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Dewetting and Hydrophobic Interaction in Physical and Biological Systems

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Abstract

Hydrophobicity manifests itself differently on large and small length scales. This review focuses on large length scale hydrophobicity, particularly on dewetting at single hydrophobic surfaces and drying in regions bounded on two or more sides by hydrophobic surfaces. We review applicable theories, simulations and experiments pertaining to large scale hydrophobicity in physical and biomolecular systems and clarify some of the critical issues pertaining to this subject. Given space constraints, we could not review all of the significant and interesting work in this very active field.

Keywords

Dewetting transition; hydrophobic interaction; protein folding; superhydrophobicity; nano-plates

1 Introduction

Hydrophobicity seems to be required for sustainable life as we know it. It gives rise to unusual properties of aqueous solutions of nonpolar solutes and plays an important role in a wide variety of chemical phenomena such as protein folding, (1-4) the self-assembly of amphiphiles into micelles and membranes(5), and the gating of ion channels (6,7). Hydrophobicity lies at the heart of so many important chemical and biophysical phenomena that considerable effort has been devoted to understanding it over the past five decades. Tanford in a paper entitled “How protein chemists learned about the hydrophobic factor” (8) gives a short and provocative history of how this concept gained common acceptance in the biochemical community. Recent reviews on various aspects of hydrophobicity have been published in Annual Reviews(9,10) and elsewhere (11-13).

Many controversies have arisen in attempts to find a generally satisfactory theory of hydrophobicity. The very recent review by Ball (13) provides a fascinating and measured historical discussion of the contentious issues along with modern developments and how they fit in with other general questions about the role of water in biological systems. Many of the controversies involve quantitative questions that cannot be resolved by taking a rigid and simplistic view based on generally useful qualitative concepts, whether of “broken hydrogen bonds”, “dry interfaces” or “hydrogen bond networks.” Experience has shown that water is a subtle and flexible medium with a fluctuating and elusive balance between many

nearly degenerate local configurations. This allows it to respond in surprising ways to different perturbations and we must be equally flexible in our attempts to understand its solvation properties.

But there does appear to be a convergence of views on certain general features of hydrophobicity involving a dependence on length scale where qualitative pictures have proved useful. This provides a conceptual framework within which we can place our present understanding and which can highlight the remaining unresolved issues. We believe that most of the remaining controversy is not so much about the physical phenomena themselves, as uncovered by experiment and careful simulations, as it is about the language used and the limitations and oversimplifications found in all current theoretical models. It is timely therefore to attempt a review of the experimental and theoretical studies leading to an emerging consensus in this specific area.

It is now generally recognized that hydrophobicity manifests itself differently on small and large length scales (14-23). For example, in the hydrophobic hydration of small hydrophobic molecules (like argon or methane) the molecules can fit into the water hydrogen bond network without destroying any hydrogen bonds (14). Since no hydrogen bonds are broken the enthalpy of solution (ΔH) is small. The formation of small cavities in the solvent to accommodate the solute is an entropically dominated process and the presence of the solute constrains the orientational and translational degrees of freedom of the neighboring water molecules. Thus the entropy of solvation (ΔS) is negative (e.g. $\Delta S_{Ar}^o = -30.2$ cal/mole-K) and is proportional to the molar excluded volume of the solute. This means that the free energy of solution (ΔG) is positive and increases both with temperature (around biological temperatures) and with the excluded volume of the solute, and that the dissolution of small apolar solutes is entropically driven.

The conventional view of hydrophobic interaction in this small-scale regime is based on a simple picture proposed by Frank and Evans (24). Small molecules are accommodated by water locally forming more ordered ice-like or clathrate-like cages with strong and relatively rigid hydrogen bonds around each molecule. Since two or more apolar molecules in contact will order fewer solvent molecules than when they are apart, the entropy change on bringing solute molecules together should be positive and should thereby lower the free energy of the solution. Small enthalpy changes are ignored in this argument.

It is now recognized that this view is overly simplistic. In particular the hydrogen bond structure is sufficiently flexible that there are still considerable fluctuations in the adjacent solvent shells (13). Simulations (25-27) and theory (28) showed that two inert gas particles would not be driven together to form a dimer but that the solvent separated pairing would be a more likely state than the contact pair. The physical reasons for the success of the theory were somewhat unclear in the original formulation (28). A deeper understanding is found in more recent work (5,29) which exploits the Gaussian nature of density fluctuations in the pure bulk solvent. This can be used to determine the probability of finding small cavities in the solvent that can accommodate the solute molecules. This approach is successful in predicting the thermodynamics of hydration of small hard spheres and provides a quantitative understanding of hydrophobicity in this regime (9).

