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# Molecular Shape and the Hydrophobic Effect

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# Abstract

This review focuses on papers published since 2000 on the topic of the properties of solutes in water. More specifically, it evaluates the state of the art of our understanding of the complex relationship between the shape of a hydrophobe and the hydrophobic effect. To highlight this, we present a selection of references covering both empirical and molecular dynamics studies of small (molecular-scale) solutes. These include empirical studies of small molecules, synthetic hosts, crystalline monolayers, and proteins, as well as in silico investigations of entities such as idealized hard and soft spheres, small solutes, hydrophobic plates, artificial concavity, molecular hosts, carbon nanotubes and spheres, and proteins.

# EXORDIUM

Water is found in many parts of the universe, but planet Earth truly is a water world. All four spheres consist to some extent of water: The hydrosphere, biosphere, atmosphere, and lithosphere all contain (respectively decreasing) proportions of water. This review pertains to the hydrosphere and biosphere. More specifically, it concerns the complex relationship between hydrophobic solutes and the solvent of life (1), for understanding how solute shape and functionality control solvation, and how this solvation folds into measurable spectroscopic and thermodynamic changes, is key to understanding our world.

The ubiquity of water is reflected in a massive literature; consequently, boundaries must be set for any review. First, although there is a continuum between a hydrophobe and a hard ion, and cases in which the hydrophobic effect (HE) and the Hofmeister effect (2–4) meet (5, 6), we avoid the latter where possible. Second, this review focuses on the molecular-scale HE (7) and mostly avoids the microscale (8). Third, temperature and pressure can have a considerable effect on the HE (9), but with only a few exceptions, this review concerns ambient conditions. Fourth, biomembranes are not discussed (10). Finally, the bulk of this review concerns work performed since 2000.

The structure of this review follows the broad types of curvature as defined by mathematics: positive curvature (convex), zero curvature (flat surface), and negative curvature (concave). Historically, because of the shape of small molecules, positive curvature has received the most attention; correspondingly, our picture of its solvation is relatively strong. Overall, there is less known about the solvation of flat surfaces or negative curvature, but the past decade or so has seen this gap begin to close.

# A Word About Water

Water is a small, highly polar molecule (1.85 D,  $\varepsilon = 78$ ), a strong hydrogen bond (HB) donor ( $\alpha = 1.17$ ), and a reasonable HB acceptor ( $\beta = 0.47$ ) (11; see the sidebar, The  $\alpha$ - and  $\beta$ -Scales). Consequently, bulk liquid water is relatively structured. There is general consensus that a bulk water molecule forms on average 3.5 HBs (12) with a lifetime on the order of 1–20 ps. Although there is strong evidence that water forms (isolated) energy minimum clusters (13, 14), the extent of such clusters in the bulk is unclear. Indeed, probing water on the subfemtosecond timescale indicates only two strong HBs between water molecules, suggesting that chains and rings are more dominant than cages (15, 16). This ambiguity means that the vague but descriptive term flickering clusters (17) is often used to describe water.

A simple way to view water solvation is to consider the six different permutations of dangling HBs (those O–H bonds that point at a solute rather than hydrogen bond to water) that can exist

# THE $\alpha$ - AND $\beta$ -SCALES

The  $\alpha$ - and  $\beta$ -scales were developed by Kamlet et al. to respectively measure the hydrogen-bond donor acidities and acceptor basicities of common organic solvents using solvatochromic probe molecules. The  $\alpha$ -scale quantifies the hydrogen-bond donor acidities of solvents with a fixed reference point of  $\alpha = 1.00$  for methanol, a strong hydrogen-bond donor solvent. Similarly, the  $\beta$ -scale, quantifying the hydrogen-bond acceptor basicities, using  $\beta =$ 1.00 for hexamethyl-phosphoric triamide, a strong hydrogen bond acceptor solvent. Values less than the reference point correspond to a weakened donating or accepting capacity and vice versa. Therefore, water represents a stronger hydrogen-bond donor than methanol but a weaker hydrogen-bond acceptor than hexamethylphosphoric triamide.



#### Figure 1

Idealized representations of the solvation of the surfaces discussed in this review: (*a*) a small convex solute, (*b*) a large convex molecule, (*c*) a flat surface, (*d*) a slightly concave surface, (*e*) a highly concave surface, and (*f*) a fully encapsulating surface (front hemisphere removed for visualization). For each of the six cases, one water molecule is shown along with the idealized number of hydrogen bonds (HBs) to donor (D) or acceptor (A) water molecules in the solvation shell or bulk. For each case, the corresponding number of dangling HBs is shown in the black box.

(Figure 1). Thus, there is a shift from no dangling HBs, in the case of bulk water and small solutes, to four dangling HBs, in the case of a totally isolated water molecule. Undoubtedly, one of the keys to unraveling the HE is to be able to correlate these changes in the water HB pattern to corresponding spectroscopic and thermodynamic data.

# Notes on the Hydrophobic Effect

As Ben-Amotz (18) pointed out in a companion review, our common notion that the HE is a powerful driver of binding and assembly in water is frequently misrepresented. Thus, there is building evidence that interactions between nonpolar molecules in the gas phase are stronger than the corresponding interactions in water (19), and like any solvent, the solvation shell of water impedes other supramolecular interactions; it is just that water is not very good at this impeding. Thus, it may be best to view the HE as pertaining to comparisons between water and other solvents. Additionally, there is evidence that the HE only becomes significant when  $\sim 1 \text{ nm}^2$  of the exposed surface area of the hydrophobic solute is buried in an assembly or complex (19).

These points noted, the hydration of small convex, hydrophobic solutes is generally enthalpically favorable (20). Thus, the dissolution of methane in water ( $\Delta G^{\circ} = 25.5 \text{ kJ mol}^{-1}$ ) is promoted by enthalpy ( $\Delta H^{\circ} = -13.8 \text{ kJ mol}^{-1}$ ) but has a large entropic penalty ( $-T\Delta S^{\circ} = 39.3 \text{ kJ mol}^{-1}$ ). Such entropic penalties were observed by Frank & Evans (21), and their interpretation of this phenomenon was that structured water, or "microscopic iceberg[s]" formed around hydrophobes. Some time later, Kauzmann (22) applied this concept to protein folding, and in combination, these two papers were largely responsible for the persisting textbook picture of the HE. This textbook description has proven to be too simplistic a viewpoint (23, 24). Indeed, a review by Blokzijl & Engberts (25) concluded that there is actually no good reason to suppose that the iceberg model is true, and there is some evidence that it is not. That stated, recent evidence highlighted here indicates that the jury is still out on the matter of icebergs around solutes.

Alternatives to this iceberg model have been proposed. For example, an entropic penalty might reflect the inability of the HB network of water to accommodate an apolar solute (25). Alternatively, this entropic penalty may be due to the small molecular volume of water (26–30). Consequently, the frequency of observing a cavity of sufficient volume to accommodate a large solute is low. In short, the most honest statement about the HE is that we are far from fully understanding it.

# **POSITIVE CURVATURE**

The key characteristic of positive curvature is that solvation is size dependent. As first suggested by Stillinger (31), the surfaces of small solutes are wetted (i.e., the first solvation layer of water is mostly in direct contact with the solute), whereas large solutes are dewetted (i.e., their solvation shell is slightly remote from the surface). Although there is now good consensus that this is indeed the case, there is still disagreement about how this phenomenon is affected by the properties of the solute.

The only molecular-scale theory that successfully describes many of the structural and thermodynamic properties of infinitely dilute solutions of small, apolar solutes was devised by Pratt & Chandler (32, 33). This model gave accurate thermodynamic properties for aqueous solutions of apolar solutes and successfully predicted the  $\Delta G^{\circ}$  of transfer of *n*-alkanes from a hydrocarbon solvent to water. However, this model could not account for observations from larger solute systems. The first model that could successfully bridge the molecular scales and macroscales and account for size-dependent wetting/dewetting was described by Lum et al. (34). To summarize, in the case of small solutes, hydrogen bonding between water molecules is hindered yet persists near the surface. The result is a hydration layer denser than the bulk with roughly the same number but different patterns of HBs. In these cases, the  $\Delta G^{\circ}$  of solvation scales with solute volume. However, solutes >10 Å in diameter cannot be accommodated by even a distorted HB network; consequently, hydrogen bonding is sterically depleted. The result is a reduction in the cohesive forces and a concomitant dewetting of the surface (20, 35, 36). In such cases, the  $\Delta G^{\circ}$  of solvation is dominated by interfacial free energetics and scales with the surface area. Dewetting is reduced as van der Waals forces between the solute and water are increased; however, there is a negligible corresponding effect on the wetting of small solutes.

Because large solutes cannot be accommodated within the HB network of water, HBs can dangle over the solute (**Figure 1**). This accounts for the scale dependence of the thermodynamics. As the breaking of HBs is dominated by enthalpy, but the spatial arrangement of HBs is dominated by entropy, it is the latter that dominates in the solvation of a small solute. Yet the entropy of solvating a large solute is favorable. Therefore, there exists not only a wetting/dewetting transition, but also an entropy crossover transition (or entropy-enthalpy crossover). These two types of solvation are fundamental to the assembly of hydrophobic solutes; the equilibrium is dominated by the difference between the entropically dominated solvation of the small solute and the enthalpically dominated solvation of the larger assembly (20).

# **Small Solutes**

A combination of neutron diffraction and hydrogen/deuterium isotope substitution in the study of the formation and decomposition of methane clathrate suggests that the HE is not caused by the release of structured water (37, 38); there are no significant differences in the water structure before, during, or upon clathrate formation or after decomposition. Additionally, ab initio molecular dynamics (MD) simulations of krypton in liquid water concurred with X-ray absorption fine-structure (XAFS) on krypton solution and were found to be significantly different from XAFS results for the radial hydration structure of krypton clathrate (39).

