

Charge, hydrophobicity, and confined water: putting past simulations into a simple theoretical framework¹

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Abstract: Water permeates all life, and mediates forces that are essential to the process of macromolecular self-assembly. Predicting these forces in a given biological context is challenging, since water organizes itself differently next to charged and hydrophobic surfaces, both of which are typically at play on the nanoscale in vivo. In this work, we present a simple statistical mechanical model for the forces water mediates between different confining surfaces, and demonstrate that the model qualitatively unifies a wide range of phenomena known in the simulation literature, including several cases of protein folding under confinement.

Key words: confinement, protein folding, water, hydrophobic effect, Potts model.

Résumé : L'eau baigne toute vie et sert d'intermédiaire aux forces essentielles au processus d'autoassemblage macromoléculaire. C'est un véritable défi que de prédire ces forces dans un contexte biologique donné car l'eau s'organise différemment à proximité de surfaces chargées et hydrophobes, les deux étant typiquement sollicitées à l'échelle nanométrique in vivo. Dans ce travail, nous présentons un modèle statistique mécanique simple des forces réglées par l'intermédiaire de l'eau entre différentes surfaces de confinement, et nous démontrons que le modèle unifie quantitativement un vaste spectre de phénomènes connus dans la littérature sur la simulation, y compris plusieurs cas de repliement des protéines en condition de confinement.

Mots-clés : confinement, repliement des protéines, effet hydrophobe, modèle de Potts.

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Introduction

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. Moreover, as biophysics shifts its focus from the in vitro behavior of proteins to their properties in the cellular environment, questions about the nature of water in the crowded cytosol become highly relevant to protein thermodynamics and kinetics. The subtlety of physics in the aqueous medium arises from the dual nature of the water molecule itself: 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 charges (Dzubiella and Hansen 2004); however, water's unrivaled ability to participate in a highly coordinated network of strong hydrogen bonds (Eaves et al. 2005) makes the free energy cost of solvating non-polar surfaces especially high, leading to the so-called hydrophobic effect (Chandler 2005).

Owing to the complexities of water and the challenges of experimental tests of the nature of water, it is natural to see what physical chemistry theory and simulation can do to shed light on these issues. 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 (Roux and Simonson 1999; Simonson 2001). 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 (Chandler 2005; Hummer et al. 1996).

A number of studies in the simulation literature focusing on confined water challenge both of these theoretical approaches (Dzubiella and Hansen 2004; Bulone et al. 1997;

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Bulone et al. 2000; Dzubiella and Hansen 2003; Vaitheeswaran and Thirumalai 2006; Vaitheeswaran et al. 2005). 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.

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. (2005) 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 polarized (Landau et al. 1993). 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 researchers (Bratko et al. 2007), 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. (2008a) 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 in a certain range of chemical potentials for the water bath, but that this transition would become slight and unnoticeable for a bath whose chemical potential too strongly favored a stable liquid phase between the plates. 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 molecular dynamics 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 (Lee and Yang 1952). 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. More recently, an augmented lattice-field theory of hydrophobic interactions was developed by Lum et al. (1999) that makes quantitative treatment of phenomena like hydrophobic dewetting possible. Here, it is our aim to follow in the traditions of these models 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, and tumbling freely so as to lower the free energy of its interaction with neighboring water molecules in the liquid phase. This leads us to posit a grand canonical lattice liquid model (Lee and Yang 1952) 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 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 Hamiltonian for a single site i and its nearest neighbors j as $H = -\frac{1}{2}\vec{s}_i \cdot (\mathbf{u}\vec{s}_j)$ and the grand canonical Hamiltonian 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 $u_{rr} \sim u_{ll} > u_{rl} > 0$, 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 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.

In this paper, we propose a framework and, in light of it, examine several recent molecular dynamics studies of mixed solvation scenarios. We show that the model reproduces basic phenomena expected from past simulations of water confined between polar and hydrophobic surfaces, and 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.

