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Charge, hydrophobicity, and confined water: Putting past simulations into a simple theoretical framework

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Water merits special attention from biophysicists, not only because it acts as the backdrop for virtually every chemical and physical reaction in nature, but also because its character as a solvent eludes simple description. The subtlety of physics in the aqueous medium arises from the dual nature of the water molecule itself: on the one hand, the molecular dipole moment resulting from the high electronegativity of oxygen makes bulk water a powerful dielectric whose density and configurational order can increase in the presence of charges¹; on the other hand, water's unrivaled ability to participate in a highly coordinated network of strong hydrogen bonds² makes the free energy cost of solvating non-polar surfaces especially high, leading to the so-called "hydrophobic effect"³.

Past attempts to give a theoretical characterization of water as a solvent have tended to focus on either one or the other of these two aspects. Studies concerned with the effects of ions, charged surfaces, and external electric fields on solvation have typically treated liquid water as a polarizable continuum whose thermodynamics are best described either by solutions to the non-linear Poisson-Boltzmann equation, or by more phenomenological approximate formulae^{4,5}. In contrast, the most successful theories of hydrophobicity and its associated phenomena have rooted themselves in the relationship that can be demonstrated between the probabilities of different density fluctuations in a pure water bath and the work required to introduce different inert solutes into that bath^{3,6}.

A number of studies in the simulation literature focusing on confined water challenge both of these theoretical approaches^{1,7–11}. In scenarios where charged and hydrophobic surfaces become separated by layers of water that are thin on the nanometer scale, solvent-mediated forces and phase transitions can arise that are difficult to account for by making reference to either electrostatic or hydrophobic effects alone. Simply put, if a relatively small number of confined water molecules find themselves under the mixed influence of both charge and hydrophobicity at the same time, the contortions of the hydrogen bond network required to satisfy one can be ill-suited to interact favorably with the other. The result is a set of simulation phenomena on the nanoscale that require a new theoretical framework capable of treating the organization of water near polar and non-polar solutes on equal footing.

In this paper, we propose such a framework and examine several recent molecular dynamics studies of mixed solvation scenarios in light of it. We begin by motivating and defining the assumptions of the theoretical model, which posits a relationship between a water molecule's orientational entropy and its ability to interact favorably with a neighboring

molecule. Following this, we show that the model reproduces basic phenomena expected from past simulations of water confined between polar and hydrophobic surfaces. Ultimately, we demonstrate the model's efficacy for explaining outcomes in different instances of protein folding under confinement that have been investigated in a series of previous works. We propose that the framework presented here may be useful in a variety of contexts in the future where water confined between chemically heterogeneous surfaces plays an important role in protein folding, macromolecular assembly, or nano-engineering.

Model

Generally speaking, the value of an effective theoretical model is that it has the power, by virtue of its ability to predict outcomes in a variety of circumstances, to show that a diverse set of seemingly disparate phenomena may actually be different manifestations of a single underlying set of rules. In this discussion, we are interested in explaining various simulations of water confined between charged and hydrophobic surfaces. Thus, we suggest a model inspired by past theoretical work that had some success in describing several simulated scenarios involving water, hydrogen bonding, and uniform electric fields.

The first of these scenarios to be studied was that simulated by Vaitheeswaran *et. al.*, in a 2005 work¹¹ that clearly demonstrated the potential for unexpected behavior in systems involving confined water and charge. The authors carried out constant pressure molecular dynamics (MD) simulations of nanometer-separated, water-immersed hydrophobic plates with a uniform electric field applied between them normal to the plates. In such a system, the bulk theory of electrostriction would predict that, as the electric field increased, water density would rise between the plates as the liquid became more polarized¹². Instead, a drastic decrease in the number of water molecules between the plates above a certain field strength was reported. In a subsequent study by other researchers¹³, however, no field-induced evacuation was observed in a nearly identical system, raising the question of whether this counter-intuitive phenomenon required an explanation.

Recently, a theoretical study by England *et. al.*¹⁴ proposed a simple, statistical mechanical model for the water between the plates that sought to provide an explanation for both of the previous plate-field studies. By describing the water as a lattice liquid in which molecules may not hydrogen bond to their neighbors when their dipoles are aligned with the applied field, the authors predicted that a sharp density drop between the plates could be observed with increasing field, but that this transition would be too mild to notice for a water bath of moderately different chemical potential. In addition to offering a possible explanation for the discrepancy between the two past studies of the plate-field system *in silico*, the model also predicted a similar density transition for a constant pressure water box in an applied field, which the authors subsequently observed in their own MD simulation.

