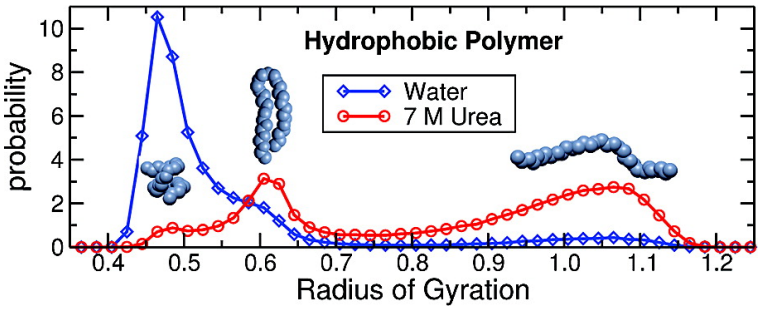
Article

Urea's Action on Hydrophobic Interactions

Ronen Zangi, Ruhong Zhou, and B. J. Berne

J. Am. Chem. Soc., **2009**, 131 (4), 1535-1541 • DOI: 10.1021/ja807887g • Publication Date (Web): 05 January 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
ARTICLES

Published on Web 01/05/2009

Urea's Action on Hydrophobic Interactions

Ronen Zangi,[†,§] Ruhong Zhou,[†,‡] and B. J. Berne*[,†,‡]

Department of Chemistry, Columbia University, 3000 Broadway, New York, New York 10027, and Computational Biology Center, IBM Thomas J. Watson Research Center, 1101 Kitchawan Road, Yorktown Heights, New York 10598

Received October 9, 2008; E-mail: bb8@columbia.edu

Abstract: For more than a century, urea has been commonly used as an agent for denaturing proteins. However, the mechanism behind its denaturing power is still not well understood. Here we show by molecular dynamics simulations that a 7 M aqueous urea solution unfolds a chain of purely hydrophobic groups which otherwise adopts a compact structure in pure water. The unfolding process arises due to a weakening of hydrophobic interactions between the polymer groups. We also show that the attraction between two model hydrophobic plates, and graphene sheets, is reduced when urea is added to the solution. The action of urea is found to be direct, through its preferential binding to the polymer or plates. It is, therefore, acting like a surfactant capable of forming hydrogen bonds with the solvent. The preferential binding and the consequent weakened hydrophobic interactions are driven by enthalpy and are related to the difference in the strength of the attractive dispersion interactions of urea and water with the polymer chain or plate. This relation scales with $\sqrt{\epsilon_b}$, where ϵ_b is the Lennard Jones (LJ) energy parameter for each group on the chain. Larger values of ϵ_b increase the preferential binding and result in a larger decrease of the hydrophobic interactions, with a crossover at very weak dispersions. We also show that the indirect mechanism, in which urea acts as a chaotrope, is not a likely cause of urea's action as a denaturant. These findings suggest that, in denaturing proteins, urea (and perhaps other denaturants) forms stronger attractive dispersion interactions with the protein side chains and backbone than does water and, therefore, is able to dissolve the core hydrophobic region.

1. Introduction

Urea as a protein denaturant has attracted extensive study in the past several decades, and it has been widely accepted that urea interacts differently with hydrophobic groups than with either hydrophilic groups or protein backbones. The latter are dominated by formation of hydrogen bonds and other polar interactions.[1−3] In an effort to cast light on urea's action, many model systems have been designed to isolate and estimate the contribution of the interaction of urea with these different groups to the denaturing process.[4−11] For example, urea was found to increase the solubility of hydrocarbons, but the mechanism by which it does this is not yet clear. Does it act as a "chaotrope"? That is, does its addition to an aqueous protein solution break the structure of water and make it a better solvent for hydrophobic groups.[12−19] This can trigger a folded protein to unfold by exposing the hydrophobic side chains to the more accommodating solvent. In this paper, instead of grappling with polar and nonpolar regions of proteins, we determine how urea affects purely hydrophobic systems, such as unfolding of hydrophobic polymers, or solvent-induced dimerization of graphene sheets. The incentive for this study was to help us interpret urea's mode of action on hydrophobic residues in our recent microsecond simulations of the effect of urea on the unfolding of a hen eggwhite lysozyme mutant[20,21] (W62G). We believe that the present study provides a simple explanation for how urea affects the purely hydrophobic side chains.

† Columbia University.
‡ IBM Thomas J. Watson Research Center.
§ Present address: Department of Organic Chemistry I, University of the Basque Country, Avenida de Tolosa 72, 20018 San Sebastian, Spain.

(1) Mirsky, A. E.; Pauling, L. *Proc. Natl. Acad. Sci. U.S.A.* **1936**, *22*, 439–447.
(2) Gill, S. J.; Huston, J.; Clopton, J. R.; Downing, M. *J. Phys. Chem.* **1961**, *65*, 1432–1435.
(3) Creighton, T. E. *Curr. Opin. Struct. Biol.* **1991**, *1*, 5–16.
(4) Robinson, D. R.; Jencks, W. P. *J. Biol. Chem.* **1963**, *238*, PC1558–PC1560.
(5) Nozaki, Y.; Tanford, C. *J. Biol. Chem.* **1963**, *238*, 4074–4081.
(6) Gordon, J. A.; Jencks, W. P. *Biochemistry* **1963**, *2*, 47–57.
(7) Weltlaufer, D. B.; Malik, S. K.; Stoller, L.; Coffin, R. L. *J. Am. Chem. Soc.* **1964**, *86*, 508–514.
(8) Robinson, D. R.; Jencks, W. P. *J. Am. Chem. Soc.* **1965**, *87*, 2462–2470.
(9) Nandi, P. K.; Robinson, D. R. *Biochemistry* **1984**, *23*, 6661–6668.
(10) Roseman, M.; Jencks, W. P. *J. Am. Chem. Soc.* **1975**, *97*, 631–640.
(11) Auton, M.; Bolen, W. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 15065–15068.
(12) Rupley, J. A. *J. Phys. Chem.* **1964**, *68*, 2002–2003.
(13) Hammes, G. G.; Schimmel, P. R. *J. Am. Chem. Soc.* **1967**, *89*, 442–446.
(14) Frank, H. S.; Franks, F. *J. Chem. Phys.* **1968**, *48*, 4746–4757.
(15) Barone, G.; Rizzo, E.; Vitagliano, V. *J. Phys. Chem.* **1970**, *74*, 2230–2232.
(16) Finer, E. G.; Franks, F.; Tait, M. J. *J. Am. Chem. Soc.* **1972**, *94*, 4424–4429.
(17) Kallies, B. *Phys. Chem. Chem. Phys.* **2002**, *4*, 86–95.
(18) Bennion, B. J.; Daggett, V. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 5142–5147.
(19) Idrissi, A. *Spectrochim. Acta A* **2005**, *61*, 1–17.
(20) Zhou, R.; Eleftherious, M.; Royyuru, A.; Berne, B. J. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 5824–5829.
(21) Hua, L.; Zhou, R.; Thirumalai, D.; Berne, B. J. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 16928–16933.