# Assembly of Small Solutes

The osmotic second virial coefficient  $(B_2)$  offers a direct means to probe the HE, but there is a discrepancy between  $B_2$  measurements and Pratt & Chandler's model. One explanation for this discrepancy is that Pratt & Chandler's theory relies on hard spheres rather than polarizable solutes. However, the former are intrinsically difficult to model in MD packages. As a step toward bridging this gap, Pratt and colleagues (40) adapted simulation data to Pratt & Chandler's atomicscale hard-core models. The results show surprisingly strong attractive and endothermic, atomicscale hydrophobic interactions between hard-sphere solutes and constitute an initial step toward a molecular theory that accounts for attractive solute-water interactions.

Scheraga and colleagues (41) examined the potential of mean force for the assembly of dimers and trimers of methane. By analyzing the packing and orientation of the solvation shell, they determined that the  $\Delta G^{\circ}$  of association depended mostly on the difference in the number of water molecules in the first solvation shell of the cluster compared to the monomers. Additionally, they noted that during assembly, unfavorable electrostatic interactions develop between water molecules in different regions of the solvation shell. These interactions make an anticooperative contribution to the potential of mean force. Thus, changes in the pattern of water interactions within the solvation shell make significant contributions to even straightforward assemblies.

Ashbaugh & Paulaitis (42) demonstrated that close-packed methane clusters and analogous hard spheres have quite different hydration  $\Delta G^{\circ}$  values. Clusters of 1–305 methanes showed no evidence of dewetting, but with hard-sphere analogs, there was a decrease in the water density as the radius increased from 3.25 to 16.45 Å. With small methane clusters, a slight decrease in the water density with increasing size was attributed to the greater surface roughness of the smaller assemblies.

# The Crossover from Small to Large Solutes

Because of the low solubility of hydrocarbons in water, verification of a crossover is hard to confirm experimentally. However, relatively recent evidence of dewetting has been obtained by high-energy X-ray reflectivity measurements of the interface between water and octadecylsilane monolayers (35, 36). Furthermore, evidence of a thermodynamic crossover has been obtained from single-molecule force spectroscopic studies of hydrophobic polymers (43, 44).

Huang et al. (45) examined crossover in more detail using hard spheres. Following Lum et al.'s (34) model, the computed solvation  $\Delta G^{\circ}$  crossover occurred at ~5–10 Å. Furthermore, when weak solute-solvent attractions were introduced, accurate predictions of the alkane-water interfacial tension were obtained (46). In the absence of these attractions, the water interface was found to be more than one solvent molecular diameter away from the surface, but in their presence, wetting increased. For typical alkane-water interactions, there is still a dewetting (decrease in water density) adjacent to large hydrophobes, but the water interface is in contact with the surface of the solute.

However, within a temperature relevant to protein folding/unfolding, these attractive interactions were of little consequence to the temperature dependence of the  $\Delta G^{\circ}$  of solvation. Parenthetically, MD simulations showed that increases in pressure and the addition of salt or ethanol resulted in a decrease in the solute's crossover radius to subnanometer dimensions (47).

The Garde group (48) examined the solvation of methane and spherical single and multisite C60 and C180 fullerenes. By examining water-solute interactions, from purely repulsive to attractive, they determined that the water structure around larger solutes is more sensitive to the strength of the solute-water attractions than that of smaller solutes. Probing this further by separating the solute-water potential of mean force into solute-water and water-water interactions revealed that as the former was increased, the latter became increasingly unfavorable. This competitive expulsion phenomenon captures the idea that the hydration shell opposes the introduction of a test water molecule to replace one already present, and this was shown to be primarily enthalpic in origin. Mittal & Hummer (49) carried out a similar study. An analysis of the radial distribution function of water around different solutes confirmed that the water density at contact gradually decreased with increasing solute size and that there was no evidence of surface wetting at a diameter of 20 Å or larger. These studies also examined the population and fluctuations in the water occupancy within the solute-water interface; for repulsive solutes, as the radius increased, the variance in the density fluctuation increased to four times the bulk. Moreover, probing the dynamics of the interfacial density fluctuations revealed a slow relaxation process attributed to transitions between locally wet and dry states, a process that slowed with increasing solute size.

There is an empirical correlation between the  $\Delta G^{\circ}$  of solvation of a molecule and the surface tension of its solution that allows the latter to be used to probe the thermodynamics of solvation. To bridge the difference between the molecular and macroscopic scales, researchers have had to introduce a curvature correction to surface tension measurements, a correction that must itself be temperature dependent to account for the fact that the entropies of hydrating small and large nonpolar molecules are negative and positive, respectively. Ashbaugh (50) derived an expression predicting the solute size at which the solvation entropy is zero based on surface tension values, its first-order curvature correction, and their temperature dependencies. For hard spheres at room temperature, the estimated diameter was 16 Å.

Also examining crossover, the Berne group (51) determined the radial and orientational distribution functions of water around argon, methane, and neopentane. Their results showed that only neopentane (with a diameter of 5.2 Å) displayed an orientational distribution of water molecules in the first hydration shell, suggesting dewetting and the presence of dangling OH bonds. The authors also determined the potential of mean force between two neopentane molecules, ascertaining that there were two energy minima: (a) the dimer and (b) the solvent separated dimer slightly higher in energy.

The Levy group (52) studied the solution thermodynamics of a series of linear, branched, and cyclic alkanes. Their results support the idea that the classical HE has its roots in the small size of water molecules rather than icebergs. They also found that their favorable hydration enthalpies arise from attractive solute-solvent dispersion interactions and by small water reorganization energies. Additionally, by separating solvation into cavitation and alkane solvation, they determined a complete thermodynamic description of the solvation of the corresponding cavity for each alkane. Their results suggest that the work of cavity formation is split roughly equally between unfavorable entropic and solvent reorganization energy effects.

The Ben-Amotz group (53) used Raman multivariate curve resolution (Raman-MCR) and simulations to quantify the extent of dangling HBs around hydrophobic groups. For a series of alcohols, they found a high-frequency OH band attributed to dangling OH bonds. Additionally, MD simulations of the vibrational spectra of water molecules in the hydration shell of neopentane and benzene revealed high-frequency OH features that closely resemble the experimentally observed dangling OH vibrational bands around (sufficiently soluble) neopentyl and benzyl alcohol. In the latter, the red shift was similar to that previously observed in benzene-water clusters. Expanding on this, the same group examined a series of different guests (54). Their results were consistent with the idea that on average a hydrophobic moiety induces less than one dangling bond and that on average alcohol molecules possessed more dangling HBs than alkyl ammonium molecules. Additionally, dangling HBs were stabilized (destabilized) by negatively (positively) charged groups. This study also revealed that both proximal and distal hydrocarbon groups were important to the probability of forming a dangling HB and that they were entropically stabilized.

Both the sign and the magnitude of  $\Delta G^{\circ}$  for the association of the alkyl groups of pairs of alcohol molecules remain unknown. To address this, researchers combined Raman-MCR and polarization-resolved femtosecond infrared (IR) experiments with random mixing and MD simulations (55). The conclusion of these studies was that from methanol to tertiary butyl alcohol, there is no association driven by the HE.

Raman-MCR has also been used to probe the hydration of linear alcohol (56), carboxylic acid and tetraalkylammonium (57). These results showed that at low temperatures the hydration shells of each class have an enhanced water structure with greater tetrahedral order than the bulk, but for alcohol molecules longer than 10 Å, this structure disappeared as the temperature increased. Additionally, at high pH, the onset of the hydration-shell structural transformation was suppressed by the carboxylate. Tetraalkylammonium cations were found to more strongly suppress this transformation.

Although short-chain alkanes are primarily in an extended conformation in water, there is still controversy about the extent of folding/hydrophobic collapse of longer chains. Simulations probing the properties of *n*-alkanes have found an approximately exponential decrease in solubility up to *n*-eicosane ( $C_{20}H_{42}$ ), in excellent agreement with empirical data up to *n*-dodecane ( $C_{12}H_{26}$ ) (58). Analysis of the  $\Delta G^{\circ}$  landscape of the alkanes revealed similarities between conformational preferences in the ideal gas and solution phases, suggesting that water does not heavily influence conformation; the authors found no evidence for hydrophobic collapse of *n*-alkane chains shorter than *n*-eicosane. However, water did increase the barriers between the minimal  $\Delta G^{\circ}$  conformations corresponding to compacted and extended chain conformations, and decreased the stability of the latter.

A recent paper examined the possibility of a hydrophobic to hydrophilic crossover for the hydration of sufficiently long *n*-alkane chains (59). The authors combined previously obtained thermodynamic data, simulations, and fundamental thermodynamic relations to determine the intermolecular contributions to  $\Delta G^{\circ}$  and concluded that the hydrophobic to hydrophilic crossover would be in the region of hectane (C<sub>100</sub>H<sub>202</sub>).

A combination of femtosecond two-dimensional (2D)-IR spectroscopy and femtosecond polarization-resolved vibrational pump-probe spectroscopy has revealed a correlated slowing down of both the vibrational frequency dynamics and orientational mobility of the water molecules around nonpolar groups (60). For different concentrations of four small solutes, the fraction of slower water scaled with the number of methyl groups. Furthermore, the obtained results suggest that a common effect of hydrophobic moieties on solvating water is to restrict the formation of bifurcated HBs.

# The Effects of Charge

Pascal et al. (61) examined the effects of introducing positive charge into spherical solutes. The intrinsic solute was given helium-like parameters and ranged in diameter from 0 to 30 Å. As

expected, when the neutral solute increased in diameter, the solvation  $\Delta G^{\circ}$  became more repulsive. By examining the incremental addition of positive charge, the authors observed a gradual shift in the solvation  $\Delta G^{\circ}$  to hydrophilic and attractive. The crossover was at 0.4*e*. Interestingly, with this charge, the solvation  $\Delta G^{\circ}$  was approximately zero over all diameters. Breaking down the solvation  $\Delta G^{\circ}$  of these solutes revealed that in all cases solvation was dominated by enthalpy and was entropically penalized. The enthalpy became more negative as the size increased, which was compensated by an increasing entropic penalty. Thus, unlike hydrophobic solutes, the 0.4*e* charged species show no entropy-enthalpy crossover.

