

FEATURE ARTICLE

A View of the Hydrophobic Effect

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Oil and water do not mix. The disaffinity of oil for water, with its unusual temperature dependence, is called the hydrophobic effect. It is important to understand the factors underlying the hydrophobic effect because they appear to play key roles in membrane and micelle formation, protein folding, ligand-protein and protein-protein binding, chromatographic retention, possibly nucleic acid interactions, and the partitioning of drugs, metabolites, and toxins throughout the environment and living systems. Here, we survey experimental and theoretical studies of nonpolar solute partitioning into water. We note that the hydrophobic effect is not just due to “water ordering” and not merely due to small size effects of water. The properties vary substantially with temperature and solute shape. Also, we discuss the limitations of using oil/water partitioning as the basis for some thermodynamic models in chemistry and biology.

I. Introduction

The hydrophobic effect has been widely studied because of its central role in chemistry and biology.^{1–8} There remains considerable debate about its molecular origin. Here, we give our view of current experimental results, and we review recent interpretations in terms of water structure. We do not attempt, nor does space permit, an exhaustive review of all aspects of hydrophobicity.

II. Historical Background

Possibly the first published oil/water experiments were those of Benjamin Franklin in 1773, who put a drop of oil on the pond at Clapham Common to test the idea, known since Pliny the Elder in the first century A.D., that when oil is poured onto water, it stills the waves.⁹ In 1890, Lord Rayleigh repeated Franklin's experiment.⁹ Knowing the volume of a spoonful of oil and the surface area of the body of water, and assuming that oil forms a monolayer film on water, Rayleigh made one of the first experimental determinations of the size of a molecule, at a time when the existence of atoms and molecules was still in doubt.

As early as 1891, Traube appreciated that amphiphilic molecules—molecules having both hydrophobic and hydrophilic parts, such as soaps and proteins—were concentrated at the air-water interface due to a disaffinity between hydrocarbon and water.¹⁰ In 1892, Agnes Pockels developed what later came to be called the Langmuir trough and began the first quantitative studies of films of oil and surfactants on water. Irving Langmuir developed his well-known adsorption equation in 1917.¹¹ The word “hydrophobic” appeared at least as early as 1915,¹² where

it was defined rather differently than it currently is: “applied to a hydrosol that readily forms a precipitate and on evaporation or cooling gives a solid that cannot readily be converted back into a sol”.

It became apparent that the disaffinity of oil for water drives many different types of noncovalent association in biology and chemistry. In 1898, Meyer and Overton observed a direct proportionality between oil/water partition coefficients and the anesthetic potencies of certain drugs.^{13,14} In 1920, J.W. McBain and C.S. Salmon demonstrated reversible formation of micelles in soap solutions.¹⁵ In 1925, Gorter and Grendel proposed that lipid molecules from red blood cells could form “bilayers”, i.e., a pair of opposed monolayers, based on careful Langmuir trough experiments that showed twice the surface concentrations of surfactant molecules as in monolayer experiments.¹⁶ In 1935, Davson and Danielli proposed that proteins associate with bilayer membranes through nonpolar interactions.¹⁷ In 1941, Henry Bull¹⁸ noted that “proteins contain a large number of nonpolar groups and it might be that upon denaturation these hydrophobic groups are exposed”. By 1945, X-ray experiments and structural studies had firmly established that polar groups are localized at micellar surfaces and nonpolar groups form the interior.¹⁹ The first X-ray structures of proteins appeared in 1958, showing cores of buried hydrophobic amino acids similar to the structures of micelles.²⁰ These examples showed that the structures of chemical and biological complexes are organized and driven, at least in part, by the avoidance of oily groups for water.

This work raised the question of whether the disaffinity of oil for water might be driven by either an affinity of oil for oil or an affinity of water for water. Either would lead to the separation of oil from water. In early models, micelle formation was assumed to be driven by an oil/oil attraction^{21,22} or even by charge interactions.²³ In contrast, Hartley's 1936 monograph

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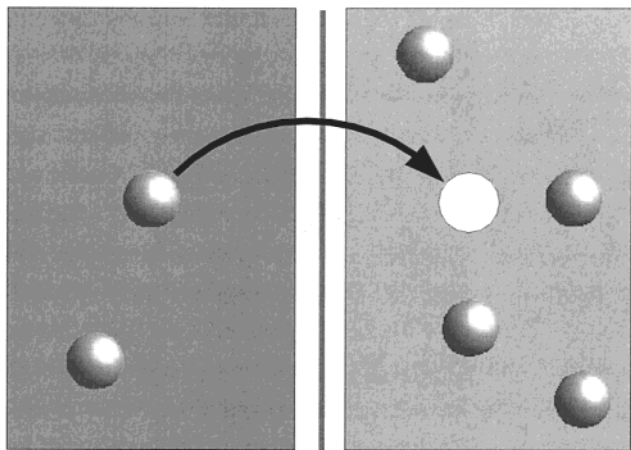


Figure 1. Partitioning is a balance. Hydrophobicity is measured by oil/water partitioning of solutes. At equilibrium, the chemical potentials in the two phases, 1 and 2, are equal $\mu_1^\circ + RT \ln C_1 = \mu_2^\circ + RT \ln C_2$. Measuring the solute concentrations c_1 and c_2 in the two phases give the difference in chemical affinities between phases, $\Delta\mu^\circ = -RT \ln C_1/C_2$.

had proposed that nonpolar molecules avoid water because they are unable to compete with the strong attraction of water for itself.²⁴ The latter view now prevails, that the disaffinity of oil for water is predominantly due to water–water hydrogen bonding, and that water interactions are stronger than the intermolecular dispersion interactions that attract oil to oil.

In 1954, Walter Kauzmann coined the term “hydrophobic bonding” to refer to the tendency of oils to associate in aqueous solutions.²⁵ Although he emphasized that the driving force was the avoidance of the aqueous phase by the oil, his terminology drew criticism. Joel Hildebrand objected to the term “bonding”, which he preferred to reserve for covalent interactions.²⁶ Hildebrand also objected to the term “hydrophobic” on the grounds that oil has a favorable enthalpy of interaction with water.²⁷ However, the alternative view has ultimately prevailed,^{4,28,29} namely (1) that it is the free energies, not enthalpies, that define affinities, (2) that “bond” is a term that is widely accepted to also refer to certain types of noncovalent interactions, and (3) that it is useful to have a word for the types of interactions between nonpolar molecules and water.^{30–32} “Hydrophobic” has now become common usage.

A. Temperature Dependence of the Hydrophobic Effect.

The hydrophobic effect is defined by the notable thermodynamics of mixing oils with water. The solubilities of oil in water were first studied by Valentiner in the 1920s.³³ The temperature dependence of these solubilities was used in the 1930s to obtain entropies and enthalpies.^{34–37} In light of the expectation from Regular Solution Theory that liquid–liquid disaffinities are usually enthalpic,^{38–40} the conclusion that oil/water disaffinities were dominated by large unfavorable entropies was regarded as surprising.

Figure 1 shows the standard interpretation of oil/water partitioning equilibria. A solute has some chemical affinity, μ° , for each phase, oil or water. The solute also has a translational entropy in each phase, represented by $kT \ln c$, where c is some measure of the solute concentration in that phase. (There has been considerable discussion of appropriate measures of concentration,^{41–43} but they will not concern us here.) The solute partitioning between the two phases reaches equilibrium when the difference of chemical affinities balances the difference of concentration, $\Delta\mu^\circ = -kT \ln c_2/c_1$. If the ratio of concentrations, c_2/c_1 depends on temperature, then μ° depends on temperature, and this is expressed in terms of a molar transfer

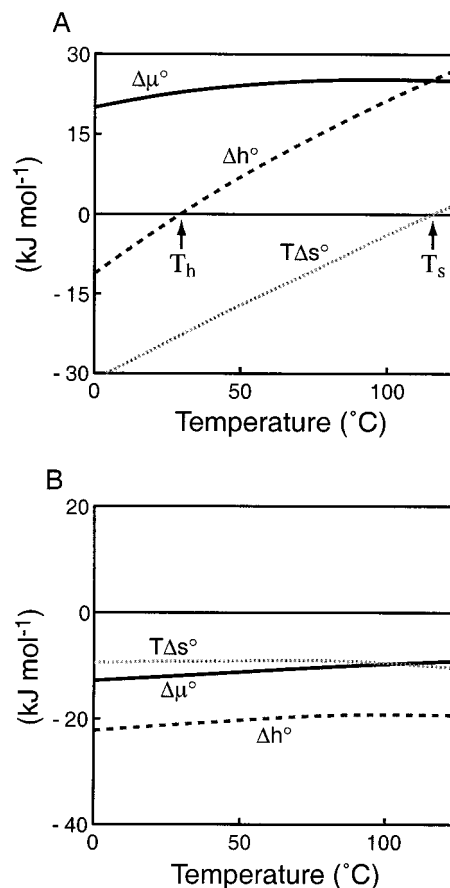


Figure 2. Hydrophobic Effect is the anomalous mixing thermodynamics of nonpolar solutes with water. (A) The transfer of neopentane from its own neat phase into water, and (B) a regular solution: the transfer of neopentane from the gas phase into a neopentane neat phase. T_s is the temperature where the entropy of transfer is zero, T_h is where the enthalpy of transfer is zero. Data adapted from Lee, B. *Biopolymers* **1991**, *31*, 993.

enthalpy Δh° , an entropy Δs° (where $\Delta\mu^\circ = \Delta h^\circ - T\Delta s^\circ$), and sometimes also a heat capacity, calculated from the slope of the enthalpy, $\Delta C_p = d\Delta h^\circ/dT$. The quantity Δs° represents *excess* entropies that are not included in the translational entropy term, $kT \ln c$.

