

Hydrophobic Effects. Opinions and Facts

By Wilfried Blokzijl* and Jan B. F. N. Engberts*

The term hydrophobic interactions denotes the tendency of relatively apolar molecules to stick together in aqueous solution. These interactions are of importance in many chemical disciplines, including the chemistry of *in vivo* processes. Enzyme–substrate interactions, the assembly of lipids in biomembranes, surfactant aggregation, and kinetic solvent effects in water-rich solutions are all predominantly governed by hydrophobic interactions. Despite extensive research efforts, the hydration of apolar molecules and the noncovalent interactions between these molecules in water are still poorly understood. In fact, the question as to what the driving force for hydrophobic interactions is shifts the study into a quest for a detailed understanding of the remarkable properties of liquid water. This review highlights some of the novel insights that have been obtained in the past decade. The emphasis is on both hydrophobic hydration and hydrophobic interactions since both phenomena are intimately connected. Several traditional views have been found to be deeply unsatisfactory, and courageous attempts have been made to conceptualize the driving force behind pairwise and bulk hydrophobic interactions. The review presents an admittedly personal selection of the recent experimental and theoretical developments, and when necessary, reference is made to relevant studies of earlier date.

1. Introduction

1.1. General Remarks and Outline of the Review

At moderate temperatures and pressures, apolar compounds are poorly soluble in water. Traditionally, the reluctance of apolar compounds to dissolve in water has been attributed to the hydrophobicity of these compounds, in other words, their fear of water. In fact, the term hydrophobicity is misleading. The London dispersion interactions between water and apolar compounds are favorable and quite substantial.^[1] It is more appropriate to point out that the apolar compound must intrude into a liquid that is characterized by an extended network of hydrogen bonds and an extremely high cohesive energy density. Each water molecule is strongly inclined not to sacrifice any of its hydrogen bonds, leading inevitably to significant reorientation of water molecules at the surface of the nonpolar solute molecule. Water is unique among solvents, because it has not only an exceptionally small molecular volume, but also the capacity to form a molecular network with tetrahedral coordination and an extremely low isothermal compressibility. This provides water with an almost unrivaled propensity to wrap itself around an apolar solute particle and simultaneously maintain most or all hydrogen bonds. But in spite of this unique structural solution, water must be considered a notoriously poor solvent for apolar compounds.

Unabated, chemists have sought a molecular interpretation of the hydrophobicity of apolar compounds. The first explanations were based on the capricious thermodynamic parameters of the hydration of these compounds in water at room temperature. The thermodynamic parameters of the solvation of gaseous methane in various organic solvents and water at 25 °C are given in Table 1. At room tempera-

Table 1. Thermodynamic parameters of solvation for gaseous methane in various organic solvents and water at 25 °C (taken from ref. [31]) [a].

Solvent	ΔG^\ominus [kJ mol ⁻¹]	ΔH^\ominus [kJ mol ⁻¹]	$-\Delta S^\ominus$ [kJ mol ⁻¹]
<i>n</i> -hexadecane	12.7	-2.30	15.0
<i>n</i> -decane	13.0	-4.31	17.3
<i>n</i> -hexane	13.1	-2.26	15.4
cyclohexane	14.2	-3.01	17.2
CCl ₄	14.5	-2.97	17.5
diethyl ether	13.4	-3.34	16.7
benzene	15.3	-1.25	16.6
acetone	15.6	-2.80	18.4
1-propanol	16.0	-10.3	26.3
ethanol	16.5	-8.78	25.3
methanol	17.5	-7.94	25.4
water	25.5	-13.8	39.3

[a] For gaseous methane (1 atm) → solution with 1 mol% methane.

ture, the dissolution of apolar gases in water is strongly promoted by enthalpy and characterized by an anomalously large change in heat capacity (for gaseous methane, the change in heat capacity (at 25 °C) amounts to 209 kJ mol⁻¹ K⁻¹^[2]). In contrast, the change in heat capacity upon solvation of gaseous methane in organic solvents is generally small. The poor solubility is completely governed by an extremely large loss of entropy. The partial molar volume of an apolar gas dissolved in water is generally similar to the molar volume of the gas at the boiling point, which is remarkable since the partial molar volume of solutes in organic solvents is significantly higher. Some of these striking thermodynamic properties were first identified by Butler and Reid.^[3] In 1945 Frank and Evans^[4] gave a molecular interpretation for these observations which would later become the archetype for further studies on hydrophobic effects. The model, generally known as the iceberg model, explained the unusually large entropy loss upon dissolution of apolar gases in terms of the structuring of water molecules in the near vicinity of the apolar solute. The emphasis on the enhanced local structure led to overinterpretation of the original idea in later studies, and frequently the hydration

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shell of apolar solute particles has been pictured as a rather rigid clathrate-like structure.^[5] The idea of a special kind of hydration, referred to as hydrophobic hydration, became generally accepted.

It was proposed that the tendency of water molecules to avoid entropically unfavorable interactions with apolar solutes constitutes a driving force for the aggregation of apolar solutes. The notion that the interactions between apolar species in aqueous solution are primarily entropy driven gave chemists a genuine feeling of discomfort. In 1959 Kauzmann^[6] introduced the concept of hydrophobic interactions, the alleged solvent-induced attractive forces between apolar molecules or moieties in aqueous solution. He suggested that the attractive interactions between apolar molecules or moieties in aqueous solution are promoted by the solvent as a result of destructive overlap of structured hydrophobic hydration shells, involving the release of structured hydration water into the bulk. Later, the idea of a hydrophobic bond received much attention, particularly in the field of protein chemistry.

Hydrophobic effects play a pivotal role in many chemical phenomena in aqueous solution. The stability of biological membranes as well as of the tertiary structure of proteins in aqueous solution depends critically on interactions between apolar moieties. Molecular recognition also relies strongly on noncovalent interactions, for which hydrophobic regions in host and guest molecules are often crucial. Apart from their importance in biological processes, hydrophobic effects are also important for various solution phenomena such as surfactant aggregation, mineral flotation, coagulation, complexation, and detergency. In addition, hydrophobic effects play a decisive role in the formation of gas clathrates and in surface phenomena such as wetting, aggregation processes at air–water interfaces, and forces between apolar surfaces. In view of the wide interest in hydrophobic effects it is essential to have a clear understanding of the molecular nature behind these phenomena.

Although the qualitative concept of the increased structuring of water in hydrophobic hydration shells has provided a

popular explanation for the unusual thermodynamic properties of aqueous solutions of apolar compounds, serious doubts have been voiced occasionally concerning the existence of hydrophobic effects. Frequent suggestions have been made to reassess the traditional ideas about hydrophobic effects and to adjust the somewhat dogmatic picture of hydrophobic hydration. More recently, rapid maturation of computational methods such as molecular dynamics and Monte Carlo calculations, the introduction of elaborated integral theories and refined versions of scaled particle theory for a description of liquids and solutions, the development of sophisticated microcalorimetry to measure highly accurate thermodynamic solvation data not only at room temperature but also at elevated temperatures, and the application of protein engineering to study the role of hydrophobic amino acid residues in protein folding and stability have yielded increasing evidence against the traditional interpretation of hydrophobic effects. In spite of growing doubts about the validity of the original models, the classic interpretation of hydrophobic effects still prevails in the recent literature. The fact that arguments against traditional ideas have been largely ignored must, at least in part, be attributed to notorious ambiguities in the nomenclature pertaining to the hydrophobic effect; this has lent recent discussions a strongly semantic flavor.

At this stage, we feel that there is a need to stand back a little and review recent experimental as well as theoretical studies of hydrophobic effects in different areas of chemistry. Because of the yearly surge of papers on hydrophobic effects, our survey can by no means be exhaustive. Our major goal is to signal remarkable results and to identify important shortcomings in current views on hydrophobic effects. Our review is therefore deliberately biased and aims at a critical reappraisal of hydrophobic effects in which the validity of the whole concept of hydrophobic hydration and hydrophobic interactions will be questioned. To avoid unnecessary ambiguities, definitions will be given for hydrophobic hydration and hydrophobic interactions in Section 1.2. Unfortunately, these definitions are as yet not unanimously accepted.



Jan B. F. N. Engberts was born in 1939 in Leiden, the Netherlands. He studied synthetic and physical organic chemistry at the University of Groningen under supervision of Prof. J. Strating and Dr. B. Zwanenburg. After graduating with a Ph.D. degree in 1967, he was a postdoctoral fellow with Prof. T. J. de Boer at the University of Amsterdam (1968–1969). He returned to Groningen in 1969 as Associate Professor and was appointed Professor of General Chemistry in 1978. Since 1992 he has held a chair in Physical Organic Chemistry at the same university. The research in his group is centered around the theme “organic chemistry in water” and includes projects in molecular recognition, surfactant aggregation, synthesis and properties of polymers and polysoaps, and vesicle fusion.



Wilfried Blokzijl received his degree in chemistry at the University of Groningen in 1987 for his research with Prof. G. Challa on the catalytic behavior of flavine-modified polyelectrolyte complexes. The same year he started as a Ph.D. student in the group of Prof. J. B. F. N. Engberts and collaborated also with Prof. M. J. Blandamer of the University of Leicester. In 1991 he completed his doctoral thesis entitled “Organic Reactivity in Mixed Aqueous Solvents”, which described a quantitative link between kinetics and thermodynamics. He is currently examining the catalysis of organic reactions in homogeneous aqueous media. He will take a position at the Research Laboratories of Unilever in Vlaardingen in 1994.

This survey focuses mainly on developments reported during the past decade. Where necessary, reference is made to relevant studies of earlier date. Many experimental studies of hydrophobic effects applying nuclear magnetic resonance, ultrasound, diffraction methods, and calorimetry were reported in the sixties and seventies. For detailed accounts of this work, we refer to the series of books, *Water. A Comprehensive Treatise*, edited by Franks.^[17] A short historical excursion into the field of hydrophobic effects presented by Franks^[8] in 1982 showed that these experimental studies were often consistent with the molecular interpretation of hydrophobic effects as given by the traditional iceberg model. More theoretical approaches to understanding hydrophobic effects have been described in textbooks by Tanford,^[9] Ben-Naim,^[10] and Luck.^[11] Following the classic review of Kauzmann,^[6] the sixties and seventies witnessed a number of attempts to quantify the concept of hydrophobic interactions. Some important contributions, briefly reviewed in Section 1.3, set the stage for ensuing theoretical studies of hydrophobic effects.

This review has two parts comprising, respectively, experimental and theoretical studies of hydrophobic effects. The criteria for defining the separation of these sections are somewhat arbitrary, and activities can show considerable overlap. Following tradition, Part A is inaugurated by a survey of results of recent calorimetric studies as well as reinterpretations of previous data for the solvation of apolar gases and liquids in water over a broad temperature range (Section 2.1). In subsequent sections the importance of hydrophobic effects for the stability of tertiary protein structures (Section 2.2), molecular recognition (Section 2.3), aggregation of amphiphiles (Section 2.4), and surface forces (Section 2.5) will be discussed. This will be followed by a selection of recent results obtained by spectroscopic techniques (Section 2.6). Finally, Part A concludes with a section in which applications of water as a medium for organic reactions are discussed (Section 2.7).

Part B starts off with a brief introduction of theoretical descriptions of hydrophobic effects that have been presented over the years (Section 3.1). Subsequently, efforts are described for the development of rather simple molecular models to account for the thermodynamic properties of aqueous solutions of nonelectrolytes (Section 3.2). In Section 3.3 more sophisticated theories are summarized that are mainly based on virial expansions of thermodynamic excess properties of aqueous solutions to quantify interactions between apolar solutes in aqueous media. Section 3.4 contains a concise summary of applications of scaled particle theories to the problem of hydrophobic effects. Part B concludes with an extensive survey of results of computational studies of both hydrophobic hydration and hydrophobic interactions (Section 3.5).

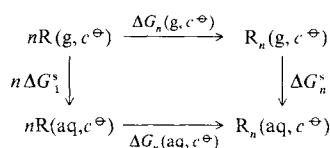
A final summary and outlook is presented in the epilogue (Section 4) which signals inconsistencies between results of experimental and theoretical studies of hydrophobic effects and also identifies aspects that are currently unanimously accepted. On the basis of these considerations, we present a molecular interpretation of the solvation of apolar compounds in water as well as their aggregation in aqueous solution which is conceptually different from traditional ideas.

1.2. Hydrophobic Effects; The Terminology

Many of the existing definitions of hydrophobic effects reflect the lack of clarity with respect to the exact meaning of the terms that have been introduced to describe the unusual thermodynamic behavior of apolar solutes in aqueous solution (see, for example, the discussion in *Science* two to three years ago)^[12–15]. Although the concept of hydrophobicity is widely used, its exact meaning is somewhat vague. We prefer to define hydrophobicity on the basis of the thermodynamics of the transfer of apolar compounds, either from their liquid state or from a solution in an apolar solvent, to water. This process involves the disruption of interactions between the apolar molecule and its apolar environment, refilling of the vacancy in the apolar medium, the creation of a cavity in water, the initiation of water–solute interactions, and the concomitant reordering of the water molecules in the near vicinity of the solute. Thus, hydrophobicity yields information about the limited solubility of apolar compounds in water and can be quantified on the basis of solubility measurements or partitioning studies.

The definition of hydrophobic hydration is even more troublesome, and several interpretations can be encountered in the literature. Hydrophobic hydration refers to the way a nonpolar solute affects the local structure of water in its immediate environment. A convenient definition of hydrophobic hydration considers the process of cavity formation in liquid water, the insertion of an apolar solute particle, the onset of solute–solvent interactions, and the concomitant reordering of the water molecules in close proximity to the solute. Unfortunately, this process is difficult to study by experimental means.

The occurrence of hydrophobic interactions is perhaps the most dramatic revelation of the limited solubility of apolar compounds in water. It is essential to make a clear distinction between pairwise and bulk hydrophobic interactions.^[16] Scheme 1 shows a thermodynamic cycle taken from a paper by Wood and Thompson,^[16] which illustrates the relation between these distinct phenomena. In this cycle, R stands for



Scheme 1. Thermodynamic cycle for the transfer of n molecules R (nR) as well as of a cluster of n molecules R_n (R_n) from the gas phase into an aqueous solution, illustrating the difference between bulk and pairwise hydrophobic interactions (taken from ref. [16]). s denotes the solvation steps.

a hydrophobic compound, c^\ominus is a standard or reference concentration (vide infra), g denotes the gas phase, and aq the aqueous phase. For $n = 2$, $\Delta G_2(aq, c^\ominus)$ is the Gibbs energy involved in the interaction of a pair of molecules R in aqueous solution and provides a measure of the pairwise hydrophobic interaction. The solvent-induced part can be computed by comparing $\Delta G_2(aq, c^\ominus)$ with $\Delta G_2(g, c^\ominus)$.^[17] Bulk hydrophobic interactions are characterized by large values of n . Interestingly, when $n \rightarrow \infty$, ΔG_n^s approaches zero. In this case $-(1/n)\Delta G_n(aq, c^\ominus)$ is the Gibbs energy for the

transfer of an apolar compound from an aqueous solution to its liquid state. ΔG_1^o is the Gibbs energy for the solvation of a gaseous compound R in water. This process is sometimes incorrectly considered equivalent to hydrophobic hydration.

The magnitudes of the thermodynamic parameters of the various processes depend critically on the choice of standard states *c*.^[18, 19] The most commonly used standard state assumes gaseous compounds at 1 atm that are dissolved in water at a mole fraction of one. The advantage of this particular choice is that the statistical part of the chemical potential (which is mainly entropic in origin) does not differ when solvents with different molecular weights and densities are considered. Other standard states such as 1 mol dm⁻³ and 1 mol kg⁻¹ suffer from this disadvantage. When standard states are explicitly known, data can be easily interconverted.^[18] As shown convincingly by Abraham,^[18] the interpretation of transfer parameters or solvation data is often seriously hampered by application of erroneous reference states. In particular, the pure liquid is an often used but very hazardous reference state. Ben-Naim^[20] enunciated the advantages of an alternative standard state based on the transfer of an apolar gas to a fixed position in water. In a subsequent step, the solute is released into the solvent, gains kinetic energy, and becomes indistinguishable from the other molecules in the solution. The so-called pseudochemical potential can be computed from known thermodynamic parameters of solvation but cannot be determined directly. The popularity of this particular standard state can be attributed to the fact that the solute molecule can always be distinguished from solvent molecules. This offers interesting possibilities for studying the solvation of organic compounds in mixed aqueous solvents.

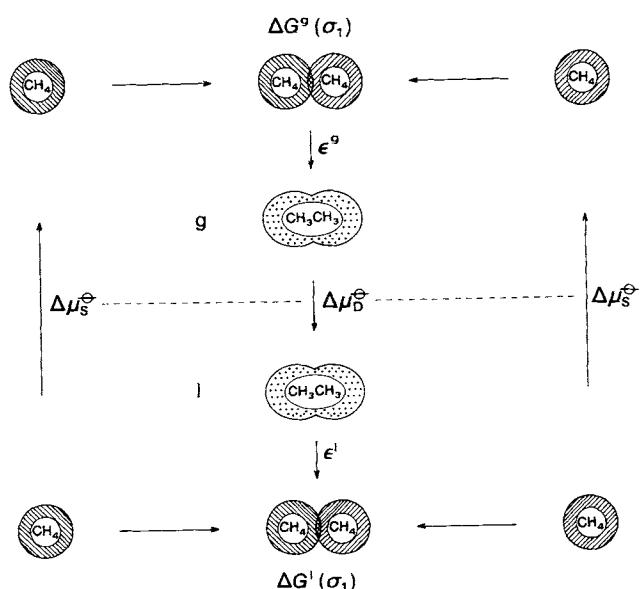
1.3. Classic Models for Hydrophobic Effects

As mentioned in the introduction, Frank and Evans^[4] were the first to speculate about the molecular origin of the thermodynamic behavior of aqueous solutions of nonelectrolytes. In their classic paper largely devoted to aqueous solutions of electrolytes, they stated that "when a rare gas atom or a nonpolar molecule dissolves in water at room temperature it modifies the water structure in the direction of greater crystallinity—the water, so to speak, builds a microscopic iceberg around it". The promotion of crystalline-like regions was rationalized by arguing that these structures contain more free space in which apolar solute particles could be accommodated. This model, based on well-known mixture models for liquid water, achieved great fame as the "iceberg model" and found a more sophisticated quantitative counterpart in a model developed by Némethy and Scheraga,^[21, 22] in which the flickering cluster model for liquid water^[*] proposed by Frank and Wen^[23] was used. An important aspect of this model is the assumed cooperativity in the formation of hydrogen bonds in liquid water. This cooperativity was attributed to the fact that the formation of a hydrogen bond by a water molecule is greatly facilitated when a second hydrogen bond is already present. As a conse-

quence, hydrogen bonds are made and broken as a collective rather than an isolated event. Thus, short-lived regions of either strongly hydrogen-bonded or isolated water molecules occur, described as flickering clusters. Insertion of an apolar solute particle causes a change in the energy levels of water molecules at the surface of the solute. A fully hydrogen-bonded water molecule will be stabilized by additional van der Waals interactions with the solute, and hence the number of these water molecules will increase in the vicinity of apolar solutes. In the original model of Frank and Wen,^[23] the nonpolar solute is assumed to act as an effective wall unable to transmit disruptive forces that tend to destruct the icelike clusters.

One of the first critical evaluations of iceberg-like models was reported by Miller and Hildebrand,^[24] who developed a model for the loss of entropy upon solution of apolar gases without assuming the formation of more rigid structures. Instead, they suggested that hydrogen bonds are destroyed when water molecules are brought into contact with air or apolar surfaces, causing the low solubility of apolar compounds in water. An interesting alternative was offered by Howarth,^[25] who reported an interpretation of the entropy of hydration of apolar compounds in terms of restricted motion of the hydrophobic parts of the solute molecules.

Classic models for hydrophobic interactions originate from the molecular interpretation given by Kauzmann.^[6] Pioneering work in this area was done by Ben-Naim^[10, 26] and Friedman et al.^[27–29] In an elegant approach by Ben-Naim^[10, 26] the process of the hydrophobic association of two methane molecules in water is considered. The indirect part of the work associated with this process (δG^{HI} or $\delta G(R)$), the part that reflects the properties of water, is evaluated by computing the difference between the thermodynamic parameters of the hydration of gaseous methane and ethane as illustrated in Scheme 2 (see also Section 3.3.1). Here, $\Delta\mu_s^\ominus$ and $\Delta\mu_d^\ominus$ are the standard chemical potentials for the solvation of gaseous methane S and ethane D, respectively.



Scheme 2. Cyclic process in which two methane molecules in aqueous solution as well as in the gas phase are brought from infinite separation to a close separation σ_1 and subsequently replaced by an ethane molecule (taken from ref. [10]).

[*] In the flickering cluster approach small regions in liquid water with a given structure are assumed to undergo sudden cooperative transitions into other structures.

tively, ε^g and ε^l account for the differences between an associated pair of CH_4 molecules and a covalent bond between CH_3 moieties. The assumption is made that ε^g and ε^l are similar in magnitude. The Gibbs energy involved in the solvent-induced part of the process of hydrophobic interaction can now be expressed as $\Delta G^{\text{HI}} = \Delta\mu_D^\ominus - 2\Delta\mu_S^\ominus$.

Having a method to estimate the solvent-induced part of hydrophobic interactions, one can study the influence of temperature or the presence of organic cosolvents on the magnitude of hydrophobic interactions. The model developed by Friedman et al.^[27–29] quantifies the thermodynamic parameters involved in the overlap of hydrophobic hydration shells upon association of apolar solute molecules and is treated in some detail in Section 3.3.3.

2. Part A. Experimental Studies

2.1. Hydrophobic Effects across a Broad Temperature Range; A Thermodynamic Approach

Traditionally, hydrophobic effects have been studied by measuring thermodynamic parameters for the solvation of gaseous or liquid organic compounds in water or, alternatively, by relating them to partition coefficients of solutes between aqueous and nonpolar phases (see, for example, Abraham et al.^[30,31] and Zaslavsky and Masimov^[32]). An excellent review on previous studies of solvation of apolar gases in water is given by Wilhelm et al.^[33] Since then, techniques for the measurement of solubility data on the basis of vapor–liquid equilibria have been improved significantly.^[34] Furthermore, in the past decade microcalorimetry has reached a high level of sophistication. Recently, Pollack devoted an interesting discussion to the general question of why gases dissolve in liquids.^[35]

The general aim of this section is twofold. First, emphasis is placed on a method to correlate thermodynamic data of solvation at 25 °C with solute parameters. This method provides a refreshing view of hydrophobic hydration and yields an interesting comparison between the thermodynamic parameters of the solution of apolar compounds in water and in organic solvents. The second part of this section describes an analysis of thermodynamic hydration parameters as a function of temperature and shows that the emphasis on the entropy-controlled nature of the solubility of apolar compounds in water cannot be maintained. Highly accurate enthalpies and heat capacities of solvation for apolar gases and liquids in water now available over a broad temperature range have seriously challenged the traditional view of hydrophobic effects.