The Bakker group (5) studied the dynamics of solvating water molecules around tetra-*n*-alkylammonium bromides using polarization-resolved femtosecond IR spectroscopy to reveal that both ions slow the orientational dynamics. In the case of the ammonium ion, they found that the number of retarded water molecules scaled with the chain length. For the bromide ion, the solvation shell experienced a partial decay of its orientation via rapid wobbling and a subsequent slower decay. The former was found to be cation dependent. For the smallest salt, Me<sub>4</sub>NBr, the slow reorientation time was concentration independent, but with longer chains, it increased dramatically with concentration, suggesting aggregation. In complementary work, dielectric relaxation spectroscopy was used to study the dynamics of water molecules around tetramethylurea (60). These authors found that the reorientation dynamics of solvating water molecules around the methyl groups to be 3–10 times slower than the bulk; as the concentration increased, the dynamics slowed. Their results also demonstrated that with increasing temperature, the fraction of water contained in this hydrophobic hydration shell decreased.

The Ben-Amotz group (62) used Raman-MCR spectroscopy to quantify the interactions between the trimethyl domains of four solutes and the ions Na<sup>+</sup>, F<sup>-</sup>, and I<sup>-</sup>. Their results suggest that Na<sup>+</sup> and F<sup>-</sup> are strongly expelled from the first hydration shells of the solute, and I<sup>-</sup> entered the hydrophobic hydration shell to an extent controlled by the partial charge on the methyl groups. However, the estimated association constants for the binding of I<sup>-</sup> suggested that its concentration in the first hydrophobic hydration shell was lower than the bulk. Contrasting this are results from recent studies utilizing more circumambient pockets of concave hosts. Thus, both host-guest complexation studies (6, 63, 64) and extensive X-ray crystallography work on the binding site of carbonic anhydrase (65) have unequivocally demonstrated that relatively hydrophobic anions associate with hydrophobic pockets.

# **ZERO CURVATURE**

Because it is hard to control the dimerization of flat molecules, the study of these and the HE is dominated by computational work. Nevertheless, in addition to more established approaches such as X-ray reflectivity, newer techniques such as vibrational sum frequency generation spectroscopy and Raman-MCR have recently been applied to their study.

#### The Aqueous–Flat Surface Interface

Jensen et al. (66) experimentally confirmed the behavior of water at a hydrophobic surface. Using X-ray reflectivity measurements and MD simulations, they observed that there was significant dewetting of the surface of a crystalline monolayer of n-C<sub>36</sub>H<sub>74</sub> that created a narrow vacuum layer 1.0 Å wide. This is smaller than that predicted for hard spheres using Lum et al.'s (34) model but is larger than that predicted in models including attractive solute-water interactions (67).

The Garde group (68) noted dewetting and dangling HBs in simulations of the water-octane interface. Additionally, the water coordination number and extent of hydrogen bonding between

interfacial water molecules decreased moving from the bulk to the surface. Correspondingly, the models suggest an increasing probability of void formation closer to the surface. The same group has also shown that both the probability of cavity formation next to a surface and the  $\Delta G^{\circ}$  of binding of a hydrophobic solute correlate with macroscopic wetting (69).

Malaspina et al. (70) probed the solvation of a rigid, planar graphene-like surface. They observe that within 8–10 Å of the surface, water dynamics slow relative to the bulk because of increased structure. This ordering was observed to be independent of temperature over the range 240–320 K. Building on this, a comparison between flat and concave graphitic surfaces revealed no evidence of dangling HBs (71).

Continuing with aromatic surfaces, the Ben-Amotz group (72) explored dangling HBs (OH– $\pi$  bonds) between benzene and water using Raman-MCR. In combination with computational studies, their results revealed that the OH– $\pi$  bond is ~20% weaker and more flexible than bulk HBs. Furthermore, their results suggest that dangling HBs are more entropically favored but less enthalpically favored than water-water HBs.

The Chandler group (73) probed the mean water density and density fluctuations around plates to reveal the expected dewetting. Additionally, they determined that near both repulsive and attractive hydrophobic surfaces, density fluctuations were larger than the bulk and akin to those at the water-vapor interface. Both these factors contribute to a destabilization of the water structure between surfaces and a driving force for dimerization.

Studies of hydrophilic and hydrophobic self-assembled monolayers have revealed that the hydration thermodynamics of hydrophobes at the former have a typical entropy-enthalpy crossover (74). In contrast, near hydrophobic surfaces, effective surface tension ruled, and hydration was enthalpically dominated and size independent. This work also revealed that small solutes bind to these surfaces entropically and increasingly more favorably with increasing temperature, whereas large solutes bind enthalpically in a temperature-independent manner. Furthermore, the driving force for hydrophobe assembly close to a hydrophobic surface was shown to be weaker than in the bulk and decreased with increasing temperature.

Vibrational generation spectroscopy demonstrated that  $D_2O$  near a hydrophobic surface has enhanced orientation, structure, and stronger HB interactions relative to the  $D_2O/air$  interface. The results were consistent with dangling HBs and water dipoles perpendicular to the surface (75), with an orientation preference that decreased with increasing temperature.

Willard & Chandler (76) compared the air-water and hydrophobe-water interfaces. Using a local instantaneous water interface as a dynamic frame of reference rather than a fixed Cartesian plane, the authors were able to define an intrinsic interface. The study revealed that the strength of attractive water-hydrophobic surface interactions had no significant effect on the intrinsic interface and had only a small effect on the mean density profile of water from the hydrophobic surface out to the bulk. In contrast, however, attractive interactions significantly affected the entropically driven capillary wave fluctuations of the instantaneous liquid interface.

# **Between Two Plates**

The Berne group (77) modeled the solvation of two ellipsoid plates as a function of their size, separation, and the strength of the attraction between them and water. They found that the critical distance for hydrophobic collapse was linearly proportional to the interfacial area. Additionally, when the two plates were held at the critical distance, an initially dry zone between them remained dry, but when initially wet, the space remained wet. There was no hysteresis when the plates were held at a distance less that the critical distance; irrespective of the starting state, drying

resulted. Hydrophobic collapse of the plates occurred only after an initial bubble nucleation and a subsequent cooperative large-scale drying fluctuation.

Electron density profiles and X-ray reflectivity data have revealed weak dewetting (wetting) between two hydrophobic (hydrophilic) surfaces (78). In both cases, the characteristic water orientation was observed, with longer-range ordering observed in the case of the hydrophilic surfaces. Additionally, the dynamic properties of water between the two pairs of surfaces were different from each other and the bulk. In particular, at the hydrophobic surface, water molecules had characteristic diffusive behavior and orientational ordering due to the lack of interactions with the surface; observations suggesting that altered water dynamics, together with partial drying, are stronger signatures of the HE than structural ordering.

Phase transitions of water between two plates have been examined by Koga (79) and Giovambattista et al. (80). Koga observed two distinct phase transitions at separations of 9.4 Å and 8.2 Å: a liquid-to-bilayer amorphous solid phase transition and bilayer amorphous solid-to-liquid phase transition. The models predicted very few dangling HBs, with a bilayer structure composed of five- to eight-membered water rings. Giovambattista et al. (80) found water to take one of two forms, monolayer ice or bilayer (either amorphous or crystalline), depending on the pressure and plate separation. For hydrophobic surfaces, decreased pressure induced capillary evaporation, whereas increasing pressure pushed water closer to the surface and increased its order. However, the average orientation was not affected.

The Berne group (81) examined the properties of two hydrophobic plates as a function of salts of various charge densities and interplate separation. In pure water, both enthalpy and entropy promoted dimerization. Consistent with Hofmeister studies, high-charge-density ions formed strongly hydrated complexes independent from the hydrophobic plates that increased the hydrophobic interaction between the charged plates via an entropic effect. In contrast, low-charge-density ions exhibited an entropic- or enthalpic-based weakening of the HE by adsorbing to the hydrophobic plates and causing a salting-in effect. The effect of 7-M urea upon a hydrophobic polymer, a particle-based hydrophobic surface, and graphene has also been examined (82). Consistent among these simulations was that the association of urea to all surfaces caused a decrease in their hydrophobicity and a diminished potential of mean force. These effects were enthalpically dominated.

The Berne group (83) also studied plates composed of both hydrophobic and hydrophilic regions. Their studies revealed the expected relationship between the degree of hydrophobicity and the presence or absence of a dewetting transition and the presence of attractive or repulsive interactions between plates. More interestingly, with these metrics, an analysis of five plate pairs containing equal amounts of hydrophobic and hydrophilic groups arranged in different patterns revealed qualitative and quantitative differences between each. For example, if all the hydrophobic areas on the plate were grouped in the center, dewetting between the plates readily occurred, whereas, if the same number of hydrophobic groups were spaced out evenly on the surface, no dewetting was observed. In pairing a purely hydrophilic and a purely hydrophobic plate, the latter dominated wetting/dewetting. In related work, the affinity of a hydrophobic probe to a pair of hydrophobic plates was examined as a function of plate charge density (84). Residency between the plates, with the probe being attracted between the plates at q < 0.37(61).

Sharma & Debenedetti (85) found that paired  $1 \times 1$  nm<sup>2</sup> surfaces were too small to accommodate a stable vapor cavity except at distances of <9 Å, whereas, for all distances examined, paired  $3 \times 3$  nm<sup>2</sup> surfaces exhibited gap-spanning tubular cavity formation. The associated barriers corresponded to the formation of a critically sized cavity for the latter and to complete emptying of the gap region for the former. Calculated  $\Delta G^{\circ}$  barriers varied approximately linearly with gap size, with 1-Å increases leading to rate drops of approximately two orders of magnitude. The authors also examined the evaporation rate of the confined water (86). For the smaller plate examined, the time necessary for nucleation of a surface-induced evaporation event showed an increase of 10 orders of magnitude from 9- to 14-Å separation, whereas the larger plates showed an increase of 6 orders of magnitude in this rate between 11 and 13 Å. The evaporation rate per unit area was also dependent on the plate size.