Figure 2 compares the transfer of a hydrophobic solute, neopentane, from gas into neopentane, and from neopentane into water.⁴⁴ For a simple system such as neopentane in neopentane, $T\Delta s^\circ$ is small, Δh° is the dominant component of $\Delta\mu^\circ$, and both the enthalpy and the entropy are relatively independent of temperature. Figure 2 shows how the mixing of oil and water is different than solvation in simpler solutions. (1) The free energy of transferring nonpolar molecules from oil into water is positive and large ($\Delta\mu^\circ \gg 0$) compared to simpler systems such as neopentane in neopentane. (2) In cold water (around room temperature), the disaffinity of oil for water is due mainly to entropy ($-T\Delta s^\circ \gg 0$, $\Delta h^\circ \approx 0$). (3) In hot water (around the boiling temperature), the disaffinity of oil for water is mainly enthalpic ($\Delta h^\circ \gg 0$, $-T\Delta s^\circ \approx 0$). (4) Over the full temperature range for liquid water, from the melting point to the boiling point, there is a large positive heat capacity change upon transfer of nonpolar solutes into water. The curvature of the free energy with temperature can be expressed in terms of this heat capacity ΔC_p

$$\Delta C_p = -Td^2\Delta\mu^\circ/dT^2 \quad (1)$$

and it accounts for the strong temperature dependence of the entropy and enthalpy

$$\Delta C_p = d\Delta h^\circ/dT = Td\Delta s^\circ/dT \quad (2)$$

B. A Subtle Point: T_h and T_s . At what temperature is the hydrophobic effect maximal? It depends.⁴⁵ On one hand, driving forces require interpretation in terms of free energies. $\Delta\mu^\circ$ is maximal at a temperature near the boiling point of water, indicating that the oil/water interaction is least favorable at that temperature. But experimental observables provide a slightly different perspective in this case. The solubility of oil in water, which is proportional to $\Delta\mu^\circ/kT$, is lowest around room temperature. Hence, there is an important distinction between the temperatures where $\Delta\mu^\circ$ and $\Delta\mu^\circ/kT$ are maximal. Between about 300 K and 380 K is the subtle and interesting region where the free energy becomes more positive with temperature, yet the solubility of oil in water increases. This distinction motivates the definition of two characteristic temperatures. It is readily shown using the Gibbs–Helmholtz equation that $\Delta s^\circ = 0$ at the temperature where $\Delta\mu^\circ$ is maximal. This defines a temperature called T_s . Similarly, $\Delta h^\circ = 0$ at the point where $\Delta\mu^\circ/kT$ is maximal. This defines a temperature T_h , where the transfer enthalpy is zero. For small molecules such as benzene and neopentane, $T_h \approx 25^\circ\text{C}$ and $T_s \approx 113^\circ\text{C}$.⁴⁶

The full thermodynamics of solute partitioning into water can be expressed in terms of these two temperatures

$$\Delta h^\circ(T) = \int_{T_h}^T \Delta C_p dT = \Delta C_p(T - T_h) \quad (3)$$

$$\Delta s^\circ(T) = \int_{T_s}^T \Delta C_p/T dT = \Delta C_p(T/T_s) \quad (4)$$

$$\Delta\mu^\circ(T) = \Delta C_p[(T - T_h) - T \ln(T/T_s)] \quad (5)$$

provided that ΔC_p is independent of temperature. One of the main fingerprints indicating when the hydrophobic effect is involved in chemical or biological processes is a temperature dependence that obeys these equations, particularly if T_h and T_s have approximately the values noted above.

III. The Hydrophobic Effect in Protein Folding

This form of temperature dependence is one of the main lines of evidence for the importance of the hydrophobic effect in folding globular proteins.⁴ Upon folding, a protein buries its nonpolar amino acids into a core, away from contact with water. Figure 3 shows that the thermal features of protein unfolding resemble those for transferring small nonpolar molecules into water. Unfolding leads to a positive free energy, and an enthalpy and entropy with a steep slope, indicating a large positive heat capacity. The earliest such measurements on proteins were the basis for Walter Kauzmann's proposal for the importance of hydrophobicity in protein folding.^{25,47} Kauzmann noted that the heat capacities of protein unfolding are huge, 0.1–0.2 cal K⁻¹ per gram of protein, the same magnitude as the heat capacity of pure water, 1 cal K⁻¹ g⁻¹, which is one of the largest known, "being approached or exceeded only by solid lithium, liquid or gaseous helium, solid, liquid, or gaseous hydrogen, or liquid ammonia".⁴⁸

Further insights into protein folding come from looking at the two corresponding temperatures, T_h and T_s (see Figure 2). Julian Sturtevant noticed that several different biomolecular processes at 25 °C have nearly identical values of $\Delta S/\Delta C_p$.^{4,49} Robert L. Baldwin found that the constancy of this ratio, taken together with $T = 298$ K substituted into eq 4, gives a universal

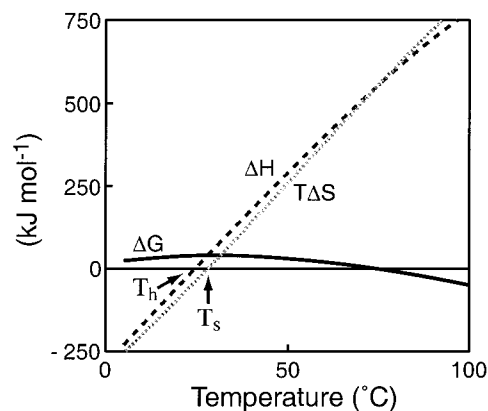


Figure 3. Protein unfolding free energy, $\Delta G = G_u - G_f$, entropy, ΔS , and enthalpy, ΔH , versus temperature. For proteins, $T_s \approx T_h$. Data on myoglobin from Makhatazde, G. I. and Privalov, P. L. *Biophys. Chem.* **1994**, *51*, 291.

temperature $T_s = 25^\circ\text{C}$ for these biomolecular processes,⁴⁶ implying a resemblance between protein folding and small molecule transfers. Murphy et al.³¹ also found a single slope of $\Delta S/\Delta C_p$ for a variety of different processes, indicating the universality of T_s for identifying hydrophobic processes in biomolecules.

However, the thermodynamics of protein unfolding is not identical to that of oil partitioning into water. First, the free energies of protein unfolding are much smaller than for transferring benzene to water, per unit nonpolar surface area. Second, although T_h and T_s differ by nearly 80 °C for small solutes, these two temperatures are nearly equal, $T_h \approx T_s$, for proteins. $T_h \approx T_s \approx 112^\circ\text{C}$ for the several proteins studied by Privalov,⁵⁰ and $T_h \approx T_s \approx 10^\circ\text{C}$ for T4 lysozyme.⁴⁵ These two differences can be explained by the large conformational entropy increase that accompanies protein unfolding that does not apply to small solute partitioning processes.⁴ Of course, other interactions such as hydrogen bonding and electrostatics also contribute to the overall free energy of folding,⁵¹ but distinctive thermodynamic signatures have not yet been identified for these types of interactions.