That urea weakens the hydrophobic effect was inferred from experiments showing that the solubility of most hydrocarbons increases upon the transfer from water to aqueous urea solution[7,22] and experiments showing an increase in the critical micelle concentration of cationic surfactants in urea solution relative to water.[23] In contrast, it was also found that the smallest hydrocarbon, methane, is more soluble in water than in urea.[7] In addition, simulations indicated that urea stabilizes the contact pairing of two methane molecules.[24−27] Computer simulations on larger hydrocarbons[25,26,28,29] obtained conflicting results in regard to whether urea increases or decreases the hydrophobic interaction. It was, therefore, assumed that this effect is not likely to be strong enough by itself to induce protein unfolding. Since urea molecules make hydrogen bonds with the protein backbone, it was suggested that urea drives protein unfolding by weakening electrostatic interactions within the protein, thereby, reducing the backbone−backbone hydrogen bonds.[24,27,28] In other studies, it was also suggested that urea does not entirely dissolve a hydrophobic cluster but acts as a bridge between hydrophobic particles, holding them together.[29]

In this paper we show, by molecular dynamics simulations, that a 7 M aqueous urea solution unfolds a model hydrophobic polymer which otherwise adopts a compact structure in pure water. The chain beads are purely hydrophobic, each of a size typical of protein residues, and linearly connected to each other. We also show that the attractive hydrophobic interaction between two hydrophobic model plates as well as graphene sheets is reduced in 7 M urea solution. In all cases, the action of urea is found to be direct, through its preferential binding to the polymer or plates. It, therefore, acts like a surfactant capable of forming hydrogen bonds with the solvent. The preferential binding of urea, and its concomitant weakening of the hydrophobic interactions, is driven by enthalpy and is related to the difference in the strength of the attractive dispersion interactions of urea and water with the polymer chain or plates. We find that this relation scales with $\sqrt{\epsilon_b}$, where ϵ_b is the LJ energy parameter for each group on the chain. Larger values of ϵ_b increase the preferential binding and result in a larger decrease of the hydrophobic interactions, with a crossover at very weak dispersion interactions. We also show that the indirect mechanism, which assumes urea acts as a chaotrope, does not play a major role in urea's action as a denaturant.

2. Methods

We used the MD package GROMACS version 3.3[30] to perform all of the computer simulations, with a time step of 0.002 ps. The flexible version[31] of OPLS-AA urea model[32] and the TIP4P water model[33] were employed to describe the solvents. The system was maintained at a constant temperature of 300 K and pressure of 1.0 bar.[34] The electrostatic forces were evaluated by the Particle-Mesh Ewald method (with grid spacing of 0.12 nm and quadratic interpolation) and the LJ forces by a cutoff of 1.0 nm. We applied geometric combination rules to calculate the LJ interaction between different particles.

2.1. Hydrophobic Polymer. The linear polymer contains 32 hydrophobic groups (beads) each connected to the covalently bonded neighbor by a harmonic potential, $V_b(r_{ij}) = {}^1/_2 k_b(r_{ij} - r_0)^2$, with an equilibrium bond length of $r_0 = 0.153$ nm (the same as the CH_2-CH_2 bond length) with a force constant of $k_b = 3.3 \times 10^5$ kJ/mol/nm^2. The angle between adjacent covalent bonds is represented by a harmonic potential, $V_a(\theta_{ijk}) = {}^1/_2 k_a(\cos(\theta_{ijk}) - \cos(\theta_0))^2$, with $\theta_0 = 111°$ (the same as the bond angle for $CH_2-CH_2-CH_2$) and $k_a = 50$, 50, 75, and 100 kJ/mol for the simulations with $\epsilon_b =$ 0.4, 0.6, 0.8, and 1.0 kJ/mol, respectively. The angular force constant needs to be increased as ϵ_b is increased to maintain the average bond angle at 111°. Nonbonded interactions between a bead and its first and second nearest neighbors were excluded, and no dihedral interaction terms were included. The LJ diameter of a hydrophobic group is $\sigma_b = 0.4$ nm. We performed four different sets of simulations with $\epsilon_b = 0.4$, 0.6, 0.8, and 1.0 kJ/mol. The number of water molecules in the simulations in pure water is 1706. The 7 M aqueous urea solution was generated by dissolving 220 urea molecules in 1200 water molecules. The results presented in this paper are averaged over 12 simulations (same initial configuration but different velocities) for each of the solvents (water and 7 M urea). The length of each trajectory was in the range 40−120 ns. To calculate the preferential binding of urea to the polymer we performed eight additional simulations (with a total simulation length of 120 ns) where we froze the polymer in different extended conformations. To calculate preferential binding/exclusion we define binding by a bead−solute (oxygen atom for water and carbon atom for urea) cutoff of 0.60 nm. It is the midpoint of the first minimum in the corresponding radial distribution functions.

2.2. Model Hydrophobic Plates. Each plate is represented by a single-layer of 49 particles, arranged in a square lattice with a bond length of 0.32 nm (thus, the size of the plate is 2.32×2.32 nm^2). The LJ diameter of each plate particle is $\sigma_p = 0.40$ nm. We performed six different sets of simulations, with $\epsilon_p =$ 0.5, 0.6, 0.75, 1.0, 1.5, and 2.0 kJ/mol. During simulations, the positions of the plate atoms are held fixed, interactions between the plate atoms are excluded, and the orientation of the two plates with respect to each other is parallel and in-registry. The simulation in pure water contained 1393 water molecules, and the 7 M aqueous urea solution is prepared by dissolving 185 urea molecules in 995 water molecules. The PMF between the two plates was computed from the mean force acting on each of the plates.[35−37] Then the mean force acting between the plates along their axis of separation was integrated as a function of the distance between the plates, r, to yield the free energy profile. As the PMF represents only relative values, it was shifted such that the free energy of the states at the largest separations correspond to zero. For each value of ϵ_p, we performed 54 simulations with different values of r, ranging from 0.36 to 1.44 nm. At each distance, the system was equilibrated for 2.0 ns and data were collected for 6.0 ns. At the associated ($r =$ 0.41 nm) and dissociated ($r =$ 1.44 nm) states, we performed additional simulations for 80 ns to reduce the statistical error when

(22) Whitney, P. L.; Tanford, C. *J. Biol. Chem.* **1962**, *237*, PC1735–PC1737.
(23) Bruning, W.; Holtzer, A. *J. Am. Chem. Soc.* **1961**, *83*, 4865–4866.
(24) Wallqvist, A.; Covell, D. G. *J. Am. Chem. Soc.* **1998**, *120*, 427–428.
(25) Ikeguchi, M.; Nakamura, S.; Shimizu, K. *J. Am. Chem. Soc.* **2001**, *123*, 677–682.
(26) Shimizu, S.; Chan, H. S. *Proteins: Struct., Funct., Genet.* **2002**, *49*, 560–566.
(27) O'Brien, E. P.; Dima, R. I.; Brooks, B.; Thirumalai, D. *J. Am. Chem. Soc.* **2007**, *129*, 7346–7353.
(28) Mountain, R. D.; Thirumalai, D. *J. Am. Chem. Soc.* **2003**, *125*, 1950–1957.
(29) Lee, M.-E.; van der Vegt, N. F. A. *J. Am. Chem. Soc.* **2007**, *128*, 4948–4949.
(30) Lindahl, E.; Hess, B.; van der Spoel, D. *J. Mol. Mod.* **2001**, *7*, 306–317.
(31) Smith, L. J.; Berendsen, H. J. C.; van Gunsteren, W. F. *J. Phys. Chem. B* **2004**, *108* (3), 1065–1071.
(32) Duffy, E. M.; Severance, D. L.; Jorgensen, W. L. *Isr. J. Chem.* **1993**, *33*, 323–330.
(33) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. *J. Chem. Phys.* **1983**, *79*, 926–935.
(34) Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; DiNola, A.; Haak, J. R. *J. Chem. Phys.* **1984**, *81*, 3684–3690.
(35) Pangali, C. S.; Rao, M.; Berne, B. J. In *Computer Modeling of Matter*; Lykos, P., Ed.; ACS Symposium Series No. 86; ACS: Washington, DC, 1978; p 29.
(36) Watanabe, K.; Andersen, H. C. *J. Phys. Chem.* **1986**, *90*, 795–802.
(37) Zangi, R.; Berne, B. J. *J. Phys. Chem. B* **2008**, *112*, 8634–8644.