The plate-field studies underlined that orientational order in water sometimes interferes with the stability of the liquid phase by disrupting hydrogen bonding. They also demonstrated that a simple lattice liquid model designed to capture this trade-off can successfully explain the outcomes for several different simulations. Lattice models of the liquid-vapor transition in fact have a long history. In 1952, Yang and Lee were the first to demonstrate that a simple,

two-state lattice model of a vapor with interactions between particles occupying adjacent sites on the lattice could reproduce the condensation transition known to occur in a van der Waals fluid. In this case, the two states of the lattice simply corresponded to the presence or absence of a molecule, and their relative probability in the grand partition function was determined by the chemical potential μ , which represents the effect of the system being in contact with a bath of molecules of a specified fugacity. The only other parameter in the model Hamiltonian was u , the energy of interaction between neighboring molecules, which determined how strongly molecules would be bound into the liquid phase at a given temperature. Here, it is our aim to augment this model in the hope of describing a broader range of phenomena using a similar lattice-based approach.

Our underlying assumption is that when water interacts with hydrophilic (i.e. charged or polar) surfaces, it faces a choice between lowering its orientational entropy to match up its partial negative and positive charges most favorably with those on the surface, on the one hand, and tumbling freely so as to lower the free energy of its interaction with neighboring water molecules in the liquid phase, on the other hand. This leads us to posit a grand canonical lattice liquid model¹⁵ of water in which each lattice site may be in one of three different states: “empty” (e), “liquid” (ℓ), and “ordered” (r). The empty state is self-explanatory; it contains no water molecule. Should a water molecule pay the thermodynamic cost and occupy a site, however, it still must choose between participating in the surrounding liquid (the ℓ state, with chemical potential μ_ℓ), or becoming more orientationally ordered (the r state, with chemical potential μ_r). Here, the ℓ state corresponds to the “normal” behavior of a molecule in the bulk phase, whereas the r state has lower orientational entropy (and a correspondingly less favorable chemical potential) that enables a molecule in that state to interact most favorably either with an adjacent polar surface *or with a neighboring ordered, r-state water molecule*. This last point requires emphasis because it is the defining assumption that provides much of the basis both for the model’s successes, and for some of its failures. The fundamental assumption is that an orientationally-ordered water molecule favors the ordering of molecules in neighboring lattice sites.

The model may be formalized by defining a vector $\vec{s}_i = (s_\ell, s_r)$ for the i th lattice site that is equal to (1,0) when the site is in state ℓ , (0,1) when the site is in state r , and (0,0) when the site is empty. In that case, defining chemical potentials $\vec{\mu}_i = (\mu_\ell, \mu_r)$ and a matrix of inter-site couplings $\mathbf{u}$, we can write the grand canonical Hamiltonian for a single site i and its nearest neighbors j as

$$\mu N - H = \frac{1}{2} \vec{s}_i \cdot (\mathbf{u} \vec{s}_j) + \vec{\mu} \cdot \vec{s}_i$$

Here, by assuming $\mu_r < \mu_\ell < 0$ we ensure that it takes positive chemical work to unbind a water molecule from the surrounding bath, and that it takes further work to reduce that molecule’s orientational entropy (i.e. to order it). Meanwhile, by setting the interactions so that $u_{rr} \sim u_{\ell\ell} > u_{r\ell} > 0$, we establish the tendency for liquid phase to bind together, and for ordered waters to promote the ordering of other waters in their neighborhood.

Finally, it should be noted that in the various applications of the model we pursue below, we always perform calculations in a one-dimensional representation, where the confining “surfaces” involved are specified by fixing the state of the lattice sites on each of the system’s two edges. The low dimensionality enables us to employ transfer matrix methods in computing partition functions, thereby simplifying the calculation enormously. Although one may not assume, in general, that the physics of one- and three dimensional-systems of this kind will be the same, we feel justified in making such an assumption in this case for at least two reasons. First, our interest is in the qualitative behavior of systems of finite size, rather than in the universal exponents of our model near a critical point. As a result, the difference between crossing a single first-order transition line in the Ising phase diagram and crossing two second-order lines in rapid succession is insignificant with respect to the question of whether or not, as in the case of dewetting, for example, there is a qualitatively cooperative change in liquid density as the result of tighter confinement. Second, the questions that interest us below involve systems that are, in fact, pseudo-one-dimensional, i.e. they only have one degree of freedom (such as the separation between two surfaces) that varies, and their finite extension in directions orthogonal to the direction of variation should not fundamentally alter the physics. That being said, it might certainly be useful and necessary in the future to apply the model in contexts where a three-dimensional representation is needed to represent all the relevant details of cavities of interest.