IV. BIPSE: Modeling Biomolecule Folding, Docking, and Partitioning Using Small Molecule Transfer Models

Many oil/water partitioning experiments have been performed on homologous series of solutes. These experiments are usually motivated by an interest in developing models of molecular and biochemical processes, such as folding, docking, assembly, and partitioning processes.^{52–54} We call them BIPSE models. BIPSE stands for "Break Into Pieces, Sum the Energies". In BIPSE models, the full thermodynamic quantity of interest, such as the free energy of folding or the free energy of micellization (see Figure 3), is broken down into a sum of component free energy quantities, such as the oil/water transfer free energies of each of the individual amino acids or individual methylene groups. For example, the free energy of folding is often modeled as the sum of the partitioning free energies of that protein's amino acids from water (representing the denatured conformations of the protein) into oil (representing the core of the native protein). The attraction of such models is that they parse a complex process into simpler component quantities that can be measured in independent experiments on model compounds.

BIPSE constitutes a huge class of models. Flory–Huggins theory is a BIPSE model that treats polymer conformational changes;⁵⁵ QSAR (Quantitative Structure–Activity Relationships) are BIPSE models that are used to predict drug interac-

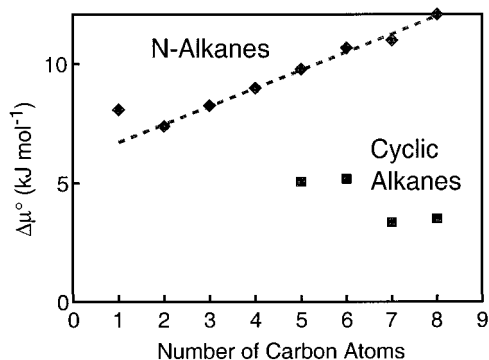


Figure 4. Alkane transfer free energies. For the normal alkanes (diamonds), except methane, there is a linear correlation between number of carbon atoms and free energy. This relationship fails to describe the cyclic alkanes (squares). From A. Ben-Naim, *Solvation Thermodynamics*; Plenum Press: New York, 1987.

tions with proteins.⁵⁶ BIPSE models have been used to treat protein folding, micelle formation, docking, chromatographic retention, solvation, and other processes.^{1,53,57–68}

However, there are some obstacles that must be overcome if BIPSE modeling is ultimately to become quantitative. Here, we discuss five problems.

(1) Additivity. BIPSE models assume the component free energies are additive. The additivity assumption may lead to errors,⁶⁹ particularly if the groups are in close proximity.

(2) Other BIPSE Components. In addition to the oil/water partitioning component, essentially every BIPSE model involves at least one unmeasurable component, usually an additional entropy that must be derived from a model of some sort. For example, protein folding requires a model of the chain entropy; micellization and bilayer formation require a model of the translational entropy of localization of the surfactant molecules, and a model for any entropies of chain restriction; drug binding to proteins requires a model for orientational entropies of drug localization; and models of polymer conformations must treat the collapse, confinement or stretching entropies. These extra contributions are seldom available from independent experiments; they must come from models, simulations, or assumptions. So it is usually not clear whether any flaws in BIPSE models are attributable to limitations in understanding the oil/water process, or in understanding the other contributions.⁶⁹

(3) What is the Right Measure of Contact? BIPSE modeling often assumes that partitioning free energies scale linearly with solute surface area. It has long been recognized that water insolubility generally increases with the size of a solute molecule.^{70,71} For example, Figure 4 shows that every methylene unit added to a hydrocarbon chain increases the free energy of transfer into water by a fixed amount,¹ estimated to be between $25 \text{ cal mol}^{-1} \text{ \AA}^{-2}$ and $33 \text{ cal mol}^{-1} \text{ \AA}^{-2}$, depending on assumptions about the oil phase.⁷² Lindenburg first suggested that solubility was roughly determined by *molar volume* of a hydrocarbon, and not otherwise by molecular structure.⁷³ This was supported by gas chromatography studies by McAuliffe, but there were large errors, and molar volumes were not predictive of the relative solubilities of methane, ethane, and propane. Also, the aromatic hydrocarbons had a different dependence on molar volume than other hydrocarbons.^{74,75} In 1972, Hermann proposed instead that the hydrophobic effect depends on the number of water molecules that surround a nonpolar solute, which is proportional to its solvent-accessible surface area.⁷⁶ Solvent-accessible surface area was found to be equally successful for predicting saturated hydrocarbon and aromatic hydrocarbon solubilities, and slightly better for branched

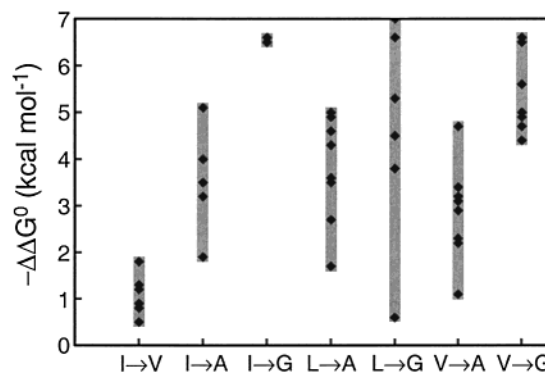


Figure 5. Mutational free energy depends as much on the context of the amino acid replaced as the identity of the amino acid replaced. Change in protein stability, $\Delta\Delta G$, from 32 mutations on fully buried hydrophobic residues in staphylococcal nuclease, bacteriophage $\phi 1$ gene V protein, phage T4 lysozyme, and barnase. [Lee, B. *Protein Science* **1993**, *2*, 733 and references therein] Amino acid abbreviations are used: I denotes isoleucine, L leucine, V valine, A alanine, and G glycine.

hydrocarbons. This gave a value of $20 \text{ to } 28 \text{ cal mol}^{-1} \text{ \AA}^{-2}$ for the hydrophobic effect, the variations arising from the methods used to calculate the surface areas for the hydrocarbons.^{77,78}

However, surface area remains an imperfect predictor of the solubilities of cyclic hydrocarbons and molecules of other shapes (see Figure 4 data for cyclohexane and cycloheptane).^{41,79} It costs 5.2 kJ mol^{-1} to transfer cyclohexane into water from the vapor phase at 298K, whereas it costs 10.7 kJ mol^{-1} for hexane. It is difficult to rationalize this difference based only on surface areas.

The justification for using surface area comes from (1) modeling solute insertion as the opening of a cavity, followed by the insertion of the solute, and (2) the assumption that the energetics of both of these steps are dominated by properties of the first shell or two of waters. Ron Levy and his colleagues have demonstrated the dominance of the first few shells of water layers around the solute.⁸⁰ Computer simulations find that the free energy of cavity formation is proportional to surface area, but that the dispersion attractions of the solute for water are not,^{81–83} hence the lack of proportionality, in some cases, between surface areas and free energies of solute insertion. Nonpolar solvation energetics depend not only on surface area, but also on molecular structure and geometry.^{84,85}

(4) Heterogeneity of the Medium. Another limitation of BIPSE modeling is that the oil phases used for small molecule partitioning, such as bulk cyclohexane or octanol, are simple, homogeneous, and amorphous. In contrast, chemical and biochemical systems, such as the interiors of proteins or lipid bilayers, are complex and heterogeneous, chemically and physically, and often at least partially structured. How transferable are the free energies obtained from data on simple oils to more complex environments? If the protein interior were an oil droplet, mutating a valine to isoleucine, for example, would change the protein stability by a fixed amount, no matter where in the protein core the mutation occurs. Figure 5 shows instead a high degree of variance, depending on where the mutation site is located in the protein.⁸⁶ Thus, the degree to which simple uniform amorphous oils can capture the essence of interiors of proteins, micelles, or bilayers may be limited.

(5) What Does the Oil/Water Experiment Measure? BIPSE modeling relies on “hydrophobicity scales”,^{47,52–54,87–90} in which solutes are rank-ordered from the most oil-like to the most water-like. Do these scales truly measure a universal property that could be called polarity, or do they measure something more

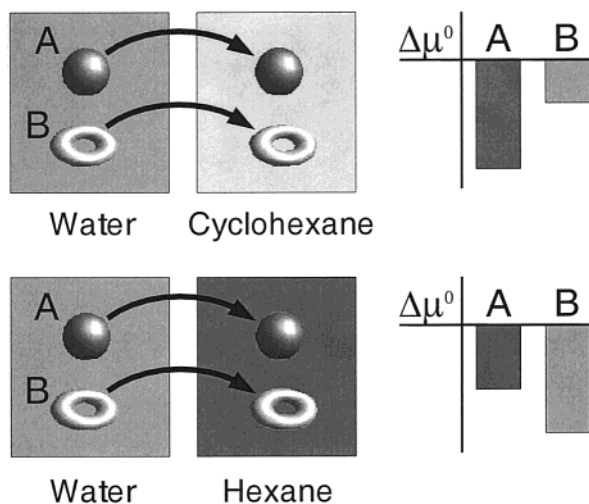


Figure 6. Thought experiment: different oils might cause different rank orderings of solutes. If so, no single hydrophobicity scale would be universal and transferable.

complex? And what is the most appropriate oil phase? Ethanol–water partitioning was the basis for the first hydrophobicity scales.⁵³ Later *n*-octanol and cyclohexane were the oil phases of choice for defining scales.^{88,91}

The oil/water partition coefficient gives a simple one-dimensional scale for rank ordering any series of solutes. Such scales rest on an implicit premise that one oil is not very different than another (provided they have the same chemical substituents). To illustrate this premise, here is a thought experiment. If a hexane/water partitioning experiment showed that solute A is more oil-like than B, while a cyclohexane/water partitioning experiment showed B more oil-like than A (Figure 6), then there would be no basis for deciding whether A or B is the more “oil”-like solute. Rather, we would be forced to attribute differences in A and B to specific interactions with hexane or cyclohexane. “Polarity” would not be a property of a solute alone, independent of the solvent. Instead, it would be necessary to label any solute rank orderings the “hexane hydrophobicity” or the “cyclohexane hydrophobicity”. This would render the concept of a polarity scale useless because it would then not be transferable, as would be required of a BIPSE model, to situations involving the interiors of micelles, proteins, or chromatographic stationary phases. There would be no value

in doing the model experiment at all, if there were not some degree of generalizability from one nonpolar medium to another.

