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Water Runs Deep

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1.1 The Control of Water

There is current consensus among archeology scholars that there are five cradles of civilization, geographical locations where the first civilizations emerged thousands of years ago. These five areas – the Fertile Crescent, the Indus River, the Yellow River, the Central Andes, and Mesoamerica – each independently gave rise to a new level of human existence [1–3]. What do these sites have in common? They all shared a combination of favorable geography and the opportunity to control water.

Because of its central role in life on Earth, the idea of controlling water manifests in many aspects of human endeavor. In agriculture, the key is irrigation – how to bring water to the fertile land and crops. "Controlling" the oceans and rivers requires harbors, moorings, and boats to open up trading routes or the possibility of fishing, while controlling sewage has been key to the success of cities. Controlling water also allowed energy production in the form of watermills and, in more modern times, dams, tidal barrages, and wave energy convertors. Unfortunately, the control of water can also be used to justify war. Conflicts between nations, states, or groups, for example, the violence in the war in Sudanese Darfur, can be partially attributed to water [4, 5].

For many different reasons, the control of water at the molecular and nanoscale levels is also key. Many scientific disciplines work toward being able to understand and control water purification for waste treatment and desalination [6, 7], water flow for microfluidics and ultra-hydrophobic materials [8], and solvation to in turn control dissolved matter. At this level, the control of water is free of famine and conflict. However, it is plagued by beguiling complexity; the solvent of life, this "matrix of the world and of all its creatures" (Paracelsus, 1493–1541 CE) is a truly complex substance possessing endless emergent phenomena [9, 10]. This means that it is easy for pathological science [11] to make wild claims about the memory properties of water [12, 13] or the existence of polywater [14, 15]. Although, at the time, the latter stoked a public fear reminiscent of Vonnegut's *Cat's Cradle* [16], polywater has passed into history. Alas the same cannot be

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said for memory water and infinitely diluted homeopathic remedies. And lest it be thought that there are no new controversies around water, consider also the relatively recent idea that water is capable of storing charge [17–19].

As the complexity of water means that it is exceedingly difficult to truly understand how water interacts with itself and how it interacts with solutes [20], the primary aim of this chapter is to outline what we do and do not know about supramolecular interactions in aqueous solution. Correspondingly, a secondary aim is to minimize situations wherein an observation might be explained away by the waving of hands. For as has been noted previously, "When confronted with unexpected experimental results water structure has commonly been used *deus ex machina* for explaining the observations." [21] We can and should do better than this.

1.2 The Shape of Water

At the heart of water and aqueous solutions lies a contentious question that goes back millennia – what is the structure of liquid water? The Greek philosopher Empedocles (490–430 BCE), like many thinkers around the world, considered water to be one of the four classical elements: earth, water, air, and fire. More helpfully, Democritus (460–370 BCE) proposed that all matter was made up of small indivisible particles called atoms (atomism). Around the same time, Plato (428–348 BCE) was being less helpful but slightly prophetic. He merged geometry with the four elements of the universe to propose that water was one of five (Platonic) solids: tetrahedron, cube, octahedron, dodecahedron, and icosahedron. Specifically, Plato proposed that water is an icosahedron, which explained its ability to flow across (cubic) the Earth.

It was not until 1781 that Henry Cavendish showed water to consist of hydrogen (inflammable air) and oxygen (dephlogisticated air) by burning a mixture of the two [22]. This discovery was built further upon by Amadeo Avogadro, who was the first to clearly differentiate between atoms and molecules, and that the latter must be represented by an empirical formula. He also hypothesized that equal volumes of gas at equal pressure contain the same amounts of molecules regardless of the nature of the gas, and from this was able to repeat Cavendish's experiment to show that water was two parts hydrogen and one part oxygen [23].

Moving into the late 1800s and the early 1900s, a combination of Röntgen's discovery of X-rays, Einstein's concept of a photon, and Compton's confirmation of X-ray scattering by electrons ultimately led to the development of X-ray crystallography. This led Bragg and others to determine the structure of ice. In the solid state, water possesses tetrahedral geometry [24] in which oxygen atoms are 2.75 Å from each other. There was, after all, an element of truth to what Plato had thought: water was not an icosahedron, but four water molecules did make a tetrahedron. That same decade saw the nucleation of the idea of hydrogen bonding (H-bonding). In 1920 Latimer and Rodebush proposed that a "free pair of electrons might be able to exert sufficient force on a hydrogen held by a pair of electrons on another water molecule to bind the two molecules together."