The Berne group (87) studied the kinetics of assembling two plates as a function of their hydrophobicity. Their models revealed that surfaces with high friction have large and slow water density fluctuations between the plates. Solvent confinement and drying played a critical role in the kinetics. The authors also found reasonable agreement between the calculated MD and Brownian dynamics. Furthermore, they observed that molecular-scale hydrodynamic interactions were essential in describing the kinetics of assembly.

Ashbaugh (88) carried out 2D Lennard-Jones solvent Monte Carlo simulations between plates. The results validated continuum thermodynamic theory and explored interfacially driven and pressure-moderated evaporation. These linked the scale of simulation to the experimental realm. There was strong quantitative correlation between theory and simulation for interplate forces and critical evaporation lengths.

Using transmission electron microscopy, Algara-Siller et al. (15) imaged water between graphene sheets. In contrast to tetrahedal bulk water, the water between the graphene had a square-ice structure, in which the hydrogen-bonding angle within and between layers was 90°. This lattice had a high packing density. MD simulations supported this picture. Nearest-neighbor measurements of oxygen-oxygen distances were in agreement for the plates that accommodated water monolayers, but for the bi- and trilayer cases, the water molecules were found to have a tetrahedral structure. This was attributed to the simulations not accounting for adhesion between the sheets imposing pressure on the water.

### **Aqueous-Protein Interfaces**

Following pioneering work by Cheng & Rossky (89), the Berne group (90) studied the hydrophobic collapse of two dimeric melittins to form the corresponding tetramer. The fastest hydrophobic collapse was observed when the dimers were separated by a critical distance of 5.5–7.0 Å. Interestingly, when isoleucine-2 was mutated to valine, alanine, or glycine, no dewetting was observed. Similar results involving a different point mutation have also been observed (91).

Work by Giovambattista et al. (92) also focused on the dimerization of melittin dimers. In this instance, instead of using the natural cupped surface of a (truncated) melittin, the authors obtained a much smoother surface by flattening and also removing the surrounding charged groups. The mutant surface was less hydrophobic than the wild type, with desolvation only observed with a separation that sterically only permitted a single water layer. Furthermore, capillary dewetting occurred at lower pressures and separations than in the case of idealized hydrophobic flat surfaces and was localized to a narrow central region between the dimers. When an amino acid residue in this central region was mutated to a polar residue, cavitation was no longer observed.

The collapse of the two domains of the BphC enzyme has been studied (93). For separations >4 Å, both wetted or dewetted initial states ultimately led to a wet state with a water density 10–15% lower than in the bulk. At a separation of 4 Å, only partial dewetting was seen unless the electrostatic, or the electrostatic and van der Waals, forces were turned off. Switching off just Coulombic forces led to rapid and almost complete dewetting, whereas switching off both forces led to a more rapid and complete dewetting. In related work with BphC, the Garde group (91)

also noted a permanently wetted interdomain space even at 4 Å separations. Similarly, turning off the charges on each protein resulted in dewetting of the interprotein space.

The Berne group (94) also examined the water dynamics in the interdomain space of the two BphC domains. With the two surfaces held apart by 12 Å, the shortest transit time for water molecules was for those near the channel center, and the longest transit time was for those at a domain surface. Even the fastest water molecules were  $\sim$ 30% slower than the bulk. A 4-Å gap showed slower water transitions, whereas a 20-Å gap exhibited bulk-like water molecules. In general, the HB lifetimes for water molecules near one of the domain surfaces were approximately three times longer than those near the channel center. Interestingly, by this metric, one surface alone only affected water molecules to a depth of 4–5 Å, but two surfaces held 20 Å apart had a similar effect to a depth of 8 Å.

The Bakker group (95) carried out vibrational sum frequency generation spectroscopic analysis of water molecules around antifreeze protein AFP-III at or above 0°C. Even above freezing, the investigators obtained strong evidence of ice-like water molecules at the flat, hydrophobic icebinding site of the protein. A point mutation of threonine-18 residue to asparagine eliminated this structure and led to complete inactivity of the antifreeze protein. These results support a preordering-binding mechanism for the recognition of ice.

# **NEGATIVE CURVATURE**

Negative curvature possesses circumambient properties; it can envelope, isolate, and simultaneously form multiple noncovalent interactions with whatever resides within. If water molecules lie there, then the cluster will possess multiple dangling HBs.

Complexation to negative curvature is commonly enthalpically driven. Ross & Subramanian (96) first noted this for proteins, and tabulation of thermodynamic data for guest binding to cyclodextrins (**Figure 2**, molecule **0**) confirms that  $\sim$ 99% are exothermic (and that 75% of these are entropically penalized) (97). In 1967, Bender and colleagues (98, p. 3252) suggested that the binding of guests to cyclodextrin was because "water molecules in the cavity cannot form their full complement of HBs as a result of steric restrictions." Consequently, when a guest is bound and the water molecules liberated, there is a release of enthalpy. Recent evidence supports this idea of (high-energy) water molecules with dangling HBs dominating the solvation and complexation properties of concavity (99).

#### Synthetic Hosts

**Figure 2** shows representative examples of water-soluble hosts of current interest in aqueous supramolecular chemistry (99–105). These range from the very open to the truly concave. The diverse cyclophane class of host (106), including the recently synthesized pillar[*n*]arenes (molecule **②** in **Figure 2**; n = 5-10; 107, 108), is open in nature, possesses bound water molecules with little in the way of dangling HBs, and correspondingly binds guests relatively weakly. That stated, the growing class of artificial water channels (109) includes pillar[5]arenes that have proven to form water wires and act as transmembrane channels for protons (110) and as transmembrane water channels (111).

Cyclodextrins (molecule ① in Figure 2) are toroidal hosts composed of 6, 7, or 8 amylose units ( $\alpha$ -,  $\beta$ -, or  $\gamma$ -cyclodextrin, respectively) (101). These are more circumambient than cyclophanes, but the presence of hydroxy groups at both portals means that bound water molecules form relatively few dangling HBs and are not exceptionally high in energy (99).



#### Figure 2

Structural representations of the host molecules discussed in the text: (①) cyclodextrin, (②) pillar[*n*]arenes, (③) cucurbiturils, (④) calix[4]arenes, (⑤) resorcinarenes, and (⑥) deep-cavity cavitands.

Cucurbiturils (CBs; molecule **③** in **Figure 2**) are bis-methylene linked oligomers of 5, 6, 7, 8, or 10 glycolurils (112–114). Their pockets comprise a hydrophobic region with two portals rimmed by imide carbonyls. In general, guest complexations are strongly enthalpically promoted. By Biedermann et al.'s (99) measure of high-energy water, the host with the most hydrophobic binding site is CB7; it contains on average 7.9 water molecules, each with an average 2.52 HBs. The resulting large number of dangling HBs is suspected to contribute to the general ability of CB7 to strongly bind guests (115). Interestingly, CB8 can bind two guests. The inclusion of the first restricts hydrogen bonding between any residual water molecules; therefore, the subsequent binding is strongly exothermic (99).

Calix[4]arenes (molecule **4** in **Figure 2**; n = 4) and resorcinarenes (molecule **5** in **Figure 2**; n = 4) are truly concave but very shallow (116). With open binding sites and proximal phenols, their hydrophobic surface is not effectively screened from the bulk. Unsurprisingly, only modest 1:1 complexations are observed. Resorcinarene-based, deep-cavity cavitands (molecule **6** in **Figure 2**; n = 4) possess truly hydrophobic pockets (100). Host molecule **6** has been shown to bind an average of 4.34 water molecules (117). The base of the cavity is occupied by a singular water molecule, which accepts an average of 1.33 HBs; water molecules in a middle layer have on average 2.65 HBs, and those in the uppermost layer at the portal have on average 3.33 HBs. These bound water molecules have higher energy than the bulk, and the deeper a water resides, the higher its energy and the more it derives stabilization from (dangling) host-water HBs rather than water-water HBs. Calculated translational diffusion constants revealed that bound water molecules possessed lower diffusion constants than the bulk, and the deeper the water molecule was located, the more it was constrained. The opposite was observed with rotational diffusion constants. Overall, the thermodynamics for hydration of the pocket were  $\Delta G_{hyd} \approx -5$  kcal mol<sup>-1</sup>,  $\Delta H_{hyd} \approx -20$  kcal mol<sup>-1</sup>, and  $-T\Delta S_{hyd} \approx 15$  kcal mol<sup>-1</sup>.

# In Silico Carbon Nanotubes

The bores of carbon nanotubes (CNTs) of  $\sim$ 8-Å diameter are solvated by a water wire (118, 119). These water molecules have only two relatively long-lived HBs and are less densely packed than the bulk. With many dangling HBs, only very small reductions of the van der Waals attraction between water and the tube are sufficient to induce emptying (118). A combination of hydrogen bonding and dipole-dipole interactions within the wire engenders rapid proton transfer via a Grotthuss relay mechanism (119).

Water flows through CNTs in bursts and is limited only by the barriers of entry and egress; the tube itself is essentially frictionless, and the flow rate is nearly independent of its length (120). Counterintuitively, as the diameter increases, transport rates decrease because of increased hydrogen bonding between water molecules (121).