In contrast, as suggested by Stillinger, (14) and confirmed by molecular dynamics simulations,(30) large strongly hydrophobic solutes behave differently. When inserted into water they must break some hydrogen bonds at the interface. One hydroxyl group of each interfacial water molecule tends to point into the hydrophobic surface so that the orientational ordering of water molecules at the solute-liquid interface resembles the ordering at a liquid-vapor interface. The “missing” interfacial hydrogen bonds give rise to a

large positive enthalpy of solvation and correspondingly to a free energy change that is proportional to the solute's surface area, A , as opposed to being proportional volume for small hydrophobes. (The pressure under most relevant conditions is small enough that the bulk $p - V$ term can generally be neglected until a solute reaches truly macroscopic size.) Stillinger argued that if the solute-water attraction is sufficiently weak, a large smooth hydrophobic solute might "be immediately surrounded by a microscopically thin film of water vapor," and its hydration free energy would then be dominated by a term like $\gamma_{lv}A$, where γ_{lv} is the vapor-liquid surface tension and A is the surface area of the solute.

Thus the hydrophobic interaction of large scale hydrophobic solutes will be different than small scale ones. Fewer water hydrogen bonds will have to be broken when two large hydrophobes are in contact than when they are apart so that there will be a negative enthalpy change when two or more such solutes are brought into contact from larger separations. Since the free energy change is dominated by the enthalpy change, it too will be negative and thus there will be a thermodynamic driving force towards aggregation. Thus small-scale hydrophobicity is entropically driven but large-scale hydrophobicity is expected to be enthalpically driven.

The suggestion that the interface between a large hydrophobic solute and water could resemble a liquid-vapor interface has itself generated much controversy (9,11,13,31-35). Certainly, there are some clear differences. A free liquid-vapor interface has long-wavelength capillary-wave fluctuations (36) that would be geometrically inhibited (damped out) at such a solute-water interface (see for example(37)), but these do not change the local interface structure (38). More importantly, even weak van der Waals attractions between the solute and bulk water (always present in realistic cases) would suppress the formation of a macroscopic vapor region (31, 34, 39, 40). And it is not so clear what is meant by a "microscopic vapor layer" or what its thickness should be (32). Ball has reviewed experimental evidence for the existence and width of this presumed vapor layer, concluding that, although many of the experiments(41-46) differ in some details and interpretation, there seems to be an emerging consensus that the depletion layer, when it exists, is only of molecular size(13). However, it still seems plausible that water molecules at the interface would tend to point one OH bond into the solute to better maintain other hydrogen bond in much the same way and for the same reasons as the OH bonds of water point into the vapor region in a free water-vapor interface.

As will be discussed below, both experiments (44, 45, 47-53) and simulations (21, 54-63) suggest that it is the similar arrangements and dynamical behavior of these partially ordered but fluctuating interfacial OH-bonds that best characterizes the analogy between the liquid-vapor and large hydrophobic interfaces in water and distinguishes them from hydrophilic interfaces. Hydrophilic solutes with strong localized attractive interactions with water, especially those arising from locally charged or polar groups, will strongly perturb and perhaps pin some interfacial OH groups in different bonding configurations, thus suppressing local fluctuations(64).

An important effect not appreciated until fairly recently(18, 19, 65, 66) is that when two large-scale strongly hydrophobic particles approach each other closer than a critical separation, there can be a large scale drying (or dewetting) transition in which the inter-particle region, although large enough to accommodate water molecules, desolvates, leading to hydrophobic collapse. As discussed below, this behavior can be correlated with the contact angle of water droplets on macroscopic hydrophobic surfaces(65-70). However even in idealized models, the critical separation is a decreasing function of the strength of the attractive interaction between the solute and water and disappears entirely for small solutes. We will review recent simulation results examining the possible relevance of such

macroscopic dewetting transitions in the folding of heterogeneous globular proteins(69-80), the collapse of multi-domain proteins, etc. In such heterogeneous dynamical environments, there is a sensitive coupling of hydrophobicity to changes in local geometry, dispersion, and electrostatic and electric field effects (81-85), and simulations offer the best method at present to help understand the final balance.

The focus of this review is for the most part on large scale hydrophobicity, that is hydrophobicity resulting from the interaction between mostly apolar mesoscopic structures such as large molecules or assemblies of small molecules, or mesoscopic enclosed spaces as might exist in protein active sites, protein cavities, nanotubes, or inside nanoparticles like C₁₈₀. Due to space limitations, a complete review of all recent work on this important subject is not possible, so we only list a few representative examples, some from our own work, in the results sections to illustrate the recent advances on large scale hydrophobicity and dewetting.

2 Theory

In this section we focus on theories describing the length scale dependence of hydrophobic interactions and in particular the role of interfaces in large scale hydrophobicity. For the special case of a liquid in a state very near liquid-vapor coexistence next to a hard wall, one can establish quite generally that a vapor-like layer must form near the wall. This drying arises from generic features of the liquid-vapor interface and the hard wall and applies equally well to liquid water, liquid argon, and other liquids. Because of the short-ranged impulsive nature of the interaction between the hard wall and the fluid, the pressure is exactly related to the contact density at the wall (86) by the same expression that would apply for an ideal gas at that same density and temperature: $P/k_B T = \rho(0)$. If the bulk liquid is very near coexistence, its pressure must be low and close to that of the equilibrium liquid-vapor system.

Since the vapor is well approximated by an ideal gas away from the critical point, it follows from the “contact theorem” above that the density near the wall must be much lower than that of the bulk liquid and close to that of the vapor phase at coexistence. More detailed arguments show that “complete drying” with the formation of a macroscopic vapor layer will occur as the system approaches two-phase coexistence(87).

