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Single Polymer Studies of Hydrophobic Hydration

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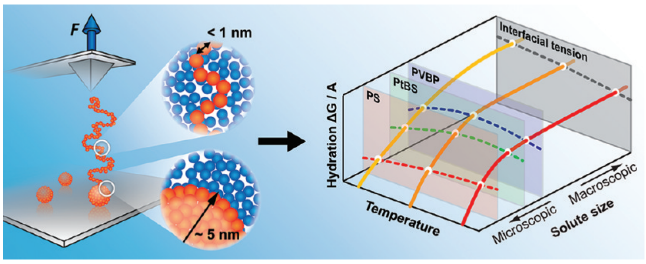

Hydrophobic interactions guide protein folding, multidomain protein assembly, receptor–ligand binding, membrane formation, and cellular transportation. On the macroscale, hydrophobic interactions consist of the aggregation of "oil-like" objects in water by minimizing the interfacial energy. However, studies of the hydration behavior of small hydrophobic molecules have shown that the microscopic (~1 nm) hydration mechanism differs fundamentally from its macroscopic counterpart. Theoretical studies over the last two decades have pointed to an intricate dependence of molecular hydration mechanisms on the length scale. The microscopic-to-macroscopic crossover length scale is critically important to hydrophobic interactions in polymers, proteins, and other macromolecules. Accurate experimental determination of hydration mechanisms and interaction strengths directly influence our understanding of protein folding.

In this Account, we discuss our recent measurements of the hydration energies of single hydrophobic homopolymers as they unfold. We describe in detail our single molecule force spectroscopy technique, the interpretation of the single polymer force curve, and how it relates to the hydration free energy of a hydrophobic polymer. Specifically, we show how temperature, side-chain sizes and solvent conditions, affect the driving force of hydrophobic collapse.

The experiments reveal that the size of the nonpolar polymer side-chains changes the thermal signatures of hydration. The sizes of the polymer side-chains bridge the length scale where theories had predicted a transition between entropically driven microscopic hydration and enthalpically driven macroscopic hydrophobic hydration. Our experimental results revealed a crossover length scale of approximately 1 nm, similar to the results from recent theoretical studies. Experiments that probe solvent dependency show that the microscopic polymer hydration is correlated with macroscopic interfacial tension. Consistent with theoretical predictions, the solvent conditions affect the microscopic and macroscopic hydrophobic strengths in similar ways.

Although the extended polymers and proteins span hundreds of nanometers, the experiments show that their hydration behavior is determined by the size of a single hydrophobic monomer. As the hydrophobic particle size decreases from the macroscopic to the microscopic regime, the scaling relationship changes from a dependence on interfacial area to a dependence on volume. Therefore, under these conditions, the driving force for the aggregation of hydrophobic molecules is reduced, which has significant implications for the strength of hydrophobic interactions in molecular systems, particularly in protein folding.

Introduction

Water actively mediates the interactions between biological molecules on the microscopic scale. In particular, the hydrophobic interaction is considered a fundamental determinant of the self-assembly of biological macromolecules into ordered structures such as protein folding.[1−3] During folding, the nonpolar amino acids induce the hydrophobic collapse of proteins into compact structures. Simulations indicate that the sequence of hydrophobic residues in a peptide alone can encode the secondary and tertiary structure of a protein.[4] Therefore, understanding molecular hydrophobic interactions in polymers and proteins is vital to understanding protein folding and achieving better engineering controls in macromolecular systems.

The thermodynamics of hydrophobic interactions are subtle, both to model and measure. At equilibrium, the free energy of hydrophobic interaction is the difference between the hydration free energies of the systems before and after the interaction. Therefore, the hydrophobic interaction strength

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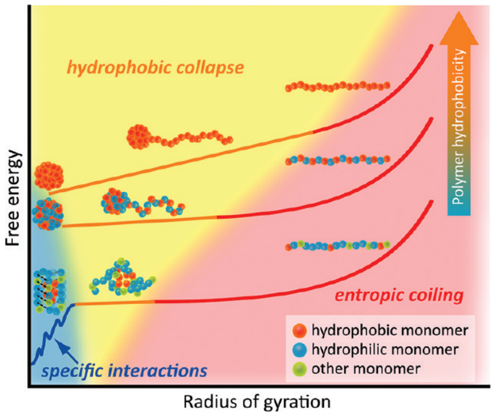

FIGURE 1. A hydrophobic polymer provides a model for hydrophobic collapse. From top to bottom, in order of decreasing hydrophobicity, are a homopolymer, a copolymer, and a protein. Starting from an extended conformation in water, chains coil under entropic elastic driving force (red). When sufficiently relaxed, hydrophobic collapse (yellow) occurs and reduces the size of the collapsed globule. Hydrophobic collapse in less hydrophobic copolymers and proteins occurs at later stages due to lower hydrophobic driving forces. For proteins, specific interactions (blue) plus the formation of secondary structures bring the protein to an energy minimum with a more compact structure. Hydrophobic polymers do not have such specific interactions and collapse to compact, random coils.

relies on accurate assessment of the hydration behavior of hydrophobic species (hence the oxymoronic term "hydrophobic hydration"). Significant theoretical efforts have advanced the understanding of hydrophobic hydration from microscopic to macroscopic length scales (see reviews in refs 1 and 5–13). A natural question is this: what are the length-scales operating in polymers and proteins? The length of extended polymers is macroscopic (hundreds of nanometers), the side-chains are microscopic (subnanometer), and the folded structure is somewhere in between. Theoretical studies on polymer hydration have been sparse[14–17] as have been direct experimental studies.[18–22] Although hydrophobic interactions in proteins can be studied by amino acid substitutions, the interactions are usually complicated by their innately complex intramolecular interactions. Furthermore, whether the hydrophobic interior of a protein can be treated as a simple nonpolar solvent is still debated, making it inappropriate to directly apply traditional amino acid transfer free energy obtained between water and organic solvents. Therefore, direct experimental measurement of polymer unfolding is needed to determine the energy of hydrophobic collapse. Hydrophobic homopolymers are good candidates for studying hydrophobic interactions and hydrophobic collapse in proteins due to their chemical homogeneity and the abundance of theoretical predictions (Figure 1). However, the polymers' insolubility in water has been an obstacle to experiments.