It has been shown that changing the diameter of the CNT induces phase transitions in water packing (122, 123). Thus, under ambient conditions, water within 8-Å CNTs is gas-like, whereas with 11- and 12-Å diameter CNTs, the water is ice-like and stacks into layers of pentagons or hexagons (124, 125). For even larger diameters (e.g., 20-30 Å), bound water molecules form multiple nanoclusters (123). It is thus not surprising that there is a nonmonotonic relationship between diameter and relative Helmholtz free energy of filling (124); diameters of 8 and 12 Å represent energy minima. Overall entropy dominates slightly for tube diameters less than 10 Å or greater than 16 Å, and enthalpy dominates for tubes between 11 and 12 Å. With the exception of 11- and 12-Å nanotubes, confinement within the CNTs leads to an increase in the translational entropy of bound water. We note that ice structures are commonly observed in CNTs at lower temperatures (126) and elevated pressures (127). Indeed, simulations of the thermodynamic states over the diameter-temperature plane reveal at least nine different ice phases (122), including stacked squares, pentagons, hexagons, and heptagons. Pentagonal and hexagonal packed water is ferroelectric (128). Interestingly, stacked octagonal water has only been observed in a coaxial-like system in which small hydrophobes form a wire running down the central axis of stacked water rings (129).

#### In Silico Hosts

In early work examining the properties of a hydrophobic pocket of 8-Å radius cut into a paraffin slab, Setney & Geller (130) observed that the average water density within the cavity was depleted relative to the bulk and that the pocket fluctuates between empty and filled states. Overall, the  $\Delta G^{\circ}$  values accompanying these fluctuations were small, suggesting that the selected radius is close to a critical size for promoting transitions between gas-like and liquid-like phases. In comparing 5-Å and 8-Å radii concavities to a corresponding flat surface, Setny (131, 132) observed vapor-like regions of significantly reduced water density in both pockets, with the smaller pocket possessing a substantial desolvation barrier to probe binding, whereas entry into the 8-Å cavity was essentially barrierless. Contrary to the idea of high-energy water molecules dominating complexation, for both concavities, dewetting was only a marginal contributor to the  $\Delta G^{\circ}$  of probe binding. Rather, the observed  $\Delta G^{\circ}$  values were related to the hydration shell of the probe.

Building on this, Baron et al. (133) estimated  $\Delta G^{\circ}$ ,  $\Delta H^{\circ}$ , and  $-T\Delta S^{\circ}$  along the concavity-guest binding trajectory as a function of the charge (neutral, +, or –) of the host and guest. They observed a broad range of thermodynamic signatures in which water enthalpic or entropic contributions drove cavity-guest binding or rejection. Thus, the binding of a neutral methane-like guest to a neutral host was driven by enthalpy and was entropically penalized, with the roots of the former pinpointed to the formation of strong water-water interactions when bound water molecules moved into the bulk. Furthermore, their data implied that, because of solvent fluctuations in the pocket, bound water was more entropically favored than the bulk. Overall, in this work, it was highenergy bound water molecules with dangling HBs that drove complexation (133, 134). Further simulations revealed that the reorientation kinetics of the water molecules around a methane-like guest increased as the host-guest distance was decreased and that this was correlated with a decrease in the number of HBs between water molecules in the guest solvation shell (135). Moreover, the authors calculated changes in the 1D and 2D IR spectra during complexation, suggesting a strategic route to correlating water structure changes and signature vibrational spectra differences. Most recently, water's role in ligand binding kinetics has been investigated (136). The empty pocket of the host exhibited solvated-desolvated oscillations whose magnitude and timescale were modulated by the approaching guest. Furthermore, these fluctuations led to a friction-induced kinetic barrier to guest entry to the pocket.

Vaitheeswaran et al. (137) used Monte Carlo simulations to study the thermodynamic stability of water clusters inside smooth graphene-like spherical cavities and the fullerenes  $C_{140}$  and  $C_{180}$ . Depending on the container size, they observed thermodynamically stable water clusters composed of three to nine water molecules. The smallest stable water cluster in a spherical cavity of 1.0-nm diameter was a trimer held together by three HBs. With a slightly smaller diameter of 0.9 nm, a thermodynamically unstable dimer was observed. Overall, the clusters observed were strikingly similar to gas-phase water clusters (13, 14).

# Proteins

Berne and colleagues (138) examined three disparate binding sites: that of streptavidin, Cox-2, and the antibody DB3. The binding site of streptavidin has a hydrophobic floor and roof yet possesses five coplanar hydrogen-bonding groups in its walls. In the case of Cox-2, its binding site is long, tubular, and hydrophobic. The pocket of DB3 has a less pronounced hydrophobic enclosure. Simulations of streptavidin revealed hydration by five water molecules that form a persistent ring, an entropically costly water structure that, in conjunction with little corresponding enthalpic gain, may contribute up to five orders of magnitude to ligand affinity. In a less extreme example, the binding site of DB3 was found to be occupied by one water molecule. Similar to those in streptavidin, this water molecule has very few energetically accessible configurations that contribute to an entropic penalty of hydration. In contrast, the binding site of Cox-2 was found to be devoid of water molecules 80% of the time; no energetically stable solvent configurations appeared to exist. Thus, Cox-2 is an example of a protein solvated by high-energy water molecules with dangling HBs. This unusual drying transition in Cox-2 was investigated further by the Berne and Friesner groups (139) to identify structural features important to drying. Screening the Protein Data Bank using two metrics of pocket hydrophobicity and one of narrowness identified 12 proteins. Except for three proteins for which it was found that water could not exchange with the bulk, whether the remaining examples underwent a drying transition was excellently correlated with a drying parameter, which was the product of the atom-based surface hydrophobicity and a measure of the narrowness of the cavity. Thus, pockets that are relatively hydrophobic and narrow possessed drying transitions, whereas the others did not.

Water wires have been shown to form in 5.2-Å diameter hydrophobic channels formed by the assembly of pentapeptides into hexameric assemblies (140). Interestingly, X-ray crystallographic analysis revealed two kinds of water wires characterized by different oxygen-oxygen distances. In both cases, the data suggested very weak interactions between the water and tube. Furthermore, reinterpretation (140) of the solid-state structure of stacked, cyclic peptides composed of alternating D- and L-residues (141) also suggests that this family of peptides can template water wires.

The pocket in a protein need not be either entirely wet or dry. Using the WaterMap program (Schrödinger), Wang et al. (142) examined the hydration properties of the binding sites of three proteins to identify wet and dry regions within their respective pockets. The authors showed that calculated affinities were excellent in cases in which a bound ligand only occupied space previously filled with water, but poor when a bound ligand displaced some or none of the water molecules. However, once the water free noncovalent contacts between ligand and protein in these dry regions were taken into account, the calculated affinities of congeneric ligands correlated with observed affinities.

Consideration of the enthalpic and entropic costs of transferring a single water molecule from the bulk to a binding site suggests that the  $\Delta G^{\circ}$  of transfer is relatively small (143, 144). Olano & Rick (145) calculated the  $\Delta G^{\circ}$  of transfer of a water from the bulk to an interior cavity of bovine pancreatic trypsin inhibitor (BPTI) and in the I76A mutant of barnase. Previous X-ray crystallography revealed the BPTI binding site to be occupied by water but gave ambiguous data concerning the barnase mutant. The former is polar and allows the formation of up to four waterprotein HBs, whereas the latter is more hydrophobic and (potentially) allows only one HB. The calculations predicted that only the polar BPTI cavity would be hydrated, that the corresponding transfer entropies for BPTI are significantly entropically unfavorable, and that the transfer to the cavity of the barnase mutant is entropically favorable.

The major domain of tetrabrachion consists of a right-handed four-helix bundle that defines a channel containing four cavities. Although the channel/cavities are lined with aliphatic residues, X-ray crystallography revealed that all are filled with water: 1, 2, 5, and 9 water molecules from the smallest to largest cavity, respectively. Remarkably, recent MD simulations have revealed that at 25°C and 92°C, the largest of these cavities was filled with clusters of seven to nine water molecules, respectively (146). Furthermore, filling of the cavity was found to be enthalpically driven and opposed by entropy. Only at 110°C did the calculations suggest cluster instability and drying.

The Homans groups (147) studied ligand binding to mouse major urinary protein (MUP) using a variety of techniques. Calculations revealed that the pockets of the wild type and mutant contained 3.74 and 4.07 water molecules, respectively, and that in accord with X-ray crystallography, the water density in the pocket was low. In the case of binding of 2-methoxy-3-isobutylpyrazine (IPMP), isothermal titration calorimetry (ITC) revealed that association was driven by favorable enthalpic contributions, even when the only ligand-protein HB was removed by mutagenesis. Furthermore, a combination of solvent isotopic substitution ITC and MD simulations suggested that neither solvation nor induced fit changes were major contributors to the overall binding enthalpy. Considering the poor solvation of the pocket, the binding thermodynamics were attributed to favorable dispersion interactions arising from the inequality of solvent-solute interactions before and after complexation. A related study of the binding of linear alcohols to MUP also supported this hypothesis (148). The Homans group (149) also examined the contribution of the entropic costs of the desolvation of IPMP and found that the overall entropic penalty arose because the favorable entropic contribution from guest desolvation did not overcome the entropic penalty due to reduced degrees of freedom. ITC studies of the MUP-n-alcohol complexes also revealed that although binding was exothermic and entropically penalized, the corresponding heat capacity change was typical of the HE (i.e., negative) (150).

# CONCLUSIONS

The theoretical transition from a small convex solute, to a larger one, to a flat surface, through increasingly concave surfaces, and finally to full encapsulation (**Figure 1**) reveals the following trends. For positively curved hydrophobes, there is an entropy-enthalpy crossover as a function of diameter. This crossover correlates with both a wetting/dewetting transition and a switch from

the water molecules of solvation being fully hydrogen bonded in the case of small solutes to a solvation shell that geometrically allows dangling HBs to point toward the solute surface in the case of large solutes.

As may be expected, the case of a large solute and that of a flat hydrophobic surface are similar. However, when water between two flat surfaces is considered, it is evident that the two surfaces work in synergy to affect water molecules in the intersurface zone. As a result, by the metrics of water molecule diffusion, the number of dangling HBs, and phase transitions from wet to dry, there is a similarity between the two-plate case and that of true concavity.