It is notoriously difficult to correlate thermodynamic parameters of solvation with some property of the solute.^[33] Correlations with the surface area^[36] and molecular volume of the solute often gave satisfactory results but also revealed some remarkable discrepancies. These were explicitly noted by Cramer^[37] but became more obvious in an excellent analysis of solvation data of a large series of organic compounds, presented by Abraham.^[18,31] Both approaches focused on a comparison of thermodynamic solvation data for a series of apolar gases. In Figure 1 the Gibbs energies, enthalpies, and entropies (expressed as $T\Delta S$) of solvation for a series of

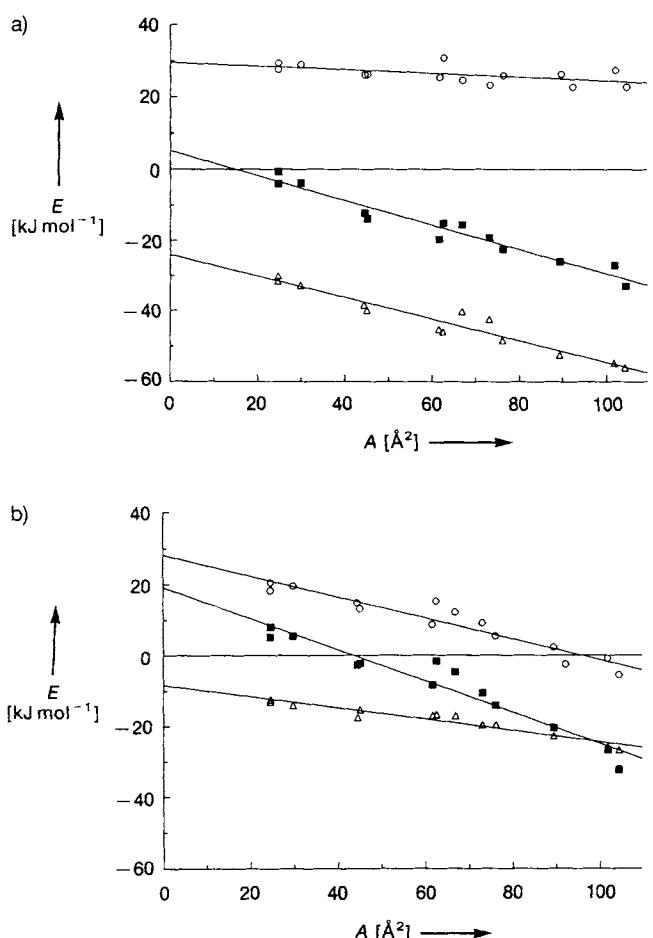


Fig. 1. ΔG^\ominus (○), ΔH^\ominus (■), and $T\Delta S^\ominus$ (Δ) of solvation for the apolar gases hydrogen (24.57 \AA^2), helium (24.65 \AA^2), neon (29.80 \AA^2), argon (44.41 \AA^2), methane (45.04 \AA^2), ethane (61.55 \AA^2), tetrafluoromethane (62.44 \AA^2), krypton (66.75 \AA^2), xenon (72.98 \AA^2), propane (76.08 \AA^2), *n*-butane (89.33 \AA^2), cyclopentane (92.04 \AA^2), *n*-pentane (101.64 \AA^2), cyclohexane (104.19 \AA^2). a) in water, b) in *n*-hexane (1 atm, 1 mol%, 298 K) as a function of the surface area A of the solute (in parentheses). Thermodynamic parameters are taken from ref. [31].

apolar gases in water and in *n*-hexane are plotted as a function of the surface area of the solute. Although the correlations are generally poor, the trends are obvious. Abraham developed a set of generally applicable solute parameters which are closely correlated with the surface area but have been optimized in order to correlate with thermodynamic solvation data in various organic solvents. These parameters have little physical meaning, and for the present purpose we prefer to use the surface area.

Strikingly, at 25 °C the Gibbs energy of solvation for apolar gases in water is only moderately dependent on the nature of the solute as compared to other solvents. Moreover, the dependence on the nature of the solute is quite remarkable. Helium is, for example, slightly less soluble than the larger (and more polarizable) xenon. In contrast, methane is slightly *better* soluble in water than in *n*-pentane. In organic solvents, the solubility increases rapidly with increasing size (or polarizability) of the solute. As suggested by Tanford^[9] and Abraham,^[18,31] it is interesting to compare the methylene increment for the dissolution of gaseous hydrocarbons in water and in organic solvents. Table 2 lists the methylene group contributions to the Gibbs energy, enthalpy, and entropy (expressed as $T\Delta S$) of solvation for gaseous $\text{C}_2\text{--C}_8$

Table 2. Methylene group contributions to the Gibbs energy, enthalpy, and entropy of solvation for gaseous *n*-alkanes (C_2 – C_8) in various solvents at 298.15 K (taken from ref. [31]).

Solvent	ΔG^\ominus [kJ mol ⁻¹]	Contribution to ΔH^\ominus [kJ mol ⁻¹]	$-\Delta S^\ominus$ [kJ mol ⁻¹]
<i>n</i> -hexadecane	-3.10	-4.81	1.72
cyclohexane	-3.18	-4.68	1.51
<i>n</i> -hexane	-3.10	-5.56	2.47
benzene	-3.10	-4.48	1.38
acetone	-2.59	-3.68	1.09
propylene carbonate	-2.01	-4.14	2.13
1-butanol	-2.76	-4.98	2.22
ethanol	-2.55	-4.90	2.34
methanol	-2.34	-4.44	2.09
water [a]	0.75	-2.80	3.56

[a] Determined for *n*-alkanes C_2 – C_7 .

hydrocarbons in various solvents at 25 °C. The Gibbs energy increment per methylene moiety in water deviates significantly. The enhanced solubility of larger hydrocarbons in organic solvents is governed by the growing importance of the enthalpy, which is only partly counteracted by the decrease in entropy. In water, the enthalpy of solvation also increases with increasing apolar surface area, but the increase is less pronounced. In contrast, the entropy loss increases more strongly with increasing alkane size. Hence, hydrophobicity becomes more pronounced as the size of the apolar solute increases, and the slight dependence of the Gibbs energy of solvation on surface area is the most eye-catching revelation of hydrophobic hydration. This phenomenon is, however, a result of subtle differences between contributions of methylene groups to the entropy and enthalpy of solvation. The exceptionally large and positive Gibbs energy of solvation of hydrocarbons in water at 298 K finds expression in the *absolute* values of the enthalpy and entropy of solvation. Figure 1 suggests that even for a solute molecule of negligible size, the entropy of solvation in water still remains highly unfavorable.

From the methylene increments listed in Table 2 the thermodynamic parameters can be calculated for the transfer of a methylene group from an organic solvent to water. For example, the transfer of a methylene group from *n*-hexane to water is an unfavorable process (the Gibbs energy increment is +3.85 kJ mol⁻¹), and it is characterized by a loss of entropy (increment is -1.09 kJ mol⁻¹) as well as by a positive

enthalpy (increment is +2.76 kJ mol⁻¹). Transfer of a methylene group from the gas phase to water is, however, *promoted* by enthalpy. This indicates that a considerable part of the unfavorable Gibbs energy involved in the transfer of an apolar methylene moiety from an organic solvent to water must be attributed to the disruption of dispersion interactions between the apolar solute and the organic solvent.

The Gibbs energies for the transfer of apolar solutes from nonpolar solvents to water have been correlated with accessible surface areas. These correlations showed that the Gibbs energy per unit surface area involved in the transfer of apolar compounds from a hydrocarbon-like solvent to water amounts to 70 to 130 J mol⁻¹ Å⁻². The exact magnitude depends strongly on the reference solvent, the computational method used to determine the surface area, and the fitting procedure.^[9, 38–43] A macroscopic measure for the hydrophobic Gibbs energy per unit surface area can be derived from liquid hydrocarbon–water surface tensions, which are about 310 J mol⁻¹ Å⁻².^[44] The discrepancy between the macroscopic and microscopic Gibbs energy relations on surfaces cannot be explained simply on the basis of differences in the curvature of the surface (see also Greco^[45]). In 1991 Sharp et al.^[46] reappraised the surface area dependence of the solubilities of hydrocarbons in water and showed that “hydrophobicity scales” significantly underestimate the magnitude of the hydrophobic effect. However, even in the revised estimate of the Gibbs energy per unit surface area, a considerable gap remains between macroscopic and microscopic surface tensions.^[46]

During the past decade the temperature dependence of the thermodynamic parameters of solvation for many apolar gases and liquids has been determined with high accuracy. The standard molar Gibbs energy, enthalpy, and entropy ($T\Delta S$) of solvation for gaseous and liquid neopentane are represented in Figures 2 a and 2 b, respectively. For comparison, the temperature dependence of the thermodynamic parameters for the transfer of gaseous neopentane to its liquid state is shown in Figure 2 c. The pseudo-chemical potential is used as a standard state (see Section 1.2). Using microcalorimetry, Naghibi et al. measured the heats of solution of methane,^[2] ethane,^[47] *n*-propane,^[47] and *n*-butane^[48] and showed that the enthalpy of solvation becomes less favorable with increasing temperature. This reflects the large and positive heat capacity of solvation observed near room temperature.

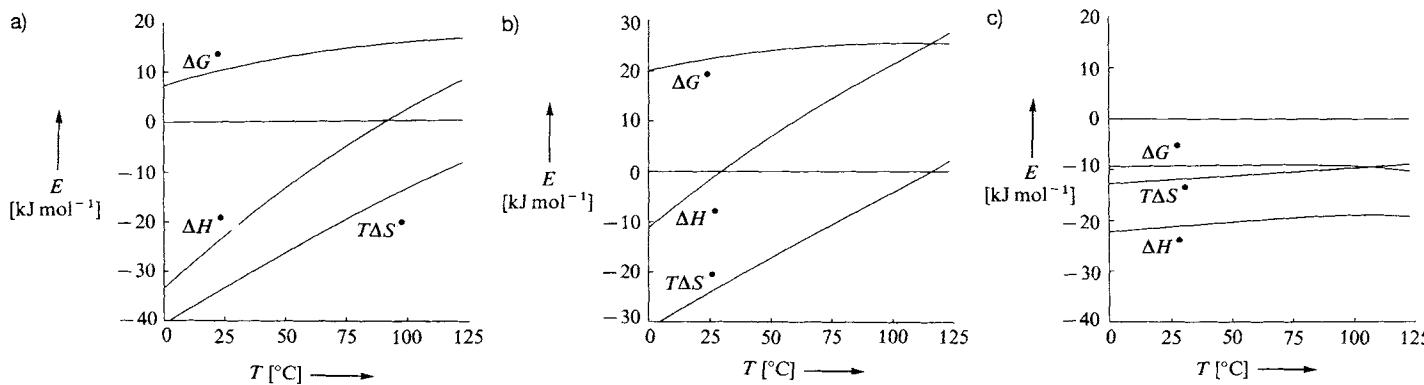


Fig. 2. Temperature dependence of ΔG^\bullet , ΔH^\bullet , and $T\Delta S^\bullet$ of the solvation for a) gaseous neopentane and b) liquid neopentane in water, and c) gaseous neopentane in its pure liquid (taken from ref. [230]).

Solubility measurements have shown that the entropy of solvation becomes less unfavorable with increasing temperature. At elevated temperatures the solubility of apolar compounds in water is, however, still exceptionally low, since it is then governed by the unfavorable enthalpy of solvation. The Gibbs energy of solvation is only slightly dependent on temperature. The heat capacity of solvation for gaseous hydrocarbons was shown to decrease significantly with increasing temperature.^[49] Unfortunately, microcalorimetric techniques did not allow accurate measurements of heats of solvation of apolar gases above 55 °C. The results, however, suggest that the heat capacity of solvation decreases continuously and eventually reaches zero at elevated temperatures. This is expected, because the hydrogen bond structure of water at these high temperatures is suggested to be completely destroyed.^[36] Makhadze and Privalov^[50] determined the specific heat capacity of solvation for liquid toluene and benzene between 0 and 150 °C. Although these data confirm the decrease of the heat capacity of solvation, even at 150 °C a considerable heat capacity change was observed.

Accurate measurements of the temperature dependence of the solubility of apolar gases and liquids in water analyzed with the van't Hoff equation also yield a temperature dependence of the thermodynamic parameters of solvation. Using this approach, Shinoda^[51] and Rettich et al.^[52–54] found that the heat capacity of solvation decreases noticeably above 80 °C and drops to zero near 160 °C. These data are in contrast with solubility data of Crovetto et al.,^[55] who did observe a considerable decrease in the heat capacity of solvation for inert gases and methane between 25 and 325 °C, but even at 325 °C the heat capacity change remained quite substantial. Unfortunately, solubility measurements at high temperatures are generally subjected to large experimental errors. In later studies Biggerstaff et al.^[56] used a flow calorimeter to measure directly the molar heat capacity of aqueous argon from 33 to 305 °C and found only a small decrease of the molar heat capacity when the temperature was raised to about 160 °C. Above this temperature, the heat capacity again *increases*. Near the critical point of water (about 250 °C), the heat capacity even increases extremely rapidly and goes to positive infinity.

The temperature dependences of the thermodynamic parameters of solvation for a large variety of apolar liquids in water show remarkable similarities. First, the enthalpy of solvation for apolar liquids is zero near room temperature, irrespective of the nature of the species (see Fig. 2 b). Near 110 °C, the entropy of solvation for apolar liquids becomes zero. Apparently, both temperatures are universal, and are now generally referred to as T_H^* and T_S^* , respectively. The temperature dependence of the thermodynamic parameters of solvation for apolar liquids strongly resembles that for apolar gases (Fig. 2 a, b). Also for the dissolution of a large series of apolar gases, temperatures can be found where ΔH^\ominus and $T\Delta S^\ominus$ reach common values but differ from zero. The occurrence of isoenthalpic and isoentropic temperatures for the thermodynamics of solvation for apolar compounds in water was rationalized by Lee.^[57] His explanation is based on the assumption that the changes in enthalpy and entropy are linear functions of some property of the solute [Eq. (1)].

$$\Delta X^\ominus = a_x \sigma + b_x \quad (1)$$

Here ΔX^\ominus is the thermodynamic quantity involved, σ the property of the solute (usually the surface area), a_x a species-independent linear function of temperature, and b_x the value of ΔX^\ominus when a_x is zero. The molar thermodynamic quantities of solvation for apolar liquids and gases can now be expressed as functions of temperature in terms of the heat capacity change, assuming that the latter is independent of temperature,^[57] according to Equations (2)–(4), where T_H^* and T_S^* are the isoenthalpic and isoentropic temperatures.

$$\Delta G^\ominus = \Delta G^* + \Delta C_p(T - T_H^*) - T\Delta C_p \ln(T/T_S^*) \quad (2)$$

$$\Delta H^\ominus = \Delta H^* + \Delta C_p(T - T_H^*) \quad (3)$$

$$\Delta S^\ominus = \Delta S^* + \Delta C_p \ln(T/T_S^*) \quad (4)$$

ΔH^* and ΔS^* are the values of the enthalpy and entropy of solvation at T_H^* and T_S^* , respectively. The solubility function [Eq. (5)] shows that the solubility of apolar compounds in

$$\Delta G^\ominus / T = \Delta G^*/T + \Delta C_p(1 - T_H^*/T) - \Delta C_p \ln(T/T_S^*) \quad (5)$$

water reaches a minimum at T_H^* . Lee^[230] argued that T_H^* and T_S^* are the temperatures at which the enthalpy and the entropy of solvation, respectively, are independent of the selected property of the solute. The heat capacity change fully accounts for the temperature dependence of enthalpy and entropy of solvation,^[58] and ΔH^* and ΔS^* are independent of temperature. According to Equations (3) and (4), a plot of ΔH^\ominus and ΔS^\ominus versus ΔC_p for a series of apolar gases or liquids at a selected temperature T should be linear. This has been experimentally confirmed by Sturtevant,^[59] Murphy et al.,^[60] and Baldwin.^[61] From these plots the values of T_H^* and T_S^* have been derived.

Interestingly (vide supra), ΔS^* and ΔH^* are zero for the solvation of apolar liquids in water. Hence T_S^* and T_H^* equal T_S and T_H (about 110 and 25 °C, respectively), and Equation (6) applies at each temperature T .^[59] Equation 6 does

$$\ln(T_S/T) = \Delta C_p/\Delta S^\ominus \quad (6)$$

not hold for the dissolution of apolar gases in water since ΔS^* and ΔH^* are *not* zero. T_S^* and T_H^* are about 124 and 94 °C, respectively.^[62, 63] Apparently, the entropy of solvation is not completely proportional to the surface area, and even a mathematical point solute gives rise to an entropy of solvation of about $-80 \text{ J K}^{-1} \text{ mol}^{-1}$.^[60] $T\Delta S^*$ is similar to the difference in translational Gibbs energy between the gas and the liquid phase. A similar phenomenon was observed in Figure 1. Although Biggerstaff et al.^[56] showed that the heat capacity of an aqueous solution of argon does not depend dramatically on the temperature, T_S^* is a hypothetical temperature. Nevertheless, the variation in T_S^* for the solvation of apolar gases and liquids with temperature is surprisingly small. In contrast, the standard deviation of T_H^* is quite substantial. The existence of a unique value of T_H^* seems to be reserved only for larger liquid hydrocarbons.

The strong temperature dependence of the enthalpy and entropy of solvation for apolar gases and liquids in water

throws a different light on the analysis of thermodynamic solvation parameters measured at or near room temperature. The analysis of thermodynamic parameters of solvation for linear alkanes by means of increments (Table 2) is substantially dependent on temperature. For example, the entropy of solvation for gaseous alkanes decreases strongly with increasing temperature and eventually becomes identical for all alkanes near T_s^* . This means that the incremental methylene contribution to the entropy of solvation at T_s^* is zero. With respect to the enthalpy of solvation, the methylene contribution is zero at T_H^* . The incremental contributions to entropy and enthalpy of solvation can adopt virtually a broad range of values dependent on the temperature. In contrast, the Gibbs energy increment is hardly sensitive to temperature changes.

2.2. The Role of Hydrophobic Interactions in Protein Conformation and Stability

The stability of native protein structures and the folding of an extended protein chain have been subjects of extensive research for more than fifty years.^[64, 65] Today, it is almost generally accepted that hydrophobic interactions constitute an important factor in the stability of the folded structure of water-soluble proteins.^[65] The spatial distribution of α -amino acid residues in the native protein shows that, on average, α -amino acids with apolar character are found in a dense hydrophobic core in which the exposure to water is minimized.^[66–68] Recently, Rose et al.^[68] showed that the average spatial requirement of the α -amino acids that make up this apolar core is related to the residue hydrophobicity. Of course, Kauzmann^[6] introduced the whole concept of hydrophobic interactions in connection with the interactions between apolar side chains of α -amino acid residues and their proposed importance for the stability of biopolymers. A controversy remains whether the stability of the native structure of proteins is caused by van der Waals interactions between apolar side chains or whether water actively induces the formation of an apolar core.^[13, 14, 69] Obviously, this discussion strongly relates to our current understanding of the molecular basis of hydrophobic interactions. Since native structures of proteins unfold upon cooling as well as upon heating,^[70] a study of both the cold and hot denaturation of native protein conformations might give valuable information about hydrophobic interactions. It must be recognized that these interactions are primarily *bulk* hydrophobic interactions.

Another topical debate centers on the question as to what extent the alleged hydrophobic interactions determine the structure of native proteins. On one hand, Dill et al.^[71, 72] reported a successful theoretical analysis accounting for the temperature dependence of stability and folding of large globular proteins based on the assumption that hydrophobic interactions are the driving force for the folding of globular proteins. According to Dill,^[64] hydrophobic interactions between apolar α -amino acid residues allow the extended protein chain to fold itself in only a limited number of ways. The ultimate and unique conformation is subsequently determined by more specific polar interactions. On the other hand, the theoretical analysis of solvent effects on protein

folding in terms of solvation energies of individual parts of the total protein reported by Ben-Naim^[73, 74] has been taken as evidence that the importance of hydrophobic interactions has generally been overestimated. The suggestion was made that correlations between hydrophilic functional groups are even more important than those between hydrophobic side chains. In particular hydrogen bonds, also at the surface of the protein, were proposed to be of paramount importance for the stability of native protein conformations.

The native structure of proteins is generally only about 50 kJ mol⁻¹ more stable than the denatured state. The formation of a compact, folded protein is strongly opposed by entropic forces. A variety of intramolecular forces between α -amino acid residues in the folded state are known that contribute to the overall stability of the native protein structure. Due to the marginal stability of the folded conformation, each of these individual contributions can be decisive. Intramolecular interactions are well established, and most authors agree that these include electrostatic interactions, torsional or other "local" interactions, polar interactions (hydrogen bonds), van der Waals interactions, and hydrophobic interactions.^[64, 75] The long-range electrostatic interactions include the classical repulsions between charged residues as well as specific electrostatic interactions due to bridging of oppositely charged α -amino acids.^[76] Ion pairing is especially important at the surface of proteins.^[77] Torsional and local interactions originate from preferential conformations of sequences of three or four α -amino acids, arising from short- and long-range interactions among these neighboring residues. Karplus and Holley^[78] were quite successful in accounting for the architecture of proteins on the basis of these local interactions.