Single molecule force spectroscopy (SMFS) of homopolymers has allowed us to measure directly their hydrophobic hydration properties in controlled environments. The results are that these polymers, despite being macromolecules, do not hydrate via macroscopic interfacial thermodynamics. On the contrary, their hydration behaviors closely resemble those of small molecules, suggesting that the hydration of individual monomers along the chain governs the hydration thermodynamics. This Account begins by briefly introducing the theoretical background and recent advances. Thereafter, we summarize our recent single molecule experiments and their significance.

Macroscopic Interfacial Thermodynamics

From the aggregation of oil in water to the lotus effect, the classic hydrophobic effect that is dominated by macroscopic interfacial thermodynamics where the minimization of the unfavorable total interfacial free energy G is accomplished by reduction of interfacial area A.

$$\Delta G = \gamma \Delta A \tag{1}$$

The phenomenological model assumes a linear relationship between the free energy and the interfacial area, where the interfacial tension γ is the scaling factor. The interfacial tension can be calculated from the surface tension of solvent $\gamma_{\mathrm{solvent}}$, solute γ_{solute}, and the work of adhesion W_{ad} describing the attractive interactions:

$$\gamma = \gamma_{\mathrm{solute}} + \gamma_{\mathrm{solvent}} - W_{\mathrm{ad}} \tag{2}$$

The work of adhesion arises from contributions from the dispersive and polar components of the solvent and solute surface tensions, according to extended Fowkes equation:[23]

$$W_{\mathrm{ad}} = 2\left(\sqrt{\gamma^{\mathrm{d}}_{\mathrm{solute}}\gamma^{\mathrm{d}}_{\mathrm{solvent}}} + \sqrt{\gamma^{\mathrm{p}}_{\mathrm{solute}}\gamma^{\mathrm{p}}_{\mathrm{solvent}}}\right) \tag{3}$$

$$\gamma = \gamma^{\mathrm{d}} + \gamma^{\mathrm{p}} \tag{4}$$

For a nonpolar solute, the dispersion component is dominant ($\gamma^{\mathrm{d}} \gg \gamma^{\mathrm{p}}$); for water, the dispersive and polar components have similar magnitudes. We derived eq 5 by splitting the γ^{d} and γ^{p} components of solute and solvent in eq 2. The mismatch (eq 5) of dispersive and polar components between the solute and solvent gives rise to large unfavorable interfacial tensions, thereby creating the solvophobic effect.

$$\gamma_{\mathrm{interface}} = \left(\sqrt{\gamma^{\mathrm{d}}_{\mathrm{solute}}} - \sqrt{\gamma^{\mathrm{d}}_{\mathrm{solvent}}}\right)^2 + \left(\sqrt{\gamma^{\mathrm{p}}_{\mathrm{solute}}} - \sqrt{\gamma^{\mathrm{p}}_{\mathrm{solvent}}}\right)^2 \tag{5}$$

This relationship describes the chemical basis of unfavorable interfacial energy. Although the effective interfacial tension is influenced by the interfacial curvature on the scale of the Tolman length, as we shall discuss later, a smooth macroscopic interface can be effectively treated as planar. In this phenomenological framework, interfacial tension is a measure of the chemical compatibility between the solvent and solute, implying that the hydrophobic effect is essentially a solvophobic effect in water. However, the unusual physical properties of water and the anomalous behavior of hydration thermodynamics of small nonpolar molecules indicate that water is significantly different from other solvents and that the phenomenological model does not apply for such molecules.

At what length does a macroscopic interfacial description begin to fail? Variations in the definition of solute–water interface location by a few angstroms have little influence on the hydration energy of macroscopic objects, but have a large effect on the interfacial area of nanometer sized solutes. However, interface definition on the microscopic scale is fuzzy: a methane-sized particle has a solvent-accessible surface area (SASA) defined by a sphere of 3.3 Å radius, whereas the van der Vaal surface is defined by a sphere of 1.9 Å,[5] resulting in an area 3 times smaller. Ashbaugh and Pratt suggested an optimal surface between the hard-sphere surface and the solvent-accessible surface that makes surface tension size independent.[5] However, whether this definition is generally applicable to complex geometries and surface chemistry is yet to be investigated. Wagoner and Baker investigated the proportionality between the hydration free energy and the SASA on molecular length scale and found that the SASA failed to discriminate the different conformation states of nonpolar solutes, which leads to inaccurate assessment of the hydrophobic interaction strength.[24] As we shall outline below, the hydration free energy at small length scale is better described by a volume scaling relationship.