With respect to negative curvature, it is evident that the large number of dangling HBs of bound water plays a major role in regard to the properties of a tube or pocket. Quite simply, dangling HBs mean high-energy water molecules that would rather not be encapsulated. And the more confining the hydrophobic binding, the more extreme is the enthalpic domination, such that in the extreme case of total water confinement, water molecules resemble gas-phase clusters.

En masse, although there is still some disagreement, the described results point to a switch from entropy-dominated solvation to enthalpy domination and then to increasing enthalpy domination as a theoretical solute is morphed from a small convex entity, to a large one, to a solute that is increasingly encapsulating. In addition, however, it is evident that the HE is profoundly affected by all chemical and physical variables. It is therefore not surprising that we do not yet fully understand the HE. That means, however, that there are rich pickings ahead for those immersed in water.

# SUMMARY POINTS

- The solvation of positive curvature is size dependent. Small solutes are solvated without the breaking of HBs between water molecules. Large solutes (>10-Å diameter) are not; it is geometrically impossible to maintain full hydrogen bonding in the solvation shell. As a result, weaker dangling HBs to the solute are formed instead. The hydration layer of a small solute is denser (the solute is wetted) than the bulk, and the ΔG° of solvation scales with the solute volume. In contrast, large solutes undergo dewetting, with a ΔG° of solvation that scales with surface area.
- 2. Because the spatial arrangement of HBs is dominated by entropy, and the breaking of HBs is dominated by enthalpy, entropy rules in the solvation of small positively curved solutes, whereas enthalpy rules in the solvation of large ones. Thermodynamically, this results in a size-dependent entropy crossover transition, or entropy-enthalpy crossover.
- 3. The solvation of negative curvature (concavity) means that bound water molecules cannot form their normal complement of HBs to other water molecules. The resulting dangling HBs increase in number as the curvature becomes more negative and reaches an extreme of four dangling HBs in the case of a singular water molecule trapped inside a hydrophobic sphere. Thus, increasing concavity is associated with increasing enthalpic domination.
- 4. Between negative and positive curvature, zero curvature possesses intermediate properties. The solvation of a singular flat plate is reminiscent of that observed with large convex solutes, whereas the solvation of paired plates physicochemically resembles that of tubes and pockets.
- 5. Much work remains to understand how subtle structural nuances such as functional group type and patterning, as well exogenous variables such as temperature, pressure, and the presence of salts, fold into the aforementioned solvation process to engender the HE.

# **DISCLOSURE STATEMENT**

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#### LITERATURE CITED

- 1. Lynden-Bell RM, Morris SC, Barrow JD, Finney JL, Harper RLJ, eds. 2010. *Water and Life*. Boca Raton, FL: CRC
- Lo Nostro P, Ninham BW. 2012. Hofmeister phenomena: an update on ion specificity in biology. *Chem. Rev.* 112:2286–322
- Zhang Y, Cremer PS. 2010. Chemistry of Hofmeister anions and osmolytes. Annu. Rev. Phys. Chem. 61:63–83
- 4. Jungwirth P, Tobias DJ. 2006. Specific ion effects at the air/water interface. Chem. Rev. 106:1259-81
- van der Post ST, Scheidelaar S, Bakker HJ. 2013. Water dynamics in aqueous solutions of tetra-nalkylammonium salts: hydrophobic and Coulomb interactions disentangled. J. Phys. Chem. B 117:15101– 10
- Assaf KI, Ural MS, Pan F, Georgiev T, Simova S, et al. 2015. Water structure recovery in chaotropic anion recognition: high-affinity binding of dodecaborate clusters to γ-cyclodextrin. Angew. Chem. Int. Ed. 54:6852–56
- Hummer G, Garde S, García AE, Pratt LR. 2000. New perspectives on hydrophobic effects. *Chem. Phys.* 258:349–70
- Berne BJ, Weeks JD, Zhou R. 2009. Dewetting and hydrophobic interaction in physical and biological systems. *Annu. Rev. Phys. Chem.* 60:85–103
- Koga K. 2011. Solvation of hydrophobes in water and simple liquids. Phys. Chem. Chem. Phys. 13:19749– 58
- Pratt LR, Pohorille A. 2002. Hydrophobic effects and modeling of biophysical aqueous solution interfaces. *Chem. Rev.* 102:2671–92
- Sharp KA, Vanderkooi JM. 2010. Water in the half shell: structure of water, focusing on angular structure and solvation. Acc. Chem. Res. 43:231–39
- Kumar R, Schmidt JR, Skinner JL. 2007. Hydrogen bonding definitions and dynamics in liquid water. *J. Chem. Phys.* 126:204107
- Liu K, Brown MG, Carter C, Saykally RJ, Gregory JK, Clary DC. 1996. Characterization of a cage form of the water hexamer. *Nature* 381:501–3
- Perez C, Muckle MT, Zaleski DP, Seifert NA, Temelso B, et al. 2012. Structures of cage, prism, and book isomers of water hexamer from broadband rotational spectroscopy. *Science* 336:897–901
- Algara-Siller G, Lehtinen O, Wang FC, Nair RR, Kaiser U, et al. 2015. Square ice in graphene nanocapillaries. Nature 519:443–45
- Wernet P, Nordlund D, Bergmann U, Cavalleri M, Odelius M, et al. 2004. The structure of the first coordination shell in liquid water. *Science* 304:995–99
- Frank HS, Wen W-Y. 1957. III. Ion-solvent interaction: structural aspects of ion-solvent interaction in aqueous solutions: a suggested picture of water structure. *Discuss. Faraday Soc.* 24:133–40
- 18. Ben-Amotz D. 2016. Water-mediated hydrophobic interactions. Annu. Rev. Phys. Chem. 67:617-38

- Ben-Amotz D. 2015. Hydrophobic ambivalence: teetering on the edge of randomness. J. Phys. Chem. Lett. 6:1696–701
- 20. Chandler D. 2005. Interfaces and the driving force of hydrophobic assembly. Nature 437:640-47
- Frank HS, Evans MW. 1945. Free volume and entropy in condensed systems. III. Entropy in binary liquid mixtures; partial molal entropy in dilute solutions; structure and thermodynamics in aqueous electrolytes. *J. Chem. Phys.* 13:507–32
- 22. Kauzmann W. 1959. Some factors in the interpretation of protein denaturation. Adv. Protein Chem. 14:1-63
- 23. Snyder PW, Lockett MR, Moustakas DT, Whitesides GM. 2014. Is it the shape of the cavity, or the shape of the water in the cavity? *Euro. Phys. J. Spec. Top.* 223:853–91
- 24. Ball P. 2008. Water as an active constituent in cell biology. Chem. Rev. 108:74-108
- Blokzijl W, Engberts JBFN. 1993. Hydrophobic effects. Opinions and facts. Angew. Chem. Int. Ed. 32:1545–79
- Lucas M. 1976. Size effect in transfer of nonpolar solutes from gas or solvent to another solvent with a view on hydrophobic behavior. *J. Phys. Chem.* 80:359–62
- Lee B. 1991. Solvent reorganization contribution to the transfer thermodynamics of small nonpolar molecules. *Biopolymers* 31:993–1008
- Lynden-Bell RM, Giovambattista N, Debenedetti PG, Head-Gordon T, Rossky PJ. 2011. Hydrogen bond strength and network structure effects on hydration of non-polar molecules. *Phys. Chem. Chem. Phys.* 13:2748–57
- Lazaridis T. 2001. Solvent size versus cohesive energy as the origin of hydrophobicity. Acc. Chem. Res. 34:931–37
- 30. Baldwin RL. 2013. The new view of hydrophobic free energy. FEBS Lett. 587:1062-66
- Stillinger FH. 1973. Structure in aqueous solutions of nonpolar solutes from the standpoint of scaled particle theory. J. Soln. Chem. 2:141–58
- 32. Pratt LR, Chandler D. 1977. Theory of the hydrophobic effect. J. Chem. Phys. 67:3683-704
- Pratt LR. 2002. Molecular theory of hydrophobic effects: "She is too mean to have her name repeated." Annu. Rev. Phys. Chem. 53:409–36
- Lum K, Chandler D, Weeks JD. 1999. Hydrophobicity at small and large length scales. *J. Phys. Chem.* B 103:4570–77
- Poynor A, Hong L, Robinson IK, Granick S, Zhang Z, Fenter PA. 2006. How water meets a hydrophobic surface. *Phys. Rev. Lett.* 97:266101
- Mezger M, Reichert H, Schoder S, Okasinski J, Schroder H, et al. 2006. High-resolution in situ X-ray study of the hydrophobic gap at the water-octadecyl-trichlorosilane interface. *PNAS* 103:18401–4
- Buchanan P, Soper AK, Thompson H, Westacott RE, Creek JL, et al. 2005. Search for memory effects in methane hydrate: structure of water before hydrate formation and after hydrate decomposition. *7. Chem. Phys.* 123:164507
- Buchanan P, Aldiwan N, Soper AK, Creek JL, Koh CA. 2005. Decreased structure on dissolving methane in water. *Chem. Phys. Lett.* 415:89–93
- Ashbaugh H. 2003. Hydration of krypton and consideration of clathrate models of hydrophobic effects from the perspective of quasi-chemical theory. *Biophys. Chem.* 105:323–38
- Chaudhari MI, Holleran SA, Ashbaugh HS, Pratt LR. 2013. Molecular-scale hydrophobic interactions between hard-sphere reference solutes are attractive and endothermic. PNAS 110:20557–62
- Czaplewski C, Liwo A, Ripoll DR, Scheraga HA. 2005. Molecular origin of anticooperativity in hydrophobic association. *7. Phys. Chem. B* 109:8108–19
- 42. Ashbaugh HS, Paulaitis ME. 2001. Effect of solute size and solute-water attractive interactions on hydration water structure around hydrophobic solutes. *J. Am. Chem. Soc.* 123:10721–28
- 43. Li ITS, Walker GC. 2011. Signature of hydrophobic hydration in a single polymer. PNAS 108:16527-32
- 44. Garde S, Patel AJ. 2011. Unraveling the hydrophobic effect, one molecule at a time. *PNAS* 108:16491–92
- Huang DM, Geissler PL, Chandler D. 2001. Scaling of hydrophobic solvation free energies. *J. Phys. Chem. B* 105:6704–9
- Huang DM, Chandler D. 2002. The hydrophobic effect and the influence of solute-solvent attractions. *J. Phys. Chem. B* 106:2047–53