Basic Confinement Phenomena

With the model defined, the first order of business is to demonstrate that it reproduces the basic qualitative phenomena we expect from confined water. As a natural starting point, we might consider two inert (i.e. hydrophobic) walls of variable separation with a layer of water confined between them. For water at temperature and chemical potential typically of interest to biophysicists, it is reasonable to assume that the liquid phase is stable in bulk, but close to phase coexistence with a vapor³. In that case, if the walls start out far apart, the space between them should fill up with liquid. As the walls come closer together, however, the weaker binding energy of molecules close to the walls makes a proportionally larger contribution to the total grand potential of the system. At a critical wall separation, this surface effect brings about a destabilization of the liquid phase and the water density between the walls drops precipitously in a phenomenon known variably as capillary evaporation or dewetting¹⁶.

The three-state lattice model we employ here (see Appendix) was, in fact, deliberately constructed to be able to describe dewetting. In the absence of any external fields producing a tendency towards the formation of r states, the three-state model effectively reduces to a two-state lattice liquid model (which is exactly equivalent to the Ising model¹⁷) that has been used in the past to study capillary evaporation¹⁸. As a result, it is unsurprising that the three-state approach readily captures the discrete drop in liquid density (and the correlative transient spike in the compressibility) when the tightness of confinement between hydrophobic surfaces reaches a critical level (Figure 1a).

What the three-state model provides that the simple lattice liquid model cannot capture, however, is a description of a mathematically analogous, but physically distinct transition

that takes place when water is confined between *polar* surfaces (Fig. 1b,c). The mathematical analogy arises because in both scenarios, the lattice sites in the system are restricted to two of the three available states, and are therefore constrained to explore Ising model-like subspaces of their total space of microstates. As Figure 1c shows, when slabs of orientationally-frozen waters are brought closer together in a molecular dynamics simulation, the free water molecules between them experience a cooperative decrease in their entropy, much like the drop in density observed in the dewetting scenario. This phenomenon is captured qualitatively by the behavior of the three-state lattice model in Fig. 1b. Thus, we have a single framework that is up to the task of separately describing the effects of hydrophobic and ordering confinement on liquid water. It is this framework that will enable us to make sense of a variety of mixed confinement scenarios, involving both polar and hydrophobic surfaces, that are important to understanding various aspects of protein folding.

Solvent-mediated forces

Typically, when the hydrophobic effect is discussed in the context of protein folding, one of two different views is taken. At the most basic level, reference is often made to the tendency amphipathic molecules have to reduce how much hydrophobic surface area they expose to the solvent¹⁹. Many analytical models of protein folding meanwhile posit a pairwise attractive binding interaction that takes place between hydrophobic parts of a protein that come into contact with each other²⁰. While both of these approaches have contributed greatly to our understanding of macromolecular assembly, they do not address an additional aspect of solvation that may also affect how proteins fold, namely that both hydrophobic and hydrophilic surfaces can act on each other at a distance via the water confined between them.

That solvent can mediate effective forces between the surfaces that surround it is straightforward to demonstrate formally in the language of statistical mechanics. Any system involving confined water can be described in terms of a set of surface degrees of freedom $\mathbf{x}_I, \dots, \mathbf{x}_M$, a set of solvent degrees of freedom $\mathbf{r}_I, \dots, \mathbf{r}_N$, and a grand canonical Hamiltonian $\mu N - H(\{\mathbf{x}_i\}, \{\mathbf{r}_i\})$. If we make the reasonable assumption that the solvent reaches thermodynamic equilibrium on a timescale much faster than the one that will dominate the dynamics of the surfaces of interest, we can integrate out the solvent degrees of free and calculate a thermodynamically averaged effective force on the system:

$$\mathbf{F}(\{\mathbf{x}_i\}) = \frac{\sum_N \int d\mathbf{r} \exp[\beta\mu N - \beta H(\{\mathbf{x}_i\}, \{\mathbf{r}_i\})] (-\nabla_{\mathbf{x}} H)}{\exp[-\beta\Omega]}$$