However, it was not until 1939 and the publication of Pauling's *The Nature of the Chemical Bond* that the H-bond began to meet with widespread acceptance. Hexagonal ice, the form of all natural snow and ice on Earth, has the perfect tetrahedral structure with bond–bond, bond–lone pair, and lone pair–lone pair angles of 109.47° [25]. The reason for the high boiling point, large heat of vaporization, high heat capacity, and high surface tension of water was evident.

Partially through the development of X-ray crystallography, liquids began to be thought of as either dense ordered gases or imperfect disordered crystals, and with its H-bonding network, water was a prime candidate for this line of thinking. For example, an influential model by Bernal and Fowler treated water as a point charge with tetrahedral geometry whose structure was akin to that of disordered quartz [26]. As we shall shortly see (*vide infra*), this idea of water possessing considerable structure quickly moved into adjacent areas of research.

The Bernal and Fowler paper influenced thinking through the 1950s. Thus, Erying [27] proposed a liquid state structure of water consisting of crystalline close packing threaded with many dislocations. According to this model, molecules escaping the close packing could wander almost gas-like between the clusters. Similarly, Pauling proposed a disordered crystal based on the clathrate structure, while Bernal proposed a model of a random H-bonded network where water molecules gave rise to connected four- and seven-membered rings.

The last half-century or so has seen a dipartite approach to studying water. On the one hand, there have been the continued improvements in established spectroscopic techniques as well as the development of new spectroscopies [28]. The suite now available is extensive and includes Raman/Raman–multivariate curve resolution (Raman-MCR), IR, 2D-IR, sum-frequency generation techniques, and multiple X-ray and neutron approaches such as scattering, small-angle scattering, X-ray absorption spectroscopy (XAS), and X-ray emission spectroscopy (XES). Complementing this approach has been the development of computational chemistry. Thus, the combination of increasingly powerful computers, more accurate water models (e.g. TIP4P-Ew) [29], and new strategies has added greatly to our understanding of water and solvation at the molecular level [30].

We now know that the mean H—O bond length in liquid water is 0.97 Å, the mean HOH bond angle 106°, and the mean negative charge on the O atom ~70% of an elementary charge, with each positively charged H atom sharing the neutralizing charge. We also know that although water makes four H-bonds in the solid state, as a liquid it forms ~3.6 H-bonds on average. However, this number varies according to the specific analytical technique used. Furthermore, there is still some debate regarding the symmetry of the H-bonds in liquid water. In the gas phase, water forms minimum energy clusters [31–33]; however, it is clear from simulations that these structures do not persist in bulk solution at ambient temperature. Rather, the current consensus is that water possesses primarily tetrahedral structure [34, 35], although this is not universally agreed upon. Thus to pick one controversial example, XAS suggests that ~80% of water molecules have one strong and one weak hydrogen-bonded O—H group, such as what may occur in cyclic pentamers or hexamers [36]. In contrast, the remaining ~20% of the molecules in this model reside in four-hydrogen-bonded tetrahedrally coordinated clusters.

We also know that water H-bonds break and form on the timescale of tens of femtoseconds to picoseconds. They are weaker than the H-bonds in ice; the oxygen atoms in liquid water are 2.8–2.9 Å apart, but X-ray and neutron scattering still show evidence of a distorted tetrahedral structure [37]. As for the remaining ~0.4 H-bonds, evidence points toward virtually all molecules with such dangling H-bonds returning to an H-bonding partner within 200 fs. In other words, dangling H-bonds are intrinsically unstable [38].

Assuming the consensus is that water does not form rings or specific clusters, but instead possesses a slightly defective tetrahedral structure, what remains to be developed is a good understanding of how this lattice, composed of exceedingly short-lived H-bonds and containing ~10% defects, leads to the physical properties of water observed at the molecular and bulk levels. In the interim, it is still common to hear enduring if rather vague terms such as "flickering tetrahedral clusters" to describe the structure of water.