calculating differences in thermodynamical properties between these states. Preferential binding was calculated by counting the number of water and urea bound to the plates in the associated state (see Supporting Information for further explanation). In this analysis we considered only particles inside a cylinder along the *z*-axis of the simulation box with a radius (in the *xy*-plane) of 1.15 nm. Binding was defined by a cutoff distance of 0.57 nm along the *z*-axis in the density profile, relative to the *z*-component of the (nearest) plate particles. This cutoff distance corresponds to the midpoint of the first minimum in the density profiles of urea and water.

2.3. Graphene Sheets. The graphene plates were prepared by cutting a rectangle with a dimension of 1.212×1.330 nm^2 from a monolayer of hexagonal graphite structure (with bond length of 0.14 nm). The carbon−water LJ parameters $\sigma_{CO} = 0.319$ nm, $\epsilon_{CO} = 0.392$ kJ/mol were taken from parametrization of the contact angle of water on graphite.[38] The interactions between graphene and urea were calculated using the geometric combination rule and the LJ parameters of urea. The PMF calculations between the two graphene sheets followed the same procedure as the one applied to the model hydrophobic plates, however, in this case we performed 72 simulations at distances in the range 0.26−1.76 nm. At each point the system was equilibrated for 4.0 ns, and data were collected for 6.0 ns.

3. Results

To eliminate any bias favoring our findings, the initial state of the polymer for all the simulations in water was taken to be an extended (unfolded) conformation, with a radius of gyration, R_g, equal to 1.115 nm, and for all the simulations in urea solution the initial state was taken to be a very compact "helical" (folded) conformation with $R_g = 0.423$ nm, a state similar to the most populated conformation found in water (see below). These conformations are shown in the top panel of Figure 1. Figure 1 also displays (bottom panel) the radius of gyration of the polymer ($\epsilon_b = 1.0$ kJ/mol) as a function of time for one of the trajectories in water (a) and a 7 M aqueous urea solution (b). After a relatively short time, the polymer in water collapses to a compact state ($R_g = 0.470$ nm), while in urea it unfolds to an extended structure ($R_g = 1.050$ nm). During the course of the simulation, the polymer, in both solvents, infrequently visits states close to its initial conformation, as well as other states ($R_g = 0.605$ nm) resembling a hairpin conformation (see the snapshots in Figure 2). The most probable value of R_g for each of these states (0.470, 0.605, and 1.050 nm for the collapsed, hairpin-like, and extended conformations, respectively) is marked by a horizontal line (magenta). Note that while in urea solution the hairpin-like conformation is kinetically stable in water, it is not.

In Figure 2, averaging over all 12 trajectories, we calculate the normalized distribution of R_g in water and in urea for polymer chains with different strengths of dispersion interaction: $\epsilon_b = 1.0$, 0.8, 0.6, and 0.4 kJ/mol. In addition, we also display snapshots of the polymer conformation which corresponds to each of the three states. The vertical brown lines denote the cutoff values applied to distinguish between these states. The simulations indicate that, for $\epsilon_b = 1.0$ kJ/mol in water, the most stable conformation (observed 70.5% of the time) is the collapsed state, while the unfolded state is scarcely sampled (observed 9.8% of the time). In contrast, in 7 M urea solution, the same polymer is stable mostly in its unfolded conformation (65.9%), while the collapsed state is visited only 7.5% of the time. Interestingly Mountain and Thirumalai found, from relatively short trajectories (a few ns), that the hydrocarbon chain unfolds slightly in 6 M urea; however they conclude that this effect is not strong enough to induce unfolding and that the dispersion interactions are not as important as electrostatic interactions in protein denaturation.[28] From the results presented in Figure 2, it is clear that the polymer chain undergoes a structural transition from folded to unfolded states upon the addition of urea. Since this polymer interacts with itself and with the solvent only through dispersion interactions, the mechanism of unfolding by urea must be a weakened solvent-induced interaction between the polymer hydrophobic groups. Figure 2 also shows the distribution of R_g in water and in urea for $\epsilon_b = 0.8$, 0.6, and 0.4 kJ/mol. In all cases, urea destabilizes the folded state and stabilizes the unfolded state. The magnitude of this stabilization and destabilization effect, i.e., the denaturing power, of urea decreases as ϵ_b decreases. However for sufficiently small ϵ_b there is a crossover behavior where urea actually induces folding instead of denaturation (see below).

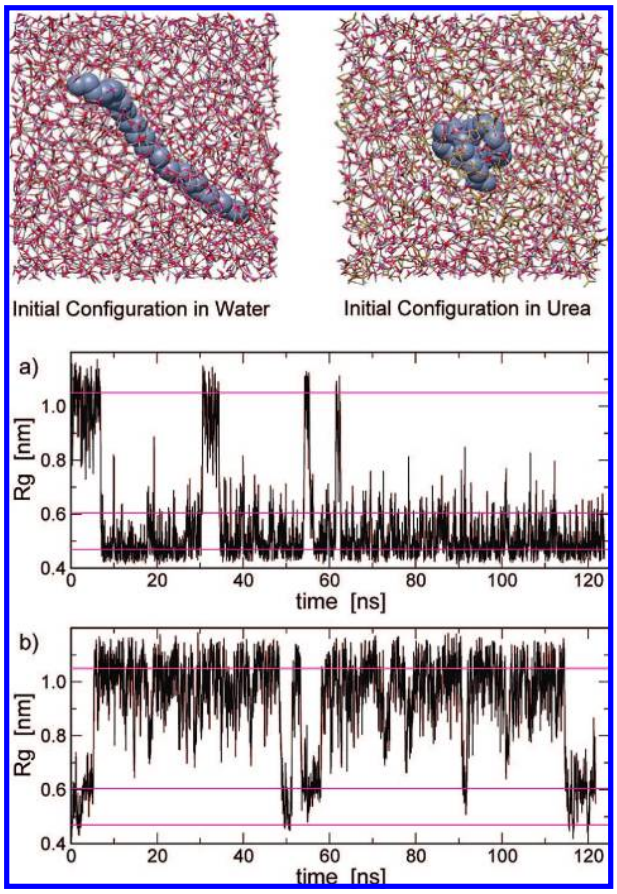


Figure 1. Top panel: Initial conformation of the hydrophobic polymer for all the simulations in water (extended state; $R_g = 1.115$ nm) and for all the simulations in aqueous urea solution (folded helical state; $R_g = 0.423$ nm). Bottom panel: Radius of gyration, R_g, of the hydrophobic polymer, with $\epsilon_b = 1.0$ kJ/mol, as a function of time for one of the simulations in water (a) and in 7 M urea solution (b). The horizontal lines in magenta at $R_g = 0.470$, 0.605, and 1.050 nm denote the most probable value of the radius of gyration for the collapsed (folded), hairpin-like, and extended (unfolded) states, respectively.