where

$$\exp[-\beta\Omega] \equiv \sum_N \int d\mathbf{r} \exp[\beta\mu N - \beta H(\{\mathbf{x}_i\}, \{\mathbf{r}_i\})]$$

is the grand partition function. The important thing to recognize here is that for each surface configuration $\{\mathbf{x}_i\}$, in general, the statistical weights of all the different solvent microstates $\{\mathbf{r}_j\}$ will differ, with the result that the solvent contribution to the total grand potential of the system will vary depending on how the surfaces are arranged. In other words, at thermodynamic equilibrium, surfaces separated in space by some distance can communicate with each other, and the water between them acts as the messenger.

The most intuitive consequence of the existence of finite-range solvation forces is that hydrophobic surfaces are attracted to each other. Whether in the case of collapsing polymers²¹ or nanoseparated plates¹⁶, and both in simulation and experiment, there is evidence that hydrophobic surfaces separated by finite distances feel solvent-mediated forces pulling them together. The three-state lattice model we consider here illustrates this point very well: as Figure 2a shows, the grand potential of a water layer trapped between two hydrophobic walls drops as the walls come closer together, indicating that an attractive force acts between the walls even before the onset of a dewetting event that would cause them to snap sharply together at close range.

Less intuitive, but equally well-illustrated in the lattice model, is the *repulsive* force water mediates between polar and hydrophobic surfaces. Figure 2b demonstrates that, as a charged surface that orders water comes closer to a non-polar surface that is inert to water, the result is that a smaller number of lattice sites (i.e. a thinner water layer containing fewer molecules) becomes increasingly frustrated trying to respond to the influences of the two different surfaces. The thermodynamic work required to bring ordered water into a region that would otherwise tend to have depleted density makes tighter confinement unfavorable, and the two surfaces thus repel.

The repulsion mediated by water between hydrophobic and hydrophilic surfaces has been amply established in *in silico*. Molecular dynamics studies by both Bulone *et. al.*^{1,7} and Dzubiella and Hansen^{8,9} have demonstrated a solvation repulsion between charged and hydrophobic groups in a water bath. More recently, Vaitheeswaran and Thirumalai¹⁰ performed simulations of a methane molecule dissolved in a water nanodroplet and found that the initial hydrophobic attraction between the methane and the non-polar surface of the droplet was converted to a repulsion once the methane became sufficiently charged. Meanwhile, two studies from Garde and co-workers^{22,23} have identified a role for this repulsion in the experimental phenomenon long known to structural biologists as the “salting-out”²⁴ of partially hydrophobic solutes in the presence of certain ionic cosolutes. Taken together what all of these past works underline is the significance that the solvent-mediated forces described in our three-state lattice model can have in a variety of contexts.

Folding Under Confinement

One of the most famous contexts in which solvation forces play a crucial role is that of protein folding. It has long been known that the hydrophobic effect helps to stabilize many folded proteins’ native states by disfavoring denatured conformations that tend to expose more non-polar surface area to the aqueous medium¹⁹. From this perspective, the importance

of the hydrophobic effect lies largely in the effective intra-chain attraction it can produce between one part of a polypeptide and another.

Such an approach suffices for describing a protein that folds in so-called “infinite dilution,” where it does not have to contend with neighboring polypeptide chains or other nearby amphipathic surfaces. In order to describe folding *in vivo*, however, a more expansive view must be taken. It is typical for a protein in the cell to undergo its search of conformational space while crammed inside nano-sized cavities (e. g. the ribosome exit tunnel²⁵, the proteasome²⁶, or a chaperonin²⁷), bound in complex with a chaperone, or jostled by non-specific interactions with neighboring macromolecules in the extremely crowded cytosol²⁷. Thus, a complete description of protein folding in its natural context must account for the forces solvent mediates not only within the protein chain itself, but also between the protein and the various surfaces that surround it. A number of different simulations have recently been carried out in pursuit of such a description, and they have yielded a diverse range of results that reflect the new layer of subtlety that confinement brings to the process of folding.