Anomalies of Small Molecule Hydration

In 1979, Tanford showed that macroscopic interfacial tension failed to explain the significantly lower hydration ΔG of small nonpolar molecules such as methane.[25] However, the discrepancy cannot be accounted for by redefining a smaller molecular surface. The temperature dependence of small molecule hydration ΔG is qualitatively different than that for macroscopic interfacial hydration, which suggests different hydration mechanisms. The macroscopic interfacial tension between water and a nonpolar solute monotonically decreases as temperature increases (Figure 2a) due to the large positive hydration entropy. The hydration ΔG of a small nonpolar molecule increases with temperature to a maximum and then decreases (Figure 2b), distinguishing it from macroscopic hydration. For small molecules, the increasing hydration ΔG is associated with negative hydration entropy, $S = -(\mathrm{d}G/\mathrm{d}T)_p$, which has traditionally been associated with formation of ordered clathrate-like water structure around small nonpolar solutes. In contrast, the temperature dependence of surface tension leads to positive hydration entropy upon forming a macroscopic interface. Experiments later found that although water molecules in the hydration shell have reduced conformational freedom, they do not form rigid clathrate structures.[26–29] Above the turnover temperature, the hydration entropy becomes positive, resembling macroscopic hydration. The turnover temperature also strongly depends on the solute size; increasing the particle size lowers the turnover temperature. This anomalous temperature and size dependence signify hydrophobic hydration for small molecules. Theories predict a transition of the hydration between molecular to macroscopic scales.

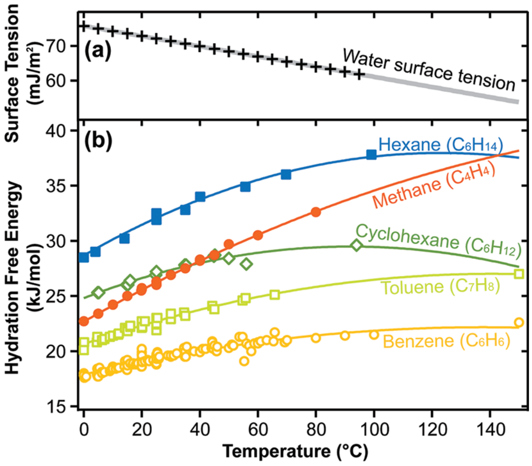

FIGURE 2. Temperature dependencies of (a) the surface tension of pure water and (b) the molar excess hydration ΔG of small molecules calculated by $\Delta G = -k_B \ln(x)$, where x is the solubility in mole fraction.[30] The small molecule data are color-coded by their relative molecular volume: methane < benzene < toluene ≈ cyclohexane < hexane.

Theoretical Treatments of a Hydrophobic Size Effect

Small molecule hydration anomalies have been the focus of significant theoretical efforts including information theory,[11,31] a revised scaled particle theory,[5] and the Lum–Chandler–Weeks theory of hydrophobicity.[6,32,33]

An early attempt to explain the lower hydration ΔG of small molecules applied a size-dependent curvature correction to the surface tension of water, giving rise to the Tolman length.[34] However, it failed to explain the temperature dependence of hydration ΔG. The classic, scaled particle theory (SPT) also failed to predict this temperature dependence because it incorporated few molecular details of water. A revised SPT[35] incorporating experimental water structural information was able to cover length scales from molecular surface tension to the macroscopic surface tension[5,36] and reproduce the turnover behavior of small molecule hydration ΔG and its size dependence, as well as the entropy convergence in small molecule hydrophobic hydration.[5] The Tolman length in the revised SPT has a temperature dependence that decreases from positive to negative, making it difficult to assign its physical meaning. For a methane-sized spherical solute, the predicted negative hydration entropy agrees with experimental results while the largely positive hydration enthalpy is the opposite of negative experimental values. In addition, the turnover temperature of an inserted particle is lower than found experimentally. These discrepancies were attributed to the lack of solvent–solute attractions in the model.[5]

Hummer and co-workers calculated the excess chemical potential μ^{ex} of cavitation using an information theory (IT) approach.[11,31] The probability density of observing water-free volumes of different sizes was used to calculate μ^{ex}. The authors showed that the prediction from IT matches the results from the test particle insertion method. Using this theory, Garde and co-workers predicted negative hydration entropies for small nonpolar molecules and the temperature dependence of their hydration ΔG. IT offered a molecular explanation for the experimentally observed convergence of entropy for small molecules. Furthermore, IT indicated that a Gaussian distribution of the density fluctuations of water is sufficient to describe hydration phenomenon,[31] which supported a Gaussian field theory.[32]

Lum, Chandler, and Weeks (LCW) developed this quantitative Gaussian mean-field theory that describes the size dependence of hydrophobicity from microscopic to macroscopic scale.[32] The unit area hydration ΔG increases linearly with solute size up to ~1 nm and asymptotically approaches the macroscopic interfacial tension as the particle size continues to increase (Figure 3a). The linear increase below 1 nm indicates an apparent volume dependence of hydration ΔG, in agreement with the lower than SASA-predicted hydration ΔG of small nonpolar solutes. The driving force behind microscopic hydration thermodynamics in the volume-dependent regime is mainly entropic, which gradually transitions into mainly enthalpic when solute size increases to the area-dependent macroscopic regime.[37] Furthermore, Huang and Chandler demonstrated that the thermal signature of hydrophobic hydration can be reproduced using the LCW theory, which is also strongly size dependent (Figure 3b).[33] The temperature at hydration ΔG turnover decreases as the particle size increases. For particles larger than 1 nm, a monotonically decreasing hydration ΔG is observed, consistent with macroscopic surface tension. By studying the density fluctuations of water near hydrophobic particles using the LCW theory, the Garde group has made significant advances in understanding hydrophobicity in polymers[16] and at interfaces.[7] In particular, they showed that the hydration ΔG of a homopolymer with 25 methane-sized repeats also exhibits turnover behavior similar to that of small molecules.[16]