To investigate the affinity of water and urea molecules to the polymer, we plot in Figure 3a the radial distribution functions $g_{B\text{-}O_W}(r)$ and $g_{B\text{-}C_U}(r)$ which, respectively, give the distributions of water oxygen atoms and urea carbon atoms around the beads

(38) Werder, T.; Walther, J.; Jaffe, R.; Halicioglu, T.; Koumoutsakos, P. *J. Phys. Chem. B* **2003**, *107*, 1345–1352.

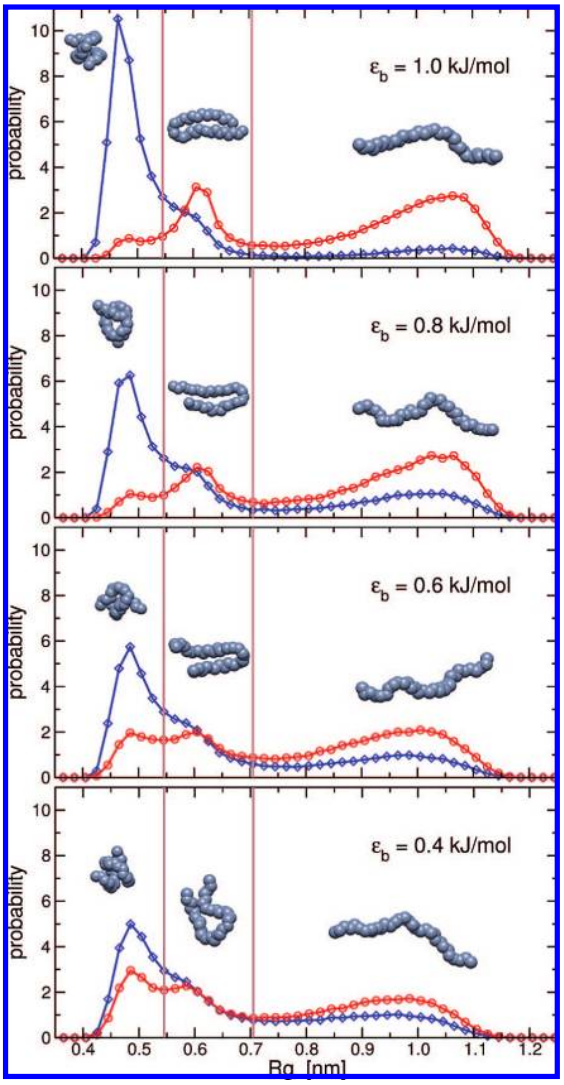


Figure 2. Normalized distribution of the radius of gyration of the polymer for the simulations in water (blue diamonds) and aqueous urea solution (red circles). The plots are displayed for different values of, ϵ_b, the polymer's dispersion interactions. The brown vertical lines mark the radius of gyration cutoff values used to classify the different states. Snapshots of the polymer conformation in each of the three states from the simulations in either water (for ϵ_b = 0.8 and 0.4 kJ/mol) or urea (for ϵ_b = 1.0 and 0.6 kJ/mol) are shown.

(B) of the polymer chain. The figure indicates that, for all values of ϵ_b, water is preferentially excluded from the vicinity of the polymer, while urea is preferentially bound to it. This behavior is similar to what we have found for protein lysozyme in 8 M urea solutions[21] and to studies that investigated the interactions of urea solution with the 20 amino-acids residues.[39] Nevertheless, the magnitude of this preferential binding/exclusion of urea/water decreases with ϵ_b. Using simple statistical mechanical arguments, it can be shown (see Supporting Information) that if the preferential binding is expressed by

$$\nu_{\text{urea}} = \frac{n_{\text{urea}}}{n_{\text{water}}} \cdot \frac{N_{\text{water}}}{N_{\text{urea}}} - 1 \qquad (1)$$

where n_X is the number of X molecules bound to the polymer and N_X is the total number of X, then

$$\ln \nu'_{\text{urea}} = \frac{\sqrt{\epsilon}_b}{RT}(\sqrt{\epsilon}_{\text{uu}} - \sqrt{\epsilon}_{\text{ww}}) + C \qquad (2)$$

where $\nu' = \nu + 1$, ϵ_{XX} is the effective interaction between the X molecules, and C is a constant that does not depend on ϵ_b. Figure 3b displays the value of $\ln \nu'_{\text{urea}}$ (computed for the extended state of the polymer) as a function of $\sqrt{\epsilon_b}/RT$. As predicted by the theory, a linear relation is observed (correlation coefficient = 0.9999) with a slope of 5.0 $(\text{kJ/mol})^{1/2}$. Positive values of $\ln \nu'_{\text{urea}}$ indicate preferential binding of urea while negative values indicate preferential exclusion. Thus, extrapolation of this line predicts a crossover to preferential exclusion of urea at $\epsilon_b \sim 0.33$ kJ/mol. In OPLSAA and Gromos96 force fields the value of ϵ_b of the united atom description for CH_2 group is 0.49 kJ/mol. Thus, urea is predicted to preferentially bind to a hydrocarbon segment in proteins, as seen in recent computer simulation studies.[21,39]

A thermodynamic relation exist between the change in the chemical potential of a macromolecule upon the addition of a cosolute to the solution and the number of cosolute molecules bound/excluded to the macromolecule.[40−43] Our system is characterized by a three-state model. The assignment of a configuration to a particular state is based on the cutoff values shown in Figure 2 which mark the minima of the distribution functions of R_g in water and urea solutions. Considering the equilibrium between the folded (collapsed; $R_g \leq 0.545$ nm) and unfolded (extended; $R_g > 0.705$ nm) states, $F \rightleftharpoons U$, the free energy change can be obtained by $\Delta G = -RT \ln([U]/[F])$. Then, the value of $\Delta\Delta G = \Delta G_{\text{urea}} - \Delta G_{\text{water}}$ is a measure of the power of urea to denature the folded state (large negative values indicate strong denaturing power). The number of urea molecules which are bound to these three conformational states of the polymer is different and is likely to be scaled by the solvent exposed surface area of the polymer conformation. Since the value of R_g of a particular conformation (state) is the same for each ϵ_b, we assume also that the solvent exposed surface area for that state is the same for each ϵ_b. Therefore, the change in the solvent exposed surface area for the unfolding reaction is the same for the different ϵ_b systems, with a value that is a fraction of that for the extended state. This mean that the change in the preferential binding during the unfolding process is linearly proportional to the preferential binding calculated for the extended state. In Figure 3c, we plot the value of $\Delta\Delta G$ as a function of the preferential binding of urea to the polymer in its extended state, ν'_{urea}. The figure indicates that the denaturing power of urea increases with ν'_{urea}. Thus, the polymer−solvent dispersion interaction plays a crucial role in the denaturing mechanism. This behavior provides an explanation of the experimental results showing that the transfer of a hydrocarbon from water to 7 M urea is more favorable as the size of the hydrocarbon increases. As the size of a hydrocarbon molecule increases, its dispersion interaction with urea increases more than with water. This is because the urea molecule has a larger density of atomic interaction sites than does the water molecule. Therefore, the preferential binding of urea is stronger for larger hydrocarbons, thereby inducing a larger decrease in the chemical potential of the hydrocarbon, stabilizing its dissolution.