Sorin and Pande²⁸ performed the first all-atom, explicit solvent simulation of confined folding in their 2006 *in silico* study of the conformational preferences of a helical peptide held inside a carbon nanotube. By using extensive sampling to obtain equilibrium helical tendencies for peptides in different sized nanotubes, they showed that, as the tube diameter decreased with water density held constant, the increased tightness of confinement drove the helix to unfold. This result ran strikingly counter to the predictions of models that assume the loss of polymeric entropy to be the dominant effect of confinement on folding. The authors explained the positive correlation between tightness of confinement and unfolding by accounting for the loss of *solvent* entropy associated with helix unfolding: as intra-chain hydrogen bonds break and more polar backbone patches are exposed, the waters in their vicinity become bound and experience a loss in translational freedom that is mitigated when the volume to which they are confined becomes smaller. More recently, Zhou²⁹ has proposed an alternative explanation, whereby local depletion of water from the hydrophobic surface of the nanotube leads to an elevation of water activity at the peptide surface, with the result that the equilibrium is tilted in favor of unfolding the helix so as to form more peptide-water hydrogen bonds.

In our approach to the question, the surface of the folded helix is assumed to be hydrophobic, since Sorin and Pande simulated a heavily alanine-rich helix-prone peptide. As Figure 3 shows, the explanations of Sorin and Zhou are not mutually exclusive, and each one fits into our theoretical framework of ordering and depletion. An *ad hoc* extension of our lattice model accounts for the scenario in which the local density of the liquid phase is allowed to vary, rather than being fixed at either one or zero molecules per lattice site. The interaction between a depleted site and its neighbor is assumed to be less strong than it would be for normal liquid state in proportion to the degree of depletion. Both when local density in the liquid is held constant (Fig. 3a) and when it is allowed to fluctuate (Fig. 3b), the solvent grand potential change associated with opening up the helix becomes increasingly favorable as the confined water layer narrows. Since the overall stability of a helix arises from the cooperative interaction of many hydrogen bonding sites along the

chain, it is quite reasonable to suppose that small, confinement-driven reductions in the favorability of the single-site solvent contribution to helix formation could lead to a pronounced decrease in the stability of helical conformations like the one observed by Sorin and Pande.

Interestingly, the lattice model we employ here suggests that Sorin and Pande might have observed the *opposite* affect if they had given their nanotubes open ends and allowed the water inside to relax at constant chemical potential. When the average density of the water inside the tube is allowed to fluctuate, the depletion of liquid from the cavity becomes more cooperative as the hydrophobic surfaces of the helix and the nanotube come closer together (Fig. 3c). Put another way, at constant chemical potential, the nanotube walls exert a solvent-mediated repulsion on the polar surface of non-helical conformations that drives the formation of a helix.

While studies of secondary structure under confinement are informative, the formation of tertiary folds follows a different logic. Whereas the “unfolded state” of a helix-forming peptide is *more* polar than the “folded” helical state, tertiary native structures generally expose less hydrophobic surface area to the surrounding solvent than their denatured conformations. As a result, one might expect confinement inside a hydrophobic cavity to have a different effect on the formation of a tertiary fold.

Lucent and Pande³⁰ confirmed this expectation when they simulated the folding of the villin headpiece in an explicit water nanodroplet. By measuring the effects of confinement on the probability of folding before unfolding in an ensemble of starting conformations, they were able to show that confinement inside a non-polar sphere disfavors folding by stabilizing a new competing ensemble of conformations that are adsorbed on the sphere surface. In addition, by also carrying out the same simulations under conditions where only the protein, and not the water molecules, was confined by the spherical potential well, they were able to show that this disruption of folding was the result of the confinement experienced by the solvent, which overwhelmed a countervailing drive in favor of folding that resulted from the decrease in the unfolded state’s polymeric entropy^{25,31}.

Figure 4 illustrates the adsorption process observed by Lucent and Pande within the three-state lattice model framework. Under infinite dilution, the folding of the protein to its native state would normally be accompanied by a decrease in the grand potential of the surrounding solvent. When confined inside a hydrophobic cavity, however, the protein both experiences an attraction to the cavity wall, and is also driven away from the folded state once bound there (Fig. 4a). As in the Lucent study, no such effect is observed in the case where only the protein, and not the solvent, is confined (Fig. 4b). Finally, It is important to note that we also expect from the model that the unfolding through adsorption reported by Lucent and Pande should also occur in an open hydrophobic cavity that is allowed to relax its number of water molecules in contact with an external bath (Fig. 4c).