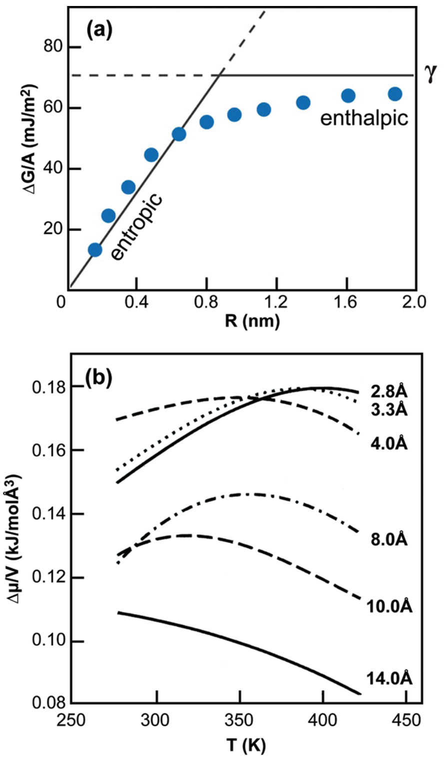

FIGURE 3. Theoretical predictions of free energy as a function of size and temperature. (a) LCW theory prediction of hydration ΔG per unit area as a function of solute radius (adapted from Chandler[6]). (b) Temperature dependence of hydration ΔG for various particle sizes (adapted from Huang and Chandler[33]).

Theories have assumed that the hydration ΔG of small molecules computed from their solubility data can be used to calculate macromolecular thermodynamics. Hydrophobic homopolymers with hundreds of repeating units are insoluble, which has previously necessitated this assumption. Experiments that force solvation can provide measurements of polymer hydrophobicity and address the following questions: Do extended homopolymers hydrate like macroscopic objects (hundreds of nanometers along the chain) or small molecules (subnanometer across the chain)? How do size, temperature, and solvent affect the hydration behavior in polymer systems?

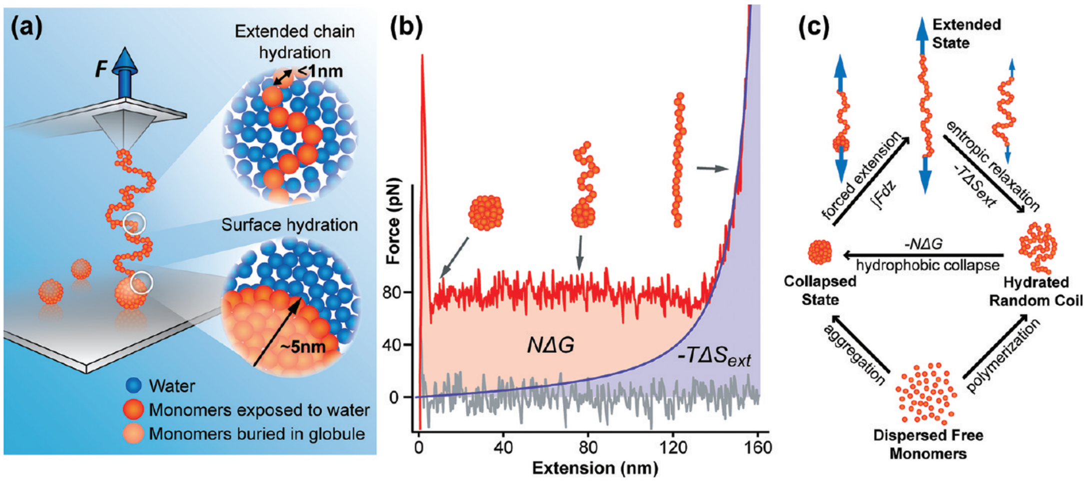

FIGURE 4. Mechanical unfolding of a single hydrophobic polymer in water. (a) Force exerted by an AFM cantilever drives the globule-to-coil transition of a single hydrophobic polymer. The insets illustrate the relative length scales associated with the hydrophobic hydration of the collapsed globule and the extended coil. (b) Single PS force curve (red) showing a force plateau followed by entropic elastic stretching and the base line (gray). The entropic elastic stretching portion is fit by the WLC model (blue). (c) Thermodynamic relationships between polymers and free monomers in various conformational states.

Hydrophobic Collapse and a Coil–Globule Transition

A polymer undergoes a coil–globule phase transition from random coil in a good solvent to compact globule in a poor solvent. In a poor solvent such as water, the polymer–polymer interaction is preferred over polymer–solvent interaction, causing the chain to collapse into a compact globule. Due to their simplicity, hydrophobic homopolymers have been used for theoretical investigation of hydrophobic collapse.[14–16,38–41] In the presence of an external force, three stages during the pulling of a hydrophobic homopolymer in poor solvent were predicted by theory.[39] In the first stage, a force below a threshold value distorts the spherical collapsed globule into an ellipsoidal shape. As pulling continues, the threshold force is reached and the polymer undergoes a first-order phase transition that dissolves the collapsed globule into an extended coil until the chain is fully exposed to the solvent. The force curve during this stage exhibits a plateau where the collapsed and extended states coexist in a dynamic equilibrium. The last stage is marked by an entropic elastic response of the chain similar to pulling a polymer in good solvent, which can be described by the wormlike chain (WLC) model. The mechanical pulling of polymers in poor solvent has been extensively investigated using scaling theory,[39] self-consistent field theory,[41] lattice models,[40] and molecular dynamics simulations.[14,38]

Single Molecule Force Spectroscopy on Hydrophobic Polymer Reveals Hydration Free Energy