Hydrophobicity scales were first justified by Tanford,⁵³ who showed that partitioning was relatively independent of the oil phase. He compared the transfer of norleucine from water into methanol, ethanol, butanol, and acetone. But since then, few systematic and extensive studies have been performed to determine the oil dependence of hydrophobicity scales. There is some evidence that partitioning does depend on the oil phase.⁸⁹ Figure 7 shows a histogram of the hydrophobicities of each of the 20 amino acids from water into various oil-like media, according to approximately 36 different hydrophobicity scales.⁹⁰ The figure shows that a given solute has very different “polarities” depending on the media and conditions. For example, phenylalanine is the most nonpolar amino acid according to 6 scales, whereas it is the fifth most nonpolar according to 3 other scales. Remarkably, 5 different amino acids are so ambivalent that they are ranked as the *most* hydrophobic by one scale and *least* hydrophobic by another. These results imply that the term “hydrophobicity” is, at best, rather loose and nonspecific. It seems reasonably justifiable to divide solutes into two or three categories—solutes are oil-like, or water-like, or ambivalent—but it is not clear that much finer distinctions define a true polarity as a solute property, independent of its solvent. For example, these scales will typically distinguish leucine from isoleucine, even when the underlying physical cause would more suitably be called “geometry” than “polarity”. Hence, polarity scales can depend on the oil phases used to determine them. The implication is that there may be limited transferability from model compound studies to BIPSE models of folding, docking, or binding.

V. Modeling the Hydrophobic Effect from Water Structure

What properties of water structure are responsible for the hydrophobic effect? The water-ordering, or “iceberg”, model was first proposed by Frank and Evans.⁹² Frank and Evans proposed that a nonpolar solute creates a weakly clathrate-like cage of first-shell waters around the solute. They concluded that “When a rare gas atom dissolves in water at room temperature it modifies the water structure in the direction of greater ‘crystallinity’”⁹² In a cage structure, the water molecules do not “waste” hydrogen bonds by pointing them at the solute;

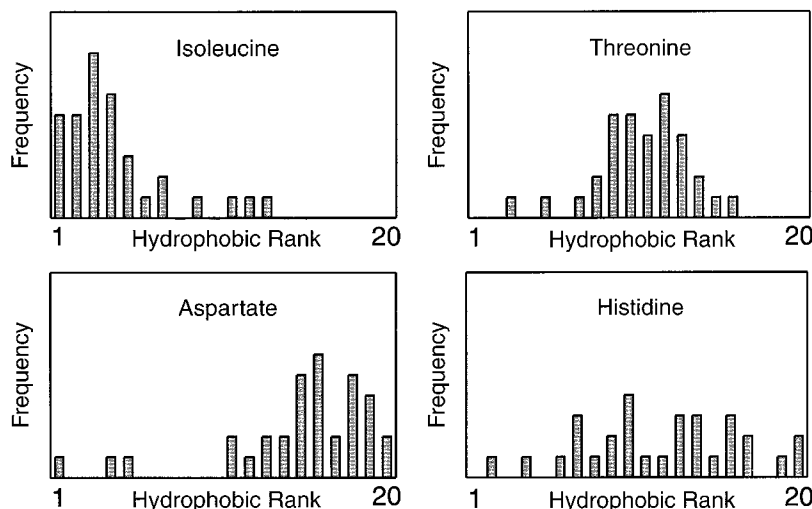


Figure 7. Rankings of some amino acids across approximately 36 partitioning scales. Specific solvent–solute interactions cause hydrophobicity rankings to vary widely between scales. Rankings are scaled from (1) most hydrophobic to (20) least hydrophobic. [D. R. Devido, Ph.D. thesis, Florida State University (1997)]

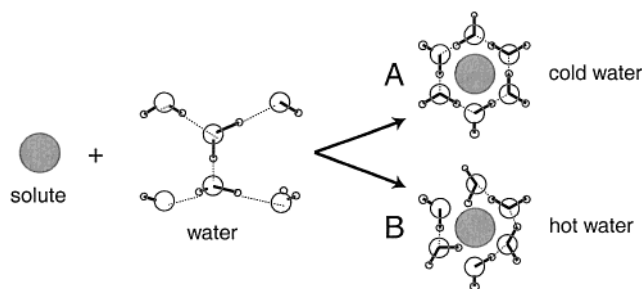


Figure 8. “Iceberg” model for the large heat capacity of transfer of nonpolar solutes into water. (A) At room temperature the water molecules surrounding a nonpolar solute adopt only a few orientations (low entropy) to avoid wasting hydrogen bonds. Most water configurations are fully hydrogen bonded (low energy). (B) In hot water, more conformations become accessible (higher entropy), but at the cost of breaking hydrogen bonds (high energy).

rather they form a hydrogen bonded fence around the solute. Accordingly, the large entropy that opposes solute transfer into water arises from the cost of ordering the waters into a more open “iceberg”-like cage structure (see Figure 8 and MB model discussion below).

However, Figure 2 shows that this large entropy, and therefore the water ordering mechanism, only apply in the narrow range of temperatures around 25 °C at 1 atm pressure. In fact, “hydrophobicity” is strongest (i.e., $\Delta\mu^\circ$ is most positive) around 100 °C, where $\Delta s^\circ \approx 0$ and where the transfer process is dominated by an unfavorable enthalpy. Hence “water ordering” is not a complete description of hydrophobicity. A more general perspective of what is unique about hydrophobicity, one that applies over the full range of temperatures for liquid water, is the large heat capacity. Hydrophobicity is entropic in cold water and enthalpic in hot water.

A. Iceberg Model. The iceberg model of Frank and Evans explains the large heat capacity of nonpolar solvation; see Figure 8. A loose explanation is based on the fundamental thermodynamic relationship, $G = H - TS$. At low temperatures, thermodynamic processes are driven to lower their enthalpies, and at high temperatures, they are driven to states of high entropy. In this case, as temperature increases, first-shell waters broaden their orientational distributions to gain entropy but doing so breaks first shell water–water hydrogen bonds, increasing the enthalpy, H . According to eq 2, increasing enthalpies and entropies with temperatures gives a positive heat capacity.

As support for the iceberg idea, Frank and Evans noted that nonpolar solutes are often surrounded by clathrate water cages in crystalline hydrates.^{92–102} Clathrates are well understood because of their commercial importance. Natural gas pipelines are sometimes clogged by such hydrates, which form a slushy “snow” at temperatures as high as 68 F.⁹⁹

And although Frank and Evans noted that the term “iceberg” should not be taken literally, subsequent work invoked a high degree of water ordering around nonpolar solutes. In 1958, IM Klotz proposed that the properties of proteins could be understood in terms of a surrounding shell of crystal-like waters, that masked the chemical activity of certain groups and affected the cooperativity of ligand binding.¹⁰³ Indeed, some waters are known to be highly ordered around proteins.¹⁰⁴

Nevertheless, the current view is that the degree of water ordering in the first solvation shell around a nonpolar solute is much smaller than the degree of ordering in ice.^{2,105} Walter Kauzmann noted that the entropy of freezing of water is 5.3 cal K⁻¹ mol⁻¹, whereas inserting a nonpolar solute into water costs about 20 cal K⁻¹ mol⁻¹, which amounts to an entropy of

only 1 cal K⁻¹ mol⁻¹ for each of the approximately 20 waters surrounding a small nonpolar solute.⁴⁷ Moreover, Kauzmann noted that freezing *increases* the partial molar volume of water, whereas inserting a nonpolar solute into water *decreases* it.⁴⁷ He concluded that water in solvation shells around nonpolar solutes is less ordered, and different than, ice. But so far, few experiments have been able to elucidate the amount of water structuring in solvation shells.