This last, seemingly minor detail makes such an adsorption process highly relevant to the process of confined folding *in vivo*. One of the best-known and most important instances of confined folding in the cell is chaperonin-assisted folding²⁷. Chaperonins, such as the *E. coli*

chaperone protein Hsp60 (also known as GroEL), are barrel-shaped protein complexes that help their substrates to fold via an ATP-driven cycle of binding, encapsulation, and release. Since the GroEL cavity is permeable to water³², it corresponds in modeling terms to an open cavity, which we would expect to be capable of driving unfolding through adsorption. It has long been thought that GroEL accelerates folding in part by using its apical domains to help unfold non-native substrate conformations²⁷, and theoretical work has suggested that the hydrophobicity of the cavity surface when GroEL is in its “open” state could destabilize the folded state of a protein inside the cavity³³. Only recently, however, has experimental evidence from single-molecule FRET studies carried out by Hartl *et. al.*³⁴ demonstrated that, even prior to any conformational change in GroEL, interactions between the chaperonin and hydrophobic parts of the substrate promote unfolding. Thus, adsorption appears to be at least one way that solvent-mediated forces contribute to chaperonin-mediated folding in the cell.

In addition to being capable of *unfolding* substrates while in its hydrophobic “open” conformation, GroEL has also been shown to *promote* the folding of some substrates trapped inside the chaperonin’s “closed” complex³⁵. The crystal structure of GroEL in complex with its co-factor lid GroES shows that upon closing, the chaperonin undergoes a pronounced conformational change that projects many more charged and polar residues into the interior cavity³². Thus, in order to understand folding inside a closed GroE complex, it is appropriate to examine the impact of confinement inside a charged cavity on the hydrophobic effect that normally stabilizes the native state. Figure 5 plots the grand potential of folding inside a lattice model of a charged cavity. As a result of the preferential repulsion between the cavity surface and the more hydrophobic unfolded state of the protein, the folded state becomes more stable with tighter confinement.

The lattice model provides a possible explanation for chaperonin function first proposed in an earlier theoretical work by England and Pande³³. In corroboration of this idea, molecular dynamics simulations of methane-water mixtures trapped inside a charged cavity carried out by Xu and Mu³⁶ and have also suggested that the solvent-mediated repulsion between the hydrophobic methanes and the charged cavity surface can produce an effective attraction between the confined hydrophobic molecules much like the one posited by England and Pande. Most recently, England *et. al.*³⁷ carried out all-atom, explicit solvent simulations of a range of GroEL mutants whose effects on the folding rate of maltose binding protein had been previously assayed. In striking confirmation of the theoretical model, the authors found a strong correlation between the hydrophilicity of the simulated cavity mutants and the experimentally measured substrate folding rate. Thus, locally enhancing the hydrophobic effect using interior cavity hydrophilicity may be yet another way in which chaperonins manipulate the aqueous solvent to help their substrates to fold.

Limitations

By employing a simple theoretical framework based on lattice liquid models from statistical mechanics, we have seen how a few basic assumptions about the interactions between water and surfaces can suggest explanations for a diverse range of phenomena in the simulation literature, and also may yet help us to better understand the processes of protein folding and unfolding *in vivo*. That being said, it is important to remember that the assumptions of the

model ignore many properties of water that may be crucial to a full picture of solvation physics. The hydrogen bond network in real liquid water is difficult to represent fully with binary distinctions such as whether a molecule is present or absent, or whether it is ordered or liquid. Both in theory and simulation, past work has demonstrated that water near hydrophobic surfaces will not deplete in local density if it is able to avoid breaking hydrogen bonds near the surface by decreasing the entropy of the hydrogen bond network there³. As a result, whether a hydrophobic surface brings about a local decrease in density or an increase in order in the surrounding water depends on the curvature of the surface in question. We would therefore expect our lattice model to be inadequate for even qualitatively describing the solvation free energies of solutes comparable in size to a water molecule.

Charge effects add an additional layer of complexity to this story. While we have represented the principal impact of charge in our model to be the local order it causes in the nearby solvent, electrostatic forces are actually long-ranged in a dielectric continuum that lacks screening. Moreover, the presence of soluble ions that provide screening only further complicates matters, since solvent density fluctuations in this case become coupled to fluctuations in the long-range electrostatic forces exerted by one part of the system on another⁵. Finally, a more explicit treatment of charge is essential if the surfaces in the system are contrived to produce electric fields with a great deal of geometric symmetry to their variation in space, such as in the study of Vaitheeswaran and Rasaiah¹¹. In a more recent work by Lu and Berkowitz³⁸ of water structure between parallel hydrophilic plates whose arrangement of atoms were each other's charge mirror image, the forces between the plates mediated by the solvent could not have been predicted by a theory that simply asked whether or not water molecules near the plates were "ordered." Such an approach will always fail in situations where the local order caused by the charges on one plate has been deliberately designed to be incompatible with the arrangement of charges on the other.