Materials and methods

Confined water entropy

The plot in Fig. 1c was generated from molecular dynam-

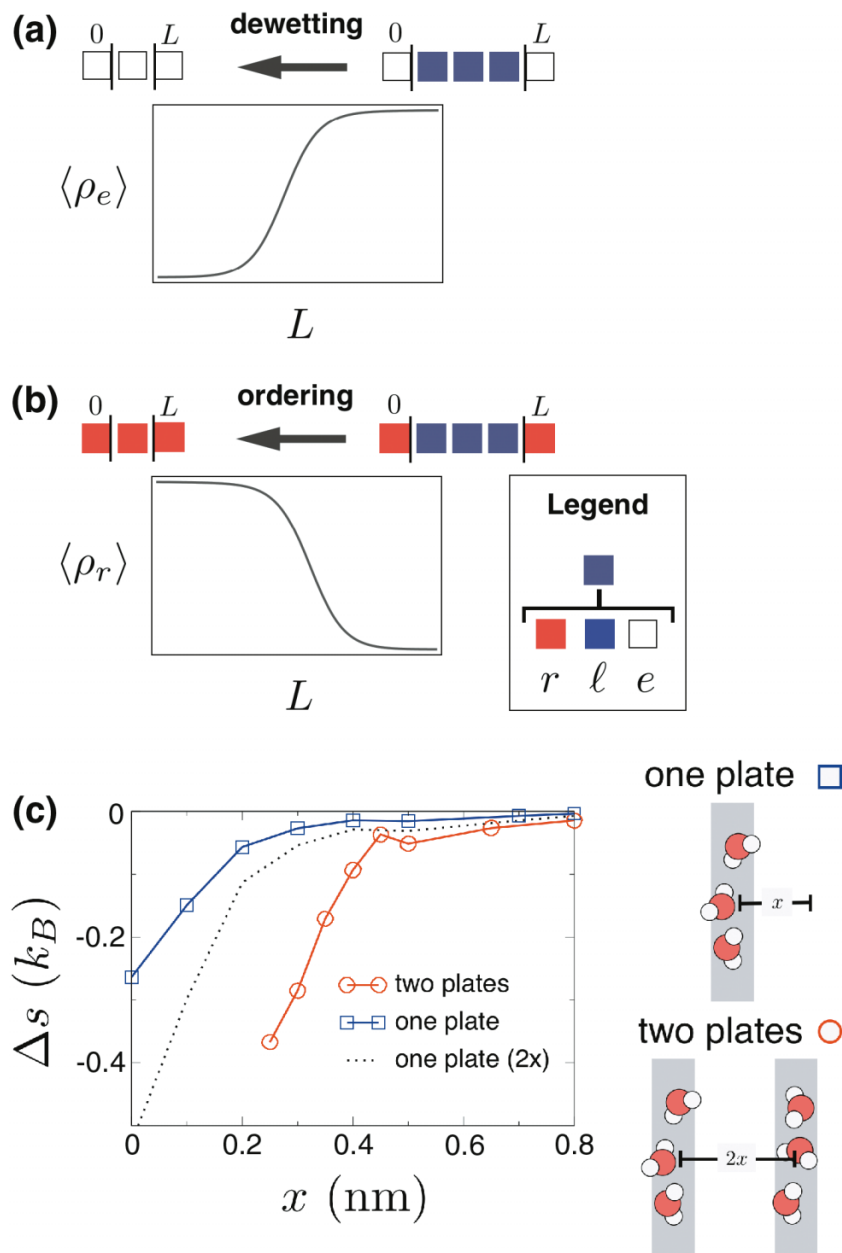
ics simulations performed in GROMACS 3.3.1 using the AMBER99 force-field and leap-frog integration (Leach 1996). A 3 nm \times 3 nm \times 6 nm box of 1773 TIP4PEW water molecules was equilibrated using a Berendsen thermostat at 298 K and particle-mesh Ewald electrostatics (Ibid.). All water molecules whose oxygen atoms were within one of two 2 Å 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 Å square grid 3 Å thick, and determining towards which quadrants each O-H bond in the molecule pointed. 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 $s = -\sum_i p_i \ln p_i$.

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 centimetre (Chandler 2005) and a molecular area A of 16 square angstroms (which would put each water molecule inside a lattice site 4 Å 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 4 hydrogen bonds are being made across the 6 faces of a three-dimensional cubic lattice. Thus, we find that at 300 K, $u_{\ell\ell} \sim 2$.