However, these simple results apply only for a true hard wall, and as mentioned before, predictions about the thickness of the vapor layer would be strongly affected by the presence of even weak attractive interactions between the wall and adjacent fluid. Complete drying or even the formation of a vapor layer of any significant extent at a single interface is hardly ever relevant in practice. But the existence of this fundamental drying limit and the idea that vapor-like interfaces could play an important role at least for idealized large hydrophobic solutes seems well established.

Indeed, the analogy between the behavior of water near an extended hydrophobic region and the liquid-vapor interface is even stronger than these generic arguments might suggest. In water there are strong hydrogen-bond forces favoring an interface with single broken hydrogen bond both for the liquid-vapor interface and near a large hydrophobic region. These local structural features, based on specific network properties of water, set an energy scale of order a hydrogen bond energy and are likely to persist even in the presence of weak solute-solvent attractions, unlike other properties of a generic liquid-vapor interface such as the interface width. This greatly extends the utility of the analogy, and offers at least the possibility that it could still be useful for more realistic solutes and in certain heterogeneous

environments. The extent of a depletion layer is a quantitative question that some of the microscopic theories discussed below could address.

Macroscopic approach

We now turn to the large scale drying that can sometimes occur between two large hydrophobic particles as a function of separation. A slight generalization of a very simple macroscopic thermodynamic model (65-67,88,89) based on Young's Equation (90) provides a context for understanding some of the key features. The model considers the change in the grand potential $\Delta\Omega(D)$ for the reaction in which the inter-plate region (the gap) between two fixed macroscopic coaxial cylindrical plates p , with separation D , goes from being completely wet to dry, $p[\text{Wet}]p \rightarrow p[\text{Dry}]p$, with the vapor occupying a cylindrical region between the plates.¹

Ignoring edge effects, the change in the grand potential for this reaction is $\Delta\Omega(D) = [(P - P_v)A_w + \gamma_{lv}C_w](D - D_c)$, and the critical separation D_c below which vapor between the disks is stable is²

$$D_c = 2\Delta\gamma / [(P - P_v) + 2\gamma_{lv}/R_m]. \quad (1)$$

Here γ_{lv} , γ_{sv} , and γ_{sl} are respectively the liquid-vapor, solid-vapor and solid-liquid surface tensions, $A_w = \pi R_m^2$ is the area of the plate face, $C_w = 2\pi R_m$ is the circumference of the cylindrical plate, P is the pressure on the liquid, P_v is the pressure of the vapor between the plates, and $\Delta\gamma \equiv \gamma_{sl} - \gamma_{sv} = -\gamma_{lv} \cos \theta_c$ is Young's equation with θ_c being the contact angle for the liquid in contact with the wall of the plate. For a hydrophobic surface the contact angle is obtuse so that $\Delta\gamma > 0$ and $D_c > 0$ and drying should occur. However for hydrophilic plates the contact angle is acute, so $\Delta\gamma$ will be positive for all separations and the inter-plate region will be wet.

For small plate sizes (small R_m), the $(P - P_v)$ term usually can be neglected compared to the surface tension term and then $D_c = -R_m \cos \theta_c$ should grow linearly with plate radius; however for sufficiently large plates the surface tension term in the denominator can be neglected, D_c should become independent of plate size, and drying should occur for very large separations $D_c = 2\Delta\gamma/(P - P_v)$.

Since the contact angle is a decreasing function of the strength of the plate-water attractive interaction, the critical distance for drying will decrease as the strength of the attraction increases. From this we expect that purely repulsive plates will be the most prone to dry,³ and substances like graphite with contact angles near 90° will not. These simple thermodynamic arguments show that macroscopic hydrophobic objects dissolved in water should expel the solvent trapped between them if brought closer than a characteristic critical distance D_c that depends on geometry and thermodynamic properties. Although thermodynamics clearly predicts the existence of this drying transitions, the free energy barrier to cross from the wet to the dry state could be very large, effectively freezing the system in a metastable state.^(54, 65, 91, 92) This free energy barrier is responsible for the

¹This is a simplifying assumption. In reality the vapor cavity will not be cylindrical but will resemble an hour-glass defined in part by the contact angle and this simple back of the envelope approach will overestimate the critical distance.

²For two different hydrophobic surfaces $2\Delta\gamma \rightarrow 2\Delta\gamma^-$ where $\Delta\gamma^-$ is the equal weight average of the $\Delta\gamma$ of each surface, a quantity equal to γ_{lv} times the average of each surface's $\cos \theta_c$. Thus if one surface has a much smaller contact angle than the other, the critical distance will be much smaller for this enclosure.

³For a hard wall $\theta_c = 180^\circ$ and from Young's equation we find that $\gamma_{sl} = \gamma_{sv} + \gamma_{lv}$, indicating that the interface is composed of a solid-vapor interface as suggested by Stillinger (14).

phenomenon of cavitation-induced hysteresis which has been observed(93, 94) experimentally in surface-force measurements.