What our theoretical approach loses in precision, however, it clearly gains back in adaptability. In a wide variety of scenarios involving confined folding or related issues in solvation physics, we have succeeded in offering explanations for observed outcomes based on one simple principle: that hydrophobic and charged surfaces prefer the water that surrounds them to organize itself in different ways, and the competition between these preferences leads to the emergence of forces that may play an important role in protein folding *in vivo*. It is our hope that applications of this simple framework in settings ranging from silica gels to the ribosome exit tunnel will lead to new insights about the stability of proteins in important contexts, both *in* and *ex vivo*.

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Appendix I: Confined Water Entropy

The plot in Figure 1c was generated from molecular dynamics simulations performed in GROMACS 3.3.1. A 3 nm by 3nm by 6 nm box of 1773 TIP4PEW water molecules was equilibrated using a Berendsen thermostat at 298 K, and all water molecules whose oxygen

atoms were within one of two 2 angstrom thick square regions 2 nm on a side of variable separation and normal to the z -axis were frozen in place. Subsequently, 16 ns trajectories were simulated, with snapshots taken every 10 ps. Solvent entropy at a location was calculated by counting all water molecules with oxygens in a 9 angstrom square grid 3 angstroms thick, and determining which quadrants each O–H bond in the molecule pointed towards. Orientational states i were defined uniquely based on what pair of quadrants were

occupied by an O–H vector. Entropy per molecule was calculated from $s = -\sum_i p_i \ln p_i$.

Appendix II: Thermodynamic Calculations

There is a certain amount of arbitrariness in choosing parameters for a coarse-grained, phenomenological model that one expects to yield qualitative descriptions of a wide variety of systems. Without the expectation of strong quantitative agreement, it is hard to insist on exact values for the couplings and chemical potentials in our model. This is especially the case because the orientational dependence of hydrogen bonds requires that our nearest-neighbor couplings in a lattice description be thought of as effective free energies of interaction whose exact calculation would involve summation over the internal orientational states of each occupied lattice site, as well as the computation of couplings between non-adjacent pairs of sites on the lattice. The relationships, however, between the different parameters in a nearest-neighbor, lattice approximation are constrained by findings in the literature. We began by assuming that the energy scale for the strength of the hydrogen bond should be set approximately by equating the surface tension of bulk water with the number of hydrogen bonds broken per unit area. Thus, with a surface tension γ of 72 milijoules per square centimeter³ and a molecular “area” A of 16 square angstroms (which would put each water molecule inside a lattice site 4 angstroms on a side), we posit that the nearest-neighbor

coupling in the liquid state should be given by $u_{\ell\ell} = \frac{4\gamma A}{6k_B T}$, where the multiplying factor of 4/6 reflects that roughly four hydrogen bonds are being made across the six faces of a three-dimensional cubic lattice. Thus, we find that at 300 degrees Kelvin, $u_{\ell\ell} \sim 2$.

The next step in the argument is to require that the liquid phase be stable, but near phase co-existence with the vapor phase³. In other words, on a thermal energy scale, the chemical potential drawing ℓ states out of the system must differ in magnitude from the binding energy $u_{\ell\ell}$ by an amount that is not large compared to unity. Thus, in our calculations, we chose $u_{\ell\ell} = 2.2$ and $\mu_{\ell} = -2$. In order to extend the model to include ordered states, we inferred from our empirical investigation of the entropy loss for ordered water trapped between polar surfaces in Figure 1b that the typical additional thermodynamic cost for moving from the liquid state to the ordered state should be on the order of $k_B T$. Thus, we set $\mu_r = -3$. From this point, what remained was to set the remaining couplings so that spontaneous ordering did not occur at equilibrium in the absence of already-ordered water, and so that the interaction between ordered and liquid states ($u_{r\ell}$) was more favorable than the interaction between either of the two states with the third, vacuum (e) state. These considerations led us to choose $u_{rr} = 2.8$ and $u_{rl} = 1$.