Hydrogen bonding is also very important in determining the structure of proteins, but if it is to be the driving force for the folding of proteins, hydrogen bonding of the unfolded protein with water must be less favorable than hydrogen bond interactions between α -amino acids in the native state of the protein. Jorgensen^[79] showed on the basis of computer simulation studies that amides tend to stack in aqueous solution, but that stable amide–amide hydrogen bonds are not very likely. Dimerization of organic compounds in water has only been observed when multiple hydrogen bonds are involved. Hydrogen bonding between α -amino acids buried in the apolar core of the native protein can, nevertheless, provide additional stability for the structure.^[80] Finally, interactions between permanent or induced dipoles, generally qualified as van der Waals forces, are of great importance for the stability of native protein structures.^[81] This has commonly been traced back to the fact that water constitutes the natural environment for most soluble proteins. Dispersion forces between the side chains of apolar α -amino acids in the native conformation of the protein replace interactions with water in the extended conformation. As argued above, it is very difficult to make a proper distinction between van der Waals interactions and hydrophobic interactions, and the active role of the solvent is still far from clear.

α -Amino acid sequences determine the native structure of a protein. It is known that these sequences are not unique, and many different sequences can code for proteins with essentially the same native conformation. Creation of cavities in the hydrophobic core of the protein by shortening the

side chains of certain α -amino acids, the introduction of α -amino acid residues with unsatisfied hydrogen bonding, and the formation of sterically crowded regions by incorporation of bulky α -amino acids are generally not favorable for the stability of the protein, but the way in which a protein can accommodate mutations commands respect.^[82]

A few years ago Sauer et al.^[82, 83] elegantly showed that the sequence of α -amino acids, which plays an essential role in the formation of the core of the protein, can be replaced by alternative α -amino acids on two conditions: first, these residues must maintain the overall hydrophobicity, and second, the new packing arrangement must satisfy steric requirements. Site-directed mutagenesis combined with sophisticated calorimetric stability measurements have shown that substitution of individual α -amino acids buried in the hydrophobic interior of the protein does lead to changes in stability of the native protein.^[84] The creation of a cavity in the interior of the protein destabilizes the native conformation to an extent that is strongly dependent on the actual site of the mutation.^[81, 85, 86] Usually, the destabilization increases with increasing cavity size.

Kellis et al.^[85, 86] and Eriksson et al.^[81] studied the effects of several side-chain mutations on protein stability and focused on the response of the protein structure to cavity-creating mutations, such as the replacement of leucine residues by alanine (which is equivalent to decreasing the hydrophobicity by removing one methylene group). Eriksson et al.^[81] showed on the basis of crystal structures of six mutants of T4 lysozyme that the packing of the core did not completely fill the created cavity, although side chains protruded into the cavity to some extent. Apparently, the protein interior consists of rigid parts alternating with more flexible regions. The presence of water molecules in the remaining cavities could not be determined. It is, however, extremely difficult to detect isolated water pools or water molecules. Generally, a considerable part of the volume of protein crystals is occupied by water molecules. Water molecules are found inside the protein at positions where they can form hydrogen bonds with the α -amino acids buried in the apolar interior. They may also be located in cavities and crevices created by insufficient packing. These interactions have been an important topic of research.^[87] The role of these isolated water molecules for the stability of the folded protein structure can be substantial.^[88]

In the last few years Monte Carlo and molecular dynamics simulations have been used to compute changes in the Gibbs energy for protein folding when a particular α -amino acid residue is replaced by another less hydrophobic moiety (e.g. leucine \rightarrow alanine) by using Gibbs energy perturbation methods (see Section 3.5).^[85, 89, 90] Prevost et al.^[90] showed that solvation of the unfolded, unsolvated wild-type protein is hardly affected by the mutation, but that folding of the dissolved chain becomes more favorable when the more apolar residue is incorporated. This phenomenon can be reconciled with the fact that the Gibbs energy of solvation for apolar gases in water is only slightly dependent on the magnitude of the apolar surface area (see Section 2.1). This emphasizes that the interactions between apolar moieties in the core of the native protein are essentially bulk hydrophobic interactions. The conclusion given by Prevost et al.^[90] that the observed decrease in stability of the mutant structure cannot be

attributed to a hydration phenomenon is not correct. The decreased stability resulting from the leucine \rightarrow alanine mutation is usually attributed to the loss of van der Waals interactions in the core of the protein.^[81, 85, 86, 91] A remarkable result of recent simulation studies is that intrinsic side-chain terms contribute significantly to the reduced stability of mutated proteins.^[90]

The packing of hydrophobic α -amino acids in a hydrophobic core leads to a highly compact conformation. Thermodynamic parameters of folding for an inactive protein chain show remarkable similarities with thermodynamic parameters of the solvation for apolar liquids in water.^[12, 36, 61, 92–94] Moreover, the ratio of the entropy and heat capacity change of denaturation is constant [see Eq. (6)].^[59] The heat capacity for the denaturation of proteins is linearly related to the fraction of hydrophobic residues^[92] but is only slightly dependent on the temperature.^[36, 70, 94] Therefore, the interior of the protein has been frequently treated as an apolar liquid or an oil drop.^[95] The process of “hiding” apolar α -amino acids from water in the hydrophobic core has been modeled by determining the relative solubilities of these α -amino acids in water and in various apolar solvents.^[9, 96–98]

An intriguing observation was made by Privalov,^[92] who found that for the unfolding of a number of globular proteins the changes in enthalpy and entropy per amino acid residue reach a common value at 110 °C. Baldwin^[61] noticed that this temperature agrees very well with the temperature at which the entropy of dissolution for apolar liquids in water becomes zero (T_s , see Section 2.1). An analogy was drawn between the thermodynamics of folding for inactive proteins in water and of the transfer of hydrocarbons from an aqueous solution to their liquid state. It was suggested^[61] to estimate the hydrophobic contribution to protein stability from the characteristic temperatures T_s and T_H (the hydrocarbon model).

By using Equations (2)–(4), the Gibbs energy of protein folding can be expressed [Eq. (7)]. This relation suggests that

$$\Delta G_{\text{folding}}^{\ominus} = \Delta G_{\text{nh}}^{\ominus} + \Delta G_h^{\ominus} = \Delta H_{\text{nh}}^{\ominus} + T \Delta S_{\text{nh}}^{\ominus} + \Delta C_{p, h} (T - T_H) + \Delta C_{p, h} T \ln(T_s/T) \quad (7)$$

the nonhydrophobic (nh) contributions to the thermodynamics of protein folding are independent of the temperature.^[61] A favorable nonhydrophobic enthalpy term is counteracted by a serious loss of entropy. At high temperatures the nonhydrophobic entropy term is large enough to cause denaturation of the protein. The analysis shows that hydrophobic interactions lead, at all temperatures, to a stabilization of the native structure of the protein. Near room temperature the stabilization is governed by a gain in entropy. Above 60 °C, however, the enthalpy becomes dominant and even prevails over the nonhydrophobic enthalpy contribution. The similarity with the solvation of apolar liquids in water is obvious.

However, much evidence indicates that it is misleading to view the interior of a protein as an apolar liquid. The packing densities of the hydrophobic core approach those of crystalline α -amino acids.^[99] In addition, the interior of proteins contains several types of polar α -amino acid residues,^[68] and exchange of certain core residues by more hydrophobic

counterparts does not always lead to higher protein stability.^[100] In 1991 Sandberg and Terwilliger^[101] showed that some polar residues reside in the core of the protein without actually interacting with each other. Apparently, the local polarity and the site-dependent packing energy are important factors for the stability of the native protein.^[102]

Application of the hydrocarbon or oil-drop model to describe thermodynamic properties for the folding of proteins is also controversial for other reasons.^[61] An important point of concern is that T_S^* and T_H^* are set equal to T_S and T_H , whereas Privalov et al.^[12, 36] found that the entropy and enthalpy for the unfolding of native protein structures reach common values at T_S^* . Replacing T_S and T_H in Equation (7) by a single temperature T_S^* (or T_H^*) leads to the remarkable conclusion that hydrophobic effects destabilize the native protein conformation. The remaining nonhydrophobic terms show striking similarities with the thermodynamic solvation data for solid cyclic dipeptides,^[12] suggesting that the structure of the interior of the folded protein is related to a solid structure.

The conclusion that hydrophobic effects destabilize the structure of proteins drawn by Privalov et al.^[12, 36] contradicts a flood of experimental evidence obtained since the classical paper of Kauzmann^[6] and recently sparked interesting discussions about the meaning of hydrophobicity.^[13–15] Lee^[57] rightfully remarked that these discussions did not provide much insight into the fundamental problem that T_S^* equals T_H^* . He argued that the assumed relation between the solvation of apolar liquids in water and the denaturation of globular proteins is based on the questionable assumption that only nonpolar groups are transferred from water to the interior of the proteins. He suggested invoking polar interaction terms as well, which would shift T_H^* without altering T_S^* . The term ΔG_h^\ominus introduced by Murphy et al.,^[12] in which T_S equals T_H [see Eq. (7)], measures not only hydrophobic contributions but also the difference between the average contributions of polar and nonpolar interactions.

The analysis reported by Lee^[57] reinforced the view of Privalov^[75, 92] that cold denaturation is reflected by the fact that the favorable nonpolar, or hydrophobic terms become too small at low temperature. The temperature dependence of the Gibbs energy of hydrophobic interactions is very modest, which emphasizes the fact that native structures of proteins are only marginally stable. The cooperative effects of bulk hydrophobic interactions and polar interactions between α -amino acid residues in the interior of the protein are sufficient only over a very small temperature range to overcome the loss of configurational entropy. Indeed, the occurrence of cold and hot denaturation is indicative of opposing hydration contributions arising from the solvation of polar and nonpolar α -amino acid residues of the unfolded protein.^[70]

2.3. The Role of Hydrophobicity in Molecular Recognition and Host–Guest Complexation

The specific binding of reactants in the active site of enzymes constitutes an important research topic. In these molecular recognition processes hydrophobic interactions

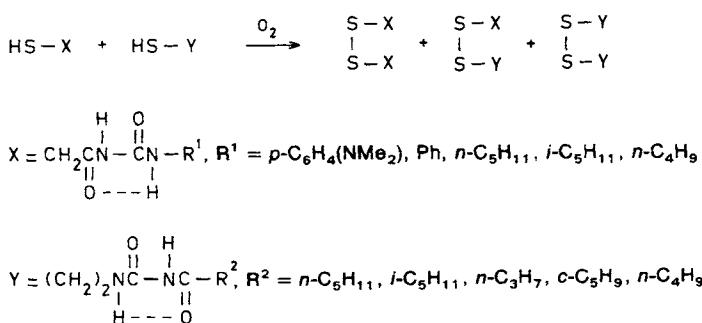
are expected to play a major role. Originally molecular recognition was a term reserved for biochemistry and biomimetic chemistry.^[103, 104] The current popularity of supramolecular chemistry has caused a shift to a more general interpretation of the term.^[105, 106] Noncovalent interactions, which lie at the heart of molecular recognition, have become a fashionable topic of research.^[107]

A convenient approach to studying basic aspects of noncovalent interactions is provided by synthetic host–guest chemistry. The formation of strong host–guest complexes is particularly feasible in aqueous solutions. Generally, this has been attributed to hydrophobic effects, although electrostatic interactions are also important.^[107] Host–guest complexes are often stabilized by π – π or stacking interactions^[108] and hydrogen bonding.^[107] Stacking interactions play a dominant role in aggregation of dye molecules and hydrophobic compounds.^[109, 110] In addition, a combination of stacking interactions and hydrogen bonding governs the strong binding of nucleotides to receptor sites.^[111]

The role of hydrophobic interactions in host–guest chemistry and molecular recognition is still ambiguous. The arguments show remarkable similarities with those that characterize discussions in the field of protein stability and mainly center on the question whether van der Waals interactions or hydrophobic interactions govern host–guest complexation. In addition, one should recognize that host–guest complexation can involve either bulk or pairwise hydrophobic interactions. To illustrate this point we note that complexation of an apolar guest molecule in an apolar cavity of a macrocyclic host is usually accompanied by complete desolvation of the guest molecule, whereas pairwise stacking of, for example, nucleotides or dye molecules involves only local dehydration. In this section, we would like to signal a few remarkable results obtained in the field of synthetic host–guest chemistry. In the light of the feverish activities in this field, we restrict ourselves to a few examples that illustrate the current debate. For extensive surveys of synthetic host–guest chemistry in aqueous solutions we refer to reviews by Schneider^[107] and Diederich.^[112]

Complexation of neutral molecules with cyclic host molecules, and molecular complexation in general, involves a variety of different binding interactions, and it is difficult to separate the hydrophobic component even if one has agreed upon the exact definition of the term. In their studies of solvent and salt effects on binding constants, Schneider et al.^[113] found evidence that hydrophobic interactions play a major role in the binding of apolar substrates to macrocyclic host compounds. An interesting contribution was reported by Endo et al.,^[114] who studied the oxidation of associated thiols having an amide-like binding site and an alkyl recognition site (Scheme 3). A small but significant selectivity was observed in the formation of symmetrical and unsymmetrical products, which was attributed to recognition of hydrophobic moieties. Unfortunately, many of these reactions were carried out with a considerable amount of organic cosolvents and not in a dilute aqueous solution.

Thermodynamic parameters of complexation processes are sometimes difficult to interpret because of conformational changes of the host molecule upon complexation.^[115] Particularly solvation of the cavity prior to complexation causes ambiguities in interpretations of thermodynamic

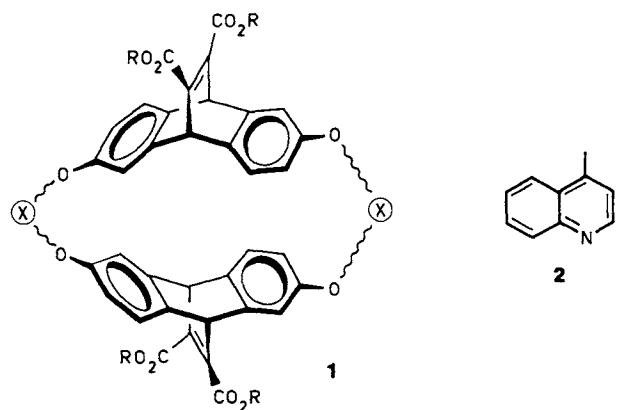


Scheme 3. Schematic representation of the oxidative coupling of thiols HSX and HSY to yield the symmetrical and unsymmetrical disulfides. The recognition sites X and Y are given as well (see ref. [114]).

parameters at the molecular level. In spite of these pitfalls, measurement of the temperature dependence of binding constants provides valuable new insights into the molecular origin of recognition processes. Frequently, a large and positive entropy change upon complexation has been found (see, for example, Stauffer et al.^[116]), which is an indication for the occurrence of hydrophobic interactions. The binding efficiency of host molecules has also been successfully enhanced by increasing the hydrophobic character of the binding site.^[112] The existence of a large and negative heat capacity change upon association of apolar molecules in water is generally considered to be a decisive criterion for the occurrence of hydrophobic interactions. Indeed, Harrison and Eftink^[117] and Stauffer et al.^[116] found a negative heat capacity change for some host–guest complexation processes.

In textbooks^[118] hydrophobic contributions to molecular recognition are attributed to positive entropy changes due to the release of structured water molecules in the hydration shells of apolar moieties. More recently, some experimental results have cast serious doubt on the active role of water molecules. Diederich et al.^[115] found that complexation of benzene in a cyclophane host molecule is *enthalpy* driven at room temperature. The process is even accompanied by a slightly *negative* entropy change. Enthalpically controlled complexation was also found by Harata et al.^[119] These observations caused some discomfort, which tempted Diederich et al.^[115] to introduce the paradoxical term “enthalpic hydrophobic effect”. The molecular origin of the complexation process was attributed to London dispersion interactions between host and guest and the binding state of water molecules in the interior of the host before binding.^[120]

Stauffer et al.^[116] showed that the enthalpy for the binding of **2** in host **1** (Scheme 4) is extremely sensitive to temperature. These heat capacity effects are more pronounced when guest molecules of increased hydrophobicity are involved. The heat capacity effects for host–guest complexation in organic solvents such as chloroform are also considerable, though less pronounced than in water.^[116] In Figure 3 the changes in Gibbs energy, enthalpy, and entropy ($T\Delta S$) are plotted as a function of temperature for the binding of compound **2** in host **1** in water. Near room temperature the complexation is governed by a favorable entropy, whereas at elevated temperatures the enthalpic contribution prevails. The striking resemblance to the data plotted in Figure 2b



Scheme 4. Electron-rich synthetic receptor molecule **1** and electron-deficient guest molecule **2**.

suggests a relation to the solvation of apolar liquids in water.^[120] The gain in entropy occasionally observed for complexation of apolar guest molecules in an apolar cavity of a macrocyclic host near room temperature^[116] cannot be considered the sole driving force for hydrophobic contributions to host–guest interactions and certainly cannot apply to those cases for which the enthalpy governs host–guest complexation even near room temperature.^[115]

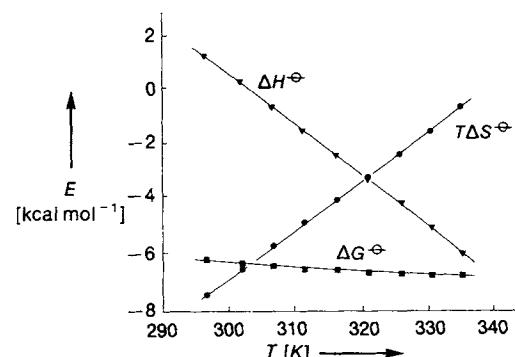


Fig. 3. Temperature dependence of ΔG^\ominus , ΔH^\ominus , and $T\Delta S^\ominus$ for the complexation of compound **2** in host molecule **1** (taken from ref. [116]).

2.4. Association of Amphiphiles

Aggregation of amphiphilic compounds in aqueous solution leads to complex supramolecular structures. Largely depending on the molecular architecture of the amphiphile, a wealth of three-dimensional structures can be formed, ranging from spherical and rodlike micelles to bilayer or multilayer structures, inverted hexagonal phases, and other sometimes very intriguing spatial arrangements. Introduction of chiral centers in the polar headgroup area can lead to beautiful structures (see, for example, Fuhrhop et al.^[121, 122]). For a survey of the relation between surfactant structure and aggregation behavior we refer to a paper by Nusselder et al.;^[123] for other reviews see Kunitake et al.^[124] and Menger et al.^[125]

It is almost generally accepted that an aqueous environment is a prerequisite for the three-dimensional assembly of amphiphilic molecules. However, examples have been found

for surfactant aggregation in other solvents such as hydrazine,^[126] formamide,^[127] ethylene glycol,^[128] and ethylammonium nitrate.^[129] Interestingly, partially fluorinated alkanes behave as amphiphilic compounds in apolar solvents in which they aggregate readily.^[130, 131] Traditionally, however, hydrophobic interactions are considered to be the major driving force for amphiphile aggregation.^[9] Results of studies on the micelle formation of cetyltrimethylammonium bromide (CTAB) and trimethyltetradecylammonium bromide in various solvents and at elevated temperatures urged a reappraisal of this traditional view.

As shown in Table 3, the Gibbs energies for the micelle formation of CTAB at room temperature in water and in hydrazine are not very different.^[132] In contrast, the enthalpy and entropy for micelle formation differ dramatically.

Table 3. Gibbs energy, enthalpy, and entropy of micelle formation for CTAB in water and in hydrazine at different temperatures (taken from ref. [132]).

Solvent	T [°C]	ΔG [kJ mol ⁻¹]	ΔH [kJ mol ⁻¹]	$-\Delta S$ [kJ mol ⁻¹]
water	25	-43.8	-12.6	-31.2
	95	-48.0	-51.9	3.9
	166	-44.4 [a]	-65.3	22.1
hydrazine	35	-29.7	-46.0	16.8

[a] A value of -43.2 is obtained by calculation; the values for ΔH and $-\Delta S$ may also be incorrect.

In water, micelle formation is driven by a favorable entropic contribution. As evident in Figure 4 and Table 3, the enthalpy and entropy of micelle formation of CTAB^[133] and other surfactant molecules^[134] are strongly dependent on temperature. At elevated temperatures the formation of mi-

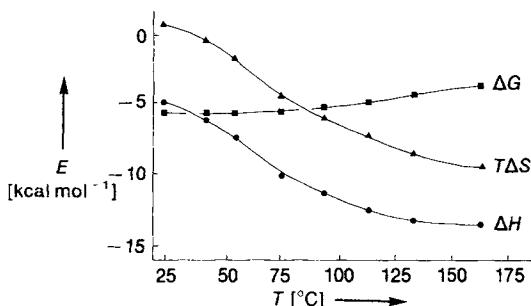


Fig. 4. Thermodynamics of micelle formation of trimethyltetradecylammonium bromide as a function of temperature, as determined by the equilibrium model (taken from ref. [133]).

celles is governed by a favorable enthalpy and even counteracted by a loss of entropy. In fact, the thermodynamic parameters for micelle formation in water at these temperatures become similar to those found for micelle formation in hydrazine near room temperature. The aggregation number decreases with increasing temperature, and at 170 °C the aggregation number is similar to that found in polar organic solvents at room temperature.^[135] Apparently, the exceptional position of water among hydrogen-bonding solvents is less evident at higher temperatures. It is furthermore remark-

able that the Gibbs energy for the formation of micelles is only moderately affected by temperature, in other words, the enthalpy and entropy changes (ΔH and ΔS) are almost fully compensating. Lumry et al.^[136] as well as Evans and Wightman^[133] have provided a firm basis for discussions about compensation behavior of thermodynamic parameters of solvation and micelle formation in aqueous solvents (see Section 3.2).

The temperature dependence of the thermodynamics of micelle formation in water is remarkably similar to that of the solvation of apolar compounds in water and of the complexation of synthetic host and guest molecules (cf. Figs. 2a, b, 3, 4). This strongly suggests that these processes have a common molecular origin described by the term hydrophobic interactions. The molecular interpretation of this term in relation to aggregation of amphiphilic molecules is still a matter of debate. In 1989 Evans and Miller^[135] emphasized the importance of van der Waals interactions for the aggregation of amphiphilic compounds. This, again, stresses the current dilemma centering on the distinction between dispersion interactions and hydrophobic interactions. A possible answer has been provided by Ben-Naim,^[120] who clearly distinguishes the solvent-induced contribution to solute-solute interactions from the van der Waals forces. A serious disadvantage of this approach is that it is very difficult to estimate the magnitude of the solvent-induced contribution.