The next step in the argument is to require that the liquid phase be stable, but near phase co-existence with the vapor phase (Chandler 2005). 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_{ll} by an amount that is not large compared with unity. Thus, in our calculations, we chose $u_{ll} = 2.2$ and $\mu_l = -2$. 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 Fig. 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

Fig. 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 (squares) and between two sheets (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.



did not occur at equilibrium in the absence of already-ordered water, and so that the interaction between ordered and liquid states (u_{rl}) was more favorable than the interaction between either of the two states with the third, vacuum state (e). 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 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 method (Pathria 1996). The plotted values for surface separations not equal to an integral number of lattice spacings were

computed by raising the eigenvalues of the transfer matrix to the corresponding fractional power.

We stress that although we have given precise values presented here for the parameters of our model used in the examples in this work, the theory is sufficiently robust that the significance of the results in a qualitative sense are not tied to the specific values quoted here.

Results

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 vapor (Chandler 2005). 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 dewetting (Wallqvist and Berne 1995).

The 3-state lattice model employed here was, in fact, deliberately constructed to describe dewetting. In the absence of any external fields producing a tendency towards the formation of r states, the 3-state model effectively reduces to a 2-state lattice liquid model (which is exactly equivalent to the Ising model (Pathria 1996)) that has previously been used to study capillary evaporation (Lum and Luzar 1997). As a result, it is unsurprising that the 3-state approach readily captures the discrete drop in liquid density (and the correlated transient spike in compressibility) when the tightness of confinement between hydrophobic surfaces reaches a critical level (Fig. 1a).

What the 3-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 (Figs. 1b and 1c). The mathematical analogy arises because in both scenarios, the lattice sites in the system are restricted to 2 of the 3 available states, and are therefore constrained to explore Ising model-like subspaces of their total space of microstates. As Fig. 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 entropy, much like the drop in density observed in the dewetting scenario. This phenomenon is captured qualitatively by the behavior of the 3-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, in-

volving 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 of amphipathic molecules to reduce how much hydrophobic surface area they expose to the solvent (Spolar et al. 1989). 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 other (Pande et al. 2000). 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 $\mathbf{x}_1, \dots, \mathbf{x}_M$, a set of solvent degrees of freedom $\mathbf{r}_1, \dots, \mathbf{r}_N$, 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 freedom and calculate a thermodynamically averaged effective force on the system:

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

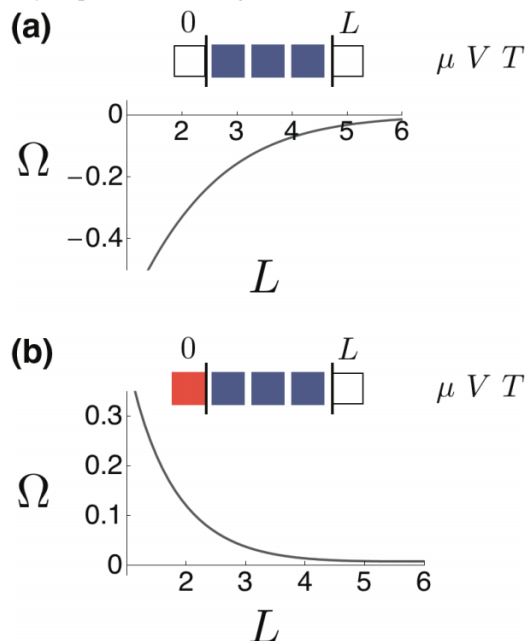
where

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

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 $\{\mathbf{r}_i\}$ 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 polymers (Miller et al. 2007) or nanoseparated plates (Wallqvist and Berne 1995), and both in simulation and experiment, there is evidence that hydrophobic surfaces separated by finite distances feel solvent-mediated forces pulling them together. The 3-state lattice model we consider here illustrates this point very well: as Fig. 2a shows, the grand potential of a water layer trapped between 2 hydrophobic walls drops as the walls come closer together, indi-

Fig. 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 2 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.