With a full set of parameters in hand, we performed our calculations for each scenario considered in this study by abstracting to a one-dimensional description in which the only relevant variables affecting the state of the confined volume are the tightness of confinement, and the character of the confining surfaces. As a result, we were able to evaluate thermodynamic potentials by numerically calculating the appropriate partition functions using the standard transfer matrix method¹⁷.

Figure 1.

The fraction ρ of confined lattice sites in a given state is plotted as a function of the separation L between two confining surfaces, whose locations are indicated in the diagrams by black vertical lines. **a** As two hydrophobic surfaces (represented by sites in e states) confine the water between them more tightly, a dewetting transition occurs in which the water density drops precipitously and the liquid phase gives way to a vapor. **b** In a

mathematically analogous transition, two polar surfaces (represented by sites in r states) bring about an ordering transition in the water confined between them as they come closer together. **c** The loss of orientational entropy for free water molecules near sheets of other waters held in fixed orientation is plotted for molecules near a single sheet (blue squares) and between two sheets (red circles). As the dotted line tracing twice the one-sheet curve's value demonstrates, the more pronounced drop in entropy at a larger distance from the sheet surface for water confined between two sheets is non-additive, indicating positive cooperativity between the sheets in ordering the water between them.

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Figure 2.

The grand potential Ω (which is minimized at equilibrium in grand canonical ensembles) for water trapped between two surfaces. As indicated in the top right of each panel, the temperature, volume, and chemical potential are held constant in this ensemble. **a** For two hydrophobic surfaces, the thermodynamic potential falls as the separation L drops, indicating a solvent-mediated attraction. **b** For a polar surface that orders water in its vicinity adjacent to a hydrophobic one, the potential rises with increased tightness of confinement, implying a repulsive force mediated by the solvent between hydrophobic and charged surfaces.

Figure 3.

In the process of helix folding, the helix surface transitions from a polar, water-ordering coil state to a more hydrophobic helical state that hides the polar peptide backbone from the solvent. **a** In a scenario with constant liquid density and negligible local density fluctuations, the free energy F is the potential that is minimized at equilibrium. The free energy change

F_f associated with folding becomes less favorable to folding as the helix is more tightly confined inside hydrophobic cavity such as a carbon nanotube (as was observed in the past molecular dynamics study of Sorin and Pande). **b** The destabilization of the helix through confinement is magnified when local density fluctuations are introduced. Fluctuations are allowed by assuming the total number of molecules per lattice site is 80% of total filling (pictured as gray-colored lattice sites). In this case, since we expect the partial depletion of density to collect on the available hydrophobic surfaces (i.e. on the nanotube surface for the coil state, and on both the peptide and the nanotube for the helical state) we fix the location of the water-depleted sites to be on the lattice sites at each bounding surface. As with constant local density, free energy of folding increases as confinement becomes more severe. **c** In contrast to the constant density ensemble, a nanotube with open ends whose global water density may fluctuate promotes folding through confinement. As the nanotube wall comes closer to the peptide surface, the solvent-mediated attraction between the hydrophobic helical state and the wall drives the formation of the helix.

Figure 4.

As a protein folds to its tertiary, native structure, it transitions from having a more hydrophobic surface in its unfolded state (pictured as a white square), to a more polar one in the folded state. Both in constant density (**a**) and grand canonical (**c**) ensembles, the confinement of the protein along with its solvent inside a hydrophobic cavity can make folding less favorable by stabilizing a new adsorbed state that excludes water from the hydrophobic cavity surface while exposing polar groups to the solvent (depicted as a half-white square that displays hydrophobic surface area to the cavity wall and polar surface area to the solvent). The exact relative stabilities of the adsorbed and folded states, (i.e. which state is favored at equilibrium) must depend on details of the two conformational ensembles that should vary depending on the protein. In a scenario where only the protein, and not the solvent, is confined (**b**), the folding protein has no way of “seeing” the surface (indicated by a vertical dotted line) and the solvent free energy is no different than it would be in bulk.

Figure 5.

When a protein folds inside a polar cavity, such as a closed chaperonin barrel, the cavity surface exerts a preferential, solvent-mediated repulsion on the hydrophobic unfolded state. As a result, tighter confinement inside the cavity makes the grand potential change associated with folding more negative, thus favoring acquisition of the native state.