B. Muller Model. A recent iceberg-like model of the hydrophobic effect was developed by Norbert Muller¹⁰⁶ based on an earlier 2-state model by Gill and others.¹⁰⁷ The model was generalized by Lee and Graziano.¹⁰⁸ The Muller model invokes four states of water–water hydrogen bonding: made or broken in the bulk, and made or broken in the first solvation shell. Each of the four states has an enthalpy and entropy. The four differences in these quantities are represented by adjustable parameters, and can then be used to calculate other properties. For example, the increased enthalpy of breaking bulk hydrogen bonds with temperature gives a heat capacity of bulk water. The heat capacity for transferring a nonpolar solute into water results from the shift in the made–broken equilibrium for the N water–water hydrogen bonds in the first shell of the solute. Using refined parameters, consistent with Raman data,^{109,110} a recent variant of this model predicts that in cold liquid water (room temperature), first-shell hydrogen bonds have lower enthalpy and lower entropy than bulk hydrogen bonds.^{111–114} In warm water, the first-shell hydrogen bonds are more broken, having higher enthalpy and higher entropy than in the bulk. Thus, heating “melts the iceberg” around the solute. A related approach attempts to quantify the relationship of perturbations in water structure to heat capacity.^{115,116}

Although the Muller model treats the solvation heat capacities, it requires additional parameters to treat the free energies.¹⁰⁸ But the Muller model considers only hydrogen bonding, which appears to be a poorer approximation for the free energy than it is for the heat capacity (see below). Computer simulations show that hydrogen bonding perturbations are not wholly responsible for the free energy of solvation and may not even represent the dominant contribution.⁸³

C. Small-Size Model: Is the Disaffinity of Oil for Water Due to Water’s Small Size? The Small-Size Model proposed by M. Lucas¹¹⁷ and B. K. Lee^{118–120} is an alternative to the iceberg model. It focuses on the free energies of solvation, rather than on the entropy and heat capacity. In the Small-Size model the high free energy cost of inserting a nonpolar solute into water is not due to orientational ordering of first-shell waters; it comes from the difficulty of finding an appropriate cavity in water, due to the small size of water molecules. That is, the dominant enthalpy and entropy of opening a cavity are the same in water as those in a liquid of Lennard–Jones molecules of small size. This raises two issues: (1) Why should water be as simple as a Lennard–Jones fluid? (2) What is the effect of molecular size on the free energy cost of creating a cavity? These are addressed in order below.

(1) Complexity. For both a Lennard–Jones liquid and water, opening a cavity leads to an entropy cost, to bring together many small packets of free volume into a single larger cavity, and it costs an enthalpy of breaking intermolecular interactions to create the cavity. There are two additional contributions for water. Opening a cavity in water should also involve an orientational entropy cost of ordering the waters at the surface of the cavity, and a corresponding reduction of the enthalpy of forming better hydrogen bonds in the first shell, relative to the bulk. But Lee argued that there is an almost perfect enthalpy–

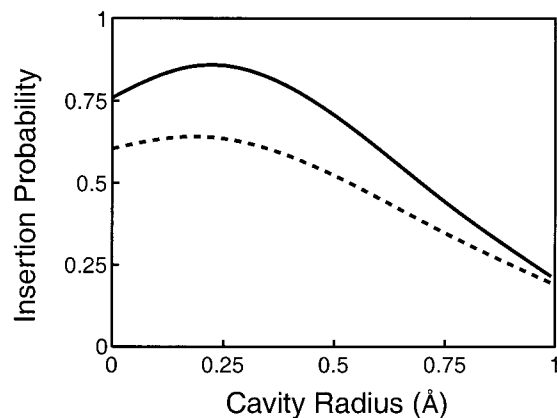


Figure 9. Free volume distributions from molecular dynamics simulations of a simple liquid, *n*-hexane (dashed line), and water (solid line). The probability of finding a cavity of a given radius is plotted. Water has more small cavities (<1 Å) than *n*-hexane. Data adapted from G. Hummer et al., *J. Phys. Chem. B* **1998**, *102*, 10 475.

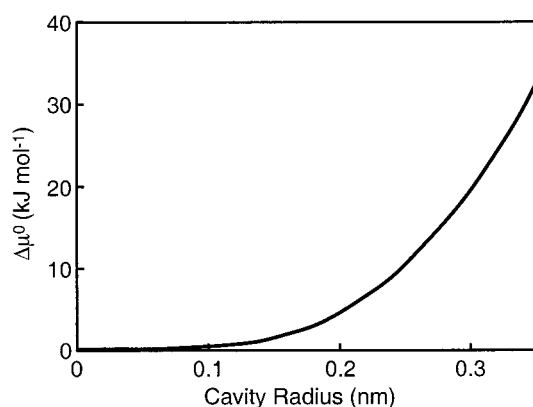


Figure 10. Free energy cost of creating a cavity, from molecular simulations. Data adapted from Hummer, G., et al. *J. Phys. Chem. B* **1998**, *102*, 10 475.

entropy compensation of these latter two contributions. He argued that the complexity that results from water orientations in the first solvation shell does not contribute much to the free energy of cavity formation in water.¹¹⁹ Some of these predictions are confirmed by computer simulations^{83,121–127} and by experiments on hydrazine.¹²⁸

(2) **Small Size.** Figure 9 illustrates the Small-Size idea. It shows a distribution of sizes of free volume in two liquids. Most cavities in liquid water are much smaller than a water molecule, and the probability of finding a larger cavity decreases rapidly as a function of cavity size.

This is explained by a simple qualitative argument. The free energy for opening a spherical cavity of radius R in a liquid is the sum of a surface term and a volume term, $f \approx 4\pi R^2\gamma + 4/3\pi R^3E$, where γ is the surface tension and E is a volumetric energy density. The Boltzmann distribution law gives the probability $p(R)$ of finding a cavity of radius R as

$$p(R) \sim \exp(-f/kT) \sim \exp(-4\pi\gamma R^2/kT - 4ER^3/3\pi kT) \quad (6)$$

which shows that $p(R)$ should drop off rapidly with increasing R . Figure 10 shows that cost of creating cavities in water increases rapidly as a function of cavity size.

Suppose the solvent molecules have a characteristic radius, R_1 . Most of the free volume will occur in packets having a characteristic dimension R smaller than the radius of the solvent molecules ($R \ll R_1$). Now compare two different solvents. If solvent molecules are small, its packets of free volume will be

small. But if the solvent molecules are large, its packets of free volume will be large. So big cavities are more likely in solvents of big molecules. According to the small-size model, water molecules are among the smallest of all solvent molecules, and the free energy cost of opening a cavity of a given size is greater in water than for other solvents. This free energy cost can be estimated by scaled-particle theory^{117–119,129–132} Some of the limitations of these arguments are discussed below (see the section on the MB model).

D. Information Theory Approach to Modeling Water. Cavities in water have also been studied by computer simulations^{83,121,122,133–137} and statistical mechanical theories.^{8,119,120,131,132,138,139} All-atom simulations are computationally expensive, and reaching convergence is often difficult, particularly for derivatives of the free energy such as the heat capacity. Studying a series of temperatures and solute sizes adds more computational expense. Integral equation methods are not yet generally available for treating orientation-dependent interactions.¹⁴⁰ For this reason, one of the most popular strategies has been to use models that start with experimentally obtainable properties, such as the pair correlation function of the solvent^{8,115,119,138,139} rather than beginning from an underlying energy function based on the structure of water.