- Rajamani S, Truskett TM, Garde S. 2005. Hydrophobic hydration from small to large lengthscales: understanding and manipulating the crossover. *PNAS* 102:9475–80
- Athawale MV, Jamadagni SN, Garde S. 2009. How hydrophobic hydration responds to solute size and attractions: theory and simulations. *J. Chem. Phys.* 131:115102
- Mittal J, Hummer G. 2008. Static and dynamic correlations in water at hydrophobic interfaces. PNAS 105:20130–35
- Ashbaugh HS. 2009. Entropy crossover from molecular to macroscopic cavity hydration. *Chem. Phys.* Lett. 477:109–11
- Huang X, Margulis CJ, Berne BJ. 2003. Do molecules as small as neopentane induce a hydrophobic response similar to that of large hydrophobic surfaces? J. Phys. Chem. B 107:11742–48
- Gallicchio E, Kubo MM, Levy RM. 2000. Enthalpy–entropy and cavity decomposition of alkane hydration free energies: numerical results and implications for theories of hydrophobic solvation. *J. Phys. Chem. B* 104:6271–85
- Perera PN, Fega KR, Lawrence C, Sundstrom EJ, Tomlinson-Phillips J, Ben-Amotz D. 2009. Observation of water dangling OH bonds around dissolved nonpolar groups. *PNAS* 106:12230–34
- Davis JG, Rankin BM, Gierszal KP, Ben-Amotz D. 2013. On the cooperative formation of nonhydrogen-bonded water at molecular hydrophobic interfaces. *Nat. Chem.* 5:796–802
- Rankin BM, Ben-Amotz D, van der Post ST, Bakker HJ. 2015. Contacts between alcohols in water are random rather than hydrophobic. *J. Phys. Chem. Lett.* 6:688–92
- Davis JG, Gierszal KP, Wang P, Ben-Amotz D. 2012. Water structural transformation at molecular hydrophobic interfaces. *Nature* 491:582–85
- Davis JG, Zukowski SR, Rankin BM, Ben-Amotz D. 2015. Influence of a neighboring charged group on hydrophobic hydration shell structure. *J. Phys. Chem. B* 119:9417–22
- Ferguson AL, Debenedetti PG, Panagiotopoulos AZ. 2009. Solubility and molecular conformations of n-alkane chains in water. J. Phys. Chem. B 113:6405–14
- Underwood R, Tomlinson-Phillips J, Ben-Amotz D. 2010. Are long-chain alkanes hydrophilic? J. Phys. Chem. B 114:8646–51
- Bakulin AA, Pshenichnikov MS, Bakker HJ, Petersen C. 2011. Hydrophobic molecules slow down the hydrogen-bond dynamics of water. *J. Phys. Chem. A* 115:1821–29
- Pascal TA, Lin S-T, Goddard W, Jung Y. 2012. Stability of positively charged solutes in water: a transition from hydrophobic to hydrophilic. *J. Phys. Chem. Lett.* 3:294–98
- Rankin BM, Ben-Amotz D. 2013. Expulsion of ions from hydrophobic hydration shells. J. Am. Chem. Soc. 135:8818–21
- Gibb CL, Gibb BC. 2011. Anion binding to hydrophobic concavity is central to the salting-in effects of Hofmeister chaotropes. J. Am. Chem. Soc. 133:7344–47
- Carnegie RS, Gibb CL, Gibb BC. 2014. Anion complexation and the Hofmeister effect. Angew. Chem. Int. Ed. 53:11498–500
- Fox JM, Kang K, Sherman W, Heroux A, Sastry GM, et al. 2015. Interactions between Hofmeister anions and the binding pocket of a protein. *J. Am. Chem. Soc.* 137:3859–66
- 66. Jensen T, Østergaard Jensen M, Reitzel N, Balashev K, Peters G, et al. 2003. Water in contact with extended hydrophobic surfaces: direct evidence of weak dewetting. *Phys. Rev. Lett.* 90:086101
- Wallqvist A, Gallicchio E, Levy RM. 2001. A model for studying drying at hydrophobic interfaces: structural and thermodynamic properties. *J. Phys. Chem. B* 105:6745–53
- Patel HA, Nauman EB, Garde S. 2003. Molecular structure and hydrophobic solvation thermodynamics at an octane–water interface. J. Chem. Phys. 119:9199–206
- Godawat R, Jamadagni SN, Garde S. 2009. Characterizing hydrophobicity of interfaces by using cavity formation, solute binding, and water correlations. *PNAS* 106:15119–24
- Malaspina DC, Schulz EP, Alarcon LM, Frechero MA, Appignanesi GA. 2010. Structural and dynamical aspects of water in contact with a hydrophobic surface. *Euro. Phys. J. E* 32:35–42
- Alarcón LM, Malaspina DC, Schulz EP, Frechero MA, Appignanesi GA. 2011. Structure and orientation of water molecules at model hydrophobic surfaces with curvature: from graphene sheets to carbon nanotubes and fullerenes. *Chem. Phys.* 388:47–56

- Gierszal KP, Davis JG, Hands MD, Wilcox DS, Slipchenko LV, Ben-Amotz D. 2011. π-Hydrogen bonding in liquid water. J. Phys. Chem. Lett. 2:2930–33
- Patel AJ, Varilly P, Chandler D. 2010. Fluctuations of water near extended hydrophobic and hydrophilic surfaces. *J. Phys. Chem. B* 114:1632–37
- Patel AJ, Varilly P, Jamadagni SN, Acharya H, Garde S, Chandler D. 2011. Extended surfaces modulate hydrophobic interactions of neighboring solutes. *PNAS* 108:17678–83
- Strazdaite S, Versluis J, Backus EH, Bakker HJ. 2014. Enhanced ordering of water at hydrophobic surfaces. *J. Chem. Phys.* 140:054711
- Willard AP, Chandler D. 2014. The molecular structure of the interface between water and a hydrophobic substrate is liquid-vapor like. *J. Chem. Phys.* 141:18C519
- 77. Huang X, Margulis CJ, Berne BJ. 2003. Dewetting-induced collapse of hydrophobic particles. *PNAS* 100:11953–58
- Jensen MO, Mouritsen OG, Peters GH. 2004. The hydrophobic effect: molecular dynamics simulations of water confined between extended hydrophobic and hydrophilic surfaces. J. Chem. Phys. 120:9729–44
- Koga K. 2002. Solvation forces and liquid–solid phase equilibria for water confined between hydrophobic surfaces. J. Chem. Phys. 116:10882–89
- 80. Giovambattista N, Rossky P, Debenedetti P. 2006. Effect of pressure on the phase behavior and structure of water confined between nanoscale hydrophobic and hydrophilic plates. *Phys. Rev. E* 73:041604
- Zangi R, Hagen M, Berne BJ. 2007. Effect of ions on the hydrophobic interaction between two plates. *J. Am. Chem. Soc.* 129:4678–86
- Zangi R, Zhou R, Berne BJ. 2009. Urea's action on hydrophobic interactions. J. Am. Chem. Soc. 131:1535– 41
- 83. Hua L, Zangi R, Berne BJ. 2009. Hydrophobic interactions and dewetting between plates with hydrophobic and hydrophilic domains. *J. Phys. Chem. C* 113:5244–53
- Wang L, Friesner RA, Berne BJ. 2010. Competition of electrostatic and hydrophobic interactions between small hydrophobes and model enclosures. *J. Phys. Chem. B* 114:7294–301
- Sharma S, Debenedetti PG. 2012. Free energy barriers to evaporation of water in hydrophobic confinement. J. Phys. Chem. B 116:13282–89
- Sharma S, Debenedetti PG. 2012. Evaporation rate of water in hydrophobic confinement. PNAS 109:4365–70
- Li J, Morrone JA, Berne BJ. 2012. Are hydrodynamic interactions important in the kinetics of hydrophobic collapse? *J. Phys. Chem. B* 116:11537–44
- 88. Ashbaugh HS. 2013. Solvent cavitation under solvophobic confinement. J. Chem. Phys. 139:064702
- Cheng YK, Rossky PJ. 1998. Surface topography dependence of biomolecular hydrophobic hydration. Nature 392:696–99
- Liu P, Huang X, Zhou R, Berne BJ. 2005. Observation of a dewetting transition in the collapse of the melittin tetramer. *Nature* 437:159–62
- Patel AJ, Varilly P, Jamadagni SN, Hagan MF, Chandler D, Garde S. 2012. Sitting at the edge: how biomolecules use hydrophobicity to tune their interactions and function. *J. Phys. Chem. B* 116:2498–503
- Giovambattista N, Lopez CF, Rossky PJ, Debenedetti PG. 2008. Hydrophobicity of protein surfaces: separating geometry from chemistry. PNAS 105:2274–79
- Zhou R, Huang X, Margulis CJ, Berne BJ. 2004. Hydrophobic collapse in multidomain protein folding. Science 305:1605–9
- Hua L, Huang X, Zhou R, Berne BJ. 2006. Dynamics of water confined in the interdomain region of a multidomain protein. *J. Phys. Chem. B* 110:3704–11
- Meister K, Strazdaite S, DeVries AL, Lotze S, Olijve LL, et al. 2014. Observation of ice-like water layers at an aqueous protein surface. PNAS 111:17732–36
- Ross PD, Subramanian S. 1981. Thermodynamics of protein association reactions: forces contributing to stability. *Biochemistry* 20:3096–102
- 97. Rekharsky MV, Inoue Y. 1998. Complexation thermodynamics of cyclodextrins. Chem. Rev. 98:1875-917
- VanEtten RL, Sebastian JF, Clowes GA, Bender ML. 1967. Acceleration of phenyl ester cleavage by cycloamyloses. A model for enzymatic specificity. *J. Am. Chem. Soc.* 89:3242–53