Single molecule force spectroscopy utilizes the force resolution and spatial manipulating power of atomic force microscopy (AFM) to probe the mechanical responses of individual molecules. In a typical experiment, a single molecule tethered between the AFM tip and the substrate is stretched from its equilibrium conformation. This approach overcomes the macromolecule insolubility problem mentioned above. SMFS has been used to study hydrophobic interactions between small hydrophobic molecules.[42,43]

We performed SMFS experiments on single polystyrene (PS) molecules in water[21,22] where collapsed PS chains adsorbed on a hydrophilic Si surface were forced to hydrate by the AFM tip (Figure 4a). PS and PS based polymers are ideal because their backbone and side-chains are nonpolar, and their size is close to the length regime of interest. Constant velocity pulling experiments reveal force curves with a plateau followed by an entropic elastic response from a single chain (Figures 4b and 5a). The force plateau corresponds to the mechanically induced globule-to-coil transition where mechanical work supplies the energy to hydrate the extended chain. Therefore, the hydration ΔG of the extended chain can be directly calculated from the work done in this process. In our studies, we separated the hydration contribution to the total unfolding free energy from the entropic contribution of the chain (Figure 4c) by using

$$\Delta G(T) = \frac{\int_{\text{plateau}} F(T)\,\mathrm{d}z + T\Delta S_{\text{ext}}(T)}{N} \qquad (6)$$

where $\Delta G(T)$ is the hydration free energy per monomer on the extended chain, $F(T)$ is the pulling force, $\Delta S_{\text{ext}}(T)$ is the entropy difference between the extended and relaxed coil, N is the number of monomers, and T is

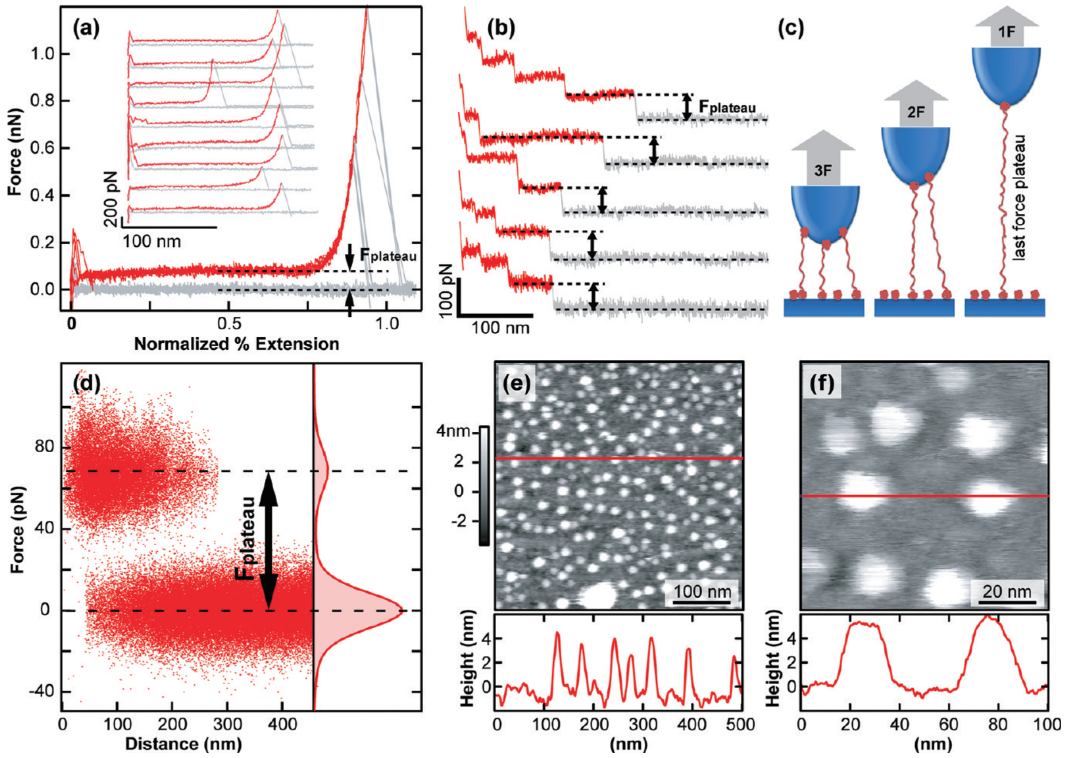

FIGURE 5. Evidence for single polymer hydration. (a) Superimposed force curves with normalized contour length. (b) Multiple steps of force plateau and the proposed mechanism (c) behind it. (d) The last plateau force magnitude for all types (a) or (b) of force curves gives the same Gaussian distribution. (e, f) Surface topography of polystyrene deposited on Si surface with a 500 × 500 nm^2 and a 100 × 100 nm^2 area. The bottom traces are cross-sectional profiles of the red lines in (e) and (f).

temperature. Graphically, the hydration contribution to the free energy is the area between the force plateau and the entropic elastic response of the chain (Figure 4b). Figure 4c illustrates the equilibrium states considered in the experiment and their thermodynamic relations. The velocity independent plateau force up to 3 μm/s and the fully reversible force curves are evidence that the polymer is at equilibrium under the applied tension.[21]