Mesoscopic and microscopic theory

Do drying transitions and the predictions of macroscopic thermodynamics still hold for proteins and other systems of nano or sub-nanometer dimensions? More detailed microscopic theories capable of describing the large scale drying limit, heterogeneous solutes, and cavity formation at small length scales are needed to address such questions. The Local Molecular Field (LMF) theory developed by Weeks and coworkers (95) is one such microscopic theory. When applied to water it would require detailed information about the water-water and water-solute interaction potentials. LMF theory can be generally characterized as a mapping that relates structural and thermodynamic properties of a nonuniform system with long-ranged and slowly-varying intermolecular interactions in a given external field to those of a simpler reference or “mimic” system with short-ranged intermolecular interactions in an effective or renormalized field. The short-ranged interactions in the mimic system are chosen to give an accurate description of the strong forces between typical nearest neighbor molecules, leading, e.g., to the local hydrogen bond network in water. The effective field is determined self-consistently and contains the direct solvent-solute interaction as well as a density-weighted average of the unbalanced forces arising from the remaining long-ranged parts of the original intermolecular interactions.

LMF theory has been very successfully applied to nonuniform LJ fluids (95-97), to ionic solutions (98-100), and very recently to bulk and confined water as described by the SPC/E model (101). Results for solvation, dielectric, and other properties of water are very promising. LMF theory also provides a framework for useful semi-empirical theories such as the LCW theory of hydrophobicity (19), which will be reviewed below.

Another very promising microscopic theory is the quasichemical (QC) theory of solvation developed by Pratt and coworkers (5, 33, 102-105). The formalism is based on Widom’s inverse potential distribution theory (90) and uses results of a computer simulation where interactions within an inner solvation shell of an appropriately chosen size around a solute are treated exactly. A Gaussian approximation is used to account for the effects of longer-ranged interactions from molecules in the outer shell. Errors in this approximation arising from high energy “collisions” between inner and outer shell molecules can be avoided by a conditioning procedure that uses configurations where there are no solvent particles in the inner shell region.

Both LMF and QC theories start from rigorous statistical mechanics foundations and make physically motivated and testable approximations to develop practically useful expressions. Both approaches are under active development and we believe that further research along these lines will shed light on the connections between these theories and on the strengths and weaknesses of related approximate theories such as the LCW theory, to which we now turn.

LCW theory (19) can be viewed as a simplification of LMF theory in which experimental structural and thermodynamic data for the liquid and vapor phases of the solvent are used to make a simple estimate for the effective field, in analogy to the classical van der Waals theory of the liquid-vapor interface (90). In the original application to water near a smooth hydrophobic solute, only the nonuniform oxygen density was considered explicitly. Thus the theory can not answer questions about changes in orientational order and bond angle correlations that could be addressed using the microscopic approaches.

Linear response theory is used to calculate the nonuniform density induced by the effective field. The linear response function (direct correlation function) is further approximated by a

crude interpolation between known results for the uniform bulk liquid (well described by a Gaussian fluctuation model (106)) and the ideal gas. This interpretation of LCW theory and its detailed connections to the full LMF theory was presented in Ref. (95).

The first application of LCW theory was to the solvation of a hard sphere (HS) solute in water as a function of its radius (19). Here simulations show that the contact density varies from much greater than the bulk density (“wetting”) at small molecular scale radii to “drying” at very large radii with a contact density much less than the bulk (107). LCW theory gave good qualitative agreement with simulations, providing in particular a very reasonable estimate for the crossover radius of about two nanometers where the solvation free energy begins to scale with surface area rather than volume.

LMF theory has not yet been applied to the water-HS system, but detailed results for both theories are available for the analogous problem of a HS solute in a LJ fluid near coexistence. Here the physics is well understood, and LMF theory gives essentially quantitative agreement with simulation results (97). The crossover radius is about a factor of 5 smaller than for the water-HS system because of the smaller surface tension of the LJ fluid. Again LCW theory provides good qualitative agreement (108), with the main errors arising from the interpolation formula.

Attractive solute-solvent interactions simply add an additional term to the effective field in both the LMF and LCW theories. For a solute with a large radius and weak attractions (where the solute-solvent attraction is much less than the solvent-solvent attraction) the resulting density and entropy is similar to that of a free liquid-vapor interface pulled close to the solute with relatively little distortion of the local interface structure (11). Strong localized solute attractions can of course disrupt this picture and induce a large contact density even for large solutes.

One possible criticism of the LCW approach is that it uses a van der Waals like theory, known to be appropriate for simple LJ fluids, to describe interfaces in water, a very different liquid. But the LCW theory makes use of experimental data that captures many anomalous features of water. In particular the large surface tension at the water-vapor interface reflects the broken hydrogen bonds that are assumed to also occur at the hydrophobic surface. The theory correctly describes both the Gaussian probability of formation of small cavities in water (106) as well as the generic drying and associated interfacial energy that must occur in the hard wall limit.

A related concern is that there is no explicit accounting for unbalanced forces arising from long-ranged dipolar interactions in water. However, detailed calculations using the full LMF theory for SPC/E water near a model hydrophobic wall with weak attractions shows that the main unbalanced force at the wall indeed arises from the LJ component of the SPC/E interaction (109). Long-ranged dipolar interactions have surprisingly little effect on the oxygen density profile, though they do strongly affect the electrostatic potential and other properties not considered in the LCW approach (101). Thus there is good reason to believe this qualitative picture holds for idealized hard sphere or for weakly attractive solutes in water.

