectively).

The properties of hydrogen bonds within the solutions in the simulations were also examined. The average number of hydrogen bonds with water for each TMAO is about three, which agrees with the results from experiment^{14,15} and ab initio calculations.^{16,17} The lifetimes extrapolated at infinite dilution of TMAO–water hydrogen bonds (~ 30 ps) are much longer than water–water hydrogen bonds in pure water (3.46 ps), whereas the hydrogen bonds from the urea oxygen to water (~ 4 ps) and from the urea nitrogen to water (~ 1.5 ps) are on a similar timescale. Interestingly, these values compare well with lifetimes estimated from recent AIMD simulations,²¹ TMAO–water (~ 20 ps), water–water (~ 4.5 ps), and the urea nitrogen to water (~ 2.5 ps) and are much better than those in the same study from MD with the Kast-OPLS parameters: TMAO–water (~ 5 ps), water–water (~ 2.5 ps), and the urea nitrogen to water (~ 2 ps). Also, a TMAO–urea hydrogen bond is shorter lived than a water–water hydrogen bond (Figure S1a–c in the Supporting Information), which is not consistent with the hypothesis that TMAO strongly interacts with urea to counteract its effects. The AIMD simulations also indicate that TMAO and urea do not form hydrogen bonds.²¹ Overall, the hydrogen bond properties using the CHARMM36 force field are in good agreement with the recent AIMD simulations compared with the Kast³³ force field tested in that study.²¹ A reason for this good agreement may be that both the dipole and the quadrupole of TMAO in the CHARMM36 force field are large (6.68 D and 10.36 D Å, respectively), while they are much smaller in the Kast (5.45 D and 7.99 D Å, respectively). Other more recent force fields also have large dipoles and quadrupoles, for instance, Nezt³⁴ (7.20 D and 10.43 D Å, respectively) and Hölzl³⁵ (6.56 D and 9.54 D Å, respectively), reflecting the partial charges for the condensed phase.

The concentration dependence of the number and lifetime of hydrogen bonds within the solutions in the simulations was calculated (Figure 3). As the TMAO concentration increases, the number of hydrogen bonds with water decreases because the concentration of water decreases (Figure 3a, left) whereas the hydrogen bond lifetime increases more than linearly (Figure 3b, left) with TMAO concentration apparently because the entire system slows down, as observed in other studies.^{11,18} Lifetimes of water–water hydrogen bonds also increase more than linearly, supporting the argument that TMAO stabilizes the overall

hydrogen bond network.^{28,29} The same trends are seen in the urea solutions (Figure 3c,d, right), although the changes in hydrogen bond lifetimes are essentially linear with solute concentration.

In the ternary solutions, the addition of TMAO to urea at constant total solute concentration slightly increases the number of hydrogen bonds between TMAO and water because water hydrogen bonds to TMAO are preferred over urea (Figure 3a, left) and, consequently, the number of hydrogen bonds between urea and water decreases slightly (Figure 3a, right). The ternary solutions also have shorter lifetimes of TMAO–water and water–water hydrogen bonds than the TMAO aqueous solution for a given total solute concentration (Figure 3b, left). Interestingly, the ternary solution also has lifetimes of urea–water and water–water hydrogen bonds that increase at a greater than linear rate unlike the urea aqueous solution (Figure 3b, right). This indicates that increasing concentrations of TMAO strengthens all hydrogen bonds with water.