Experimental Evidence of Single Molecule Hydration Events

We performed control experiments to ensure the pulling events correspond to the hydration of single chains. Unlike mechanical unfolding of multidomain proteins that display sawtooth patterns, the force plateau of these single polymer pulling events is featureless. However, we can identify the number of attached molecules from the magnitude and shape of the plateau forces. Force profiles with multiple steps of the same height (Figure 5b) indicate that the AFM cantilever is simultaneously attached to several noninteracting molecules; as the cantilever retracts, the molecules detach from it sequentially and give rise to the steps (Figure 5c). The Gaussian distribution of the last plateau force centered at $\sim$75 pN is clearly separated from the zero-force baseline and corresponds to the unfolding of a single PS molecule (Figure 5d). The discrete force distribution is a strong indication that single molecule events are probed. In addition, when scaled by their contour lengths, the force curves that contain the entropic elastic responses are superimposable (Figure 5a),[22] indicating that the hydration mechanism being probed is insensitive to the length of the polymer in these studies. Dilute PS solutions were spin-coated on a hydrophilic Si surface to ensure that the hydrophobic polymers were well separated and exhibited a globular conformation on the surface,[44,45] as confirmed by AFM surface topography scans[21] (Figure 5e, f). The height of individual PS molecule on Si surface was $\sim$5 nm, which matches the predicted height of the collapsed single PS molecule.[21]

Two possible mechanisms can contribute to force plateaus:[21,22,46–49] the desorption of a trainlike polymer from the surface[46,47] and the hydration of a collapsed polymer in poor solvents.[21,22,48,49] Polymers can form extended structures on surfaces if the polymer–surface adhesion is greater than the polymer–polymer and solvent–polymer

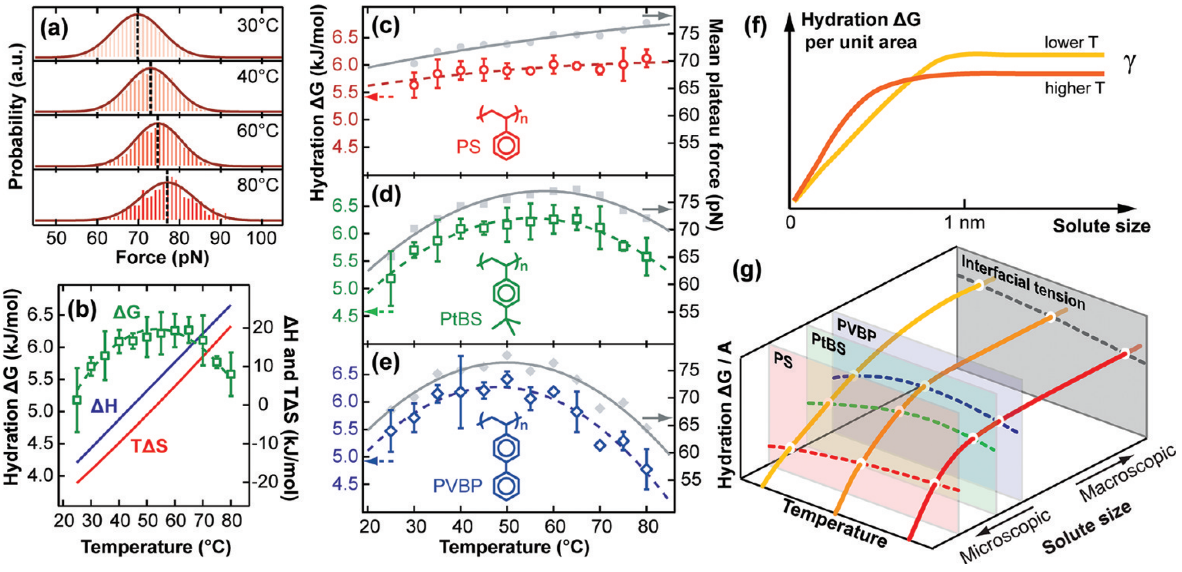

FIGURE 6. Temperature and size dependence of polymer hydration ΔG. (a) PS plateau force histograms show a steady increase of the mean force value as temperature increases.[22] (b) Temperature dependencies of hydration entropy (red), enthalpy (blue), and free energy (green) of PtBS. Temperature dependencies of plateau force (solid gray markers) and hydration ΔG (open colored markers) are shown in (c) PS (circle), (d) PtBS (box), and (e), PVBP (diamond).[22] Parabolic fits to the force (solid line) and hydration ΔG (dashed line) data illustrate their distinct dependencies on temperature. While the profile of hydration ΔG for PS is monotonically increasing, the profiles for PtBS and PVBP peak at 55.1 and 47.8 °C, respectively. The vertical error bars reflect the standard deviations of the mean from different sets of measurements. (f) Effect of temperature on the size-dependence of hydration ΔG/A (adapted from Chandler[6]). (g) Observed temperature dependent hydrations ΔG/A are signatures of hydrophobic hydration at different length scales.

interactions combined, such as in polymers with charged groups. For these polymers, the desorption force is extremely sensitive to small changes in ionic concentration; this is not the case for PS. In addition, the hydrophobic driving force favors a globular conformation of PS rather than a trainlike structure as AFM surface scans show (Figure 5e, f). We observed two distinct force plateau distributions in force curves showing multiple steps. Force curves with integer step sizes indicate the pulling of several noninteracting chains in parallel.[21,22,48] On the other hand, force curves with non-integer step sizes correspond to pulling a bundle of interacting chains,[49] in which hydration is responsible for the plateaus. In either case, the last plateau remains at the same magnitude. We found identical last plateau forces on Au, Si, and Si_3N_4 surfaces, indicating that surface–polymer interactions did not contribute significantly to the observed force plateau and that hydration provided the main contribution.[21]

Temperature Dependence of Hydration Free Energy Shows Hydration of Polymer Chains Belong to Microscopic Regime

We investigated the effect of polymer side-chain size on the hydration ΔG of an extended polymer.[22] Evidence from small molecule hydration ΔG illustrates that molecular size does not necessarily correlate with the magnitude of hydration ΔG (Figure 1) due to differences in solvent–solute attraction. However, the temperature dependence of hydration ΔG contains rich information on hydration entropy and enthalpy, which allows us to probe the effect of molecular size on hydrophobic hydration.