Much more difficult is the objection that smooth homogeneous objects of varying sizes offer a very poor model for real hydrophobic solvation in water. Here molecular granularity and heterogeneity involving local polar or charged groups(81) presents a new set of challenges beyond the scope of the simple LCW theory. More general microscopic descriptions capable of describing of describing angular and electrostatic correlations are needed; further development of the LMF and QC approaches may provide some insights here. At present,

these quantitative questions can best be addressed by careful simulations. This is the focus of the rest of this review.

3 Dewetting and Hydrophobic Interaction in Large Scale Physical Systems

Drying vs. Physical Interaction Strength

Theory shows that the dewetting transition is fairly sensitive to the strength of the solute-solvent interaction. A simple explanation follows from the realization that the stronger the attraction between the plate and water (i.e. the larger the LJ ϵ) the smaller will be the contact angle (see for example ref.(110), and correspondingly the smaller will be the critical distance D_c (see Eq.(1)). Once the contact angle becomes small enough, dewetting should not occur because D_c becomes too small to sterically allow any water molecules.

Simulations of two parallel nanoscale graphite plates show no capillary drying (dewetting) transition for any separation under standard conditions(68, 111), a finding in agreement with Eq.(1) and the small contact angle, $\theta \approx 90^\circ$, of graphite. For example, if the carbon atom interacts with water with an LJ interaction parameter $\epsilon_{CO} = 0.1156$ kcal/mol, a realistic value, no dewetting(68) transition is observed. The smallness of the contact angle probably arises from the high surface density of carbon atoms in graphite (the aromatic C-C bond is short). Interfacial water molecules can thus interact with several carbon atoms at a time so that the attractive dispersion interaction is sufficiently large to lead to a contact angle small for a hydrophobic material. Similar results have been found by Pettitt and coworkers(111) with graphite plates and Hummer et al. with carbon nanotubes (112). If the water-carbon ϵ_{CO} is reduced to 0.0611 kcal/mol, however, a drying (dewetting) transition is observed (68) with a critical distance of 6.8Å. Alkane chains have in general weaker interactions with water than graphite plates as seen from their water contact angles. When the water-carbon interaction is further reduced for graphite, the dewetting becomes stronger. The system can also fluctuate between the “dry” and “wet” states when ϵ_{CO} is near 0.0647kcal/mol, indicating the system is reaching a critical distance for the microscopic “phase transition” from liquid to vapor. In some systems even a decrease of 10% in ϵ can cause dewetting as we have found in biological proto-filaments (see next section). Thus it seems likely that many realistic systems are close to the transition region and interface formation represents one possible reaction channel that should be considered.

Superhydrophobic fluorocarbon surfaces have experimental contact angles (113) as large as 135° and behave very differently from graphite plates. Two similar-sized nanoscale plates constructed from Langmuir-Blodgett films with terminal CF_3 groups were found by simulation to dewet with a critical distances of $\sim 10\text{\AA}$ (114). The attractive interaction between the fluorinated carbons atoms and water molecules in the nearest solvation shell was found to be 10-12% weaker than in plates made of their hydrogenated counterparts. Even though the fluorocarbons have a stronger electrostatic interaction with water in the nearest shell than do the hydrocarbon chains because of their larger partial charges, the van der Waals interactions dominates over the electrostatic interactions, contributing up to 90% of the total interaction energy. The fluorocarbons have a noticeably weaker van der Waals interaction with water in the nearest shell by 10-15%, than do the alkane chains. Both this and its larger surface area per chain lead to a stronger dewetting transition plates(114). Model hydrophobic plates (66), with a water contact angle of 148° , exhibit even larger critical distances ($\approx 14\text{\AA}$) than fluorocarbon plates of similar size, but it is difficult to find such large contact angles in nature.

So far we have discussed drying in regions between identical plates. What happens when the plates are different? The highly approximate macroscopic theory discussed above shows that the critical distance for drying between plates with different contact angles will depend on

the average of the cosines of their contact angles. Thus two very hydrophobic plates will have a larger critical distance than one very hydrophobic and one less hydrophobic one. An extreme case would be that where one plate is highly hydrophobic and the other hydrophilic, then the average would be very small and drying would not be expected.

In an intriguing experiment Granick and coworkers investigated just such a system. They studied a “Janus” interfaces in which a water slab is trapped between a hydrophobic wall on one side and a hydrophilic wall on the other(47). They found that the latter prevents any macroscopic drying or cavitation of the liquid, which in any case would be strongly affected even by relatively weak van der Waals forces. This allowed them to focus on more intrinsic local properties of interfacial water near extended hydrophobic and hydrophilic surfaces, and to compare and contrast behavior in the different regions. Shear deformations produced by moving the hydrophobic surface resulted in very large noisy fluctuations consistent with the picture of damped capillary waves at the hydrophobic surface arising from partial dewetting. Film-spanning fluctuations that might lead to macroscopic dewetting between hydrophobic surfaces were suppressed by pinning of water at the hydrophilic wall. No such fluctuations were observed when both interfaces were hydrophilic, as expected. Though some interesting questions remain open, this picture of qualitatively different fluctuation behavior at extended hydrophobic and hydrophilic surfaces is broadly consistent with a growing body of experimental and simulation data(42, 44-46, 48-53, 115). We believe it offers a consistent framework within which one can address many questions of current interest, such as the effects of patterned hydrophobic and hydrophilic regions(59) or consequences of different geometries. In particular it should be no surprise that the more hydrophobically enclosed the water is, that is the more hydrophobic surface the water is surrounded by, the greater will be the thermodynamic driving force towards dewetting.