The above suggests that the lifetimes of hydrogen bonds are influenced by the solution dynamics. In the simulations, a direct relationship is seen between the diffusion coefficient of a solute and its average hydrogen bond lifetime (Figure 4). For all three solutions, the diffusion coefficient of TMAO or urea has an inverse power relationship with its average hydrogen bond lifetime, $D_i/D_i(0) = (\tau_i(0)/\tau_i)^\beta$, where $D_i(0)$ and $\tau_i(0)$ are the values at infinite dilution condition for species i . This is similar to the inverse power relationship for ions noted by Cheatham⁵⁴ and Rick.⁵⁵ From the data in Figure 2a,b, $D_t(0) = 1.090 \times 10^{-9}$ m²/s and $D_u(0) = 1.720 \times 10^{-9}$ m²/s, and from data in Figure 3a,b, $\tau_t(0) = 29.6$ ps and $\tau_u(0) = 2.7$ ps. The fitted values for the binary solutions were $\beta_t = 1.78$ ($R = 0.995$) and $\beta_u = 1.86$ ($R = 0.996$) [$\beta_u = 2.33$ ($R = 0.948$) for only O_u–H_w], and for the ternary solution were $\beta_t = 1.90$ ($R = 0.999$) and $\beta_u = 2.28$ ($R = 0.998$) [$\beta_u = 2.29$ ($R = 0.9995$) for only O_u–H_w]. Thus, $\beta \sim 2$, although the significance of the power is not clear. The power relationship for the diffusion coefficient of water is not as good especially when TMAO is present (Figure S2 in the Supporting Information), which is consistent considering the relative concentrations of solute and solvent. However, the nature of the relationship is not as important as demonstrating that diffusion and hydrogen bond lifetimes are correlated.

The diffusion of water slows greatly as the concentration of TMAO increases whereas the reduction as a function of urea concentration is less (Figure 2a,b). For TMAO, the change in $D_{\text{bulk}}(c)$ can be seen by the large decrease in $D_t(c)$ with TMAO concentration, which reaches about a third of the initial value by 4 M. For urea, the change in $D_{\text{bulk}}(c)$ is much less because $D_u(c)$ only decreases from its initial value by about 15% at ~4 M urea, which is in the range seen for ions in water.³¹ In addition, because $D_w(c)/D_w(0) \approx D_s(c)/D_s(0)$ (eq 2) holds for the simulation data (Figure 5), the decrease in diffusion with the concentration of solute can be estimated by either the concentration dependence of the solute or water diffusion coefficient.

The concentration dependence of D_w in the solutions as a function of total concentration of the different cosolutes, which is the osmolyte concentration, was analyzed in terms of eq 3 with $D_w(0) = 2.789 \times 10^{-9}$ m²/s. For the binary aqueous solutions of either urea or TMAO, the relationship between D_w and the number ratio of solute to water is fit well with an

exponential equation (Figure 6), in which the fit gave $\alpha_t = 12.71$ with $R = 0.999$ and $\alpha_u = 2.12$ with $R = 0.997$. However, for urea, the difference between the fit to eq 3 and a linear dependence is small ($R = 0.997$ vs $R = 0.998$, respectively). In addition, D_w for TMAO + urea solutions predicted by eq 3 using α_t and α_u from the fits to the binary solutions only slightly overestimates the decrease in the diffusion coefficients calculated from the simulation (Figure 6). The good agreement indicates that the effects of TMAO and urea are additive and thus uncoupled.

The large slow-down of water diffusion as the concentration of TMAO increases can be attributed to the strong interactions between TMAO and water found in vibrational spectroscopy.³² This also implies that hydrogen bonds with water are less likely to break if they cannot immediately reform a hydrogen bond with another water. However, while the strong TMAO–water hydrogen bonds can explain why the hydrated water slows, they do not necessarily explain the greater effect on bulk water in TMAO than urea solutions. The origin of the difference in the effects of TMAO and urea on bulk can be found by examining the mean-square displacement (MSD) of water in different situations (Figure 7a,b). As expected, hydration water moves slower than bulk water because both TMAO and urea are larger molecules than water. Also as expected, bulk water moves faster than the average of all water in TMAO solutions, but surprisingly, bulk water moves slightly more slowly than the average in urea solutions. Now consider only the water molecules that are either breaking or forming a hydrogen bond with a solute molecule or in other words, moving between being a hydration water to a bulk water, which will be referred to as “transition” water. The MSD of transition water is higher than bulk water in urea solutions (Figure 7b), whereas it is higher initially in the TMAO solutions but quickly becomes less (Figure 7a). In urea, transition water in the short-lived nitrogen–water hydrogen bond is mostly responsible for the higher MSD of transition water, but transition water in the oxygen–water hydrogen bond is also somewhat higher. This indicates that forming and breaking hydrogen bonds with water requires and adds, respectively, kinetic energy to water initially. Because urea–water hydrogen bonds are on the same time scale or shorter than water–water hydrogen bonds, the transition water keeps on moving in the urea solution because it keeps on getting “kicks” of kinetic energy. However, the long lifetime of the hydrogen bond slows down transition water, so it begins to diffuse more slowly as time increases. Thus, the short-lifetime urea–water hydrogen bonds appear to increase diffusion, whereas the long-life time TMAO–water hydrogen bonds appear to decrease diffusion.