cating 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 inert to water, 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. (1997, 2000) and Dzubiella and Hansen (2003, 2004) have demonstrated a solvation repulsion between charged and hydrophobic groups in a water bath. More recently, Vaitheeswaran and Thirumalai (2006) 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 (Kalra et al. 2001; Ghosh et al. 2005) have identified a role

for this repulsion in the experimental phenomenon long known to structural biologists as the salting-out (Cacace et al. 1997) of partially hydrophobic solutes in the presence of certain ionic cosolutes. Taken together, these past works underline the significance that the solvent-mediated forces described in our 3-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 medium (Spolar et al. 1989). 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. 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 tunnel (Ziv et al. 2005), the proteasome (Baumeister et al. 1998), or a chaperonin (Hartl and Hayer-Hartl 2002)), bound in complex with a chaperone, or jostled by non-specific interactions with neighboring macromolecules in the extremely crowded cytosol (Ibid). 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 Pande (2006) performed the first all-atom, explicit solvent simulation of confined folding in their *in silico* study of the conformational preferences of a helical peptide held inside a carbon nanotube. 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, Zhou (2007) 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 Fig. 3 shows, the explanations of Sorin and Pande (2006) and Zhou (2007) 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.

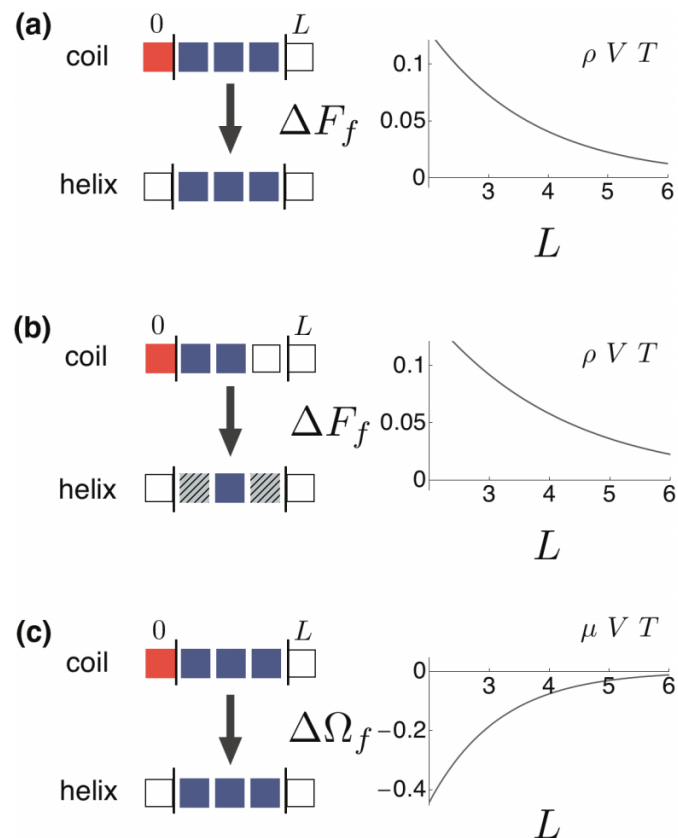
Interestingly, the lattice model we employ here suggests that Sorin and Pande might have observed the opposite affect if they had given their nanotubes open ends and allowed the water inside to relax at constant chemical potential. When the average density of the water inside the tube is allowed to fluctuate, 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 et al. (2007) 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

Fig. 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.

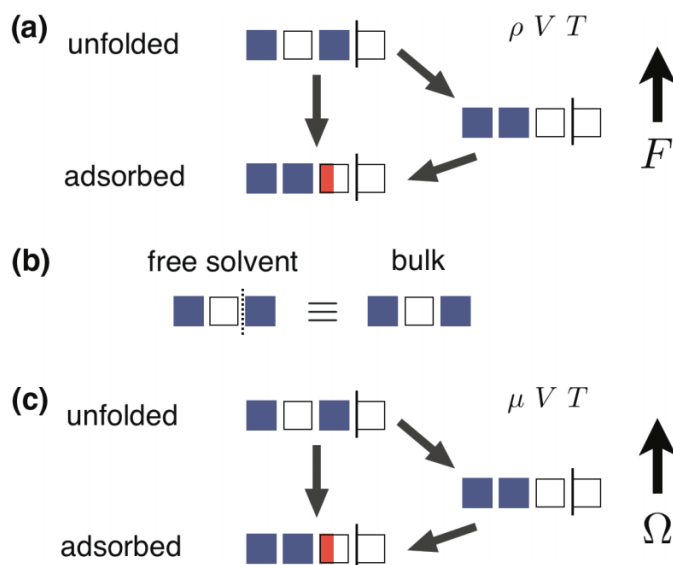
(a) In a scenario with constant liquid density and negligible local density fluctuations, the free energy F is the potential that is minimized at equilibrium. The free energy change ΔF_f associated with folding becomes less favorable to folding as the helix is more tightly confined inside a hydrophobic 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 unaffected when local density fluctuations are introduced. Fluctuations are allowed by assuming the total number of molecules per lattice site is 80% of total filling (indicated with crosshatching). 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.