Thus far we have discussed how water behaves in regions enclosed between two plates, but it is equally, if not more, important to understand how more extreme enclosures such as cavities (addressed in the next Section) or nanotubes affect water. One can for example study water in holes made through silicon or in the interior of single walled carbon nanotubes (SWCNT), in cavities inside folded proteins, or, even more importantly, in active sites of enzymes. A recent review(10) in this series focused on some of these topics (see also next section), and we will discuss only a few aspects here.

Hummer *et al.* (112), in a pioneering study, simulated a short SWCNT in a box of liquid water, and interpreted their findings by calculating the local excess chemical potential of water, using potential distribution theorems(116). An initially dry nano-channel of the SWCNT was observed to rapidly fill and no dewetting was found with the normal carbon-water interaction. Given the loss of hydrogen bonding, and the weak attraction of water to the nanotube carbon atoms, this persistent hydration of the nanotube interior was very surprising (as was the wetting between graphite plates). Hummer *et al.* also found that when the carbon-water van der Waals parameter ϵ_{CO} was reduced by 44% the water occupancy fluctuates in sharp transitions between empty and filled states, behavior similar to the aforementioned simulations of graphite-like plates with reduced carbon-water interactions(64) or even the fluorocarbon plates(114). Maibaum and Chandler(117) showed that very similar fluctuation behavior could be reproduced in a simple confined lattice gas (Ising) model at states close to coexistence. Fang and coworkers also studied a similar system under the influence of a mobile external charge as a way to mimic the electrostatic gating of a water nanopore. They found the designed nanopores show an excellent on-off gating behavior by only one single external charge.(118-120)

Pressure Effect on Dewetting

Rosky and coworkers (121) have recently simulated the pressure effect on the dewetting between two hydrophobic (dehydroxylated) nanoscale silica plates for different pressures. These studies show the following: (a) For hydrophobic plates, increasing the pressure enhances water structure and pushes water molecules towards the plates and into the interplate region; but the average orientation of water molecules next to the hydrophobic plates does not change upon pressurization, suggesting local stability of the interfacial hydrogen bond network. (b) The water molecules next to hydrophilic plates are orientationally constrained and capable of only small amplitude orientational fluctuations and the water structure is insensitive to changing pressure. Even at negative pressures for which neat bulk liquid forms cavities, the confined water remained in the liquid state. These results suggest that upon pressurization, hydrophobic plates behave as “soft” surfaces (in the sense of accommodating pressure-dependent changes in water structure) while still allowing the partially ordered water molecules with their OH bonds pointing into the solute to fluctuate as they do in the liquid gas interface. In contrast, hydrophilic plates behave as “hard” surfaces with strong localized attractive interactions with water which suppress water fluctuations.

Garde *et al.* have also studied pressure effects on drying and find, as expected, that decreasing the pressure stabilizes drying, and lowers the free energy barrier to the formation of the vapor bubble, thereby speeding up the kinetics of drying in molecular dynamics simulations(21). Under negative pressures neat water will cavitate and it is no surprise that capillary drying will be facilitated at negative pressures.

Effect of Cosolutes on Hydrophobicity

In general, hydrophobic interactions can be altered by dissolving cosolutes in water. This subject has been treated in part in the excellent review by Ball(13).

Since 1888 it has been known that the solubility of globular proteins can be increased or decreased by adding different ions and these ions have been rank-ordered according to the effect they have on protein solubility through the Hofmeister series. Because ions can affect the polar and nonpolar regions of proteins differently, recent work has aimed at understanding how these operate on nonpolar hydrophobic particles. It has been found that hydrophobic association can be decreased or increased, respectively, by preferential binding or exclusion of the ions from the hydrophobic surface(122-124). This has been illustrated recently in a model electrolyte system(125). Experiments and simulation have elucidated the effect of a wide range of ions on the pair pmf of spherical solutes.(126) Recent studies are clarifying the effect of ions on the structure of the water interface with hydrophobic surfaces (see for example refs. (127-129))

Similarly hydrophilic cosolutes like urea denature proteins presumably by altering the hydrophobic interaction and various mechanisms have been proposed for this action. This is a very interesting subject, but must be left to another review because space does not permit a treatment of this here. Suffice it to say that significant work has been done recently to determine the mechanism by which urea and other cosolutes reduce hydrophobicity in simple hydrophobe(130-133) and proteins(134-136) and we expect that much will be learned in the near future about these relatively ancient subjects which are still not completely understood.