To isolate changes due to side-chains only, we chose three hydrophobic polymers with identical backbones but increasing sizes of nonpolar side-chains: polystyrene (PS), poly(4-tert-butylstyrene) (PtBS), and poly(4-vinylbiphenyl) (PVBP). Single molecule pulling experiments were performed from 25 to 80 °C, well below the melting point of all three polymers. The force curves for all three polymers show similar force–plateau profiles indicating the hydration mechanism behind their unfolding. While the force curves look similar, the plateau force magnitudes change with temperature. Thousands of force curves at each temperature were collected in order to achieve the necessary Gaussian statistics to resolve plateau force magnitude changes below the thermal noise floor (Figure 6a). In addition, a single AFM cantilever was used in each set of temperatures to minimize calibration errors from one cantilever to the next, which can be as large as 5%.

Interestingly, the temperature dependences for the three polymers are different (Figure 6c–e). We found that the unfolding force and hydration ΔG of PS monotonically increase with temperature (Figure 6a, c) while PtBS and PVBP show turnover behavior at different temperatures (Figure 6d, e). These signatures of hydrophobic hydration are similar to those of small molecule hydration (Figure 1b) and are distinct from macroscopic interfacial thermodynamics (Figure 1a), indicating that the polymer hydration mechanism is determined by the microscopic scale of their side-chains. Fitting the hydration ΔG as a function of temperature to eq 7[5] yields turnover temperatures of 92 ± 45 °C, 55.1 ± 0.9 °C and 47.8 ± 0.9 °C for PS, PtBS, and PVBP, respectively. The turnover temperatures decrease as the size of the hydrophobic side-chain increases from 7.2 Å for PS to 9.5 Å for PtBS and 11.4 Å for PVBP, in agreement with theoretically predicted length scales[5,16,33] (Figure 3b). The vanished hydration entropy at the turnover temperature indicates a characteristic hydrophobic hydration length scale that is directly related to the microscopic–macroscopic crossover length scales associated with this temperature.

Chandler showed that in the microscopic regime, the hydration free energy per unit area ($\Delta G/A$) increases linearly with particle size, indicating an apparent volume-dependent scaling relation, which strengthens with temperature[6] (Figure 6f). On the other hand, $\Delta G/A$ in the macroscopic regime plateaus at the surface tension value, indicating an area-dependent scaling relation, which weakens with temperature[6] (Figure 6f). Our observation of the thermal signature of hydration ΔG at different molecular sizes is the evidence for this view, suggesting that an entropically driven, volume scaling relationship exists for the microscopic length scale, as illustrated in Figure 6g.

$$G(T) = G(T_0) + (T - T_0)(C_P - S(T_0)) - T\ln\left(\frac{T}{T_0}\right)C_P \quad (7)$$

$$H(T) = H(T_0) + (T - T_0)C_P \quad (8)$$

$$S(T) = S(T_0) + \ln\left(\frac{T}{T_0}\right)C_P \quad (9)$$

The entropic and enthalpic contributions to the hydration ΔG can be separated based on eq 15a–c from Ashbaugh and Pratt,[5] assuming the heat capacity is constant over the temperature range (Figure 6b). Both hydration entropy and enthalpy are negative at room temperature, in agreement with experimental small molecule (e.g., methane) data. The negative entropy indicates the local restructuring of water, while the negative enthalpy is due to the dispersive attractive interaction between polymer and water. The temperature and monomer size dependence of hydration ΔG are consistent with hydrophobic polymer unfolding simulations from Athawale et al.[16] Their work suggests that the presence of the turnover behavior of hydration ΔG is the result of Lennard–Jones attraction between polymer and water. Ashbaugh and Pratt also showed that increasing attraction lowers the hydration ΔG and shifts the turnover point to higher temperature.[5] A study from Huang and Chandler[50] showed that attraction between the solute and water does not significantly alter the turnover temperature of hydration ΔG, as the attraction does not contribute greatly to hydration entropy. In addition, the turnover temperature is much more sensitive to the size of the solute than the attraction between the solute and solvent.[50]

We found that the overall free energy of collapse per monomer ~6 kJ/mol is lower than the hydration ΔG of similarly sized monomers in the range of 20–30 kJ/mol. This is mainly due to the hydrophobic and dispersive interactions between adjacent side-chains (Figure 3c), which are sufficient to offset the reduction of solute configurational entropy going from free monomers to polymers.

Macroscopic Interfacial Tension Provides Solvent Condition on Microscopic Scale

We also tested how the hydrophobic interaction strength in a polymer depends on the solvent condition. Ethanol was used to decrease the strength of hydrophobic interaction, while NaCl salt was used to increase it. Ethanol effectively decreases the surface tension of water (Figure 7a) by disrupting water–water hydrogen bonds and adsorbing at interfaces,[51] thereby weakening the hydrophobic interaction. Indeed, SMFS on PS in aqueous ethanolic solutions revealed plateau forces whose magnitude decreased with increasing ethanol fraction (Figure 7a). Ethanol is a poor solvent for PS, and a low plateau force in pure ethanol was observed (Figure 7a inset). The magnitude of the decreasing plateau force can be correlated to the interfacial tension at the bulk PS–water interface (Figure 7b). This linear correlation was observed for other solvents too. Pulling experiments in good solvents for PS such as toluene and benzene generated force curves that exhibit purely entropic elastic response, indicating that PS was initially not in a solvent-collapsed state; hence, the energy associated with the unfolding is zero. Furthermore, NaCl solutions increased the magnitude of the plateau in the same way it increases the surface tension of water and thereby strengthens the hydrophobic interaction. Therefore, we found that the macroscopically measured interfacial tension describes the strength of hydrophobic interaction well even on the microscopic level. Another single molecule experiment drew a similar