(39) Stumpe, M. C.; Grubmüller, H. *J. Am. Chem. Soc.* **2007**, *129*, 16126–16131.
(40) Wyman, J. *Adv. Protein Chem.* **1964**, *19*, 223–286.
(41) Tanford, C. *J. Mol. Biol.* **1969**, *39*, 539–544.
(42) Parsegian, V. A.; Rand, R. P.; Rau, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3987–3992.
(43) Timasheff, S. N.; Xie, G. *Biophys. Chem.* **2003**, *105*, 421–448.

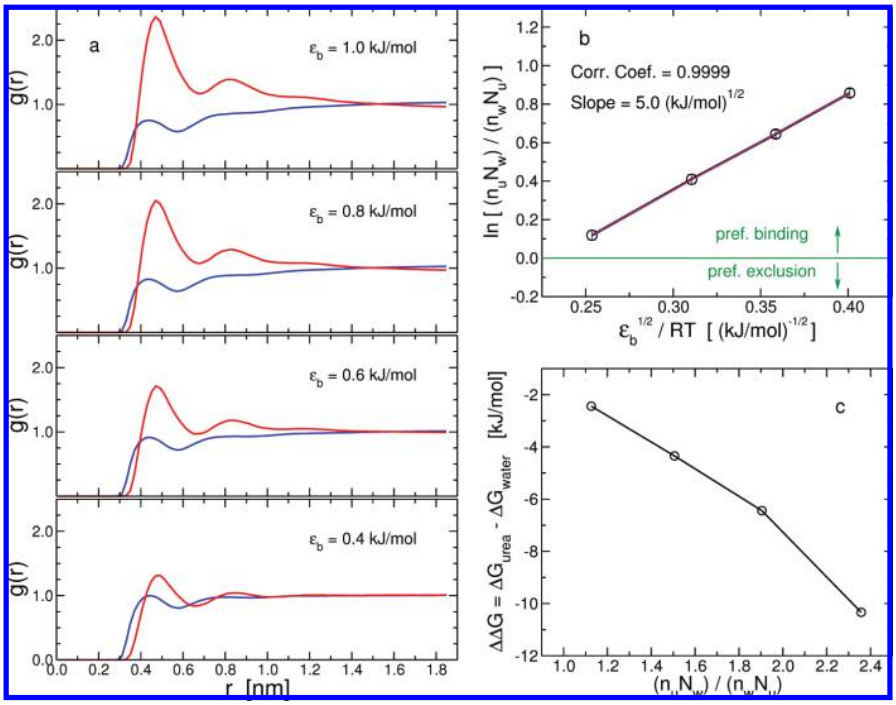


Figure 3. Radial distribution function of the hydrophobic groups of the polymer with water (oxygen atom; blue) and with urea (carbon atom; red), in 7 M aqueous urea solution, for different bead dispersion interactions, ϵ_b (a). The preferential binding of urea to the polymer, defined by ln ν'_{urea}, as a function of $\sqrt{\epsilon_b}/RT$ (b). The relation between $\Delta\Delta G$, for the unfolding reaction in urea relative to water, and the preferential binding of urea, ν'_{urea} (c).

The mechanism for destabilizing the hydrophobic interactions by urea found in this study is similar to what we found for the salting-in behavior of hydrophobes by low charge density ions.[44,45] Low charge density ions preferentially bind to the hydrophobe because their interaction with water is weaker than the water−water interaction. In this case, salting-in is driven by enthalpy; the preferentially bound ions act as surfactants reducing the unfavorable energetic penalty between water and the hydrophobe. To investigate the driving force in the case of urea and to examine quantitatively the relationship between denaturing power and preferential binding, we calculate the potential of mean force between two large hydrophobic plates, in 7 M urea solution and in pure water. The plates are represented by a square lattice array of 7 × 7 hydrophobic particles (see Figure 4a). We varied the magnitude of the LJ dispersion interaction of each particle site, ϵ_p, for different simulations. This results in a different magnitude for the preferential binding of urea. As predicted by the simple theoretical model presented in the Supporting Information, Figure 4b indicates that a linear relation (correlation coefficient $R^2 = 0.9990$) exists between the value of ln ν'_{urea} and $\sqrt{\epsilon_p}/RT$, similar to the behavior found for the hydrophobic polymer shown in Figure 3b. The slope of the linear relation is 4.2 (kJ/mol)$^{1/2}$. Considering the dimer plate dissociation process, $P_2 \rightleftharpoons 2P$, the denaturing power of urea can be expressed by $\Delta\Delta G = \Delta G_{urea} - \Delta G_{water}$. As for the hydrophobic polymer system, there is a strong relation between $\Delta\Delta G$ and the magnitude of the preferential binding of urea to the polymer (Figure 4c). In addition, here we also find that a decrease in the dispersion interaction of the plates below a certain value induces a crossover in the behavior. That is, strengthening, as opposed to weakening, of the hydrophobic interaction is observed for $\epsilon_p < 0.23$ kJ/mol. This is consistent with the explanation based on hydrocarbon size[26] for why the transfer free energies of methane and ethane from water to urea solution are positive while for larger alkanes the free energies are negative[7] (due to the fact that the attractive dispersion interaction between urea and the solute grows with alkane size until it saturates). The existence of this crossover is likely to rule out the possibility for an indirect mechanism, in which urea breaks the structure of water. Furthermore, in previous studies we[21] and others[46−49] observed that urea mixes well with water with only minor effects on the structure of water (as measured by water's radial distribution functions). Therefore we do not believe that urea induces unfolding by breaking the structure of water.

It is shown for the theoretical model (see Supporting Information) that $\Delta\Delta G$ for plate dissociation depends on the preferential binding of urea to the plates ν_{urea} by the following relation:

$$\Delta\Delta G/RT = -M \ln(\nu_{urea} \cdot X_u + 1) \quad (3)$$

where M is the change in the number of binding sites on the hydrophobic plates for the dissociation reaction and X_u is the mole fraction of urea in solution. Note that this relation applies only for sufficiently large ϵ_p that urea preferentially binds to the plate such that it weakens the hydrophobic interactions. Taking into account the solvent molecules that are bound to the plates, we find the mole fraction of urea in solution to range

(44) Zangi, R.; Berne, B. J. *J. Phys. Chem. B* **2006**, *110*, 22736–22741.
(45) Zangi, R.; Hagen, M.; Berne, B. J. *J. Am. Chem. Soc.* **2007**, *129*, 4678–4686.
(46) Kuharski, R. A.; Rossky, P. J. *J. Am. Chem. Soc.* **1984**, *106*, 5786–5793.
(47) Tsai, J.; Gerstein, M.; Levitt, M. *J. Chem. Phys.* **1996**, *104*, 9417–9430.
(48) Klimov, D. K.; Straub, J. E.; Thirumalai, D. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 14760–14765.
(49) Stumpe, M. C.; Grubmüller, H. *J. Phys. Chem. B* **2007**, *111*, 6220–6228.