A recent and elegant descendant of scaled-particle theory is the information theory approximation developed by the Los Alamos group,⁸ following on the work of Pohorille and Pratt.¹²¹ The chemical potential for creating a cavity is given by

$$\Delta\mu_{\text{ex}} = -kT \ln p_0 \quad (7)$$

where p_0 is the probability of finding an empty cavity in water of a given size and shape. The probability p_n for finding a cavity containing n molecules is

$$p_n = \exp(\lambda_0 + \lambda_1 n + \lambda_2 n^2) \quad (8)$$

where the λ 's are Lagrange multipliers that force the zeroth, first, and second moments of the number distribution function to agree with experimental observables such as the mean density and pair correlation function.⁸ This model predicts that the probability of finding a cavity containing n molecules is a Gaussian function

$$p_n = [\exp(-(n - \bar{n})^2/(2\sigma^2))/(2\pi\sigma^2)] \quad (9)$$

where \bar{n} is the mean number density of molecules in the solution, and σ is the standard deviation of the observed density, leading to

$$\Delta\mu_{\text{ex}} = \frac{kT\rho^2 v^2}{2\sigma^2} + \dots \quad (10)$$

It is found that small density fluctuations in water are well modeled by such Gaussian distribution functions.¹³⁶ The model also successfully reproduces the transfer entropies, isotope effects, pair hydrophobic interactions, and the salt dependence of transfer,^{8,141} although not the heat capacity.^{142,141}

These models all have some limitations. The Muller model requires many parameters, including an enthalpy and entropy for each of the 4 states of hydrogen bonding, and does not explain how those properties arise from the structure of water and the solute. The Small-Size model only attempts to explain the free energies, and not the entropies, enthalpies, or heat capacities, and it gives no explanation for the physical origin of compensation. The information theory model predicts proper-

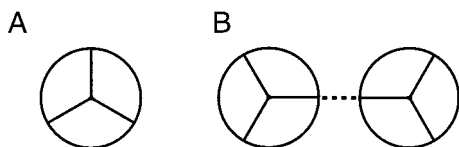


Figure 11. MB model of water. (A) One MB water molecule: a Lennard-Jones disk with three directional hydrogen bonding arms, and (B) two MB water molecules that form a hydrogen bond.

ties of water from other properties of water (pair correlation function and density), rather than from the structure of water and solute. The MB model, described below, aims to circumvent some of these limitations by providing a microscopic theory, but it does so at the expense of atomic detail and a simplified energy function.

E. MB Model. In the MB model, each water molecule is a 2-dimensional circular disk that interacts with other molecules through: (1) a Lennard-Jones 6-12 interaction, and (2) through three hydrogen bonding arms arranged like the Mercedes-Benz logo, hence the name^{85, 111,112,143,144} (see Figure 11). This model has been studied in (N,P,T) Monte Carlo simulations. It predicts qualitatively the volume anomalies of water and the thermodynamic signatures of the hydrophobic effect (see Figures 12 and 13). This model has been used to seek a structural basis for the hydrophobic effect and to test some of the model assumptions and approximations described above. As in the

iceberg model, it predicts that the hydrophobic effect in cold water results from cage-like waters around nonpolar solutes. Inserting a small nonpolar solute orders cold waters into low-entropy, low-enthalpy cages. In hot water, the first-shell waters have higher entropies and enthalpies. This model has motivated a strategy for obtaining hydrogen bond strengths in (real, three-dimensional) water from experimental spectroscopy^{109,110} and heat capacity data. It is found from Raman data that the breaking of a water-water hydrogen bond in the bulk gives $\Delta G = 480 \text{ cal mol}^{-1}$, $\Delta H = 1.9 \text{ kcal mol}^{-1}$, and $\Delta S/k = 2.4$, and it is predicted that in the first shell around argon, $\Delta G = 620 \text{ cal mol}^{-1}$, $\Delta H = 2.4 \text{ kcal mol}^{-1}$, and $\Delta S/k = 3.0$.¹¹² These results provide both a quantitative measure of the degree of water organization in water cages around nonpolar solutes, and a testable prediction about the microscopic reorganization of water by a solute.

The MB model has been used to study how hydrophobicity depends on solute radius.⁸⁵ In cold water, growing the radius of a *small* spherical solute (smaller than a water molecule) increases the free energy by increasing the structure of the cage, lowering the enthalpy and entropy and increasing the heat capacity. But inserting a *large* solute (at least twice the diameter of water) into either hot or cold water increases the free energy by a different mechanism, namely by breaking hydrogen bonds in the cage, increasing the enthalpy but with little effect on the

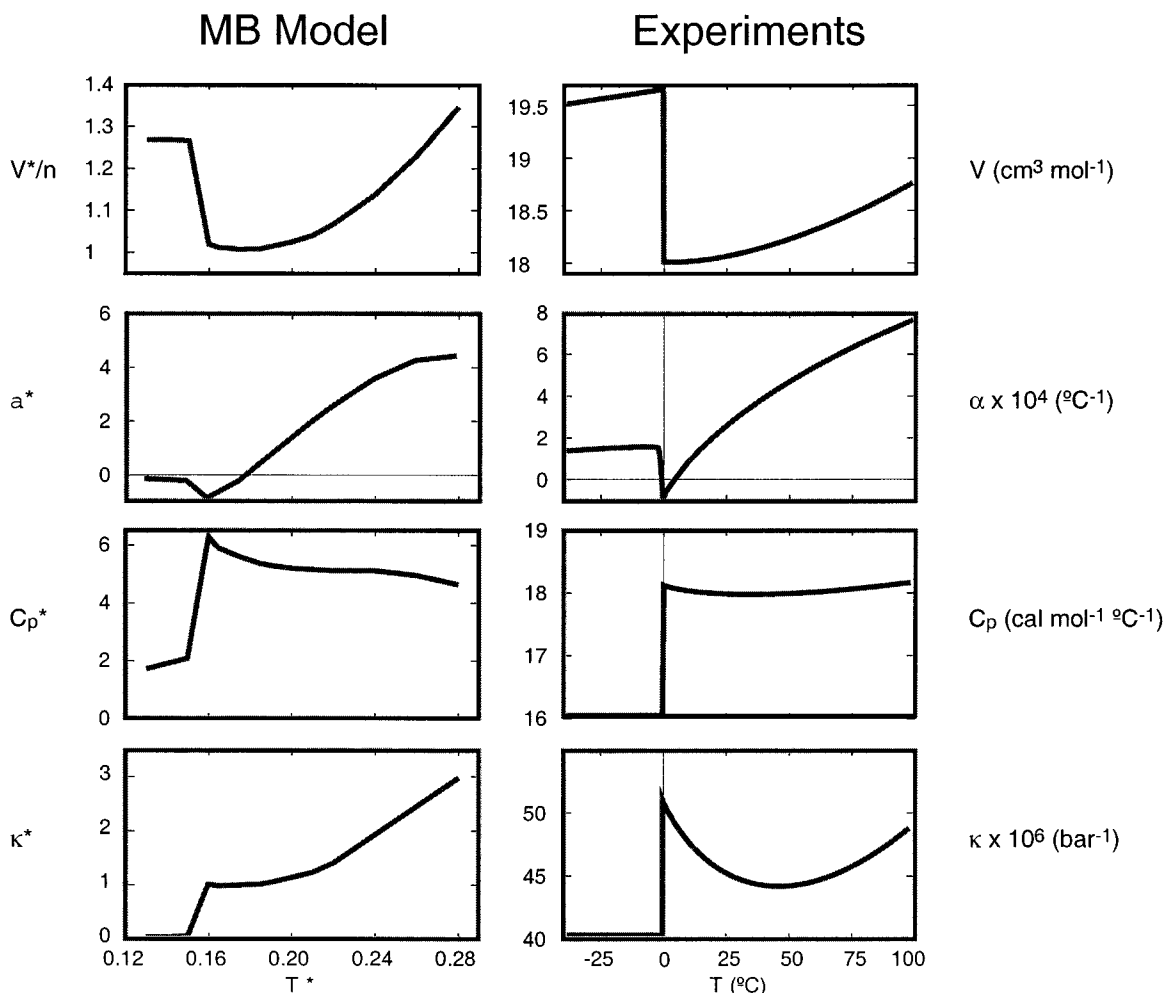


Figure 12. MB model predictions versus experiments for molar volume (V), thermal expansion coefficient (α), heat capacity (C_p), and isothermal compressibility (κ) versus temperature. Experimental data are replotted from D. Eisenberg and W. Kauzmann, *The Structure and Properties of Water*; Oxford University Press: Oxford, 1969. Simulated quantities are in reduced units, relative to the energy (ϵ_{HB}) and length (l_{HB}) of a hydrogen bond in the MB model ($T^* = k_B T / \epsilon_{\text{HB}}$, $V^* = V / l_{\text{HB}}^2$).