2.5. Surface Forces and Hydration of Flat Surfaces

In the past decade, much attention has been paid to the forces between aggregates of amphiphilic compounds in aqueous solution (for recent surveys, see Israelachvili and Wennerström^[137, 138]). These so-called surface forces play an important role in biological processes as well as in a number of industrial applications such as mineral flotation, colloid stability, wetting of surfaces, and coating. In order to understand the molecular origin of surface forces between, for example, bilayer structures, forces between apolar surfaces were also considered. These forces exhibit a quite remarkable dependence on the distance between the macroscopic bodies.^[139] In particular, forces between apolar surfaces show extremely long-range attractions unknown for polar surfaces.^[140–145] In contrast with previous experimental results obtained by Israelachvili and Pashley^[140] and Pashley et al.,^[141] who found an exponential decay of attractive forces between apolar surfaces when the separation was increased from 0 to 15 nm, it has recently been shown that attractive forces between charge-free surfaces are measurable even at separations of 70–90 nm.^[143–146] This means that the attractive forces are transmitted over distances corresponding to more than 300 interfacial water molecules! Claesson and Christenson^[143] showed that the force between fluorocarbon surfaces in water increases exponentially until they are separated by a distance of about 10 nm. Beyond 10 nm the attractive force is even more amplified. A typical example is shown in Figure 5.

Today, much is known about the predominantly repulsive interactions between amphiphilic and polar surfaces.^[137, 138, 147, 148] Despite the increasing interest in the

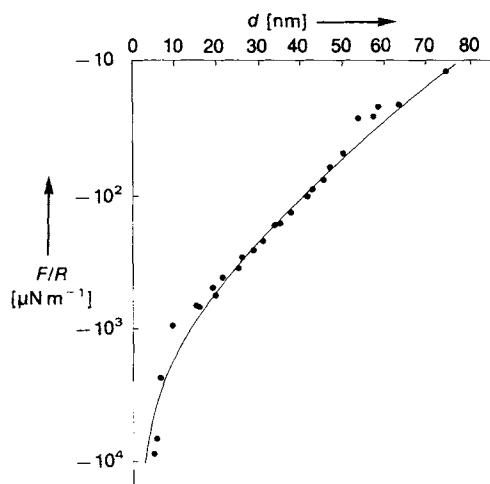


Fig. 5. Attractive surface force F/R plotted versus the separation d of fluorocarbon-covered mica surfaces (taken from ref. [167]).

anomalous forces between nonpolar surfaces in water, the origin of these forces is a matter of continuing discussions. Clearly, hydrophobicity of the surface areas is essential for the observed long-range forces. Long-range attractions have not been found between uncharged and nonhydrophobic surfaces such as carbohydrates^[149, 150] nor between surfaces coated with a mixture of hydrophobic and hydrophilic compounds.^[150] Claesson et al.^[142] showed that the measured force is significantly stronger than would be expected on the basis of simple van der Waals forces. Moreover, van der Waals forces are only effective at surface separations of at most 10 nm. Some attempts have been reported to explain these amazing hydrophobic attractive forces. A possibility that has been considered is the formation of vapor cavities between the surfaces at distances less than a few hundred nanometers. The presence of such cavities has been established repeatedly.^[146, 151, 152] On account of these cavities the force between the apolar surfaces is not an equilibrium force. Claesson et al.^[152] showed, however, that these cavities can never give rise to the observed attractive forces. An alternative explanation is based on the possible role of electrostatic forces, but the fallacy of this view was demonstrated by Christenson et al.^[145]

Several approaches focus on the properties of water present between the apolar surfaces. The hydrophobic forces are thought to arise from the alleged metastable character of water between the surface. The structure of water molecules near a large flat apolar surface has been a topic of extensive computer simulation studies. These studies^[153–166] show clearly that water molecules adjacent to a hydrophobic surface are not capable of forming the optimal number of hydrogen bonds, although near the hydrophobic surface they clearly tend to form a clathrate-like structure.^[157, 161] This structural response does not penetrate far into the medium, and the order in the fluid persists only over a few molecular diameters.^[157, 159]

In 1989 Eriksson et al.^[167] presented a phenomenological theory for the long-range attractive forces. The model was based on the variational mean-field theory developed by Marçelja et al.^[168] to account for the interactions between small hydrophobic solute molecules. Eriksson et al.^[167] sug-

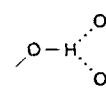
gested a structural mechanism based on a layer-to-layer propagation of the force due to ordering of the water molecules at the surface. Unfortunately, the question of how the force can be passed through so many water molecules remains unanswered. In the same year Attard^[169] attributed the long-range attractions to van der Waals forces. Since these forces can normally not account for the observed magnitude and distance dependence of the attractive forces, he invoked an anomalous electrostatic response of the fluid between the surfaces. The large electrostatic response was assigned to the structural changes of liquid water at the surface. The molecular origin of this effect has, however, not been identified yet. In summary, although some efforts have been made to understand the inexplicably long-range action of the hydrophobic attractive forces between apolar surfaces in water, these attempts only rephrase the intriguing question of how water can mediate these forces across such a great distance.

2.6. Spectroscopy, Ultrasound, Light Scattering, and Diffraction Studies: Selected Results from the Last Few Years

The application of spectroscopic techniques to study hydrophobic effects peaked more than a decade ago. Because of the limitations of these techniques, these studies focused mainly on average changes in the organization of water molecules induced by apolar solute particles or, alternatively, on the preferential solvation or bulk hydrophobic interactions occurring at higher concentrations of the apolar solutes. Readers interested in applications of NMR spectroscopy are referred to publications of Zeidler^[170] and Hertz and Leiter.^[171] Results in the field of IR spectroscopy have been reviewed in detail by Walrafen^[172] and Luck.^[173] For more recent applications of IR spectroscopy we refer to the work of Symons et al.^[174–176] and recent work of Engberts et al.^[177] Most of the ultrasound studies of aqueous solutions also date from the sixties and seventies and have been reviewed thoroughly by Blandamer.^[178] ESR spectroscopy has not been applied frequently, but it has been used almost exclusively to probe the environment of the solutes by inducing a spectroscopic response of the solute itself.^[179] Most of these spectroscopic studies gave results that were consistent with the traditional picture of hydrophobic hydration. The question whether the structure of water is enhanced by the formation of more hydrogen bonds, stronger hydrogen bonds, or a reduction of free OH bonds has not been unambiguously answered.

In 1989 Raman scattering studies^[180] confirmed the presence of bifurcated hydrogen bonds (Scheme 5), the existence

Scheme 5. Schematic representation of a bifurcated hydrogen bond.



of which had been proposed several times. Water molecules with five neighbors cause defects in the tetrahedral hydrogen bond network of liquid water and lower the energy barriers

between different arrangements of the network.^[181] The “extra” water molecule acts as a catalyst by making fast restructuring possible without disrupting hydrogen bonds. Recently, Sciortino et al.^[182] tentatively suggested a link with hydrophobic hydration. The inert hydrophobic solute prevents water molecules in the hydrophobic hydration shell from being approached by a fifth neighbor, thus slowing down the molecular motion of the water molecules involved. The decreased mobility of water molecules in the hydrophobic hydration shell is therefore attributed to an *increased* local density of water. The opposite, increased mobility in water of decreased density, was observed by Pottel et al. a few years ago.^[183]

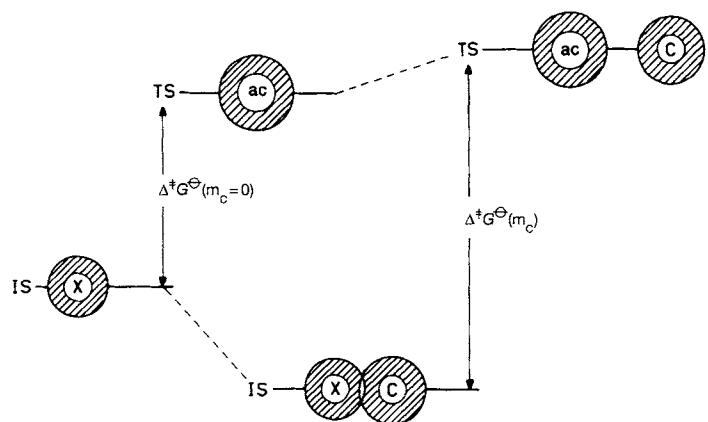
The most direct information about the structure of hydrophobic hydration shells can be obtained from diffraction studies, since these experiments yield the total pair correlation function $G(R)$, which is the averaged sum of a large number of partial distribution functions $g_{ij}(R)$ representing the average distribution of atom type j relative to atom type i in solution (see also Section 3.3.1).^[184] Unfortunately, it is extremely difficult to obtain accurate information concerning solute–water and solute–solute interactions in hydration shells present in highly dilute aqueous solutions, since the total $G(R)$ function is dominated by the water–water terms.^[185] Isotopic labeling of components of the aqueous solutions provides a possible solution of this problem.^[185] In 1990 Turner et al.^[186] reported a neutron diffraction study of the hydration of tetramethylammonium chloride (TMACl) in aqueous solution ($0.5\text{--}2\text{ mol kg}^{-1}$) with isotopic substitution of nitrogen, chlorine, and hydrogen. The results indicate that the TMA ion is surrounded by water molecules that tend to orient their OH bonds tangentially to the surface of the solute. In this respect, the results confirm the general picture of a somewhat distorted, clathrate-like solvation cage. The water correlations, however, suggest that the relative orientation of water molecules in the hydration shell is not significantly different from that in bulk water. Moreover, no evidence was found for aggregation of the TMA ions, and the structure of the hydration shell appeared to be independent on the concentration of TMACl. Results of more diffraction studies of aqueous solutions of isotopically labeled solutes are eagerly awaited.

2.7. Organic Reactions in Water

Although living organisms depend in a unique way on water as a solvent and reagent for biochemical transformations, synthetic organic chemists do not particularly like water as a solvent for chemical reactions. This “hydrophobic” attitude stems from the fact that water is a bad solvent for many organic compounds and is, in addition, highly reactive towards many organic reagents. Nevertheless, the past decade has witnessed a remarkable reappraisal of water as a solvent for organic reactions. The pioneering work of Breslow et al.,^[187] who reported intriguing solvent effects of water on notoriously solvent-insensitive Diels–Alder reactions constituted a major source of inspiration.

Our interest in organic reactivity in water and mixed aqueous solvents goes back some time and was mainly excited by the unpredictable effects of the solvent on the thermo-

dynamic parameters of activation in the water-rich region of mixed aqueous solvents.^[188, 189] Solvent effects in highly aqueous media directly reflect the interactions between solute particles, in other words, the interactions between the reactant(s) and the cosolvent, and between the activated complex and the cosolvent. In dilute solutions the rate effects are mainly caused by pairwise interactions, and the results indicate that these interactions are governed by the apolar character of the reacting species and the cosolvent (from a thermodynamic point of view, it is more appropriate to speak of a cosolute). In Scheme 6 a general example is given; the rate-decreasing effect of an apolar cosolvent C on a reaction involves a loss of apolar character during the activation process, schematically represented by the transformation of reactant X into activated complex ac.



Scheme 6. Schematic representation of the effect of cosolvent molecule C on a reaction involving reactant X and activated complex ac in aqueous solution. IS = initial state, TS = transition state, $\Delta^{\ddagger}G^{\Theta}$ = standard Gibbs energy of activation.

Towards the end of the eighties Blokzijl et al.^[190–194] reported a quantitative approach to explain solvent effects in dilute aqueous solvents in terms of pairwise interactions of cosolvents with reactant(s) and activated complex, respectively. By using the additivity principle developed by Savage and Wood (Section 3.3.2), contributions of functional groups in cosolvent molecules to the overall solvent effects could be determined. This approach does full justice to the complexity of intermolecular interactions in aqueous solutions. The additivity of functional group interactions was critically analyzed, and it was shown that the hydration shells of functional groups in close proximity are not independent of each other.^[191] Hydrophobic methylene moieties were shown to generally retard organic reactions involving a decrease of apolar character during the activation process, whereas hydrophilic groups such as hydroxyl functionalities counteract this effect.^[193] In the solvent systems examined, preferential solvation or solute aggregation does not occur. The solvent effects are therefore a consequence of hydrophobic encounters between apolar moieties in solution. These encounters are akin to molecular recognition processes but do not lead to aggregation or complexation.

Interactions between apolar groups in water are accompanied by the destructive overlap of hydrophobic hydration shells. Curiously, even apparently hydrophilic cosolutes like

carbohydrates and polyols induce a significant reduction of the rate constant of reactions involving apolar reactants and a more polar activated complex.^[193, 195, 196] The hydration of polyols and carbohydrates is predominantly governed by hydrogen bonding. The rate-retarding effects suggest that apolar groups are always “noticed” by reacting species, even in the absence of hydrophobic hydration shells, whereas hydrophilic groups can be camouflaged and become almost indistinguishable from the OH groups of water.^[195] Tentatively, we have made the suggestion^[195, 196] that nature uses this feature in molecular recognition processes that involve polysaccharides at the surface of living cells.^[197]

Diels–Alder reactions are dramatically accelerated in water.^[198–203] The reaction of cyclopentadiene and 5-methoxynaphthoquinone (Scheme 7) has been studied in detail. The rate constants in *n*-hexane and in water are



Scheme 7. Intermolecular Diels–Alder reaction of cyclopentadiene with 5-methoxy-1,4-naphthoquinone. R = OCH₃.

7.73×10^{-4} and $5.26 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$, respectively. An interesting aspect of a bimolecular Diels–Alder reaction of apolar reactants in water is that the formation of the activated complex necessarily models the pairwise hydrophobic interaction between the apolar reactants. According to Breslow,^[198] the hydrophobic packing of diene and dienophile is the most likely explanation for the observed rate effects. This idea is mainly based on a series of kinetic experiments in solutions of “salting-in” or “salting-out” materials.^[198] The enhancement or disruption of the water structure is used as a criterion for the respective promotion or inhibition of hydrophobic packing. The finding that Diels–Alder reactions are also accelerated in the presence of β -cyclodextrin by complexation of the reactants in the hydrophobic interior, appears to support this view.^[198, 201] In addition, rate constants for Diels–Alder reactions in mixed aqueous solvents were successfully related to solvent parameters such as the solvophobicity parameter S_p ,^[201] the polarity parameter $E_T(30)$,^[202, 204] and the cohesive energy density.^[199, 200] These correlations strongly suggest a relation with the hydrophobicity of diene and dienophile. Schneider et al.,^[201] however, did not find a correlation between the hydrophobicity of the dienophile and the observed rate effect.

These explanations for the remarkable effect of water on the rate of Diels–Alder reactions in water are doubtful and leave some important questions unanswered. On the basis of a combined quantitative and qualitative study of solvent effects in a series of Diels–Alder reactions in dilute aqueous solvents, Blokzijl et al.^[205, 206] found that the rate effect arises primarily from changes in the Gibbs energy of the initial state. The activated complex appeared to be only moderately sensitive towards solvent effects. The formation of a complex between diene and dienophile could not be detected.

A study of solvent effects on a series of intramolecular Diels–Alder reactions confirmed also that hydrophobic packing of diene and dienophile in the aqueous medium cannot be held responsible for the exceptional rate effects. Therefore, the high rate constant was attributed to the fact that the hydrophobicity of diene and dienophile is considerably relieved during the activation process, a process which has been termed “enforced hydrophobic interaction”.^[205, 206] The term “enforced” has been introduced to stress the fact that under the employed reaction conditions diene and dienophile do not aggregate spontaneously in water. The reactive encounter is subjected to serious geometrical constraints and in water is *less unfavorable* in terms of Gibbs energy than in organic solvents. Apparently, the activated complex is a highly adaptable species that can adjust itself to the aqueous environment. Hydrogen bonding to the polarized carbonyl bond of the dienophile appears to contribute significantly to the interaction between water and the activated complex (see also Desimoni et al.^[207]). A computer simulation study of Diels–Alder reactions in aqueous solution reported by Jorgensen et al.^[208] suggests a similar explanation.

Other organic reactions are also accelerated when conducted in aqueous solvents.^[209–214] In most cases, a reduction of the hydrophobic surface of the reactants during formation of the activated complex can largely account for the observed rate effect, although polarization effects are important as well. It is remarkable that the low solubility of the reactants enhances their reactivity, whereas complexation or aggregation only renders them less reactive. A few examples have been given, mainly photochemical processes, for which aggregation of reactants in water is favorable for the rate and product distribution.^[215]

Up to this point we have discussed primarily organic reactions in dilute aqueous solvents. But mixed aqueous solvents are particularly popular reaction media in physical organic chemistry. When high proportions of an organic cosolvent are used, the preferential solvation of the reagents due to bulk hydrophobic interactions becomes a dominant factor for the observed solvent effects. The onset of bulk hydrophobic interactions is a consequence of the occurrence of highly dynamic, cosolvent-rich domains. The absolute solvent effect is then a measure of the extent of pseudo-phase separation. Recently, we have shown that these solvent effects can be analyzed quantitatively by using an inverse Kirkwood–Buff treatment^[216] (see also Section 3.3.1).

3. Part B. Theoretical Studies

3.1. An Overview of the Theoretical Approaches to Explain Hydrophobic Effects

Theoretical studies of hydrophobic effects can be divided into four major categories. Most straightforward are those studies in which a model is developed that accounts for the observed thermodynamic parameters of solvation of apolar compounds in water on the basis of thermodynamic considerations; these include the liquid hydrocarbon model, multiple-step models for solvation, compensation models, and mixture models. Most of these models aim at a better under-

standing of hydrophobic hydration, and approaches along these lines will be reviewed in Section 3.2. Many of the theoretical approaches to describe hydrophobic effects center on virial expansions of thermodynamic properties of aqueous solutions containing apolar solute particles. As will be shown in some detail in Section 3.3.1, virial coefficients can be related to interactions between solute particles in aqueous media. Hence, these studies mainly deal with hydrophobic interactions either pairwise or bulk. Theoretical studies have either aimed at an empirical documentation of virial coefficients for aqueous solutions of organic solutes (Section 3.3.2), or, alternatively, have been devoted to a (semi)-quantitative description of these virial coefficients on the basis of intermolecular forces (Section 3.3.3). As will be discussed in Section 3.3.1, virial coefficients can also be interpreted in terms of the distribution of solute particles over the entire solution. Computation of these distribution functions can therefore yield both interaction potentials and virial coefficients. The accuracy of the applied methods can be tested by comparing the results with experimentally obtained virial coefficients. During the past fifteen years highly sophisticated integral theories have been developed for the solute–solute and solute–solvent distribution functions. These interaction site models (ISM) are equally suited for studying both hydrophobic hydration and hydrophobic interactions. Some approaches that have received particular attention will be described in Section 3.3.4.

A very elegant but often criticized approach to the study of hydrophobic effects uses the scaled particle theory (SPT).^[*] Although the SPT is inherently a rather rigorous simplification of reality, applications of SPT yield surprisingly accurate results, and researchers in the field constantly draw attention to this remarkable fact. Some intriguing results of improved versions of SPT in the past few years are summarized in Section 3.4. The rapid maturation of theories concerning molecular liquids and solutions during the past fifteen years has made computer simulations one of the most valuable tools in the study of hydrophobic effects. Monte Carlo (MC) and molecular dynamics (MD) calculations have provided chemists with detailed information on hydrophobic effects on a molecular level that has been largely unaccessible by experimental methods. The molecular picture that has emerged from computational studies has reinforced some aspects of the traditional ideas about hydrophobic hydration, but has caused growing doubts about the validity of the whole concept of hydrophobic interactions. A detailed discussion will be given in Section 3.5.

3.2. Thermodynamic Properties of Aqueous Solutions of Apolar Compounds; Some Models

Since the introduction of the concepts of hydrophobic hydration and hydrophobic interactions, theories have been developed to account for the remarkable thermodynamic properties of aqueous solutions of apolar compounds. As will be shown in this section, thermodynamic parameters are a notoriously unspecific starting point for developing struc-

[*] In the scaled particle theory all particles are assumed to be spherical with diameters defined such that thermodynamic quantities of solutions and pure liquids are reproduced.

tural models. Many models can account for the thermodynamics of the solvation of apolar compounds in water, and most have a strongly qualitative character. Although each model emphasizes different aspects of hydrophobic hydration and hydrophobic interaction, most models can be categorized as mixture models. In these, water is considered to be a mixture of two or more species characterized by some differentiating property such as the number, strength, or length of hydrogen bonds. Introduction of apolar solute particles is thought to shift the equilibrium between the species. The classic models of Frank and Evans,^[4] Frank and Wen,^[23] Neméthy and Scheraga,^[21, 22] Kauzmann,^[6] and Ben-Naim^[10, 26] are in fact mixture models (Section 1.2).

The geometric relaxation model developed by Lumry et al.,^[217] a more recent example of a mixture model, is a hybrid of the classic model of Frank and Evans^[4] and the flickering cluster model of Frank and Wen.^[23] It is based on the water model of Sceats and Rice^[218] and considers the water structure as composed of pairwise-bonded water molecules with continuously distributed energies, bond angles, and bond lengths. Two limiting structures are defined on the basis of bond length. One is characterized by short bonds, low entropy and enthalpy, and a large free volume available for solute particles; the other is characterized by long bonds, a large librational freedom of individual water molecules, and little free space for solute particles. Lumry et al.^[217] expected small clusters of water molecules to undergo spontaneous “cluster contractions and expansions”, thereby fluctuating between the limiting structures. An apolar solute particle was thought to promote the structure with short bonds by occupying the free volume and thus shifting the equilibrium.

The thermodynamic aspects of mixture models can be illustrated by a recent mixture model developed by Muller.^[219] This model clearly finds its precursor in the classic model of Neméthy and Scheraga^[21, 22] and is based on the assumption that every hydrogen atom in an aqueous solution can either participate in a hydrogen bond or is not involved in hydrogen bonding at all. An additional distinction is made between hydrogen atoms of bulk solvent molecules and hydrogen atoms that are perturbed by the presence of an apolar solute particle. The formation and disruption of hydrogen bonds is considered an equilibrium process, on the basis of which the enthalpy and heat capacity can be defined. A crucial aspect of the model is that it is not necessary to make any statements about the extent of structuring in the hydration shell (hs) in relation to the bulk (b) other than that $\Delta H_{hs}^\ominus \neq \Delta H_b^\ominus$ and $\Delta S_{hs}^\ominus \neq \Delta S_b^\ominus$. In this way the heat capacity of the bulk and the hydration shell can be analyzed separately. When n^h is the number of hydrogen atoms in the hydrophobic hydration shell, the heat capacity increment is given by Equation (8), in which $C_{p,b}^h$ and $C_{p,hs}^h$ are the heat

$$\Delta C_p^\ominus = n^h(C_{p,hs}^h - C_{p,b}^h) \quad (8)$$

capacity contributions per mole of hydrogen atoms in the bulk and the hydration shell, respectively. The heat capacities can be quantified on the basis of the enthalpy of the equilibrium between broken and intact hydrogen bonds.