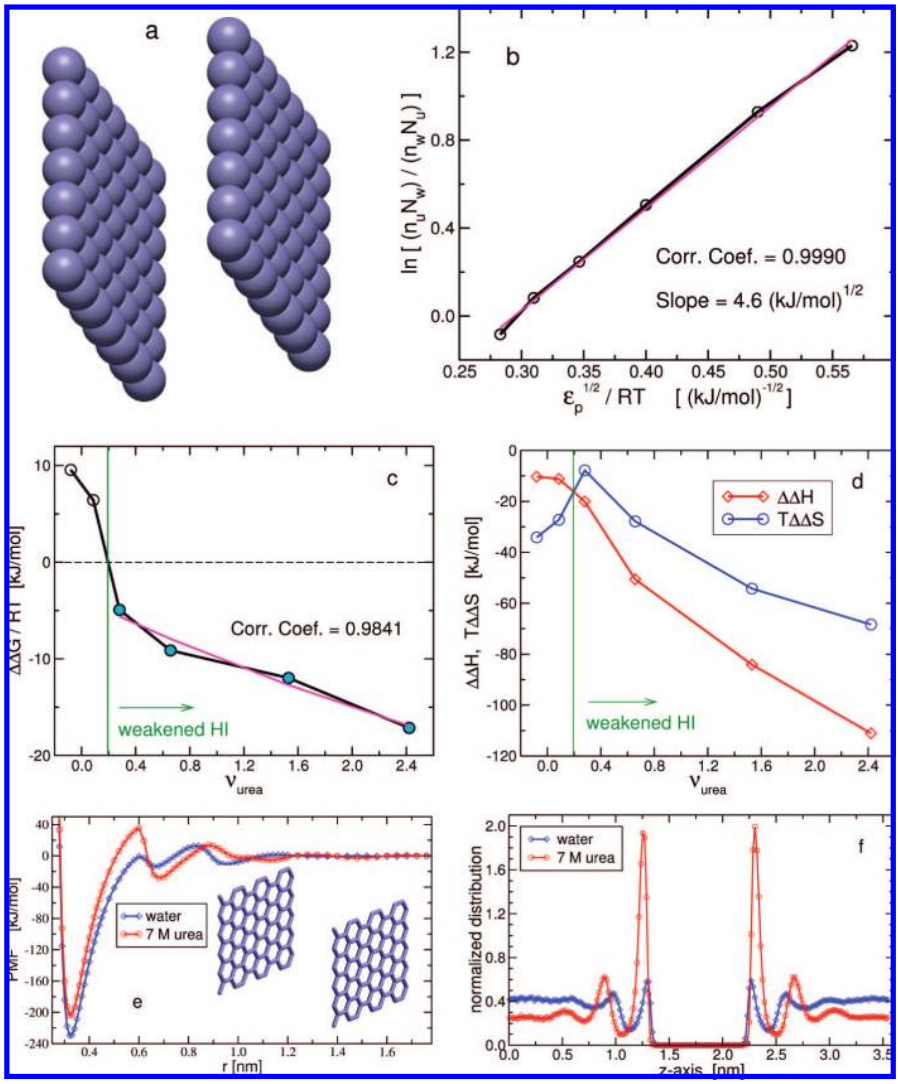


Figure 4. Model plates used in the simulations (a). The value of ln ν'_{urea} as a function of $\sqrt{\epsilon_p}/RT$ (b). The value of $\Delta\Delta G = \Delta G_{urea} - \Delta G_{water}$ (c), and the corresponding values of $\Delta\Delta H$ and $T\Delta\Delta S$ (d) as a function of $\nu_{urea} = (n_{urea}N_{water})/(n_{water}N_{urea}) - 1$. The potential of mean force between two, 1.58 × 1.53 nm^2, graphene sheets along the axis of their separation (e). Normalized density profile of water and urea along the z-axis (inside a cylindrical shaped volume with radius of 0.76 nm from the plate center of mass in the xy plane) around the graphene plates at contact (d = 0.33 nm and centered at 1.78 nm) in 7 M aqueous urea solution (f).

from 0.154 to 0.144 for $\epsilon_p = 0.75$ and $\epsilon_p = 2.00$ kJ/mol, respectively. Using these values of $X_u(\epsilon_p)$ for each ϵ_p, we fit the curve of $\Delta\Delta G/RT$ to the function $y = -A\ \ln(X_u(\epsilon_p) \cdot \nu + B)$, where A and B are fitting parameters (Figure 4c). The values obtained after fitting (correlation coefficient $R^2 = 0.9841$), $A = 47.3$ and $B = 1.08$, represent M and 1. Since the sizes of urea and water molecules are different, the effective number of binding sites is not easy to estimate. From the calculation of the preferential binding, we find that, for $\epsilon_p = 0.75$ kJ/mol, the average number of bound urea molecules is 13.1 and that of water is 55.1. For $\epsilon_p = 2.00$ kJ/mol the number of bound solvent molecules are 23.7 and 37.2 for urea and water, respectively. Therefore, the value we obtain for M is a reasonable measure for the effective number of binding sites on the two faces of one plate. In Figure 4d we plot the corresponding difference for the enthalpy and entropy changes. Since weakening the hydrophobic interaction is characterized by negative values of $\Delta\Delta G$, it is evident that the driving force for this process is enthalpic, since the value of $-T\Delta\Delta S$ is positive. This means that the binding of urea to the plates releases enthalpy that stabilizes the monomeric state of the plates. On the other hand, strengthening the hydrophobic interaction is characterized by positive values of $\Delta\Delta G$, and in this case the driving force is entropic.

Given the qualitative and quantitative differences in the denaturing power of urea as a function of the dispersion interaction in the model plate system, what behavior should we expect for a realistic hydrophobic surface? To this end, we prepared two graphene sheets of 77 atoms each and calculated the potential of mean force (PMF) in 7 M urea solution and in pure water (Figure 4e). Figure 4e clearly indicates that urea destabilizes the hydrophobic interaction between these two graphene sheets. The magnitude of the destabilization, i.e., the difference in the PMF at the equilibrium (contact) distance, is 26 kJ/mol. In Figure 4f we plot the density profile of the water molecules (oxygen atoms) and urea molecules (carbon atoms) along the z-axis (for an equilibrium contact configuration of the plates, d = 0.33 nm) in the 7 M urea solution. The figure indicates that the weakened hydrophobic interaction is due to the preferential binding of urea to the graphene sheets, as we

found for the hydrophobic polymer and model hydrophobic plates systems.