4 Dewetting and Hydrophobic Interaction in Biological Systems

Dewetting in Protein Folding

Proteins are often characterized by surfaces containing extended nonpolar regions, and the aggregation and subsequent removal of waters between these hydrophobic surfaces is believed to be central to their self-organization. Because the amino acid residues range from being strongly hydrophobic to strongly hydrophilic (charged residues) and because the carbonyl and amide groups along the peptide backbone are polar and capable of participating in hydrogen bonds it is a challenge to analyze the role of small and large scale hydrophobicity, and especially dewetting, in folding and aggregation of proteins. Important work on the nonpolar confinement of water in nanopores and cavities was recently reviewed(10, 13) so that we will focus on the complimentary issue of dewetting, although we will briefly discuss the powerful effect hydrophobic enclosure has on ligand binding to proteins.

The phenomenon of large scale capillary drying between approaching strongly hydrophobic plates suggests that fluctuations of water, in particular, bubble creation and annihilation, should be greatly implicated in the kinetics of plate association. Another way of saying this is that the kinetics of hydrophobic collapse involves a multidimensional reaction coordinate, at least one component of which must be a collective coordinate of water. The implications of this for hydrophobic chain folding were poignantly suggested by Chandler and coworkers(137) in their study of the collapse from an extended coil state to a compact globule state of a string of twelve purely hydrophobic beads (each of volume similar to the volumes of amino acids in solution), interacting with a coarse-grained lattice-gas model of water through purely repulsive forces. They found that the evaporation of water in the vicinity of the polymer is implicated in the collapse. Once a sufficiently large cluster of beads formed, a Stillinger-like vapor interface formed and seemed to embrace the growing cluster until collapse was complete. They concluded that length-scale-dependent hydrophobic dewetting is the rate-limiting step in the hydrophobic collapse of the model chain system. Chandler and coworkers recently performed MD simulations on a similar chain, with larger beads, interacting repulsively with an all-atom model of water and found very similar results, although in this latter study the vapor seems to appear in hydrophobically enclosed regions rather than by surrounding a cluster as before(138). This behavior is reminiscent of drying in the region between plates and in other enclosed regions, and not a Stillinger-like vapor layer as was seen in the lattice-gas model of water. In addition, they determined the path of maximum likelihood for the dynamical trajectories which confirmed the mechanism of hydrophobic collapse proposed in the earlier study(137). The behavior observed is not surprising since the system consists of large beads with no attractions for water. Athawale *et al.* made a detailed study of the effects of length scales and attractions on the collapse of hydrophobic polymers and presented a nuanced view of the subject.(139)

While suggestive, these studies do not directly show that hydrophobic collapse is involved in mechanisms of biological assembly such as protein folding. Because of the time scales involved, to date there has been no successful molecular dynamics simulation of folding from an extended state of realistic proteins in explicit solvent. Nevertheless, it is possible to study the role of water in the collapse of multi-domaine proteins like the BphC enzyme, or aggregation of proteins, like the formation of tetramers of melittin, since one can investigate how water behaves as the domains, or monomers are brought together. The first such study involved bringing the two domains of the BphC enzyme together to see if there is a critical distance for drying between these domains(71). It was found that when either the van der Waals interaction or both it and the electrostatic interactions between the protein and water were turned off the region between the domains exhibited a drying transition, but when the

full force field was turned on, no complete drying transition was found. Based on this study it was at first thought that dewetting would not occur in proteins because the van der Waals and electrostatic attractions would be too strong. Of course it is expected that this will be sensitive to temperature (and pressure). For example in recent studies of the assembly of Alzheimer Amyloid-beta A β 16-22 Protofilaments no drying was observed at room temperature but was observed at a slightly higher temperature(140). When the strength of the $1/r^6$ terms in the protein-water LJ potential was decreased by 10% the protofilaments exhibited a drying transition. Given the uncertainties in the force fields for proteins and the sensitivity of dewetting to the force field parameters it is difficult to make firm conclusions at present. Several studies have shown that lowering the pressure can also cause dewetting(73, 141, 142). Anything that brings the liquid closer to coexistence with the vapor should have this effect.

Capillary Drying in Melittin Aggregation—A good place to look for dewetting in protein folding is probably in the protein complex formation during the final stages of folding. Liu et al. (69) recently studied the water drying transition inside a protein complex - the collapse of the melittin tetramer. Cheng and Rossky previously studied the structure of water nest to a melittin monomer.(143)

The two dimers of the melittin tetramer were next separated by a distance to create a “nanoscale channel,” then solvated in a water box. The authors found that there is a sharp dewetting transition and essentially all water molecules are expelled from the nanoscale channel after the transition. Simulations with many other separation distances show that the critical distance for for the melittin tetramer system is approximately 5.5–7.0 Å, which is equivalent to 2-3 water molecule diameters. The reasons that the melittin tetramer channel exhibits a drying transition while the previously studied two-domain protein BphC does not (71) appears to be two-fold. First, the melittin channel is more enclosed, like a tube, while the inter-domain region in the two domain protein is like the region between two plates (See the previous discussion). It is less costly with respect to free energy to disrupt the hydrogen bonds in a tube-like channel. Second, the unique surface topology springing from the isoleucine residues in melittin disrupts the water hydrogen bonds in the channel, thus destabilizing the wet state. When certain isoleucines are mutated to glycine or alanine the channel wets. Interestingly, Debenedetti, Rossky and coworkers(144) have recently studied the structure and thermodynamics of water confined between two slightly modified melittin dimers. They found that the density and compressibility of water adjacent to a melittin dimer is intermediate between that observed adjacent to idealized hydrophobic or hydrophilic surfaces. Meanwhile, solvent cavitation between dimers is observed when the separation between the dimers has closed to a distance about a single water layer.(144) This cavitation occurs at smaller pressures and separations than in the case of idealized hydrophobic flat surfaces. The cavitation is also found to be localized in a narrow central region between the dimers around hydrophobic residues. When that hydrophobic residue is replaced by a hydrophilic one, the cavitation disappears. These results seem to be consistent with the previous findings by Berne and coworkers.(69)