The heat capacity contribution of hydrogen atoms in the hydration shell was shown to be described by Equation (9),

$$C_{p, hs}^b = (\Delta H_{hs}^\ominus)^2 K_{hs} / RT^2 (1 + K_{hs})^2 \quad (9)$$

in which K_{hs} is the equilibrium constant. If $K_b = K_{hs}$, a positive heat capacity change can only occur when $\Delta H_{hs}^\ominus > \Delta H_b^\ominus$. Alternatively, if $\Delta H_{hs}^\ominus = \Delta H_b^\ominus$, then K_{hs} must be larger than K_b . Hence, the heat capacity effect can either be caused by increasing the strength of the hydrogen bonds or by increasing the number of broken bonds. A cooperative effect observed if $\Delta S_{hs}^\ominus > \Delta S_b^\ominus$ can lead to a very large heat capacity effect. A realistic choice of the parameters in Equation (9) yields a satisfactory description of the temperature dependence of the thermodynamic parameters for the solvation of apolar compounds in water. This mixture model clearly shows that statements about structure of the hydration shell with respect to bulk water are irrelevant without a proper definition of structure. Muller^[219] stressed the fact that even at elevated temperatures, structural reorganization in hydrophobic hydration shells is still substantial and that the heat capacity change does not become zero; this was proven experimentally as well (see Section 2.1).

A similar but more general two-state model has been used by Gill et al. and Wadsö et al.^[58, 63, 220–222] According to this model of a noncooperative hydration shell, each water molecule exists in two thermodynamic states separated by an enthalpy difference ΔH . These different states are caused by the fact that a water molecule in the solvation shell is affected by the presence of a solute boundary and cannot participate in the maximum number of hydrogen bonds. Each water molecule is assumed to behave independently of its neighbors, and cooperative effects are not taken into account. The heat capacity for the solvation process is now given by Equation (10), in which N is the number of water molecules in the solvation shell, τ the reciprocal temperature ($1/T$), and τ_m the

$$\Delta C_p^\ominus = N \frac{(\Delta H)^2}{RT^2} \frac{\exp[-(\Delta H/R)(\tau - \tau_m)]}{[1 + \exp[-(\Delta H/R)(\tau - \tau_m)]^2} \quad (10)$$

reciprocal temperature at which the concentrations of water molecules in the two states are equal. Shinoda^[51] estimated that this mean temperature is 370 K. Equation (10) is rather similar to Equation (9).

Unfortunately, thermodynamic analyses based on mixture models do not aid the molecular interpretation of hydrophobic effects, as long as water is considered as a mixture of two or more species with different characteristics. Although two-state models are somewhat simplistic, they usually do provide an adequate quantitative description of the temperature dependence of the thermodynamics of solvation of apolar compounds in water. The molecular interpretation of these mixture models remains a matter of assumptions.

Closely related to two-state or mixture models are models that we will describe as compensation models. Lumry et al.^[217, 223] showed that the Gibbs energy for the transfer of argon from cyclohexane to water, hydrazine, and ethylene glycol are remarkably similar (Table 4). The low solubility of argon in the hydrogen-bonded solvents glycol and hydrazine is due to the unfavorable enthalpy change. The transfer of argon from glycol or hydrazine to water is accompanied by significant changes in enthalpy and entropy which, however, show almost complete compensation.

This observation led to the suggestion that the thermodynamics for the solvation of apolar compounds in water result

Table 4. Thermodynamic parameters for the transfer of one mole of argon from solvent I to solvent II at 298 K (taken from refs. [217, 218]).

Solvent I	Solvent II	ΔG^\ominus [kJ mol ⁻¹]	ΔH^\ominus [kJ mol ⁻¹]	$-T\Delta S^\ominus$ [kJ mol ⁻¹]
cyclohexane	water	10.4	-11.2	21.6
cyclohexane	hydrazine	11.9	9.5	2.4
cyclohexane	ethylene glycol	10.3	0	10.3
ethylene glycol	water	0	-11.3	11.4
hydrazine	water	-1.5	-20.7	19.2

from two contributions. First, the low solubility of apolar compounds in water, hydrazine, and glycol is caused by the extensive hydrogen-bonded structure of the liquid;^[224, 225] this contribution to the Gibbs energy of solvation is defined as the “motive” part. The second contribution is attributed to the characteristic fluctuation behavior of liquid water that distinguishes water from hydrogen-bonded organic solvents. Obviously, this aspect originates from classic mixture models (vide supra). The fluctuation of the hydrogen bond network leads to the remarkable heat capacity effects and compensation of enthalpy and entropy. This contribution is thus identified as the “compensative” part. According to Lumry,^[217] the fluctuation behavior can be explained on the basis of a simple two-state, geometric relaxation model, and he attributed the low solubility of apolar compounds in water to the distortion of the hydrogen bond network. A detailed theoretical description of compensation laws was given by Grunwald^[226, 227] in the mid-eighties.

In 1985 Lee^[228] reported an interesting analysis of the compensation model of Lumry based on a dissection of the Gibbs energy of solvation into a contribution arising from the creation of a cavity in the solvent (G_c) and a contribution accounting for the attractive forces between the solute and the solvent (G_i). Both the cavity formation term and the interaction term were used to express the enthalpy and the entropy of the solvation process [Eqs. (11) and (12)]. The

$$\Delta h = G_i - \sigma_{v, H}^2 \quad (11)$$

$$T\Delta s = -G_c - \sigma_{v, H}^2 \quad (12)$$

factor $\sigma_{v, H}^2$ is a compensation term affecting both the entropy and enthalpy of solvation.^[*] A statistical mechanical approach to the fluctuation behavior of liquid water has been offered by Oguni and Angell,^[229] Lumry et al.,^[217] and Lee.^[228, 230] The compensation terms are related to the mean square fluctuations in H and S , $\langle \sigma_H^2 \rangle$ and $\langle \sigma_S^2 \rangle$, and can be expressed in terms of temperature and heat capacity. An important parameter determining the magnitude of the compensation term is the isothermal expansion coefficient α . The compensation term originates from the loss of solvent–solvent interactions and counteracts the favorable G_i term. For organic solvents, $\sigma_{v, H}^2$ and G_i are similar and Δh is small. The isothermal expansion coefficient α of water is extremely small, which implies that the enthalpy of water is not affected when the volume of water changes; in other words, water can accommodate large volume fluctuations without a significant enthalpy loss. Hence the compensation term for water is small, and Δh is similar to G_i , which is large and favorable.

[*] In this review the symbol σ is used in several contexts, in analogy to the literature usage.

The cavity formation term G_c results from the restricted motion of the solvent molecules in the presence of the solute particles and is governed by entropy. A large number of configurations available to the molecules in the pure solvent are unavailable in solution since they produce solvent–solute hard-core overlap. The compensation term reflects the increase in volume that leads to a loss of solvent–solvent interactions and an increase in entropy. In water the hydrogen bond network is hardly affected by the apolar solute, and the compensation term is small. According to Lee^[228] it is misleading to say that apolar solute particles enhance the structure of liquid water. The low solubility of apolar compounds in water is thus mainly due to the unfavorable cavity term, which arises from the extremely small molecular volume of a water molecule (Section 3.4).^[228, 230]

In the hydrocarbon model proposed by Baldwin^[61] and Privalov et al.,^[36, 60, 231] the thermodynamic parameters for the solvation of apolar compounds in water are also a result of two contributions—from the disruption of dispersion interactions between the hydrocarbon molecules in the liquid state and from the solvation of the hydrocarbon molecule in water. The model is based on the temperature dependence of the thermodynamic parameters of solvation for liquid hydrocarbons in water and was inspired by the role of hydrophobic effects in the folding and stability of proteins.

The crucial assumption in the hydrocarbon model is that at T_s^* (Section 2.1) water has lost its capacity to form a hydrophobic hydration shell. By applying Equation (3) the enthalpy of solvation of an apolar liquid at T_s^* can be expressed as Equation (13).^[*] Combining Equations (2) and (13) provides Equation (14), in which the Gibbs energy of

$$\Delta H^\ominus(T_s^*) = \Delta C_p(T_s^* - T_H^*) \quad (13)$$

$$\Delta G^\ominus = \Delta H^\ominus(T_s^*) - [\Delta C_p(T_s^* - T) + T\Delta C_p\ln(T/T_s^*)] \quad (14)$$

solvation of liquid hydrocarbons is expressed as a function of T_s^* . $\Delta H^\ominus(T_s^*)$, the enthalpy of solvation at T_s^* , and the term in brackets are labeled as nonhydration and hydration terms, respectively. The hydration term is negative at all temperatures and counteracts the positive nonhydration term (Figs. 2a, b). In order to interpret Equation (14) in terms of a molecular model, Privalov et al.^[14] introduced a somewhat artificial description of the solvation of a liquid hydrocarbon in water as a two-step process. At T_s^* the apolar liquid is first transformed into a compressed gas that has the same entropy and heat capacity properties as the liquid state but the enthalpy properties of the gas state. The second step involves the transfer of the compressed gas into water and is defined as the hydration step. The introduction of this fudged two-step process was tempting, because the nonhydration term $\Delta H^*(T_s^*)$ so beautifully equals the enthalpy of evaporation of the apolar liquid. This approach leads to the at first sight paradoxical conclusion that hydrophobic hydration increases the solubility of apolar liquids in water. The favorable Gibbs energy of hydration is small because of the compensating contributions from entropy and enthalpy. The low solubility of apolar liquids in water is fully account-

ed for by the nonhydration term. Hydrophobic interactions (bulk hydrophobic interactions) are most pronounced at T_s^* .

The practical value of the two-step model is questionable. It is not clear whether the contribution identified as the hydration term completely accounts for the process of hydrophobic hydration. An inherent contradiction in the approach is that the hydrocarbon model is based on the assumption that the heat capacity change is independent of temperature, whereas the two-step interpretation strongly relies on the absence of heat capacity effects at T_s^* . But the change in heat capacity of solvation at T_s^* is still considerable. In addition, at temperatures above T_s^* the entropy of solvation becomes positive, which would imply “reversed” hydrophobic hydration.

Equation (15) is a modified version of Equation (14) in which T_H^* is used instead of T_s^* . The compensative or hydra-

$$\Delta G^\ominus = [\Delta C_p(T - T_H^*) - T\Delta C_p\ln(T_H^*/T)] - T_H^*\Delta S^\ominus(T_H^*) \quad (15)$$

tion part in brackets is small and positive. The temperature-independent part describes the low solubility of apolar liquids and is comparable to the enthalpy term in Equation (14). This approach requires a different two-step model which is more difficult to interpret^[36] and finds a detailed molecular expression in the scaled particle theory.^[228]

Recently, Ruckenstein^[232] proposed a mechanistic picture of hydrophobic hydration with a two-step model that distinguishes the process of cavity formation and the subsequent reorganization of the water molecules into “icebergs” based on earlier ideas of Shinoda.^[51] The features of this model closely resemble those of the compensation models of Lumry et al.^[217] and Lee.^[228] Alternative two-step models for the solvation of apolar compounds in water have been proposed by Hvilsted^[233] and Ben-Naim.^[10] From a thermodynamic point of view, two-step descriptions of solvation processes can be very convenient, but the definition of the separate steps remains an arbitrary exercise. In general, introduction of artificial intermediate states provides a deceptive sensation of clarity with respect to the molecular nature of hydrophobic effects.

3.3. Potentials of Mean Force, Virial Coefficients, Distribution Functions; Probing Hydrophobic Effects

3.3.1. Introduction

Another class of theoretical approaches to describe hydrophobic effects centers on theoretical models of molecular liquids and solutions based on statistical thermodynamics. In this section some basic terminology will be introduced to provide the context for the discussion of results in the following sections. Hydrophobic hydration is most conveniently described based on the distribution of water or, alternatively, oxygen and hydrogen atoms around a solute particle. The corresponding radial distribution function $g_{AO}(r)$ is defined as the average number of oxygen nuclei in a thin spherical shell of width Δr and radius r , which is centered on solute A [Formula (16)]. In this formula, ϱ_w is the average number of

[*] In the literature T_s^* and T_H^* have been replaced by T_s and T_H , respectively, when the solvation of apolar liquids in water is considered. To avoid unnecessary confusion, we prefer to use T_s^* and T_H^* without exception.

$$4\pi r^2 \varrho_w g_{AO}(r) \Delta r \quad (16)$$

water molecules per unit volume. The excess chemical potential of the solute particle can be computed by an integration of $g_{AO}(r)$ over a well-specified radius (at large r the integral diverges).^[234] The aim is now to determine the radial dependence of the distribution function, an exercise which obviously requires detailed knowledge of the structure of liquid water. Comparison of the distribution functions of hydrogen and oxygen atoms gives insight into the orientation of the water molecules with respect to the surface of the solute particle.

When two or more apolar solute particles are present in water, the question arises as to the forces between these particles as a function of their relative distance. The chance of finding a second solute particle at a distance R from the center of the first directly measures the magnitude of the interactions between the solute particles. The potential of mean force between two solute particles A, $\Delta G^{[*]}$ is defined as the work required to bring two molecules of A, which were originally at infinite separation, to a separation R (which is on the order of the diameter of A). Following statistical thermodynamics, ΔG can be expressed^[10, 17] by Equation (17), where $U(R)$ is the direct pair interaction between

$$\Delta G(R) = U(R) + \delta G(R) \quad (17)$$

the solute particles in the absence of solvent and $\delta G(R)$ the contribution of the solvent. The force acting between the particles is given by the first derivative of ΔG with respect to distance. Occasionally, $\delta G(R)$ is taken as a measure for hydrophobic interactions.^[10, 235] Although this definition is formally more appropriate than the definition given in Section 1.2, a major disadvantage is that $\delta G(R)$ is difficult to determine experimentally.

Solvent-induced interactions result from changes in the solvation of solute particles when they approach each other. Even solute particles considered as hard spheres dissolved in a featureless solvent composed of hard spheres are subjected to solvent-induced interactions. This phenomenon is well established and is a consequence of partial shielding of solute surfaces when the particles are in close proximity.^[26] When solute and solvent do not consist of hard spheres but have soft potentials, shielding leads to repulsive forces. These repulsive forces are, however, counteracted by attractive contributions caused by solvent molecules that simultaneously interact with two solute particles.

The potential of mean force acting on two solute particles is related to the distribution of the solute particles in the aqueous solution by Equation (18), where $g_{AA}(R)$ is the pair

$$g_{AA}(R) = \exp[-\Delta G(R)/k_B T] = \exp[-U(R)/k_B T] + \exp[-\delta G(R)/k_B T] \quad (18)$$

distribution function giving the chance of finding a second particle A at a distance R from the center of reference particle A [Eq. (16)]. The art of theoretical approaches to explain of hydrophobic interactions is to give a description for the behavior of either $\Delta G(R)$ or $g_{AA}(R)$.

Qualitatively, the pair distribution of spherical solute particles dissolved in a solvent containing only spherical solvent

molecules can be rationalized on the basis of a number of preferred solute–solute arrangements. As shown in Figure 6, the contact arrangement and the solvent-separated arrangement give rise to well-defined maxima in the pair distribution function. More elaborate treatments also yield preferred arrangements at greater solute–solute distances.

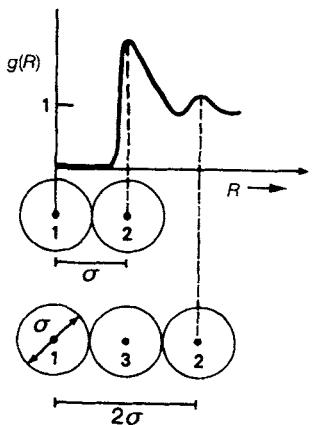
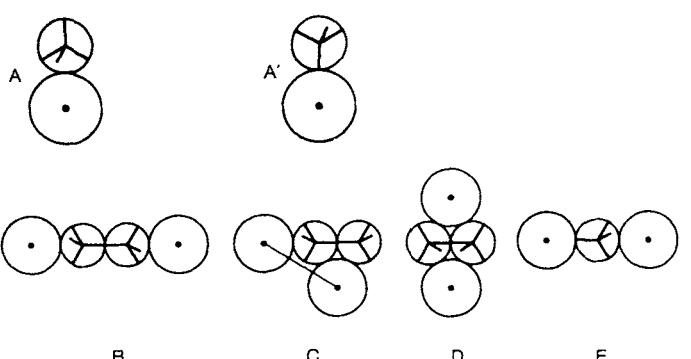


Fig. 6. Pair distribution function $g(R)$ for spherical solute particles 1 and 2 in solvent 3. Solute and solvent are shown as spherical particles with the same diameter σ . The first and second maximum in the pair distribution function correspond to the preferred arrangements (taken from ref. [17]).

Any solute–solute distribution function therefore exhibits oscillatory behavior as a function of the solute–solute distance. Unfortunately, a water molecule can hardly be taken as a spherical particle with a homogeneous charge distribution. An elegant and illustrative semiquantitative solution to this dilemma has been provided by Ben-Naim.^[17] According to his approach, water is viewed as a Lennard–Jones sphere equipped with four additional binding sites, two hydrogen bond donor sites and two hydrogen bond acceptor sites. The binding energy B_W of each water molecule may be divided into a Lennard–Jones contribution and a hydrogen bond contribution [Eq. (19)].

$$B_W = B_{LJ} + B_{HB} = B_{LJ} + \sum_{l=1}^4 B_l^{HB} \quad (19)$$

As shown in Scheme 8^[17] each water molecule will be inclined to maintain hydrogen bond interactions. Thus, a water molecule near an apolar solute particle is far more likely to adopt orientation A rather than A'. Some preferred solvent-separated arrangements of two solute particles in an aqueous medium are shown by B–E. The shortest solute–



Scheme 8. Schematic representations of various arrangements of water molecules with respect to one or two apolar solute molecules (taken from ref. [17]).

[*] From this point on ΔG is used to denote the potential of mean force.

solute distance, about 6 Å, is found in arrangement C. This arrangement seems more probable than E, which has no hydrogen bonds. However, this preference has not been unambiguously confirmed by computational studies (Section 3.5). Although these qualitative and semiquantitative considerations are instructive, it remains a challenge to develop more sophisticated and quantitative molecular theories to account for the thermodynamic behavior of aqueous solutions of nonelectrolytes in terms of intermolecular potentials and distribution functions.

A convenient way of thinking about the distribution of solute particles in a solution is provided by the solution theory of McMillan and Mayer.^[236] According to this theory, solutes in a solution are distributed in the same way as in a hypothetical gaseous state. The concentration of this gas equals that of the solution, and the interaction potentials in the hypothetical gas are related to the solute–solute potentials of mean force in a solution of infinite dilution. The chemical potential of the hypothetical gas is given by Equation (20), where w_2 and w_N are the pairwise and higher-

$$W(r_1, \dots, r_N) = \sum w_2(r_{i,j}) + \sum w_3(r_{i,j,k}) + \dots \quad (20)$$

order potentials of mean force acting between the solute molecules. The potentials of mean force can be calculated from the distribution at infinite dilution and used to compute the distribution of the solutes at finite concentration.

Traditionally, computer simulations only consider two-body interactions and are therefore perfectly suited for a study of pairwise hydrophobic interactions. It is, however, questionable whether the pair potential w_2 is sufficient to account for bulk hydrophobic interactions occurring in aqueous solutions of finite concentration. The theory of McMillan and Mayer^[236] offers a relation between experimentally accessible thermodynamic parameters of solution and intermolecular potentials by the expression of the osmotic pressure Π in terms of a virial density expansion [Eq. (21)]. Here B_2^* is the second osmotic coefficient and directly related to the potential of mean force according to Equation (22).^[236] The pointed brackets indicate that orientational averaging has been performed.

$$\Pi/kT = \rho + B_2^* \rho^2 + B_3^* \rho^3 + \dots \quad (21)$$

$$B_2^* = 2\pi \int \langle 1 - \exp[-\Delta G(R)/kT] \rangle r^2 dr \quad (22)$$

In thermodynamic studies of aqueous solutions, thermodynamic excess functions are more widespread than osmotic pressures. The Gibbs excess function, for example, can be expressed in a molality expansion [Eq. (23)]. Here g_{AA} and

$$G^E = g_{AA}[m_A^2/m_0^2] + g_{AAA}[m_A^3/m_0^3] + \dots \quad (23)$$

g_{AAA} are the parameters for the interaction of two and three particles, respectively; they are also known as the Lewis–Randall coefficients and should not be mixed up with $g_{AA}(R)$. Friedman^[237, 238] derived a formal link between g_{AA} and the second osmotic virial coefficient B_2^* [Eq. (24)]. Here

$$g_{AA} = (RT/V_w^\ominus)(NB_2^* - V_A^\ominus + 1/2\kappa RT) \quad (24)$$

V_w^\ominus is the volume of 1 kg of pure water, V_A^\ominus the partial molar volume of the solute at infinite dilution, κ the isothermal compressibility, and N Avogadro's number. The second osmotic coefficient and the Lewis–Randall coefficient of aqueous solutions of nonelectrolytes are therefore a direct measure of the magnitude of pairwise hydrophobic interactions, since they reflect the number of encounters between both molecules beyond that expected statistically.