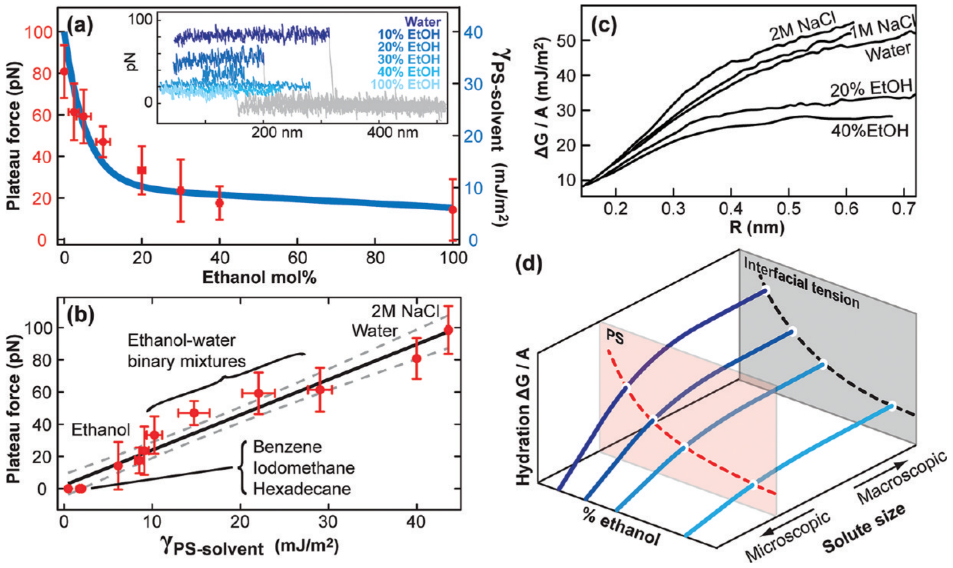

FIGURE 7. Solvent dependence of hydrophobic hydration. (a) Plateau force magnitude and interfacial tension as a function of the mol % ethanol in water. (b) Force plateau magnitude plotted against the interfacial tension between the solvent and polystyrene for various solvents. (c) Effects of salt and ethanol on the size dependence of the hydration ΔG per unit area (graph adapted from Rajamani et al.[37]). (d) Ethanol alters the hydration $\Delta G/A$ on the microscopic and macroscopic scales similarly. This gives rise to similar solvent-dependent profiles between the microscopic polymer solvation free energy and the macroscopic interfacial free energy.

conclusion, that the unbinding force between hydrophobic small molecules (β-cyclodextrin and adamantine) linearly depends on the surface tension of the water–ethanol mixture.[43]

One might ask whether the proportionality with macroscopic interfacial tension contradicts our finding that the hydration of hydrophobic polymers is dominated by the microscopic scale. Theoretical studies of solvent additives indicate that ethanol decreases, while NaCl increases, the hydration ΔG on both small and large length scales (Figure 7c).[37] Therefore, it is expected that even though the hydration ΔG to unfold a hydrophobic polymer is on the microscopic scale, the strength of the hydrophobic interaction scales with the macroscopic counterpart, the interfacial free energy. Therefore, the correlation between microscopic hydration and macroscopic interfacial tension can be expected (Figure 7d).

Conclusion and Perspective

Due to their relatively simple structures, hydrophobic homopolymers have been used to study hydrophobic collapse in both theories and experiments. Single molecule force spectroscopy has advanced the understanding of the size, temperature, and solvent dependent hydration behavior of hydrophobic polymers. Our results indicate that although the length of a polymer can be hundreds of nanometers long, its hydration behavior is dominated by the dimension of its monomers on the subnanometer length scale. The temperature dependence of hydration ΔG is highly sensitive to molecular size. From the temperature dependence of hydration ΔG, we identified that the length scale crossing over from microscopic to macroscopic hydration is on the order of 1 nm, which is consistent with theoretical predictions. The apparent volume scaling relationship in this length regime implies that the hydrophobic driving force for small molecules to aggregate is smaller than what a surface area dependent model would predict. For instance, the stability of hydrophobic clusters in the folding intermediates may not be as stable as might be assumed, which may impact the folding pathway predictions. In addition, the turnover points at a particular temperature signify the characteristic length scales where the entropic contribution of hydration is zero, which is indicative of the crossover length scale of hydrophobic hydration at that temperature.

Moving forward, several critical questions remain to be answered. As molecules are not spherical, we need to understand how their shapes and surface heterogeneity affect their hydration. In addition, interfaces represent important loci for biomolecular interactions; the effect of surface chemistry and its patterning on hydrophobic collapse is important for understanding protein interactions, denaturation and aggregation. Garde et al predict striking behavior of hydrophobic polymers at hydrophobic interfaces,[7] which provides a challenge to experimentalists. We have entered a golden age where single molecule experiments and simulations can be compared. We expect that the synergy between experiment and theory in the area of hydrophobic hydration will accelerate our understanding of molecular hydrophobicity and improve our ability to include it in biomolecular engineering.

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FOOTNOTES

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The authors declare no competing financial interest.

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