Dewetting in Protein-Ligand Binding

When a ligand binds to a protein, the water solvating the active site is expelled into the bulk fluid. This expulsion of the active site solvent makes enthalpic and entropic contributions to the binding free energy of the complex. The less energetically or entropically favorable the expelled water, the more favorable its contributions to the binding free energy. The active sites of proteins provide very diverse environments for solvating water. Water solvating narrow hydrophobic enclosures such as the Cox-2 binding cavity is energetically unfavorable because it cannot form a full complement of hydrogen bonds. Similarly, water

molecules solvating enclosed protein hydrogen bonding sites are entropically unfavorable since the number of configurations they can adopt while simultaneously forming hydrogen bonds with the protein and their water neighbors is severely reduced(145). The expulsion of water from such enclosed regions has been shown to lead to enhancements in protein binding affinity(146, 147). From these observations Abel *et al.* determined that a computationally derived map of the thermodynamic properties of the active site solvent could be used to rank the relative binding affinities of certain classes of congeneric compounds(147).

Young *et al.* has recently studied the water drying inside the protein-ligand binding active sites using molecular dynamics simulations(145). Three binding cases, including streptavidin, antibody DB3, and COX 2, are examined. In all the cases, water seems to be simply eager to get out of the protein binding cavity. For example, in the streptavidin case, water molecules in the cavity do manage to form some hydrogen bonds with each other and with protein residues, but they are largely trapped on top and bottom of the hydrophobic surroundings, thus making them less stable both enthalpically and entropically. On the other hand, the Cox-2 active site was found to contain no persistent hydration sites and is in fact entirely devoid of solvent in 80% of the simulation, despite the cavity being sterically able to accommodate approximately seven water molecules. The high excess chemical potential of the binding-cavity solvent is due to an inability of the water molecules to make hydrogen bonds with the surrounding hydrophobic protein residues and other water molecules. This results in an extreme enthalpic perturbation, 8 kcal/mol, which drives the dewetting of the cavity. The active site water molecules of an artificially hydrated Cox-2 structure were evacuated within 100 ps. The active site of Cox-2 is predominantly a narrow paraffin-like tube and is therefore in line with other studies of hydrophobically induced dewetting(69,148). A similar effect has been reported for bovine β -lactoglobulin (BLG) (149). Halle and coauthors show that an extreme case of a completely dehydrated free binding site is realized for the large nonpolar binding cavity in protein BLG. They use a combination of water ^{17}O and ^2H magnetic relaxation dispersion (MRD), ^{13}C NMR spectroscopy, molecular dynamics simulations, and free energy calculations to establish the absence of water from the binding cavity—the 315 Å³ binding pore is completely empty. The apo protein is thus poised for efficient binding of fatty acids and other nonpolar ligands. (149)

In subsequent paper, Abel *et al.* continue the line of research described in the ref(145) by applying the inhomogeneous solvation approach to study ligand binding in factor Xa, an important drug target in the thrombosis pathway, several inhibitors of which are currently in Phase III clinical trials(147). They use a clustering technique to build a map of water occupancy in the factor Xa active site, and assign chemical potentials to the water sites using the inhomogeneous solvation theory discussed above(150). They then construct a semiempirical extension of the model which enables computation of free energy differences ($\Delta\Delta G$ values) for pairs of congeneric fXa ligands that differ by deletions of atoms, and thus will displace different portions of the solvent. These free energy differences are shown to correlate exceptionally well with experimental data with few or no adjustable parameters. They hypothesized that the contributions to the binding free energy of adding a *complementary chemical group*—ie chemical groups that make hydrogen bonds where appropriate and hydrophobic contacts otherwise—to a given ligand scaffold could largely be understood by an analysis of the solvent alone.

5 Final Remarks

Our survey of length scale effects on hydrophobicity and dewetting is necessarily incomplete and has at best touched on a few of the issues that have captivated a vast array of

workers with many different perspectives from many different disciplines. This diversity has produced its share of conflicts, but more importantly, has illuminated the problem from many different directions. Because of the subtle nature of the hydrophobic effect itself and its interplay with an array of other forces in realistic biophysical environments, much more work is needed before we can claim with any confidence to have even a qualitative understanding in many relevant cases. But we believe that a picture of a soft fluctuating interface structurally similar to the liquid-vapor interface near an extended hydrophobic region is well established, and can serve as one cornerstone as we try to extend our understanding from idealized theoretical models to realistic biophysical systems.

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