The existence of substantial triplet and higher-order terms reflects the occurrence of bulk interactions. Although it is theoretically feasible to calculate these higher-order terms from excess functions at elevated concentrations, this is not commonly done. To account for solute–solute (or solvent–solvent) interactions in more concentrated solutions or even in binary solvents, application of the theories of Kirkwood and Buff^[239] is more convenient and provides insight into the molecular organization of these solutions. The crucial parameter in this theory is the Kirkwood–Buff integral G_{AA} [Eq. (25)], which is a measure of the mutual affinity of mol-

$$G_{AA} = \int_0^\infty (g_{AA}(r) - 1) 4\pi r^2 dr \quad (25)$$

ecules A and yields, when multiplied with the number density of A, the excess or deficiency of particles A in the proximity of a solute particle A. The integral decays to unity beyond a certain distance R . In practice, the integral can be cut off at a distance of a few molecular diameters; the volume element is then defined as the correlation volume.^[240] The integral can be related, along with other thermodynamic quantities, to the excess chemical potential of the aqueous solution (vide supra).^[240] In fact, the Kirkwood–Buff integral collects all virial coefficients in the density expansion of the McMillan–Mayer theory [Eq. (21)] in one single integral function and becomes equal to the second osmotic coefficient in the limit $m_A \rightarrow 0$ [Eq. (23)]. For a recent treatment of the Kirkwood–Buff theory, see Matteoli et al.^[241]

Theoretical approaches to account for structural and thermodynamic properties of aqueous solutions require knowledge of the distribution functions $g(R)$ and intermolecular potential functions. Here the previously discussed approaches run into serious difficulties.^[242] First of all, various relations have been proposed to express the distribution of molecules in solution in terms of potentials of mean force acting between these molecules. In solutions in which the concentration of the solute approaches zero, the pair correlation function for the solute particles can be effectively described by Equation (18). As the density is large, which is always the case for solvent molecules, the situation is far more complicated. A number of so-called integral equations have been proposed which express the distribution of the components present in the solution in terms of potential functions. In the theoretical treatments of hydrophobic effects, the Ornstein–Zernicke equation and the Percus–Yevick equation are frequently encountered.^[242] These integral equations can be solved only by numerical methods. By using computers and simple pair potential functions, such as hard-sphere potentials or Lennard–Jones potentials, solutions can be obtained^[242] (see also Section 3.3.4).

3.3.2. Virial Coefficients and Distribution Functions; (Semi)empirical Approaches

In Figure 7 the second osmotic coefficients (at 25 °C) for homotactic interactions in aqueous solutions of noble gases and methane are plotted as a function of Henry's law constant K_H . The polarizability of the solute affects the sign and

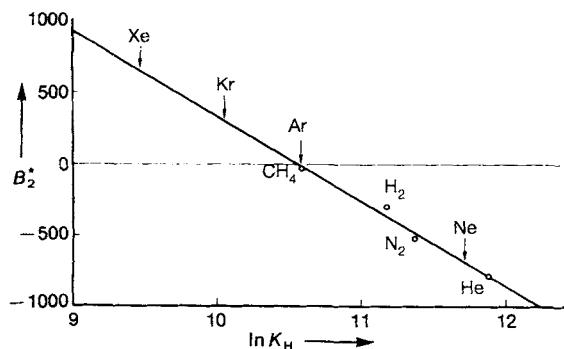


Fig. 7. Correlation between the logarithm of Henry's law constant and the osmotic virial coefficient (in Å³) for small nonpolar solutes in water at 25 °C (taken from ref. [246]).

the magnitude of the virial coefficient. Apparently, krypton atoms repel each other in an aqueous environment, whereas methane molecules attract each other, though only slightly. On the basis of a limited number of accurate osmotic coefficients, Clark et al.^[243] suggested that the association of alcohols in water is less than in the gas phase. This was also established for aqueous solutions of pure hydrocarbons such as cyclohexane and benzene^[244, 245] and for noble gases such as krypton.^[246] In Table 5 the second virial coefficients (at 25 °C) are given for some organic compounds in aqueous solution and in the gas phase. Remarkably, the Lewis–Randall coefficients differ quite substantially from the second osmotic coefficients and sometimes even have opposite signs; this can be reconciled by Equation (24). The results summarized in Table 5 emphasize that apolar molecules in a dilute aqueous solution show less tendency to associate than in the gas phase. Water apparently shields apolar solutes from each other and does not seem to promote their associ-

Table 5. Experimental values of the second virial coefficients g_{AB} and B_{AB}^* at 25 °C (taken from ref. [16]).

Solute(s)	g_{AB} [J kg mol ⁻¹]	B_{AB}^* (aq) [a] [cm ³ mol ⁻¹]	B_{AB}^* (g) [cm ³ mol ⁻¹]
methanol	−52	17	
ethanol	−125	4	
2-propanol	−203	−11	
2-methyl-2-propanol	−277	−25	
cyclohexanol	−772	−209	
<i>myo</i> -inositol	−199	20	
cyclohexane		−402 to −527 [b]	−1734
benzene		−388	−1480
cyclohexanol + cyclohexane	−1163	−368	
cyclohexanol + <i>myo</i> -inositol	390	259	

[a] McMillan–Mayer standard state. [b] Strongly dependent on the method of calculation.

ation.^[16, 247] A few years ago Archer^[248] studied the effect of temperature on the second virial coefficients of aqueous alcohols and showed that the “screening” effect of water weakens with increasing temperature.

Second virial coefficients in the Lewis–Randall representation of thermodynamic excess functions for solvation of nonelectrolytes in water have been frequently treated by empirical models. Solute–solute interactions reflect the interactions between the functional groups that form the solute molecules and are able to interact with their surroundings. In 1976 Savage and Wood introduced a model that has received much attention during the past decade, in which the enthalpies^[249] and later also Gibbs energies^[273] of interaction, represented by the second Lewis–Randall coefficients h_{AB} and g_{AB} , are expressed in terms of a summation of pairwise functional group interactions [Eq. (26)]. Here $G_{i,j}$ repre-

$$g_{AB} = \sum_{i,j} n_i^A n_j^B G_{i,j} \quad (26)$$

sents the interaction between n_i^A groups i in solute A and n_j^B groups j in solute B in the Lewis–Randall representation. Similar equations can be set up for enthalpy and entropy. The Savage–Wood additivity of groups is based on three major assumptions: 1) Each group i of solute A interacts with each group j of solute B, 2) each of these interactions makes a characteristic contribution to the parameter for the interaction between A and B, and 3) each group interaction is independent of other groups and their relative position in the solute. A slightly different group additivity approach has been proposed by Bloemendal and Somsen.^[250]

The group additivity approach can be criticized for being too simplistic.^[251–253] The importance of neighboring group effects and stereochemistry have been recognized but purposefully neglected. In addition, some doubtful assumptions have been made in dissecting solute molecules into separate groups (see, for example, Tasker et al.^[254] and Spitzer et al.^[255]). Group additivity approaches are therefore considered to be first approximations. Theoretically, the potential of mean force acting between the solutes must be small in comparison to the thermal energy kT , since only in this case is linearization of the exponential term $\exp[W_{AB}/kT] − 1$ according to Equation (27) a valid approxima-

$$\exp[W_{AB}/kT] − 1 = \exp[(W_1/kT + W_2/kT)] \cong W_1/kT + W_2/kT \quad (27)$$

tion.^[256] This exponential term expresses the solute–solute interactions as shown in Equation (18).

In spite of these shortcomings, the additivity approach has been applied to calculate pairwise interaction parameters (both Gibbs energies as well as enthalpies) for many organic compounds and functional groups (see for recent examples, Suri et al.,^[257] Bloemendal et al.^[258] Check et al.,^[259] Borghesani et al.,^[260] and Barone et al.^[261, 262] and references cited therein). A selection of Gibbs energies and enthalpies of pairwise functional group interactions in aqueous solution at 25 °C is given in Table 6. In a pragmatic sense, the breakdown of the additivity rules may be used to signal atypical effects.

In studies of both heterotactic^[263] and homotactic interactions^[264] between a large variety of organic compounds in

Table 6. Gibbs energies and enthalpies of pairwise functional group interactions in aqueous solution at 25 °C [a].

<i>i</i>	Group	G_{ij} [b] [J kg mol ⁻²]	H_{ij} [b] [J kg mol ⁻²]
CH ₂	CH ₂	-34	33
OH	OH	-24	-19
OH	CH ₂	27	9
CONH	CONH	-115	-252
CONH	OH	-34	-
CONH	CH ₂	55	66
CHOH	CHOH	-3	-14
CHOH	CONH	-6	-47
CHOH	CH ₂	10	44

[a] Taken from refs. [254, 255]. [b] Standard errors are large, often 20–40%.

aqueous solution researchers concluded that hydrophobic solutes are characterized by $g_{AB} < 0$ and $Tg_{AB} > h_{AB} > 0$. The classification of compounds as hydrophobic or hydrophilic is somewhat ambiguous, and some compounds such as carbohydrates^[264] clearly show both hydrophobic and hydrophilic behavior. Because of the lack of solubility, interactions between unsubstituted hydrocarbons or noble gases can unfortunately not be studied. Hence, the approach does not provide insight into the molecular origin of pairwise hydrophobic interactions but yields a straightforward inventory of single group contributions to interactions of solutes more complex than noble gases and alkanes.

The relation between pairwise and bulk hydrophobic interactions is notoriously vague and difficult to describe in a theoretical model. To account for bulk hydrophobic interactions, Kirkwood–Buff integral functions have been frequently used.^[265, 266] A typical example representing the distribution of 2-propanol and water in their binary mixture as a function of composition is shown in Figure 8. Apparently

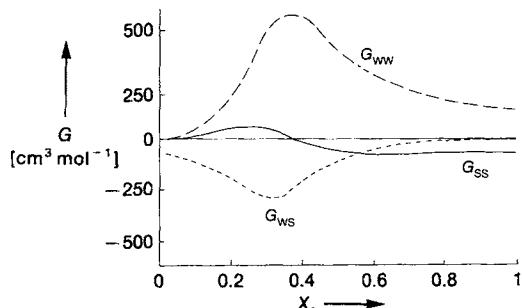


Fig. 8. Kirkwood–Buff integral functions G for mixtures of water (W) and 2-propanol (S) as a function of the mole fraction X_s of the organic cosolvent at 25 °C (taken from ref. [265]).

the preference of the organic component for alike molecules becomes greater with increasing concentration, and G_{ss} reaches a maximum in the water-rich region. At this concentration, the thermodynamic behavior of the mixture deviates strongly from that of an ideal mixture. A relevant question in this respect is whether the pairwise interactions predominantly present in dilute solution are additive or cooperative in more concentrated solutions. As shown above, water reduces the attractive forces between pairs of some apolar gases, but evidence suggests that it enhances the interactions

between many solute particles. The molecular origin of this phenomenon is still unclear.

3.3.3. Virial Coefficients; The Models

During the past twenty years a number of approaches have been developed to calculate second osmotic coefficients on the basis of (semi)quantitative potential models for interactions between apolar solute particles in aqueous solution. A classic approach was reported by Kozak et al.,^[267] who used lattice theories and the McMillan–Mayer theory to account for trends in second osmotic coefficients. Their main conclusions were that solute–solvent interactions decrease the magnitude of attractive forces between apolar solute particles and that the magnitude of the osmotic coefficient strongly depends on the size of the solute.

Friedman et al.^[27–29] developed a model for solute–solute interactions on the basis of a repulsion term for hard spheres (hard-core repulsion term) and a term for the overlap of hydration spheres (cosphere overlap term) which became very popular in qualitative explanations of hydrophobic effects. The cosphere overlap term, often referred to as the Gurney contribution (in honor of Gurney^[268] who first suggested the importance of overlapping hydration spheres for ion–ion interactions in aqueous solution), is estimated from the overlap volume of two solvation spheres. Following the classic ideas of Kauzmann,^[6] the overlap of hydration shells leads to the release of structured water molecules into the bulk.

The basis of the model is schematically shown in Scheme 9. Water molecules in the hydrophobic hydration



Scheme 9. Schematic representation of the pairwise hydrophobic interaction between two apolar solute particles S, involving overlap of hydrophobic hydration shells and the concomitant release of structured hydration water to the bulk, according to the Gurney overlap model.

shells have a less favorable Gibbs energy than bulk water molecules; this is expressed in the Gurney coefficient. As the solute particles approach each other, the unfavorable situation of water molecules in overlapping hydration shells is gradually lost, and an attractive force increases as a function of the decreasing distance between the solute particles. The magnitude of the Gurney coefficient can be estimated from experimental data. However, it is questionable whether the Gurney overlap term measures only hydrophobic interactions, since the Gurney overlap term is a repository for all forces that are not hard-core repulsions.^[269] The model has, however, been quite successful in accounting for experimental osmotic coefficients.^[270, 271]

Various attempts^[269, 272] to improve the cosphere overlap model have been reported. Clark et al.^[269] suggested to exclude attractive solute–solute dispersion interactions from the Gurney overlap terms. The solvent-induced contribution to the solute–solute interactions was computed by compar-

ing the interactions in solution with those in the gas phase. Interestingly, the model also suggested that the attraction between apolar compounds in the gas phase and in the absence of solvent molecules appears to be stronger than in aqueous solution (vide supra).^[269]

Calculation of second osmotic coefficients for interactions between hydrocarbons is hampered by the low solubility of these compounds in water and the concomitant lack of experimental data. Wood et al.^[16, 273, 274] applied several additivity approaches to calculate second osmotic coefficients for interactions between medium-sized hydrocarbons such as cyclohexane. More recently Kang et al.^[275–277] developed a sophisticated hydration shell concept for a quantitative description of thermodynamic parameters for the solvation of a large series of organic compounds in water. An improvement of the cosphere overlap approach was reported by Marčelja et al.^[278] based on the Pople model for water. Their approach predicted a smooth exponential decay of the attraction between two apolar solutes with increasing separation.

In the late seventies and early eighties, however, it became increasingly clear that the potential of mean force between apolar solute particles is characterized by an oscillatory dependence on the separation between the particles. Using this knowledge, Wood et al.^[247] developed an interaction site model (ISM) similar to that used by Clark et al.^[269] However, Wood et al. employed a number of simple square-well potential functions to fit experimental osmotic coefficients. These square-well potential functions were chosen to represent both the contact arrangement and the solvent-separated arrangement, the latter by introduction of a barrier to avoid association of the solutes. In addition, nonpairwise additive contributions were introduced. The results reinforced the notion that water decreases the attractive forces that are present between (solute) particles in the gas phase under conditions of equal concentrations. Unfortunately, these models merely rephrase the question regarding the molecular origin of hydrophobic interactions.

3.3.4 Interaction Site Models; Integral Theories

One of the major problems in theoretical studies of hydrophobic effects is the description of liquid water. In water, strong attractive interactions between molecules compete with short-range repulsive forces, which is quite unusual for organic solvents. In spite of these difficulties, computer simulations of hydrophobic effects have been quite successful. To circumvent the inherent problem of having to develop a theoretical water–water potential, semiempirical approaches have been advanced that employ experimental data for liquid water. The ST2 model developed by Stillinger and Rahman^[279–281] is a typical example of this approach (Section 3.5). Almost simultaneous to the appearance of the first computer simulation studies of hydrophobic effects in the literature, Pratt and Chandler^[234, 282] developed an alternative microscopic theory, now known as the Pratt and Chandler theory (PC). This approach is more ambitious than the scaled particle theory and is essentially a statistical mechanical treatment using an interaction site model represented by a sum of pairwise, additive, spherically symmetric potentials

acting between specified sites located on each molecule. A similar approach had already been considered useful in accounting for properties of nonassociated organic solvents. In these fluids the molecules interact predominantly through hard-core repulsions. The integral equation formulation for these solvents is generally known as the repulsive interaction site model (RISM)^[283]. For a description of liquid water Pratt and Chandler^[282] used the experimentally determined, orientationally averaged, pair correlation function for the oxygen–oxygen distribution. Subsequently, an Ornstein–Zernike-type integral equation (Section 3.3.1) was used to obtain the solute–solvent and solute–solute distribution functions after insertion of a solute molecule in the pure liquid.

Figure 9 a shows the radial distribution of water molecules around a methane-like particle and reveals that the water molecules occupy a distinct position in the hydration shell of methane.^[282, 284] As shown in Figure 9 b, the PC theory pre-

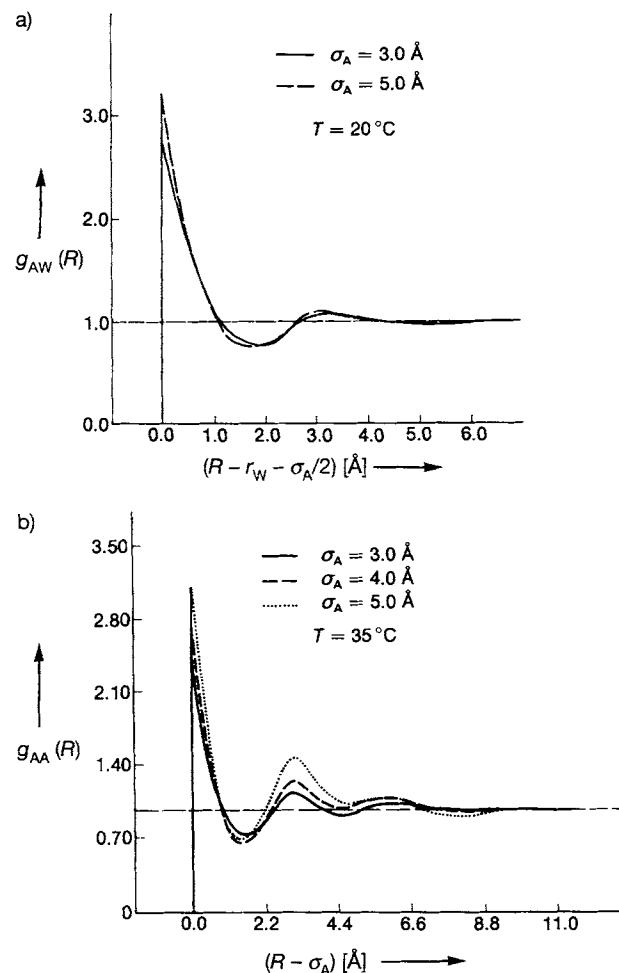


Fig. 9. Radial distribution functions $g_{AW}(R)$ (a, infinite dilution) and $g_{AA}(R)$ (b) for hard-sphere solute particles A dissolved in water W at 1 atm and 20°C (a) and 35°C (b). σ_A is the diameter of the solute and r_W , the radius of water. g_{AA} was calculated based on the theory of Pratt and Chandler (taken from ref. [282]).

dicts an oscillatory dependence of the solute–solute pair correlation function $g_{AA}(R)$ on the separation of the solute particles.^[285] Simultaneously, computer simulation studies yielded similar results (see Section 3.5). The occurrence of

preferred arrangements of solute particles was anticipated on the basis of packing phenomena (see Section 3.3.1). Surprisingly, however, the populations of the contact and the solvent-separated arrangement appeared to be similar. The solute–solute pair correlation function was shown to be significantly altered by introducing attractive forces between the solute particles.

Unfortunately, as a result of the application of an orientationally averaged pair correlation function for water, information about the orientation of water molecules surrounding apolar solutes is lost. Tani^[286] recognized this inherent weakness of the PC theory and generalized the PC model by introducing orientational aspects of water–water interactions. Application of the improved PC theory reinforced the traditional idea of clathrate-like solvation of apolar solutes in water. Tani found^[286] that quantitative features of the calculated radial distribution function critically depend on the applied pair correlation function for liquid water and applied both experimental and theoretical potential models for liquid water. Generally, theoretical models for liquid water tend to exaggerate pair correlations between apolar solutes and water molecules.

Pratt and Chandler^[284] and Tani^[286] studied the hydration of solutes with more than one interaction site (non-spherical solutes) and found that the hydration characteristics of each site are qualitatively the same as that for an isolated interaction site. The solute–solute distribution function obtained by Tani^[287] is qualitatively similar to that obtained by Pratt and Chandler.^[234, 282] Remarkably, water–solute attractive forces hardly affect the quantitative features of the solute–solute distribution function. In contrast to its predecessor, the improved interaction site model^[287] completely excludes the occurrence of contact interactions between apolar solutes. The size dependence of hydrophobic interactions is suggested to be small, but with increasing solute size the proportion of contact arrangements appears to be even less, which is in contrast with results obtained by other theoretical approaches (see, for example, Thompson et al.^[247]).

Another extension of the PC theory was provided by Rossky et al.,^[288, 289] who introduced a nonuniform charge distribution by employing an interaction site model (ISM) with charged interaction sites for dipolar and quadrupolar fluids. The extended repulsive interaction site model (XRISM) equation was used to determine the potential of mean force acting between rigid, polyatomic solutes in polar molecular solvents such as water.^[290] To describe the interactions between water molecules, Rossky et al. used a theoretical model for water developed by Jorgensen et al.^[291] (Section 3.5). Interestingly, Rossky et al.^[290] confirmed that in both a solvent consisting of diatomic molecules and in water the potential of mean force between apolar solute particles is characterized by an oscillatory dependence on their separation. The XRISM was also used to determine internal motions of polyatomic molecules in aqueous solution.^[292] More recently, Tanaka et al.^[293] made a comparison between Monte Carlo simulations of aqueous solutions of apolar compounds and the results obtained by the XRISM. They confirmed the intuitively wrong result suggested by interaction site models that the solvent-separated arrangement is favored with increasing solute size.

3.4. Scaled Particle Theory (SPT); The Formation of Cavities in Liquid Water

An interesting attempt to explain thermodynamic data of solvation for apolar compounds in liquid water is the scaled particle theory (SPT). Thanks to the pioneering work of Pierotti, application of SPT to the study of hydrophobic effects should probably be considered one of the first theoretical approaches to hydrophobic effects.^[294–296] In this model the dissolution of a particle is separated into two steps. The first step is the creation of a cavity in the solvent (see also Section 3.5.2) which has the appropriate size to accommodate the solute particle. The second step is the onset of interactions between the solute and the solvent. The Gibbs energies of these two processes are generally referred to as G_c and G_i respectively. The structural implications of solute–solvent interactions for the solvent in close proximity to the solute cannot be treated by the SPT. The theory uses statistical arguments based on pair distribution functions of solvent molecules in the pure liquid and yields expressions for the work that is involved in introducing a spherical particle into a solvent of spherical particles. An essential feature of the SPT is that the creation of a volume element in a liquid to accommodate a solute particle requires exclusion of solvent particles, a process that involves a certain amount of work. This can be expressed in a Boltzmann equation [Eq. (28)].^[296]

$$p_o(r,\varrho) = \exp \{ - W(r,\varrho)/kT \} \quad (28)$$

Here ϱ is the number of solute particles per unit volume (N/V), $W(r,\varrho)$ the work required to create a cavity of radius r , and $p_o(r,\varrho)$ the probability that such a cavity arises from statistical fluctuations in the solvent network. The second osmotic coefficient B_2^* is directly related to $p_o(r,\varrho)$. The aim of the SPT is now to determine $p_o(r,\varrho)$ as accurately as possible on the basis of statistical mechanical and geometrical arguments. Essential is that the solvent is considered to be a spherical particle with a radius σ . The general approach followed is to start off with a cavity radius of zero after which the cavity size is gradually expanded.