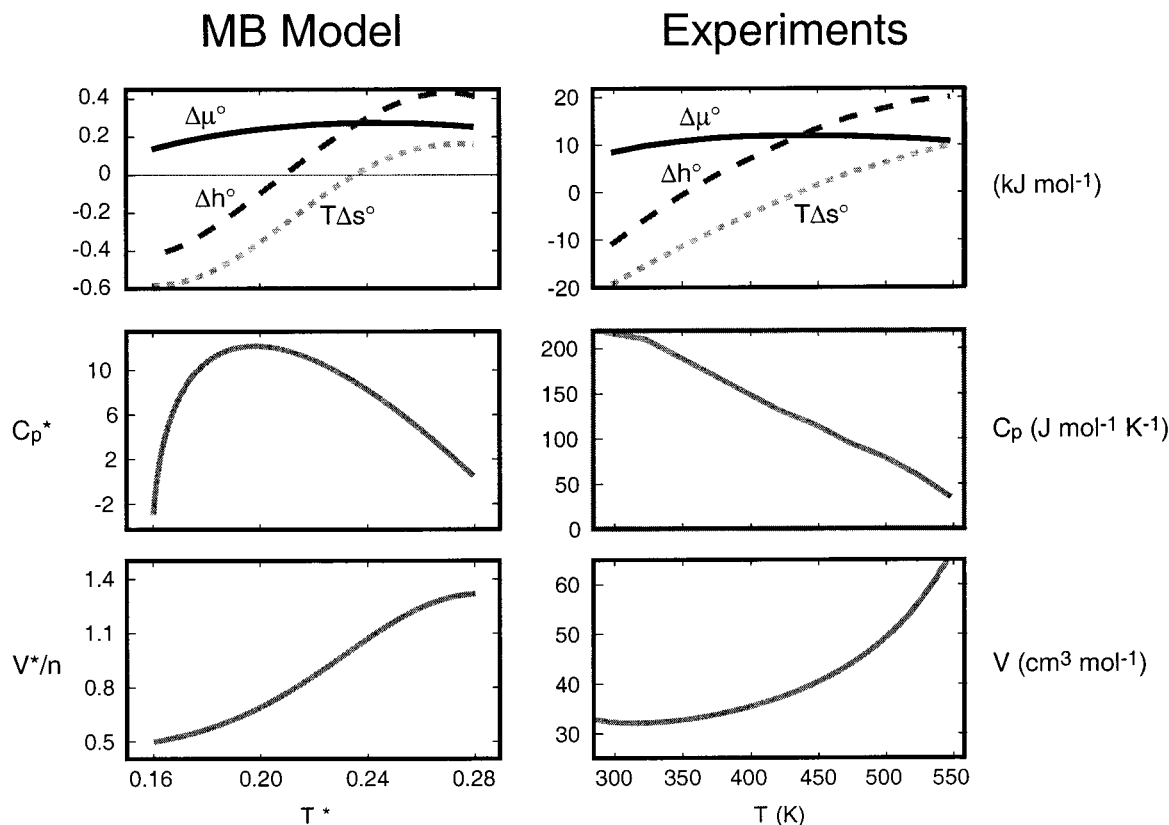


Figure 13. MB model predictions for nonpolar solvation, versus experiments on the transfer of gaseous argon into water, taken from A. Ben-Naim, *Solvation Thermodynamics*; Plenum Press, New York, 1987. The experimental molar volume data was taken from A. H. Harvey, J. M. H. L. Sengers, and J. C. Tanger, *J. Phys. Chem.* **1991**, 95, 932.

entropy or heat capacity (see Figure 14). The latter mechanism arises because water is geometrically unable to form its maximal number of hydrogen bonds to other waters in the vicinity of a large inert surface, so it wastes hydrogen bonds by pointing them at the nonpolar surface.^{145,146} This changeover in mechanism explains⁸⁵ a long-standing puzzle about why it costs only about 25 cal mol⁻¹ Å⁻² to transfer small oil molecules into water, while it costs about 75 cal mol⁻¹ Å⁻² to create planar oil/water interfaces.^{27,29,145–147} In short, small solutes order neighboring waters, leading to an entropy cost, whereas large solutes break water–water hydrogen bonds, leading to an enthalpy cost. And the latter free energy is 3-fold greater than the former. This model also accounts for why planar oil/water interfaces are thermally more similar to regular solutions than to hydrophobic thermodynamics.^{85,146,148–151} Simple thermal behavior is also seen in other large-curvature systems, such as micelles and inclusion systems.^{152–157}

The MB model has also been used to study the enthalpy–entropy compensation assumption underlying the Small-Size model.¹²⁷ For small solutes at room temperature, MB model studies confirm that free energies are well-modeled by simple hard disk fluids. But it also shows that the enthalpy–entropy compensation assumption is quite limited. Enthalpy–entropy compensation applies only when nonpolar solutes are small; only applies approximately with temperature; and only applies to free energies, not to enthalpies, entropies, or heat capacities. For example, inserting a large nonpolar solute breaks water–water hydrogen bonds, which *increases* the enthalpy, and involves no compensating entropy. Hence, while the energetic and entropic complexities of water may cancel in the transfer free energies of small solutes in cold water, water’s complexities appear to be important in most other circumstances.

Tests have also been made of the information theory model. MB model tests have shown that the IT model predicts qualitatively but not quantitatively the dependence of hydrophobic heat capacity on temperature, a key signature of the hydrophobic effect.¹⁴² Adding additional moments does not improve the calculation.⁸ Second, it is now clear that large cavity fluctuations in water are much more probable (by several orders of magnitude) than the IT model Gaussian distribution predicts.^{127,158}

The Gaussian distribution assumption in the IT model applies to small fluctuations in water, but not to the creation of large cavities. There are two cavity costs (see eq 6): a surface term and a volume term. Most of the cost of creating small cavities comes from the volume term. The cost of creating large cavities is dominated by the surface term.^{127,159}

Fluids with a vapor–liquid transition exhibit two mechanisms of creating cavities; the free energies of both are well described by a polynomial equation with a volume term, a surface term, etc. (see eq 6). Small cavity free energies are dominated by a large volume term (where the pressure coefficient is related to hard disk pressure), but large cavity free energies have a small volume term (where the pressure coefficient is related to ambient pressure) and a large surface term.¹²⁷ The latter mechanism may have important implications for protein folding and the behavior of large systems.^{139,160}

Analysis of the full, angle-dependent multiparticle correlations in the MB model show that the entropy contribution to the hydrophobic effect¹⁶¹ is the sum of two terms of opposite sign: a pairwise-additive, highly ordering, term of the form $g \ln g$, and a nondecomposable three- and higher-body term which is *disordering*, especially in cold water. The differing temperature