We would like to emphasize that the driving force for the denaturing power of urea is its stronger (total) binding energy to the hydrophobe relative to the water−hydrophobe binding energy. These binding energies are correlated with the molecular sizes of the solutes. For example in alkane chains, the larger the molecule the larger the difference in binding energy between urea−alkane and water−alkane (until it saturates beyond a certain size because the dispersion interactions at large distances will not contribute significantly). Thus, the denaturing power of urea will increase with the size of the alkane molecules up to a threshold, as found in previous studies.[7,26] However, for very small hydrocarbons like methane, urea has the opposite effect and increses the hydrophobic interaction. This is consistent with the crossover we find at very small ϵ_b. It has been conjectured by Chan and co-workers[26] that urea's ability to enhance the hydrophobic interaction for small solutes can partially explain why some denatured proteins in 8 M urea can still exhibit residual hydrophobic clustering.[50,51]

4. Discussion

In this paper, we demonstrated the ability of urea to unfold a purely hydrophobic polymer and to significantly weaken the solvent induced attraction between two realistic hydrophobic surfaces. Although, the simulation results shown in this paper do not exclude additional unfolding mechanisms that may take place in the denaturation of proteins, we do show (in the hydrophobic polymer system) that the magnitude of the destabilization (due to weakening the hydrophobic interactions) is strong enough to induce a substantial change in the conformational preference and to induce a transition from a folded to an unfolded state. There has been much debate over the past few decades on whether the hydrogen bonds between amide groups (e.g., intraprotein or urea−protein hydrogen bonds) are stronger or not than the hydrogen bonds formed by either the carbonyl or amine group with water.[2,6,52−55] If we suppose that they are not stronger, then it is plausible that the mechanism of protein denaturation by urea is due to weakening the hydrophobic interaction, as demonstrated in our recent work on lysozyme in 8 M urea.[21] The uniqueness of urea (and other denaturants) is that, in addition to its character as a "salting-in agent", it is also able to substitute for the intraprotein hydrogen bonds (present in the folded state of the protein) and, thereby, avoid a (large) loss of enthalpy during unfolding. The stronger dispersion interactions between the hydrophobic particles (both the polymer and plate) and urea, as compared to water, are responsible for the preferential binding of urea to the surfaces of these species and thereby to the denaturing process itself. This binding allows urea to penetrate into the unfolding polymer and act as a surfactant (as it does for the plates) by hydrogen bonding to water and urea in the next shell. We expect preferential binding to be the key for the protein denaturing mechanism as well. The stronger dispersion interactions of urea (relative to water) with the hydrophobic sidechains and backbone of the protein drives the preferential binding of urea as well as partly allowing for its intrusion into the core of globular proteins and, as such, provides a plausible explanation for urea's strong denaturing power.[21]

Acknowledgment. This research was supported by the National Science Foundation via Grant NSF-CHE-1689.

Supporting Information Available: We present a simple model, based on the Langmuir adsorption isotherm, that provides some perspective on how the preferential binding of urea over water to hydrophobic plates or chain molecules alters their hydrophobic interactions. This material is available free of charge via the Internet at http://pubs.acs.org.

JA807887G

(50) Shortle, D.; Ackerman, M. S. *Science* **2001**, *293*, 487–489.
(51) Klein-Seetharaman, J.; Oikawa, M.; Grimshaw, S. B.; Wirmer, J.; Duchardt, E.; Ueda, T.; Imoto, T.; Smith, L. J.; Dobson, C. M.; Schwalbe, H. *Science* **2002**, *295*, 1719–1722.
(52) Kauzmann, W. *Adv. Protein Chem.* **1959**, *14*, 1–63.
(53) Klotz, I. M.; Franzen, J. S. *J. Am. Chem. Soc.* **1962**, *84*, 3461–3466.
(54) Dill, K. A. *Biochemistry* **1990**, *29*, 7133–7155.
(55) Mitchell, J. B. O.; Price, S. L. *Chem. Phys. Lett.* **1991**, *180*, 517–523.

Supporting Information for "'Urea's Action on Hydrophobic Interactions"

Ronen Zangi[1]*, Ruhong Zhou[1,2], and B. J. Berne[1,2]†

[1]*Department of Chemistry, Columbia University, 3000 Broadway, New York, NY 10027.*

[2]*Computational Biology Center, IBM Thomas J. Watson Research Center*

1101 Kitchawan Road, Yorktown Heights, NY 10598

October 9, 2008

In the following we present a theoretical analysis of the effect of preferential binding of urea on the hydrophobic interaction that is refered to in the acompanying paper.

Relation between preferential binding of urea and the polymer's dispersion interactions

A simple model provides some perspective on how the preferential binding of urea over water to hydrophobic plates or chain molecules affects the hydrophobic interaction. We consider a hydrophobic plate with M binding sites at each of which either a urea or water molecule can bind with binding energies $-\epsilon_u$ and $-\epsilon_w$ respectively, where

$$\epsilon_u = \sqrt{\epsilon}\sqrt{\epsilon_{uu}} \qquad \text{and} \qquad \epsilon_w = \sqrt{\epsilon}\sqrt{\epsilon_{ww}} \quad . \tag{1}$$

Here ϵ represents the LJ energy parameter for the interaction between sites on different plates and ϵ_{uu} (and ϵ_{ww}) are the LJ energy parameters for the interactions between urea molecules (or water molecules). This reflects the usual combining rules used in most force fields and in our simulations. Because urea is larger than water we expect that $\epsilon_{uu} > \epsilon_{ww}$ and

$$\epsilon_u - \epsilon_w = \sqrt{\epsilon}(\sqrt{\epsilon_{uu}} - \sqrt{\epsilon_{ww}}) > 0 \quad , \tag{2}$$

represents the difference in binding energy of urea and water.

*Present address: Department of Organic Chemistry I, University of the Basque Country, Avenida de Tolosa 72, 20018 San Sebastian, Spain.

†To whom correspondence should be addressed. E-mail: berne@chem.columbia.edu (B.J.B.).

We assume that an ideal solution of urea in water is placed in contact with the plate and calculate the grand canonical partition function for the molecules bound to the plate in terms of the chemical potentials (μ_u, μ_w) of urea and water in the ideal solution and from this the average fraction of sites on the plate occupied by urea and water. These fractions are designated by θ_u and θ_w respectively, and the ratio is found to be:

$$\frac{\theta_u}{\theta_w} = \frac{z_{u,ads} e^{\beta\mu_u}}{z_{w,ads} e^{\beta\mu_w}} \quad . \tag{3}$$

Here $z_{u,ads}$ and $z_{w,ads}$ are the single site molecular partition functions for the adsorbates, which can be written

$$z_{u,ads} = z'_{u,ads} e^{\beta\epsilon_u} \qquad \text{and} \qquad z_{w,ads} = z'_{w,ads} e^{\beta\epsilon_w} \quad , \tag{4}$$

where the primed partition functions involve sums over internal energies.