the unfolded state's polymeric entropy (Ziv et. al 2005; Zhou and Dill 2001).

Figure 4 illustrates the adsorption process observed by Lucent and Pande within the 3-state lattice model framework. Under infinite dilution, the folding of the protein to its native state would normally be accompanied by a

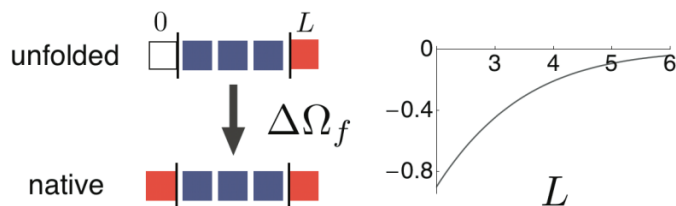
Fig. 4. As a protein folds to its tertiary, native structure, it transitions from having a more hydrophobic surface in its unfolded state (depicted as a white square), to a more polar one in the folded state (depicted as a white square). Both in constant density (*a*) and grand canonical (*c*) ensembles, 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.



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 folding (Hartl and Hayer-Hartl 2002). 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 water (Braig et al. 1994), 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

Fig. 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.



thought that GroEL accelerates folding in part by using its apical domains to help unfold non-native substrate conformations (Hartl and Hayer-Hartl 2002), 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 cavity (England and Pande 2008). Only recently, however, has experimental evidence from single-molecule FRET studies carried out by Sharma et al. (2008) 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 complex (Tang et al. 2006). 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 cavity (Braig et al. 1994). Thus, 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 Pande (2008). In corroboration of this idea, molecular dynamics simulations of methane–water mixtures trapped inside a charged cavity carried out by Xu and Mu (2008) 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. (2008b) 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 hydrophi-

licity 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.

Discussion

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. Both in theory and simulation, past work has demonstrated that 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 with a water molecule.

The failure of our model to handle microscopic solutes of high surface curvature is connected to the simplicity of its treatment of density fluctuations. In a 3-state lattice-liquid description of the aqueous environment, each lattice site is either fully occupied by a molecule, or empty. In fact, real water is capable of subtler modulations of its local density that have to do with the organization of the hydrogen bond network, which means that it can undergo decreases in local free energy density next to a hydrophobic surface without decreasing the local particle density. As a result, several independent studies (Mittal and Hummer 2008; Godawat et al. 2009; Sarupria and Garde 2009; Willard and Chandler 2009) have established that in many scenarios where water density near a hydrophobic surface does not appear to decrease, the signature of the unfavorability of solvating the surface can still be seen in the elevated density fluctuations in the interfacial region. Our model, on the other hand, lumps all unfavorability of being near a hydrophobic surface into an enthalpic loss of binding opportunities, which means that any destabilization of the interface is registered in a local depletion of density. The importance of fluctuations, as opposed to depletion, to the nature of hydrophobicity suggests an augmentation of our model, in which the presence of a hydrophobic surface is allowed to weaken site-to-site attractive coupling u_{ij} , thereby bringing about an increase in local compressibility.

Charge effects add an additional layer of complexity to this story. Although 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-range 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 the spatial distribution of ions, which in turn affects the long-range electrostatic forces exerted by one part of the system on an-

other (Simonson 2001). Finally, a more explicit treatment of charge is essential if the surfaces in the system are contrived to produce strong electric fields that vary slowly along one or more coordinate axes, such as in the study of Vaitheeswaran et al. (2005). In a more recent work by Lu and Berkowitz (2006) 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; 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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