An important parameter for the calculation of the work involved in formation of a spherical cavity of radius R is the probability of finding a center of a spherical solvent molecule in this spherical volume element. This probability is defined in a distribution function describing the conditional distribution of solvent particles in the solution. The theory provides an expression for this conditional probability function $G(r,\varrho)$ in which experimental properties of the solvent such as the density and isothermal compressibility appear. Thus, structural information for the solvent, absent in basic forms of the SPT, is introduced and provides the theory with a semiempirical character. Nonetheless, the application of SPT to liquid water has been vigorously criticized.

In a classic paper, Stillinger^[297] developed methods to incorporate the strongly directional character of water–water interactions and made the SPT suitable for applications to aqueous solutions by incorporating some rudimentary aspects of the hydrogen bond structure of liquid water. This approach led to the development of the famous ST2 model

for liquid water (see Section 3.5). A pragmatic application of SPT yields very good results in calculating thermodynamic parameters for the solvation of apolar gases in water.^[294, 296] In 1988 Langlet et al.^[298] reported a modified SPT with excellent results for nonaqueous solvents.

During the past decades, application of the SPT to the study of the solvation behavior of apolar gases in liquid water has led to the remarkable suggestion that the low solubility of apolar gases in water is mainly due to the small molecular volume of the water molecule. In other words, because of this feature, the frequency of observing a cavity of sufficient volume to accommodate an single-atom solute or larger particle in water is much lower than in organic solvents. Lucas^[299, 300] was one of the first to make this suggestion after a careful study of the role of the molecular size and the isobaric expansion coefficient α for the thermodynamics of solvation. Later Lee^[228] developed a model for the solvation of apolar gases in water on the basis of cavity formation and the compensative behavior of enthalpy and entropy terms (Section 3.2) and stressed the fact that the contribution of G_c is similar for all solvents. The low solubility of apolar compounds in water is reflected almost exclusively by the G_c term, which deviates strongly from that for organic solvents. According to the SPT as well as to the theory of Pratt and Chandler (Section 3.3.4), G_c is directly correlated with the number of solvent molecules that hit the surface of the cavity during its growth. Obviously, this number is related to the size and the density of the solvent. The small molecular size of water enhances the unfavorable character of G_c , whereas the low packing density of liquid water, which is a consequence of the open hydrogen-bonded structure of liquid water, counteracts this effect. The packing density of hydrazine, whose molecular volume is somewhat larger than that of water, is considerably higher. Indeed, the solubility of apolar compounds in hydrazine is just as bad as in water and in some cases even worse. As noted by Lee,^[228] the low density caused by the hydrogen bond network in liquid water promotes the dissolution of apolar gases.

Recently, Lee^[230] used the experimental thermodynamic data of solvation for hydrocarbons in water and their pure liquid state in combination with solute–solvent interactions obtained from computer simulation studies to compute the enthalpy change upon solvent reorganization. In addition, the cavity term H_c was determined for the solvation of hydrocarbons in their liquid phase. These data were compared with parameters obtained from the SPT. It was shown that the thermodynamic parameters for solvent reorganization and cavity formation, in particular the enthalpy and the entropy, are strongly dependent on temperature. The SPT appeared to reproduce the temperature dependence of the Gibbs energy of cavity formation very accurately. The temperature dependence of the enthalpy and entropy of cavity formation was not reproduced as satisfactorily.

In Figure 10 the differences between the Gibbs energy for cavity formation G_c , the energies of the solute–water interactions E_s , and the enthalpic solvent reorganization enthalpies H_r are plotted versus temperature for the solvation of neopentane in water and in its neat phase. In addition the total Gibbs energy change ΔG^* (‘ indicates that the standard state advocated by Ben-Naim is used (see Section 1.2)), enthalpy change ΔH^* , and entropy change $T\Delta S^*$ for the transfer

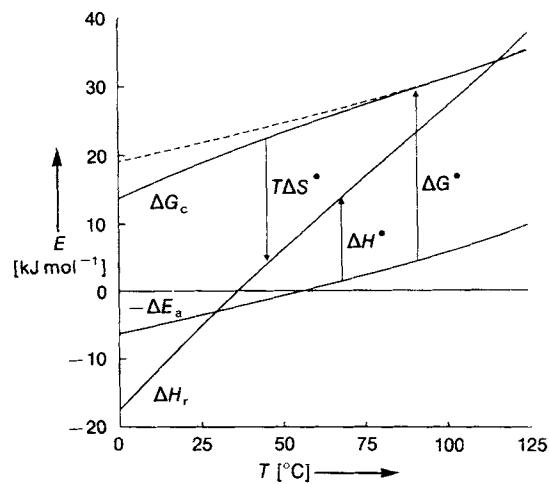


Fig. 10. Temperature dependence of changes in the Gibbs energy of cavity formation ΔG_c , the solute–solvent interaction energy ΔE_s , and the solvent reorganization enthalpy ΔH_r for the solvation of neopentane in water as opposed to in its neat phase. Thermodynamic parameters based on the Ben-Naim standard state are indicated as well (taken from ref. [230]).

of liquid neopentane to water are indicated. Figure 10 is very revealing and shows that ΔG^* is rather insensitive towards temperature, because the temperature dependence of changes in solute–solvent interactions and the changes in the cavity term are similar. By contrast, the change in the enthalpy of solvent reorganization is dramatically dependent on the temperature and causes a separation of ΔG^* into entropy and enthalpy terms that are equally temperature dependent. These terms compensate each other almost completely (see also Section 3.2). ΔH_r for the transfer of gaseous neopentane to water increases rapidly with temperature, whereas ΔH_r for the transfer of gaseous neopentane to its neat phase decreases with temperature (for solvation of apolar compounds in water, the assumption is made that $\Delta H_r = \Delta H_c$). Even near room temperature, the enthalpy of solvent reorganization is positive for both solvation processes. This observation seriously conflicts with the idea of increased solvent structure due to enhanced hydrogen bonding around the inert solute particles. In addition, solute–solvent interaction energies are remarkably similar and attractive. Thus, the low solubility of apolar compounds in water is fully governed by the Gibbs energy of cavity formation G_c , which is accurately reproduced by the SPT. According to Lee,^[230] hydrophobicity must therefore be attributed to an excluded volume effect and the compensation of enthalpy and entropy to changes in the volume of the solvent.

The SPT has been criticized on various grounds, in particular for its results in applications to hydrogen-bonded solvents.^[301] In fact, in addition to the SPT and the hybrid approach followed by Lee (vide supra), two other approaches are available for calculating the thermodynamic parameters of cavity formation in liquids. A semiempirical method going back to publications of Sinanoglu^[302, 303] considers the formation of a cavity in a liquid in terms of the work against counteracting surface forces. The theoretical approach is based on the concept of microsurface tension in the solvent (see Section 2.1). A few years later Reisse et al.^[304, 305] drew an analogy between the process of creating a molecule-sized cavity and the evaporation of the liquid.

Unfortunately, the Sinanoglu–Reisse approach is less suited for hydrogen-bonded solvents. More recently, Moura-Ramos et al.^[306] showed that changes in the molecular order during the evaporation process hamper the Sinanoglu–Reisse analysis. Thus they proposed to describe the process of cavity formation in analogy to the evaporation of a disordered liquid by using the corresponding state principle.^[306, 307] Entropies and enthalpies for cavity formation in various solvents, unfortunately not including water, were calculated. In contrast to the data reported by Lee^[228, 230] and Sinanoglu,^[303] negative entropies were found for the creation of cavities in organic solvents near room temperature.

During the past decade, computer simulations have become increasingly popular for the calculation of thermodynamic parameters for the formation of solute-sized cavities in water (see Section 3.5). Since it has been generally questioned whether the solubility of apolar compounds in water can be interpreted in terms of a packing phenomenon for spherical solvent molecules, and since even incorporation of some structural aspects of real water did not enhance the confidence in the results of SPT, the main goal of these computer simulation studies was to test the results of the SPT. The traditional idea that water is a liquid with a remarkably open structure argued against the conclusions from SPT. Computational approaches were reported by Postma et al.,^[308] Jorgensen et al.,^[309] Tanaka,^[293] and recently by Guillot et al.^[310] Particularly interesting was the approach reported by Guillot et al.^[310] in which a comparison was made between the work of creating a cavity in water, described by a realistic water model, and in a liquid composed of hard spheres. The calculations show unequivocally that the Gibbs energy for the creation of cavities in liquid water is not primarily determined by the specific structure of water but by factors such as packing density, size of the solvent molecule, and the equation of state.

Recently Pohorille and Pratt^[311] used molecular dynamics (MD) and Monte Carlo (MC) simulations to calculate the thermodynamics of formation of a cavity in various organic liquids and water in order to study the impact of the structure of hydrogen-bonded liquids. They found that water has a larger fractional free volume which is, however, more disperse than that of organic solvents. They also found that the work required for cavity formation predicted by the SPT is about 20% below the value obtained with the TIP4P model (see Section 3.5) for liquid water. As shown by Guillot et al.,^[310] a solvent composed of hard spheres of similar size to water molecules has a significantly better capacity to accommodate apolar solute particles in its free volume. Unfortunately, the sizes of the cavities considered were still rather small. In a more detailed study of the so-called transient cavities in molecular liquids, the statistics for cavity formation in water, *n*-hexane, and *n*-dodecane were compared in relation to the density of the solvent at the surface of the spherical solutes, referred to as the contact density $\rho_s G(\lambda)$.^[312] The contact density at the surface of a hard sphere is significantly greater in water than in the organic solvents; in other words, water squeezes the solute out of solution more strongly.^[312] This force reaches a maximum for cavities roughly 2.4 Å in diameter. According to Lee,^[228, 230] this conclusion is essentially a different way of

saying that the low solubility of apolar compounds in water is mainly an excluded-volume effect.

3.5. Computer Simulation Studies of Aqueous Solutions; An Introduction

During the past fifteen years, computer simulation studies have provided a rich source of detailed information on the molecular organization of aqueous solutions. Structural aspects of hydrophobic hydration and hydrophobic interactions became apparent that were not easily accessible by alternative methods. Results of computer simulation studies have confirmed the traditional ideas on hydrophobic effects to some extent, but have also raised a number of important questions. A typical simulation considers a limited number of water molecules (usually 100–500) at equilibrium in a box confined by periodic boundaries. One or more of these water molecules are replaced by a solute particle. The subsequent structural and thermodynamic changes are monitored by sophisticated sampling methods. The new equilibrium properties of the solution are calculated either by averaging over a large number of selected configurations (MC), or by solving the Newtonian equations of motion and taking a time average (MD). The choice of intermolecular potential functions describing the interactions between the components of the system is therefore of paramount importance.

For liquids like water with strong, attractive intermolecular interactions, the development of potential functions describing the interactions between the molecules in the liquid has been notoriously difficult. Computer simulation studies of hydrophobic effects have employed a number of well-known potential models for liquid water. Most of these potential models are (semi)empirical.^[313] Charge distributions and Lennard–Jones parameters have been optimized in order to reproduce experimental properties of water with high accuracy. Stillinger and Rahman^[279–281] developed a pair potential for rigid water molecules on the basis of scaled particle theory. This ST2 model became very popular and was used frequently with or without adjustments for the system investigated. Other popular mean field models are the simple point charge model (SPC) developed by Berendsen et al.^[314] and the transferrable intermolecular potential functions (TIPS, TIPS2, and TIP4P) developed by Jorgensen et al.^[291] A popular analytical potential model based on ab initio calculations has been advanced by Matsuoka, Clementi, and Yoshimine (MCY model).^[315]

In most computer simulation studies, the total potential energy of an aqueous solution is assumed to be the sum of individual *two-body* interactions. Polarizabilities and the corresponding induced dipoles, nuclear polarization, and intramolecular flexibility are not normally included in simulations of liquid water. This is regrettable, since polarizability effects involving cooperativity of a large number of water molecules might be significant in the molecular origin of hydrophobic effects. Recently, some important progress has been made by incorporating electric polarizabilities, intramolecular degrees of freedom, and many-body effects in empirical^[316, 317] as well as in nonempirical^[318–321] potential models for liquid water. Unfortunately, this knowledge has not yet been employed in computer simulation studies of

hydrophobic effects. Since water–water interactions are so exceptionally strong, solute–water interactions are not a major factor for the structure of water molecules in the near vicinity of apolar solutes and are usually described by simplified interaction laws. Occasionally, quantum mechanical potentials are used to model these interactions, though ab initio calculations are not necessarily an improvement in this respect.^[322]

The decisive thermodynamic quantity in chemical processes is the free energy change (either Gibbs energy or Helmholtz energy). Unfortunately, calculations of these quantities for the solvation of apolar compounds in water are still extremely difficult, and the results are subject to large errors.^[79, 323–328] Alternatively, the free energy difference between two systems, for example, two aqueous solutions containing different solute particles, can be calculated by stepwise perturbations of one system by which it is slowly converted into the other. For example, aqueous solutions of propane and ethane can be compared by stepwise elimination of a methylene group. Although this might seem like a bizarre kind of alchemy to an organic chemist, it is a very convenient procedure and a valid thermodynamic path between these two states. Free energy perturbation methods are a relatively new, important improvement in the calculation of relative free energies of processes in solution on the basis of computer simulations.^[79, 329]

3.5.1. Description of Hydrophobic Hydration from Computer Simulations

In hydrophobic hydration the position, orientation, mobility, and potential energy of water molecules in close proximity to single apolar solute particles all play a role. In qualitative studies of hydrophobic hydration, these parameters have been associated with the structure of water in the solvation shell. In order to determine to what extent water molecules are affected by the presence of an apolar solute particle, it is convenient to make a clear distinction between water molecules in the solvation shell and in bulk water. One useful method is the “partitioning of the configuration space”. According to this method, water molecules whose oxygen nucleus is found within a certain radius R from the center of the solute particle are considered part of the hydration shell.^[330, 331] The exact value of radius R is rather arbitrary and obviously depends on the diameter of the solute particle. Usually, only the first hydration shell is taken into account by including merely the first peak of the solute–solvent radial distribution function. In an alternative approach, distribution functions for the aqueous solution are simply compared to those for pure liquid water.^[332] One drawback of this method is the fact that the number of water molecules that are affected by the apolar solute particles is relatively small in comparison to the total number of water molecules.

In 1974 Dashevsky and Sarkisov^[333] were the first to report a MC computer simulation of a solution of methane in 64 water molecules at constant volume and temperature. The computational methods applied in this simulation study were severely criticized and the results considered unreliable.^[334, 335] Three years later Owicki and Scheraga^[330] performed a more sophisticated MC simulation of an aqueous

solution of methane at fixed temperature and pressure. The first extensive MD simulation of an aqueous solution of two Lennard–Jones particles was published by Geiger et al.^[335] almost two years later. Almost simultaneously Rossky and Karplus^[331] carried out a detailed MD study of a dilute aqueous solution of an alanine dipeptide with both polar and nonpolar regions. These investigations might be considered the precursors of many computer simulation studies of the hydration of organic compounds in water.

With the aim of studying hydrophobic hydration and hydrophobic interactions simultaneously, most simulations deal with the behavior of water molecules in the presence of more than one solute particle. The solvation of prototypes of apolar solutes such as methane^[163, 310, 324, 326, 330, 332, 336, 337] and noble gases^[325, 334, 335, 338–343] has been studied extensively. Since *n*-butane is a popular model for studies of solvation effects on conformational equilibria, studies of the solvation of *n*-butane have been conducted frequently.^[344–347] In addition to computer simulations of the solvation of other alkanes,^[309, 348, 349] fundamental studies of the hydration of formaldehyde,^[350] methanol,^[349, 351–353] ethanol,^[309, 349, 354] 2-methyl-2-propanol,^[355] and a series of α -amino acids and nucleotides^[356] have also been reported. Occasionally solute–water interactions have been described by potential functions based on ab initio calculations.^[330, 332, 351, 352] In other cases, solute–water interactions were represented by relatively simple Lennard–Jones potentials^[293] or even by merely short-range repulsive forces.^[357] Recently Cramer and Truhlar^[358] reported self-consistent field calculations to compute absolute free energies for the solvation of apolar organic and small inorganic molecules in water.

Although different potential functions, simulation times, and computational methods have been used, the results are qualitatively remarkably similar. Pair distribution functions representing the distribution of hydrogen and oxygen nuclei with respect to argon atoms in aqueous solution as reported by Guillot et al.^[310] are shown in Figure 11. The fact that

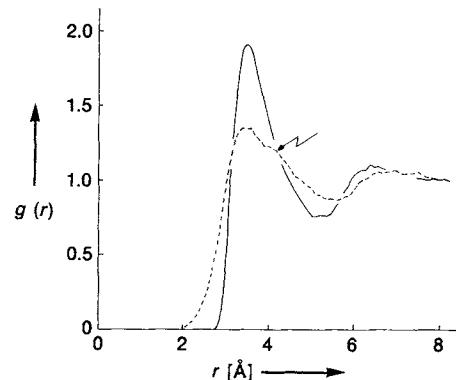


Fig. 11. Argon–oxygen and argon–hydrogen pair distribution functions $g_{AO}(r)$ (solid line) and $g_{AH}(r)$ (dashed line), respectively, for a dilute aqueous solution of argon (taken from ref. [310]). For further explanation see text.

$g_{AH}(r)$ and $g_{AO}(r)$ almost coincide indicates that water molecules adjacent to the solute particle are orientated such that their O–H bonds are tangential to the surface of the solute. This conclusion has been almost unanimously confirmed by

other computer simulation studies (see, for example refs. [310, 330, 332, 335, 338, 357]). Water molecules clearly avoid pointing their O–H bonds directly toward the nonpolar solute. The second maximum in the distribution functions shows that most water molecules in the second hydration shell point their oxygen atom toward the solute and their hydrogen atoms toward the bulk and, on average, orient themselves tangentially on the first hydration layer. An interesting feature revealed by some computer simulation studies^[310, 335, 339] is the weak shoulder in the peak of the hydrogen–solute pair distribution function (see arrow in Fig. 11). The increased probability of finding hydrogen atoms of water molecules participating in the second hydration shell at a shorter distance from the solute particle gives reason to infer that some water molecules point their O–H bonds in the direction of the first hydration layer and appear to couple the two hydration shells.

The number of water molecules involved in the solvation of an apolar solute particle depends strongly on the definition of the size of the hydration shell. On the basis of “partitioning of configuration space”, several computer simulation studies report hydration numbers.^[330, 359] They tend to be large and vary from 20 for methane to 34 for *n*-pentane. Jorgensen et al.^[359] reported an excellent correlation between the number of water molecules in the first hydration layer of a series of linear and branched alkanes and the entropy of solvation. Curiously, a Lennard–Jones solute with a diameter corresponding to the hard-sphere diameter of a water molecule is surrounded by significantly more water molecules than a water molecule itself.^[357] Some evidence indicates that the coordination number of water molecules in the solvation shell is preferentially four, but differences to the coordination numbers in pure water are small.^[332, 337, 348]

Although differences between binding energies of water molecules in the bulk and in the hydration shell are small, discernible shifts have been observed towards stronger binding among water molecules in the hydration shell. Most simulation studies reveal that the total binding energy distribution of water molecules generally becomes narrower after insertion of the apolar solutes and shifts towards lower energy (see, for example, refs. [330, 332, 338, 341]). In contrast, Jorgensen^[346] reported a slightly lower binding energy for water molecules in the solvation shell of *n*-butane.

Okazaki et al.^[348] found that the number of hydrogen bonds increases significantly when an alkane is dissolved in water, but they did not observe a relation between the size of the apolar solute particle and the observed shift in the hydrogen bonding distribution function. Geiger et al.^[335] and Pangali et al.^[338] observed only a modest increase in the number of hydrogen bonds in aqueous solutions of apolar compounds. Introduction of an apolar solute particle into liquid water is apparently accompanied by a small increase in the number of stronger, less distorted hydrogen bonds.^[335, 341, 348]

Significant effects of an apolar solute particle on the hydrogen bonding properties of water molecules should be revealed in the librational motion of the water molecules, which can be obtained from an analysis of the dynamic pathway of these water molecules (see, for example, refs. [331, 335, 336, 342, 351, 355]). Dynamic parameters are difficult

to interpret when a water molecule spends only a fraction of the total run time in the hydration shell of an apolar solute particle. The translational motion can be monitored within a very short period of time, during which relatively few water molecules exchange between the hydration shell and the bulk. Unfortunately, rotational motion of molecules in a strongly hydrogen-bonded fluid such as water is considerably slower than translational motion. The average angular reorientation of a water molecule while it is in the solvation shell is quite small, and a comparison of rotational motions of water molecules in bulk water and in a hydrophobic hydration shell is hazardous. Rossky and Karplus^[331] were the first to conclude that water molecules near apolar moieties have longer translational and rotational relaxation times than bulk water molecules. A study of the shell occupancy of the hydration shell of a hard-sphere solute as a function of time showed that the exit rate of water molecules from the solvation shell is some 20 % less than that from the solvation shell of bulk water.

The restricted motion of water molecules in the hydration shell was later corroborated by Zichi and Rossky,^[342] who showed that the translational mobility of water molecules adjacent to Lennard–Jones particles is reduced by about 30 %. The reduced translational and rotational motion was shown to be reflected in an increase of the average lifetime of hydrogen bonds. It has been suggested that structural changes in the solvation shell are more concerted than in the bulk. In contrast, Postma et al.^[308] did not observe a significant reduction of the translational motion of water molecules near a hydrophobic solute. On the basis of computer simulations it has been found that molecules in the hydration shell oscillate at somewhat higher frequencies than molecules in the bulk.^[360] This implies that the restoring forces following a slight perturbation of a single water molecule in the hydration shell are larger than those for a perturbed molecule in bulk water. An analysis in terms of hindered rotations and hindered translations presented by Zichi and Rossky^[342] and Rossky and Karplus^[331] indeed suggests a strong correlation of the movement of water molecules in close proximity to an apolar surface. However, it must be recognized that a considerable reduction in the translational motion in the hydration shell can be explained by the fact that the solute particle acts as a fixed wall.

Recently Wallqvist^[163] presented evidence that the number of hydrogen bonds at the interface between an apolar phase and liquid water is significantly reduced and that the average attractive energy between the water molecules at a water–hydrocarbon interface is also reduced considerably. This observation should be considered in light of the hydration of large apolar molecules or surfaces without sufficient curvature for all hydrogen bonds of the adjacent water molecules to be maintained. Furthermore, Wallqvist showed that interactions between water and methane molecules at the interface between bulk water and a hydrocarbon phase are enhanced by the orientation of water molecules at the interface.