Overall, these results indicate that at concentrations of urea used to denature proteins (4 to 6 M urea), TMAO may counteract the denaturing effects of urea by making longer-lived urea–water hydrogen bonds (Figure 3b, left and right) via slowing down bulk water (Figure 6). In addition, the reduction by TMAO of the energizing effects of urea on water could play a role. This does not mean a dynamic effect per se because the diffusion coefficient of water is determined by the strong hydrogen bond network, and increases in the hydrogen bond strength lead to decreases in the diffusion coefficient. Thus, these results indicate that TMAO may counteract the weakening of the hydrogen bond network by urea. At concentrations used by elasmobranchs (0.6 M total solute concentration), the effects are smaller and will also be more complicated because of the presence of other molecules in the intracellular environment. However, given the interplay of hydrogen bond lifetimes and

solution dynamics, investigating these effects at different concentrations and with protein as well as comparing with other force fields is warranted.

4. CONCLUSIONS

The simulations here indicate that solute–water hydrogen bond lifetimes, which are long in aqueous solutions of TMAO but are close to water–water lifetimes in aqueous solutions of urea, are important in understanding the differing effects of TMAO and urea on proteins as well as the counteraction of these effects in mixtures with both TMAO and urea. In the binary aqueous solutions, both solute–water and water–water hydrogen bonds increase more than linearly with TMAO concentration but increase linearly with urea concentration. Also, the diffusion rates of the solute decrease exponentially with TMAO concentration at a much faster rate than with urea concentration. In the ternary solutions, the addition of TMAO to urea aqueous solutions results in not only water–water but also urea–water hydrogen bonds, increasing more than linearly with total solute concentration. The lifetimes of solute–water hydrogen bonds are directly affected by the diffusion rate of the solute, and the solution itself moves more slowly with the added TMAO. In addition, while hydration water moves slower than bulk water because both TMAO and urea are larger molecules than water, the longer lifetime of TMAO–water hydrogen bonds means that hydrogen-bonded water is moving slower as it leaves (or arrives) whereas the lifetimes of urea–water hydrogen bonds, which are comparable to and shorter than water–water hydrogen bonds, mean that hydrogen-bonded water is accelerated. Thus, urea appears to impart motion into bulk water, whereas TMAO slows down bulk water. Either or both lengthening the lifetime of urea–water hydrogen bonds and slowing down bulk water may play roles in the counteraction by TMAO of urea denaturation of proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Density as a function of solute concentration for aqueous solutions of (a) TMAO (red) and (b) urea (blue) from simulation and experiment^{13,52} (black).

Figure 2. Diffusion coefficients as a function of solute concentration for aqueous solutions of (a) TMAO (red) and (b) urea (blue) from simulation and experiment^{32,53} (black).

Figure 3.

(a) average number and (b) lifetime of hydrogen bonds (left) between a TMAO and water (Ot-Hw) versus total solute concentration for aqueous solutions of TMAO (red) and TMAO + urea (green) and (right) between a urea and water (Ou-Hw and Hu-Ow) versus total solute concentration for aqueous solutions of urea (blue) and TMAO + urea (green).

Figure 4.

$D_s/D_s(0)$ vs $\tau_s(0)/\tau_s$ (symbols) and fit to $D_i/D_i(0) = (\tau_i(0)/\tau_i)^\beta$ (dashed lines). For τ_u , the average lifetime of hydrogen bonds with both the urea oxygen and nitrogens (eq 1) is used.

Figure 5. Ratio of diffusion coefficient at different concentrations to the diffusion coefficient at zero concentration.

Figure 6. Diffusion coefficients from simulation (symbols) and fits to eq 3 (dashed line) as a function of total solute concentration for aqueous solutions of TMAO (red), urea (blue), and TMAO + urea (green).

Figure 7. Average MSD of all water (black), hydration water (red), bulk water (blue), and “transition” water (green) for (a) TMAO solutions and (b) urea solutions. See text for the definition of transition water. Concentration is ~ 1 M in both.