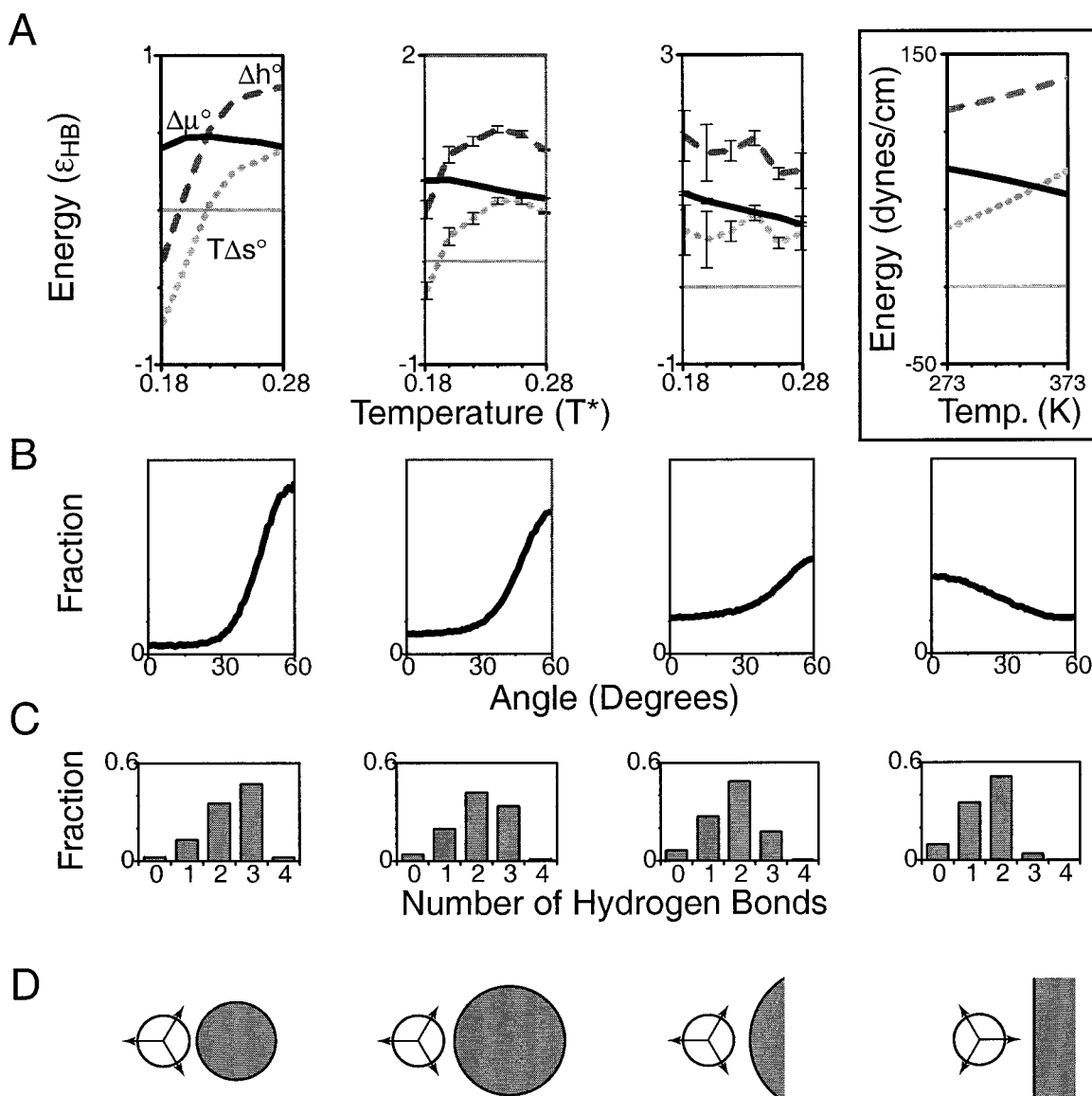


Figure 14. Dependence of the hydrophobic effect in the MB model on the size of the nonpolar solute. (A) Transfer thermodynamics and experimental surface tension of water (inset box), (B) angular orientations of first-shell waters, (C) histograms of the numbers of hydrogen bonds among first-shell waters, and (D) the most probable structure of a water adjacent to a hydrophobic solute for a series of solutes, sized (from left to right) 1.07, 1.5, and 2.0 (l_{HB}) and a plane. As solutes grow in the MB model, transfer thermodynamics change from (A) being entropically unfavorable at low temperatures to enthalpically unfavorable at low temperatures, which correlates with (B) a disordering of first shell waters, and (C) a decrease in the number of fully hydrogen bonded shell waters.

dependences of these two terms give rise to the richness of the hydrophobic effect.

F. Pairwise Hydrophobic Interactions. The term “hydrophobic effect” refers to inserting a single nonpolar solute into water. “Hydrophobic interaction” refers to the association of two nonpolar moieties in water. In the simplest view, two nonpolar solutes are driven to associate in water by the reduction in surface area of solute-water contact.^{7,162} This macroscopic continuum explanation assumes that the free energy cost of creating cavities in water depends only on cavity surface area and not otherwise on cavity geometry. This explanation neglects detailed water structure, which, at least in some instances, may not be warranted.^{163,164}

The pair interaction of two nonpolar solutes in water is described by a *potential of mean force* (pmf). Figure 15 shows how the pmf depends on solute separation. The two prominent minima in this figure indicate two favored states: (1) the “contact pair”, where the two nonpolar solutes are in contact, and (2) the “solvent-separated pair”, where the two solute

molecules are separated by a molecule of water. States that are intermediate between these two, having less than one full water layer between the two solutes, are unfavorable. Computer simulations show that the contact between two methanes is favored by entropy and disfavored by enthalpy at room temperature.¹⁶⁵ With increasing temperature, the contact pair becomes enthalpically favorable and entropically unfavorable, whereas the overall free energy changes little.¹⁶⁶ This thermodynamic signature is readily explained by the surface-area-reduction model, where opening cages is entropically unfavorable and enthalpically favorable, the traditional hydrophobic effect.

However, this surface-area argument does not explain recent simulation results on heat capacities of pairing.^{167–169} Surface-area arguments would predict a large decrease in heat capacity when two nonpolar molecules come into contact in water. Instead, extensive computer simulations find nearly zero change in the heat capacities. The contact pair of nonpolar solutes has the same heat capacity as the infinite dilution pair.¹⁶⁹ The

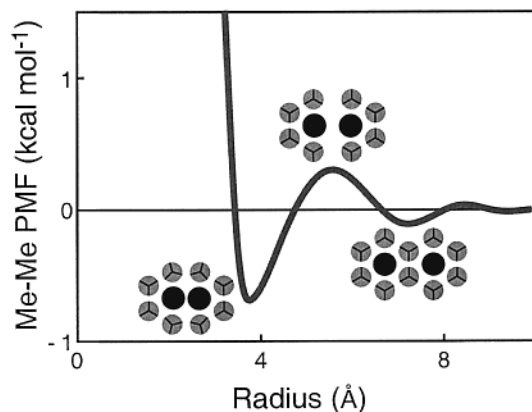


Figure 15. Methane–methane potential of mean force. Idealized potential of mean force between two methanes as a function of distance in angstroms. Left: methanes (in black) at contact, middle: at the barrier distance (which has an unfavorable free energy), and right: in a solvent-separated configuration. Adapted from D. E. Smith, and A. D. J. Haymet, *J. Chem. Phys.* **1993**, 98, 6445.

implication is that hydrophobic interactions depend on more complex aspects of water structure than just surface areas of cages.

G. Pressure Dependence of Hydrophobic Interactions and Protein Folding. When a nonpolar solute is transferred from oil to water at atmospheric pressure, the partial molar volume decreases. The solute induces first-shell waters to contract around it. For example, the partial molar volume of methane decreases from 60 to 37.3 mL mol⁻¹ upon transfer from hexane to water.¹⁷⁰ Since $d \ln K/dp = -\Delta v/kT$, it follows that applying pressure drives nonpolar solutes into the water phase. Space is filled more efficiently in water cages containing nonpolar solutes than in the empty cages in pure water.

Also, nonpolar liquids are more compressible than is water that contains nonpolar solutes. With increasing pressures, the partial molar volume of transfer approaches zero and then becomes positive. At pressures of 1–2 kbar, applying further pressure drives nonpolar solutes out of the water phase.¹⁷¹

Pressures also affect the relative stabilities of contact-pairs vs solvent-separated pairs, but this delicate balance is not yet fully understood. One computer simulation study shows that low pressures dissociate contact pairs of nonpolar molecules, giving a positive volume of dissociation, whereas high pressures have little effect.¹⁷² In contrast, the information theory model predicts a negative volume of dissociation.¹⁷³ More recent simulations support the IT model prediction, but show also that the solvent-separated state is also sensitive to pressure.¹⁷⁴ Another series of simulations found that the solvent-separated state is the most sensitive to pressure,¹⁶⁷ and recent simulations appear to confirm this.¹⁷⁵

Protein folding is also affected by pressure. Proteins often have a small negative volume of unfolding at atmospheric pressures.^{176–178} As pressure increases, the volume of unfolding becomes increasingly negative. Typically, proteins can be unfolded by 1–2 kbar pressure,^{176–183} even though nonpolar solutes are less soluble in water at these pressures.

Differences between the transfer of model solutes and the pressure dependence of protein folding have been used to argue that protein unfolding is not the same as transferring nonpolar molecules into water.^{48,167,172–174,176,177,184} However, differences in compressibilities between the oil phase and protein cores appear to be partly responsible for the discrepancy.^{4,180} Nonpolar compounds in hydrocarbon liquids are much more compressible than a native protein by a factor of 10.¹⁸⁵ As pressures increase,

the molar volume of the folded state does not change. At the same time, increased pressure compresses the unfolded protein state, and the volume of unfolding becomes increasingly negative. For hydrophobic transfer, the oil phase is more compressible than water, and the volume of transfer into water becomes positive with increasing pressure.

Pressure also affects protein folding kinetics,^{182,186} in a way that mimics its effect on hydrophobic interactions.^{167,173,174} In particular, increasing pressure slows folding, raising the free energy barrier, in a manner that would be consistent with pressure-induced dissociation of nonpolar contacts.

VI. Conclusions

We have surveyed some aspects of the hydrophobic effect. Experiments show that for simple hydrocarbons, the disaffinity of oil for water is largely entropic at room temperature, and is accompanied by a large heat capacity of transfer. Oil/water partitioning is widely used in combination with additivity schemes (BIPSE models) to predict noncovalent equilibria in chemistry and biology. However, there are limitations to other components of these models and to underlying assumptions of additivity, homogeneity of the medium, dependence on surface area, and of the nature of polarity, that must ultimately be solved to increase their predictive power. The underlying molecular basis for the hydrophobic effect is that opening cavities in any solvent is costly, in addition to water orientation effects which contribute to the thermal fingerprints. A key point is that models that explain hydrophobic free energies are often not sufficient to explain entropies and heat capacities, which are hallmarks of hydrophobicity. The physics of hydrophobic interactions changes with temperature and with solute size and shape.

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