For an ideal solution

$$e^{\beta\mu_u} = e^{\beta\mu_u^\circ} X_u \qquad \text{and} \qquad e^{\beta\mu_w} = e^{\beta\mu_w^\circ}(1 - X_u) \quad , \tag{5}$$

where X_u, and $X_w = 1 - X_u$ are the mole fractions of urea and water molecules, respectively, so that

$$\frac{\theta_u}{\theta_w} = \frac{z'_{u,ads} X_u}{z'_{w,ads} X_w} e^{\beta(\mu_u^\circ - \mu_w^\circ)} e^{\beta(\epsilon_u - \epsilon_w)} \quad . \tag{6}$$

We now define a coefficient ν_{urea} that measures the preferential binding of urea over water as

$$\nu_{\text{urea}} \equiv \frac{\theta_u N_w}{\theta_w N_u} - 1 \quad , \tag{7}$$

where N_w/N_u is the ratio of water to urea molecules in the ideal solution. Note that $\nu_{\text{urea}} > 0$, $\nu_{\text{urea}} < 0$, and $\nu_{\text{urea}} = 0$ if the ratio of bound ureas to waters is larger than, smaller than, or equal to the ratio of ureas to waters in the solution. Using this definition and Eq. 6, we see that,

$$\nu'_{\text{urea}} \equiv \nu_{\text{urea}} + 1 = \frac{z_{u,ads}}{z_{w,ads}} e^{\beta(\mu_u^\circ - \mu_w^\circ)} = \frac{z'_{u,ads}}{z'_{w,ads}} e^{\beta(\mu_u^\circ - \mu_w^\circ)} e^{\beta(\epsilon_u - \epsilon_w)} \quad , \tag{8}$$

or

$$\ln \nu'_{\text{urea}} = \ln(\nu_{\text{urea}} + 1) = a + \beta\sqrt{\epsilon}(\sqrt{\epsilon_{uu}} - \sqrt{\epsilon_{ww}}) \quad . \tag{9}$$

where a is independent of the binding energies. Thus we expect $\ln(\nu_{\text{urea}} + 1)$ to increase linearly with $\sqrt{\epsilon}$.

Dependence of equilibrium constant and free energy for dimer plate dissociation on preferential binding

We can use this simple model above to predict how the preferential binding of urea to hydrophobic plates might affect the equilibrium constant for the dissociation of a plate dimer (when the plates are stacked in contact)

$$P_2 \rightleftharpoons P + P \quad . \tag{10}$$

The dimer has half the number of facial binding sites exposed to solvent , but the same number of edge binding sites, as does the separated plates, so that the net change in the number of binding sites in the reaction is equal to the number of binding sites on the two faces of one plate.

Following the arguments given by Tanford for the dependence of equilibrium constants on cosolute concentration,[1] we find that the rate of change of the equilibrium constant for the dissociation reaction ($P_2 \rightleftharpoons 2P$) with chemical potential of urea is

$$kT\frac{d\ln K}{d\mu_u} = \Delta\nu_u - \frac{N_u}{N_w}\Delta\nu_w \quad , \tag{11}$$

where $\Delta\nu_u = 2\nu_{u,P} - \nu_{u,P_2}$ and $\Delta\nu_w = 2\nu_{w,P} - \nu_{w,P_2}$, are the changes in the number of bound ligands of each kind in the reaction. From our discussion of the changes in number of binding sites during the reaction we find that $\Delta\nu_u = \nu_{u,P} = M\theta_u$ and $\Delta\nu_w = \nu_{w,P} = M\theta_w$, so that

$$kT\frac{d\ln K}{d\mu_u} = \Delta\nu_u - \frac{N_u}{N_w}\Delta\nu_w = M(\theta_u - \frac{N_u}{N_w}\theta_w) \quad , \tag{12}$$

or

$$kT\frac{d\ln K}{d\mu_u} = M\frac{(z_u e^{\beta\mu_u} - \frac{N_u}{N_w}z_w e^{\beta\mu_w})}{(1 + z_u e^{\beta\mu_u} + z_w e^{\beta\mu_w})} \quad . \tag{13}$$

Using the Gibbs-Duhem ($d\mu_w = -(N_u/N_w)d\mu_u$) equation, where we assumed that the solution is infinitely dilute in P and P_2, we find that

$$d(1 + z_u e^{\beta\mu_u} + z_w e^{\beta\mu_w}) = \beta(z_u e^{\beta\mu_u} - \frac{N_u}{N_w}z_w e^{\beta\mu_w})d\mu_u \quad , \tag{14}$$

so that

$$kTd\ln K = MkTd\ln(1 + z_u e^{\beta\mu_u} + z_w e^{\beta\mu_w}) \quad . \tag{15}$$

It is a simple matter to integrate this expression from $X_u = 0$ to X_u,

$$\ln\left[\frac{K(X_u)}{K(X_u=0)}\right] = M\ln\left[\frac{(1 + z_u e^{\beta\mu_u} + Z_w e^{\beta\mu_w})_{X_u}}{(1 + z_u e^{\beta\mu_u} + z_w e^{\beta\mu_w})_{X_u=0}}\right] = M\ln\left[\frac{(1 + z_u e^{\beta\mu_u} + z_w e^{\beta\mu_w})_{X_u}}{(1 + z_w e^{\beta\mu_w^\circ})}\right] \quad . \tag{16}$$

Simple algebra allows us to write this in a simpler form as follows. First we note that,

$$\frac{(1+z_u e^{\beta\mu_u}+z_w e^{\beta\mu_w})_{X_u}}{(1+z_w e^{\beta\mu_w^\circ})} = \frac{(1+z_w e^{\beta\mu_w^\circ} X_w(1+\frac{\theta_u}{\theta_w}))}{(1+z_w e^{\beta\mu_w^\circ})} \quad , \tag{17}$$

where we have used Eq.(3) and Eq.(5). Also recognizing that $\theta_w^\circ = z_w e^{\beta\mu_w^\circ}/(1+z_w e^{\beta\mu_w^\circ})$ is the fraction of sites that have bound waters when there is no urea in solution, and that $\nu_{\text{urea}}+1 = X_w\theta_u/\theta_w X_u$, we finally find that

$$-\Delta\Delta G/RT = \ln\left[\frac{K(X_u)}{K(X_u=0)}\right] = M\ln\left[\theta_w^\circ \nu_{\text{urea}} X_u + 1\right] \quad , \tag{18}$$

where left hand side of this last equation can also be expressed as $-\Delta\Delta G/RT = -(\Delta G_{\text{urea}} - \Delta G_{\text{water}})/RT$, a quantity that is plotted in the paper. We note that both θ_w° and ν_{urea} depend on $\sqrt{\epsilon}$ such that when ϵ gets smaller both will get smaller, whereas when ϵ is large $\theta_w^\circ \to 1$. In this case, the fraction of binding sites with no water or urea bound is very small, $\theta_w^\circ \approx 1$ we find that

$$-\Delta\Delta G/RT = \ln\left[\frac{K(X_u)}{K(X_u=0)}\right] = M\ln(\nu_{\text{urea}} X_u + 1) \quad , \tag{19}$$

and we see that if urea preferentially binds to the plates, adding urea increases the dissociation of the hydrophobic plates.

One last point. It is a simple matter also to show that

$$-\Delta\Delta G/RT = \ln\left[\frac{K(X_u)}{K(X_u=0)}\right] = M\ln\left[\frac{\theta_{\text{unocc}}^\circ}{\theta_{\text{unocc}}}\right] \tag{20}$$

where $\theta_{\text{unocc}} = 1/(1+z_u e^{\beta\mu_u}+z_w e^{\beta\mu_w})$, and $\theta_{\text{unocc}}^\circ = 1/(1+z_w e^{\beta\mu_w^\circ})$ are the fractions of sites that are unoccupied on the plate in urea solution and in pure water respectively. Thus if there are fewer unoccupied sites in urea solution than in water, the right hand side of this equation is greater than zero and the dissociation constant for the plates will be larger in urea solution than in pure water.

References

[1] Tanford, C. Extension of the theory of linked functions to incorporate the effects of protein hydration. *J. Mol. Biol.* **39**, 539–544 (1969).