- Biedermann F, Nau WM, Schneider HJ. 2014. The hydrophobic effect revisited—studies with supramolecular complexes imply high-energy water as a noncovalent driving force. *Angew. Chem. Int. Ed.* 53:11158–71
- Jordan JH, Gibb BC. 2015. Molecular containers assembled through the hydrophobic effect. *Chem. Soc. Rev.* 44:547–85
- 101. Crini G. 2014. Review: a history of cyclodextrins. Chem. Rev. 114:10940-75
- Appel EA, del Barrio J, Loh XJ, Scherman OA. 2012. Supramolecular polymeric hydrogels. *Chem. Soc. Rev.* 41:6195–214
- Oshovsky GV, Reinhoudt DN, Verboom W. 2007. Supramolecular chemistry in water. Angew. Chem. Int. Ed. 46:2366–93
- Biros SM, Rebek J Jr. 2007. Structure and binding properties of water-soluble cavitands and capsules. Chem. Soc. Rev. 36:93–104
- Corbellini F, Knegtel RM, Grootenhuis PD, Crego-Calama M, Reinhoudt DN. 2004. Water-soluble molecular capsules: self-assembly and binding properties. *Chemistry* 11:298–307
- Meyer EA, Castellano RK, Diederich F. 2003. Interactions with aromatic rings in chemical and biological recognition. *Angew. Chem. Int. Ed.* 42:1210–50
- Xue M, Yang Y, Chi X, Zhang Z, Huang F. 2012. Pillararenes, a new class of macrocycles for supramolecular chemistry. Acc. Chem. Res. 45:1294–308
- Strutt NL, Zhang H, Schneebeli ST, Stoddart JF. 2014. Functionalizing pillar[n]arenes. Acc. Chem. Res. 47:2631–42
- Barboiu M, Gilles A. 2013. From natural to bioassisted and biomimetic artificial water channel systems. Acc. Chem. Res. 46:2814–23
- Si W, Chen L, Hu XB, Tang G, Chen Z, et al. 2011. Selective artificial transmembrane channels for protons by formation of water wires. *Angew. Chem. Int. Ed.* 50:12564–68
- 111. Hu XB, Chen Z, Tang G, Hou JL, Li ZT. 2012. Single-molecular artificial transmembrane water channels. J. Am. Chem. Soc. 134:8384–87
- 112. Freeman WA, Mock WL, Shih N-Y. 1981. Cucurbituril. J. Am. Chem. Soc. 103:7367-68
- Lagona J, Mukhopadhyay P, Chakrabarti S, Isaacs L. 2005. The cucurbit[n]uril family. Angew. Chem. Int. Ed. 44:4844–70
- 114. Moghaddam S, Yang C, Rekharsky M, Ko YH, Kim K, et al. 2011. New ultrahigh affinity host-guest complexes of cucurbit[7]uril with bicyclo[2.2.2]octane and adamantane guests: thermodynamic analysis and evaluation of M2 affinity calculations. *J. Am. Chem. Soc.* 133:3570–81
- Cao L, Sekutor M, Zavalij PY, Mlinaric-Majerski K, Glaser R, Isaacs L. 2014. Cucurbit[7]uril-guest pair with an attomolar dissociation constant. *Angew. Chem. Int. Ed.* 53:988–93
- 116. Zouhair A, Böhmer V, Harrowfield J, Vicens J, Saadioui M, eds. 2001. *Calixarenes 2001*. Dordrecht: Kluwer Acad.
- 117. Ewell J, Gibb BC, Rick SW. 2008. Water inside a hydrophobic cavitand molecule. *J. Phys. Chem. B* 112:10272–79
- Hummer G, Rasaiah JC, Noworyta JP. 2001. Water conduction through the hydrophobic channel of a carbon nanotube. *Nature* 414:188–90
- 119. Kofinger J, Hummer G, Dellago C. 2011. Single-file water in nanopores. *Phys. Chem. Chem. Phys.* 13:15403–17
- Kalra A, Garde S, Hummer G. 2003. Osmotic water transport through carbon nanotube membranes. PNAS 100:10175–80
- 121. Ohba T, Kaneko K, Endo M, Hata K, Kanoh H. 2013. Rapid water transportation through narrow one-dimensional channels by restricted hydrogen bonds. *Langmuir* 29:1077–82
- Takaiwa D, Hatano I, Koga K, Tanaka H. 2008. Phase diagram of water in carbon nanotubes. PNAS 105:39–43
- 123. Ohba T. 2014. Size-dependent water structures in carbon nanotubes. Angew. Chem. Int. Ed. 53:8032-36
- 124. Pascal TA, Goddard WA, Jung Y. 2011. Entropy and the driving force for the filling of carbon nanotubes with water. *PNAS* 108:11794–98
- Mashl RJ, Joseph S, Aluru NR, Jakobsson E. 2003. Anomalously immobilized water: a new water phase induced by confinement in nanotubes. *Nano Lett.* 3:589–92

- 126. Kyakuno H, Matsuda K, Yahiro H, Inami Y, Fukuoka T, et al. 2011. Confined water inside single-walled carbon nanotubes: global phase diagram and effect of finite length. *J. Chem. Phys.* 134:244501
- 127. Koga K, Gao GT, Tanaka H, Zeng XC. 2001. Formation of ordered ice nanotubes inside carbon nanotubes. *Nature* 412:802–5
- Mikami F, Matsuda K, Kataura H, Maniwa Y. 2009. Dielectric properties of water inside single-walled carbon nanotubes. *Nano* 3:1279–87
- 129. Tanaka H, Koga K. 2005. Formation of ice nanotube with hydrophobic guests inside carbon nanotube. *J. Chem. Phys.* 123:94706
- Setny P, Geller M. 2006. Water properties inside nanoscopic hydrophobic pocket studied by computer simulations. J. Chem. Phys. 125:144717
- Setny P. 2007. Water properties and potential of mean force for hydrophobic interactions of methane and nanoscopic pockets studied by computer simulations. J. Chem. Phys. 127:054505
- Setny P. 2008. Hydrophobic interactions between methane and a nanoscopic pocket: three dimensional distribution of potential of mean force revealed by computer simulations. *J. Chem. Phys.* 128:125105
- 133. Baron R, Setny P, McCammon JA. 2010. Water in cavity-ligand recognition. J. Am. Chem. Soc. 132:12091-97
- Setny P, Baron R, McCammon JA. 2010. How can hydrophobic association be enthalpy driven? J. Chem. Theory Comput. 6:2866–71
- 135. Baron R, Setny P, Paesani F. 2012. Water structure, dynamics, and spectral signatures: changes upon model cavity-ligand recognition. *J. Phys. Chem. B* 116:13774–80
- Setny P, Baron R, Kekenes-Huskey PM, McCammon JA, Dzubiella J. 2013. Solvent fluctuations in hydrophobic cavity-ligand binding kinetics. *PNAS* 110:1197–202
- 137. Vaitheeswaran S, Yin H, Rasaiah JC, Hummer G. 2004. Water clusters in nonpolar cavities. *PNAS* 101:17002–5
- Young T, Abel R, Kim B, Berne BJ, Friesner RA. 2007. Motifs for molecular recognition exploiting hydrophobic enclosure in protein-ligand binding. *PNAS* 104:808–13
- Young T, Hua L, Huang X, Abel R, Friesner R, Berne BJ. 2010. Dewetting transitions in protein cavities. *Proteins* 78:1856–69
- Raghavender US, Kantharaju, Aravinda S, Shamala N, Balaram P. 2010. Hydrophobic peptide channels and encapsulated water wires. *J. Am. Chem. Soc.* 132:1075–86
- Ghadiri MR, Kobayashi K, Granja JR, Chadha RK, McRee DE. 1995. The structural and thermodynamic basis for the formation of self-assembled peptide nanotubes. *Angew. Chem. Int. Ed.* 34:93–95
- 142. Wang L, Berne BJ, Friesner RA. 2011. Ligand binding to protein-binding pockets with wet and dry regions. *PNAS* 108:1326–30
- 143. Dunitz JD. 1994. The entropic cost of bound water in crystals and biomolecules. Science 264:670
- 144. Ladbury JE. 1996. Just add water! The effect of water on the specificity of protein-ligand binding sites and its potential application to drug design. *Chem. Biol.* 3:973–80
- 145. Olano LR, Rick SW. 2004. Hydration free energies and entropies for water in protein interiors. J. Am. Chem. Soc. 126:7991–8000
- 146. Yin H, Hummer G, Rasaiah JC. 2007. Metastable water clusters in the nonpolar cavities of the thermostable protein tetrabrachion. J. Am. Chem. Soc. 129:7369–77
- 147. Barratt E, Bingham RJ, Warner DJ, Laughton CA, Phillips SE, Homans SW. 2005. Van der Waals interactions dominate ligand-protein association in a protein binding site occluded from solvent water. *J. Am. Chem. Soc.* 127:11827–34
- Malham R, Johnstone S, Bingham RJ, Barratt E, Phillips SE, et al. 2005. Strong solute-solute dispersive interactions in a protein-ligand complex. J. Am. Chem. Soc. 127:17061–67
- Shimokhina N, Bronowska A, Homans SW. 2006. Contribution of ligand desolvation to binding thermodynamics in a ligand-protein interaction. *Angew. Chem. Int. Ed.* 45:6374–76
- Syme NR, Dennis C, Phillips SE, Homans SW. 2007. Origin of heat capacity changes in a "nonclassical" hydrophobic interaction. *Chembiochem* 8:1509–11