Computer simulations of aqueous solutions of organic compounds containing both apolar and polar groups show without exception that water molecules in close proximity to the apolar moieties exhibit characteristics similar to those of the surrounding perfectly apolar solute particles.^[309, 349–355]

As indicated by Jorgensen et al.,^[309, 353] the introduction of a polar hydroxyl group strongly facilitates the solvation process by increasing the solute–water interactions. This is reflected by the bimodal energy distribution function for water–alcohol interactions. Hydrogen bonding leads to a low-energy interaction band, while London dispersion interactions involving many water molecules contribute to a large interaction band with close zero energy. As might be expected, structural analysis of the solvation shell of amphiphilic molecules indicates that solvation of apolar moieties and polar functional groups is mutually obstructive.

The results obtained from computer simulations of the hydration of apolar particles in water present a coherent picture of clathrate-like hydration, and the presence of pentagonal structures has been observed.^[310, 332, 361, 362] The slightly reduced mobility of the water molecules and the moderate shift to the formation of stronger hydrogen bonds reinforce the traditional idea that water molecules in the solvation shell of an apolar solute particle behave like bulk water at lower temperature. The existence of “frozen patches” can be safely excluded, since the clathrate-like solvation shell appears to be extremely labile and permits solvent exchange on a picosecond timescale.

Most computer simulation studies of hydrophobic hydration were performed to trace differences between water molecules in hydrophobic hydration shells and in the bulk near room temperature. Interestingly, Jorgensen et al.^[346, 359] emphasized that the remarkable property of water molecules in the hydrophobic hydration shell is that they are almost indistinguishable from water molecules not participating in hydrophobic hydration shells. Furthermore, Jorgensen et al.^[309, 359] showed that the large and favorable enthalpy of solvation of apolar gases in water near room temperature can be fully attributed to solute–water interactions. The qualitative features of the solvation shell surrounding an apolar solute particle are, however, hardly affected by removing the attractive part of the solute potential.^[308, 332] Recently, Rashin et al.^[363, 364] drew similar conclusions from continuum-based calculations of hydration enthalpies and entropies of a series of inert gases. Thus, the crucial question remains to what extent the thermodynamic parameters of hydration for apolar compounds in water are affected by the moderate changes in the hydrogen-bonding properties of water molecules in the hydration shell.

3.5.2. Simulations of Pairwise and Bulk Hydrophobic Interactions

Computer simulation studies of hydrophobic interactions aim at calculating the pair distribution function or, alternatively, the potential of mean force between two or more apolar solute particles in an aqueous environment. In 1974 Dashevsky and Sarkisov^[333] used a MC procedure to compute the potential of mean force acting between two methane molecules in 64 water molecules and found a monotonic decay of $w_{\text{CH}_4-\text{CH}_4}(r)$ to zero as a function of the distance between the particles. The computational methods were ill-defined, and the results generally considered unreliable.

In 1979 Geiger et al.^[335] reported the first MD simulation study of a system comprising two Lennard–Jones solute

particles in 214 ST2 water molecules. Surprisingly, the Lennard–Jones solutes, initially placed in contact and dissolved in water, were almost immediately forced slightly apart in order to be hydrated in separate solvent “cages” that shared one layer of water molecules tangential to the surfaces of both solute particles.^[335]

Swaminathan and Beveridge^[365] carried out a MC simulation on a solution of two methane molecules in a similar number of water molecules but applied ab initio methods to describe the interactions between the components in solution. Their computer simulation confirmed the existence of a contact- and a solvent-separated hydrophobic bond.^[365] Since then a series of computational studies have appeared with the aim of studying hydrophobic interactions between a variety of solute particles, for example, relatively simple Lennard–Jones solutes,^[293, 334, 335, 338, 341, 342, 357, 362] noble gases,^[246] and methane^[163, 328, 336, 361, 365, 366] as well as more complex systems such as methanol,^[351] 2-methyl-2-propanol,^[355] and quinuclidine.^[367] The interactions of these solute particles have been simulated by using relatively simple Lennard–Jones potentials. Incidentally, more sophisticated potentials have been applied based on ab initio calculations.^[351, 355, 365] Water–water interactions have usually been modeled by empirical models such as the ST2 model,^[246, 334, 335, 338, 341, 342, 362] the SPC model,^[163, 366, 367] and the TIP4P model;^[328, 336] the nonempirical MCY model^[351, 355, 357, 361, 365] has been used as well. The run time of the simulations varied from only 7 ps (Geiger et al.^[335]) to 450 ps (Andersen and Watanabe^[246]).

Regardless of the computational methods and the applied potential functions, computer simulation studies generally yield potentials of mean force between apolar solute particles that oscillate as a function of the distance between the particles. Thus these results reinforce the ideas that emerged from integral theories (Section 3.3.4). A representative example, shown in Figure 12, is taken from the work of Wata-

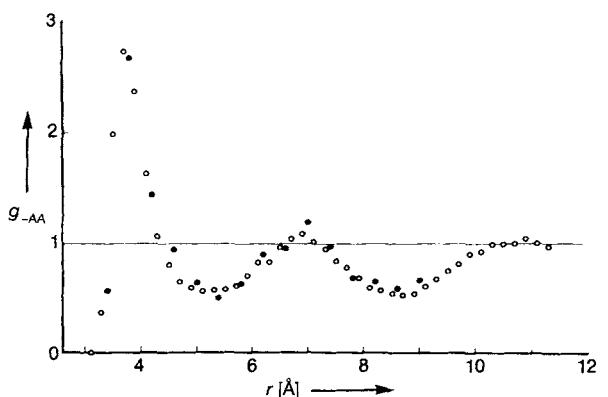


Fig. 12. Solute–solute pair distribution function $g_{\text{AA}}(r)$ for a solution of 6 solute particles (krypton-like Lennard–Jones particles; $\sigma = 3.44 \text{ \AA}$) in 394 water molecules (\circ) and for a solution of 5 solute particles in 195 water molecules (\bullet) (taken from ref. [246]).

nabe and Andersen,^[246] who carried out an extensive MD simulation of hydrophobic interactions between krypton atoms in an aqueous solution of finite concentration. The position of the first maximum corresponds to the expected contact pair, and the second maximum to an arrangement in

which the solute particles are separated by one water molecule. Even a third maximum can be distinguished, suggesting an arrangement in which the particles are separated by two layers of water molecules. The exact distance between the solute particles in the solvent-separated arrangements depends on the choice of potential models. Computer simulations by Ravishanker et al.^[361] indicate that arrangement C (Scheme 8) is preferred, whereas other simulations^[334] as well as the theory of Pratt and Chandler (see Section 3.3.4) suggest a preference for arrangement E.

The equilibrium solvation structure for the solvent-separated arrangement has been studied in more detail by Zichi and Rossky;^[362] they placed two Lennard-Jones particles at a solvent-separated distance (arrangement E) and studied the orientation and dynamic properties of the water molecules in close proximity to the solute particles. The MD simulation revealed a variety of clathrate-like structures surrounding the two apolar particles.

Conflicting results have been reported with respect to the height of the barrier between the solvent-separated and contact pair.^[342] Generally, the solvent-separated arrangement is found at higher free energy; however, this arrangement has the greater population because of the greater volume of its potential well. The relative and absolute values of the minima depend strongly on the solvent–solute interaction potential. When the interactions between solute and water decrease, the preference for the formation of hydrophobic contact pairs increases. Pangali et al.^[338, 334] presented evidence that the contact and solvent-separated arrangements are equally populated, whereas Ravishanker et al.^[361] and Andersen and Watanabe^[246] noted a strong preference for the solvent-separated arrangement.

In order to determine the importance of attractive solute–solute interactions, Rapaport and Scheraga^[357] simulated an aqueous solution of four essentially hard-sphere solutes. Solute–solute interactions were reduced to simple short-range repulsive forces by removing the long attractive tail of the full Lennard-Jones potential. The solute particles were initially not brought in contact distance. Although the solutes were shown to be highly mobile, no evidence was found for their association during a 70 ps simulation. The results of the MD simulation performed by Andersen and Watanabe^[246] were similar. These authors even observed that krypton atoms tended to avoid each other in an aqueous solution of finite concentration. A compact cluster of five krypton atoms appeared to have a lifetime of about 180 ps, but this was not the dominant equilibrium arrangement. Hence, a prerequisite for reliable results are extended-time simulations.^[246]

Jorgensen et al.^[328] obtained the potential of mean force between two methane molecules in water by using statistical perturbation theory. The free energy for the binding of two methane molecules in water was estimated as $-1.8 (\pm 1.4)$ kJ mol⁻¹, of which -1.2 kJ mol⁻¹ arises from simple Lennard-Jones attractions between the solute particles. Recently, Smith et al.^[366] applied traditional thermodynamic integration methods to compute the free energy and the entropy of the association of two methane molecules in water on the basis of an extensive MD simulation. They showed that the solvent-induced forces between methane molecules in water are small but attractive at short separa-

tions as a result of a favorable entropy change.^[366] In contrast to Ravishanker et al.,^[361] who performed a preliminary MC study of the effect of temperature on the pair distribution of methane in an aqueous solution, the results of Smith et al.^[366] confirmed the experimental observation that attractive interactions between apolar solutes at short distance increase and the potential well for the contact arrangement well becomes deeper as the temperature increases.

Computational studies of pairwise hydrophobic interactions have often been frustrated by the fact that spontaneous association of two apolar solute particles has never been observed on the simulation timescale. Wallqvist and Berne^[158] have shown that the stability of the solvent-separated arrangement even holds for the interaction of one apolar solute with a large flat apolar surface. A plausible explanation for this might be that the barrier between the contact arrangement and the solvent-separated arrangement is too high and the solute particles are trapped inside a solvent cage. During the simulation time, escape from this cage can be an extremely rare event. Furthermore, the formation of a contact pair from randomly distributed particles can be an unfavorable process in terms of Gibbs energy, despite substantial attractive forces between the solute particles. The existence of solvent-separated solute pairs appearing as local minima on a potential energy map is, strictly speaking, unrelated to the hydrophobic interactions. The occurrence of distinct arrangements of the solute particles is the result of a well-established packing phenomenon and occurs even in the most simple solvent systems (see Section 3.3.1). The additional arrangement of water molecules separating the solute molecules is a consequence of the directional character of the hydrogen bonds connecting the water molecules.

The fact that two apolar solute particles in dilute aqueous solution do not spontaneously aggregate on the simulation timescale has also seriously obstructed studies of bulk hydrophobic interactions. Recently Laaksonen and Stilbs^[336] studied the distribution and the dynamics of methane molecules in aqueous solutions with increasing concentrations of methane. At higher concentrations more methane molecules were able to overcome the barrier between the solvent-separated and the contact arrangement and occasionally stuck together. Eventually, the solvent-separated arrangement disappeared completely.

A similar computational study was reported by Wallqvist,^[163] who studied the behavior of a system comprising 18 Lennard-Jones solutes, representing methane, dispersed in 107 water molecules. Obviously, the concentration is extremely high, and spontaneous aggregation of the solute particles is inevitable. Indeed, phase separation was observed which resulted in hydrocarbon-rich domains dispersed in water. This result led Wallqvist to emphasize the necessity of invoking higher-body interactions for a successful simulation of a spontaneous aggregation of many apolar solutes.

Computer simulations of intramolecular hydrophobic interactions have generally been carried out by simulating the effect of water on the conformation of simple apolar molecules.^[368] *n*-Butane is the simplest alkane for which the effect of the solvent on the conformation in solution is not a trivial problem and might be considered the prototype for conformational studies in water. Several computer simulation studies showed that the population of the more compact *gauche*

conformer increases when *n*-butane is transferred from the gas phase to an aqueous solution.^[344–347] Although the applied computational methods are different, most authors find a shift of 10–35% at the expense of the extended *trans* conformer. Recently Tobias and Brooks^[345] reported that the *gauche* conformer is also favored in CCl₄. In water, however, the effect is significantly larger. A thermodynamic analysis showed that the *gauche* conformer is energetically destabilized but strongly favored by entropy.

Some computer simulation studies focused on interactions between solute particles having both polar and apolar regions. MD simulations of a 3 mol % solution of 2-methyl-2-propanol and a 5 mol % solution of methanol in water using the MCY potential model for water and an ab initio description of solute–solvent interactions showed that these alcohols tend to associate in water; this is consistent with experimental findings.^[351, 355] Unfortunately, it was not explicitly reported whether solvent-separated or contact pairs were formed. Hydrogen bonds between the alcohol molecules were not detected. Maliniak et al.^[367] reported aggregation of quinuclidine in a 6 mol % solution using MD simulation methods. In a more dilute solution (2 mol %), the solutes did not show any tendency to form aggregates. Recently, MD methods have even been successfully applied to simulate aggregation of surfactant molecules.^[369]

4. Epilogue

Traditionally, chemists have attributed a pivotal role to the structure of water in the vicinity of apolar solutes or apolar moieties in aqueous solutions as expressed in the concept of hydrophobic hydration and hydrophobic interaction. Inextricably wrapped up with the concept of hydrophobic hydration is the notion of stronger (or more) hydrogen bonds in hydrophobic hydration shells. In particular, the idea of a clathrate-like hydration layer led to the exaggerated picture of iceberg formation at the surface of apolar solute particles in water. Recent computational studies have yielded little evidence for a significant increase of either the number or the strength of hydrogen bonds in aqueous solutions of apolar compounds when compared to pure water.

The large and favorable enthalpy of solvation of apolar compounds in water, presented in classic papers as a convincing piece of evidence for enhanced hydrogen bonding in hydrophobic hydration shells, can be conveniently explained on the basis of interactions between water and the apolar solute without assuming additional interactions among water molecules. Various studies, however, have confirmed that O–H bonds of water molecules adjacent to an apolar solute are predominantly oriented tangentially to the apolar surface. Current evidence strongly indicates that water does not undergo a structural enhancement in the presence of apolar solute particles but *maintains* its original structure by accommodating the apolar solute in its original hydrogen bond network. The unique property of water is that it can dissolve an apolar solute of limited size without sacrificing a significant number of hydrogen bonds.

London dispersion interactions between water and the apolar solute create an additional source for enthalpy, resulting in a large and negative enthalpy for the solvation of

apolar gases in water. The orientation of the water molecules in the first hydration layers might even give rise to a bulk polarization phenomenon induced by “proton hopping”. Thus, it might be speculated that solute–water interactions are enhanced by the orientation of the water molecules in the immediate surroundings of the solute particle. Computational chemistry would be the most appropriate tool to validate or disprove this idea.

The tendency of water molecules not to sacrifice any (or only a small number) of their hydrogen bonds and to interact favorably with apolar solute particles does not mean that water is a good solvent for apolar compounds. At lower temperatures the loss of entropy hampers solvation. The interpretation in terms of “structure-making” properties of apolar solutes or related terminology is in our opinion not very elucidative and may be even wrong. As shown elegantly by applications of scaled particle theory, a large part of the negative entropy of solvation can be attributed to the small molecular volume of the water molecules; in other words, many water molecules are involved in dissolving a single solute particle. In fact, the hydrogen bond structure of water, which provides the solvent with an exceptionally low density, is even favorable for dissolving inert solutes. However, it must be noted that scaled particle theories do incorporate some structural information for the solvent such as the isothermal compressibility. As argued by Lee,^[228, 230] reduction of rotational freedom and possibly compression of water molecules in the first hydration layers will contribute to the loss of entropy as well. Nonetheless, one crucial point emerges: the orientation of the water molecules in the hydration shell of apolar solute particles, generally referred to as hydrophobic hydration, *aids* the dissolution of apolar compounds in water and is not the cause for their low solubility.

At higher temperatures water gradually loses its capacity to maintain hydrogen bonds upon intrusion of apolar solute particles. This leads to less favorable solute–water interactions, expressed in a gradual reduction of the enthalpy of solvation, which even becomes positive at elevated temperatures. Since hydrogen bonds break, the entropy increases as well. At high temperatures the increase in entropy is very significant and counteracts the reduction of entropy that stems from the small molecular volume of the water molecules. Apparently, the Gibbs energy accompanying the disruption and formation of hydrogen bonds in the hydration shell of apolar solute particles is small. The entropy and enthalpy characterizing the changes due to variation in temperature are almost fully compensative. This does not necessarily imply that this compensation behavior is a result of an intrinsic property of the water structure, as is often assumed in “compensation models”. Changes in the orientation and binding properties of water molecules in the hydration shell due to changes in temperature can also affect solute–water interactions. It is notoriously difficult to separate the thermodynamics of water–water interactions in hydrophobic hydrations shells from those of solute–water interactions. Although the capacity of water to dissolve apolar solutes decreases as the temperature is raised, the influence of temperature is remarkably small. This fact supports the notion that structural changes in liquid water are not the main cause for the low solubility of apolar compounds in water.

A more effective way of preventing water molecules from maintaining their hydrogen bonds upon intrusion of foreign solute particles is to increase the concentration of the solute. The hydrophobic hydration shell is apparently quite extensive. At a certain concentration of solute particles, the number of water molecules is not sufficient to form a complete hydrophobic hydration shell, which leads to interference and mutual obstruction of hydration shells and the inevitable sacrifice of hydrogen bonds. An alternative but related way of destroying hydrogen bonds is to increase the size of the apolar solute particle. In the limiting situation of a hydrophobic wall, water molecules are not able to salvage all hydrogen bond interactions, even when their O–H bonds are tangentially aligned towards the hydrophobic surface. Finally, introduction of polar functionalities leads to mutual obstruction of hydration shells. A hydroxyl group will contribute strongly to the solubility of a predominantly apolar solute particle and will considerably reduce the necessity and possibility for the formation of (local) hydrophobic hydration shells. This phenomenon governs the hydration of polyols and carbohydrates. Strikingly, the apolar methylene functionalities remain available for interactions with other apolar moieties in solution; this might be crucial for molecular recognition phenomena at the surface of living cells, in which polysaccharides play a prominent role.

Hydrophobic interactions, both pairwise and bulk, have traditionally been treated as a natural consequence of hydrophobic hydration. It was, however, alarming that computer simulations of dilute aqueous solutions of apolar solute particles do not reveal any tendency of the apolar solutes to form long-lived pairwise or higher-order aggregates on a typical simulation timescale. The concept of partial destruction of hydrophobic hydration shells involving the release of structured hydration water to the bulk, which plays a dominant role in the traditional interpretation of hydrophobic effects, has lost much of its attraction as a molecular model of hydrophobic interactions. Following the argument that hydrophobic hydration aids the dissolution of apolar compounds in water, it cannot also be considered as a cause for the association of apolar solutes in aqueous solution. In contrast, hydrophobic interactions are *counteracted* by the formation of a hydrophobic hydration shell. Apolar solute particles are “screened” in dilute aqueous solution as compared to the gas phase.

Pairwise and bulk interactions between apolar moieties do lead to partial desolvation of the apolar surface and to a concomittant reduction of the number of water molecules involved in the solvation of the solute particles. Inherently, this leads to a gain of entropy. In dilute solutions the gain in entropy is insufficient to overcome the unfavorable enthalpic effect and the loss of entropy due to the association of solute molecules and, consequently, the formation of long-lived pairs of apolar solute particles is an infrequent event. The molecular origin of the slightly unfavorable enthalpy involved in bulk hydrophobic interactions near room temperature can be explained in terms of a loss of solute–water interactions which are not completely counteracted by a gain in solute–solute interactions, while water–water interactions remain effectively unchanged. Recent computational studies of hydrophobic interactions gave rise to arguments about the preference of solvent-separated over contact solute

pairs. In our opinion, this is not a very relevant question. As shown in several studies, the existence of well-defined geometrical configurations of pairs of solutes in solution is an inherent property of all solvent systems and not restricted solely to water. More interesting is the question as to the actual distance between the solute particles in the solvent-separated configuration. As shown in Section 3.3.1, the directionality of hydrogen bonds might have a significant effect on this distance.

Following the arguments given above, destruction of hydrophobic hydration shells *increases* the frequency of hydrophobic contacts. This can be accomplished by either increasing the temperature or, more effectively, by increasing the concentration of apolar compounds. Increasing temperature is not very effective in increasing hydrophobic interactions, as has been clearly demonstrated by studies of host–guest complexation and micelle formation at elevated temperatures. However, when the number of solute particles in solution prevents the formation of complete hydrophobic hydration shells, hydrogen bonds are sacrificed and an increasing number of O–H bonds will point towards the apolar surface; this is an energetically unfavorable event. As a result, the tendency to form aggregates of two or more apolar particles increases rapidly. This behavior has been repeatedly confirmed by computational studies. When the apolar solute particles can form a well-defined aggregate by means of directional bonding, for example, a micelle or an aggregate with a more complex morphology, the concentration at which aggregation takes place can be very critical. Additional factors such as surface forces, headgroup effects, and aggregate size play a decisive role, and it is too simplistic to consider only hydrophobic effects. Even aggregation of less complex organic compounds such as monohydric alcohols into small, short-lived clusters is very difficult to describe theoretically. It is still a considerable challenge to relate pairwise interactions between solutes in dilute aqueous solutions to the occurrence of bulk interactions in more concentrated solutions.

In conclusion, we believe that hydrophobic interactions are not so much a result of a structural property of the hydrophobic hydration shell of apolar compounds but rather reflect the limited capacity of liquid water to accommodate the apolar solute and maintain its original network of hydrogen bonds. It is surprising that apolar compounds dissolve in water at all; this must be attributed to the formation of a hydrophobic hydration shell.

Answers to pertinent questions regarding hydrophobic effects must be expected from either sophisticated neutron diffraction studies or from accurate computer simulations. Incorporation of polarization effects is highly desirable if one wishes to test the proposition that solute–water interactions are enhanced by cooperative polarization effects in the hydrophobic hydration shell. Polarization effects must also be considered in investigations of the molecular origin of the exceptionally long-range interactions between large apolar surfaces.¹³⁷⁰¹ Furthermore, it would be most instructive to perform an extensive comparative computational study on solutions of apolar compounds in water and in a solvent of similar size but without hydrogen bonds, for example liquid methane. In general, comparative studies of the solvation behavior of apolar compounds in various organic solvents

and water are scarce. In particular, the structural response of solvation shells to changes of temperature and concentration has been rather neglected by computational studies. Advances in this area are eagerly awaited.^[37,1]

Received: November 30, 1992 [A 919 IE]
German version: *Angew. Chem.* **1993**, *105*, 1610

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