1.3 The Matrix of Life as a Solvent

Many common organic solvents are large and, in a structural sense, relatively homogeneous; they are relatively nonpolar, and correspondingly the range of their different solvent–solute interactions is relatively small and intrinsically similar to their own solvent–solvent interactions. As a result, chemists can frequently ignore the existence of organic solvents, but not water. Water is exceptionally small: a third of the volume of dichloromethane and one sixth of the volume of toluene. Even dissolved oxygen is large relative to water. Moreover, water is highly polar; recall the −0.7 charge on the oxygen and +0.35 charge on each hydrogen atom. These lead to an agile, nimble, and cohesive pack animal and *en masse* an excellent solvent; it can dissolve both simple ions and hydrocarbons with a solubility range of ~50 orders of magnitude [39].

The small size, high polarity, and presence of two H-bond acceptors and two H-bond donors mean that water is uniquely dense with supramolecular motifs. This combination of features leads to a unique combination of properties, some of which are extreme and many of which are not. For example, water has a significant dipole moment of 1.84 D and a high dielectric constant of $\varepsilon_r = 78$; but hexamethylphosphoric acid triamide (HMPT) has a dipole moment of 5.55 D, and *N*-methylformamide has a dielectric constant of 182. Similarly, water has an α-value (Kamlet–Taft solvent parameter) of 1.17, indicating that it is a weaker H-bond donor than many halogenated alcohols, and a very middling β-value of 0.47, indicating it is a weaker H-bond acceptor than solvents such as pyridine. That stated, by the measure of solvatochromic dyes, water is *the* extreme polar solvent. For example, it has the largest E_T^N of common solvents (a measure of polarity based on the transition energy for the longest wavelength absorption band of a betaine dye, which for water is by definition 1.00) . The extensive H-bonding network in liquid water means that it also has an exceptionally high heat capacity: its value of 75.2 J mol^{-1} K^{-1} (at 25 °C) is second only to ammonia

($80.8\,J\,mol^{-1}\,K^{-1}$) for heteroatomic species. This has ramifications not only for aquatic life on Earth but also for the fundamental properties of solvation.

Water is also highly cohesive. This means that it has remarkably high melting and boiling points for its size (cf. the boiling points of H_2S, H_2Se, H_2Te, and H_2Po are −60.3, −41.3, −4, and 36.1 °C, respectively). It also has an exceptional surface tension: $72.8\,mN\,m^{-1}$, a value much higher than any other common liquid with the exception of mercury ($472\,mN\,m^{-1}$). This high surface tension means that water is not a good wetting agent, which is good news for the platypus but frustrating for the analysis of the solvation of nonpolar solutes. Compared to common organic solvents, water is also quite viscous, although its value (8.94×10^{-4} Pa s) is dwarfed by larger molecules with multiple H-bonding groups such as glycerol.

Water also has a small but significant ionization constant: at 25 °C and zero ionic strength, $K_w = 1 \times 10^{-14}$. This means that even at neutral pH water is not chemically pure; there is hydronium (H_3O^+) and hydroxide (OH^-) or their corresponding higher ions such as $H_5O_2{}^+$ (Zundel cation) or $H_9O_4{}^+$ (Eigen cation) to consider also. This adds another level to the complexities of water. For example, it has been known for a long time that, via proton hopping processes, hydronium diffuses at twice the rate of hydroxide ion. Why is this? Very recent work suggests that it is because hydronium diffusion is concerted, whereas hydroxide diffusion is stepwise [40]. However, this has yet to be confirmed by others.

So, by many metrics water is an exceptional solvent, yet by others, less so. The ineffable uniqueness of water is that it is composed of the first and third most abundant elements in the universe, which combined lead to a molecule of unusual physical and chemical properties. As a result, given a suitable temperature window, water can be expected to be the ubiquitous solvent of life.

There is a problem however. Many of the aforementioned properties cannot easily be scaled to the molecular level, and it is down at the molecular or atomistic level that supramolecular chemists would really like to fully understand water. How are solutes solvated? How extensive is the water–water H-bonding around a solute? How does this solvation influence the thermodynamics or kinetics of host–guest complexation? There is no easy way to "translate" surface tension, wettability, cohesive energy density, etc. to the molecular level. Yet this is an eternal problem with studying aqueous supramolecular chemistry (and one reason why computational chemistry has been such a boon for the field). This means that given sufficient time hypotheses can become fact. To pick one classic exemplar, consider a 1945 landmark from Frank and Evans [41]. In this paper the authors describe examining the solvation thermodynamics of small nonpolar molecules in water. In expanding their own and other's work, they concluded that the entropic cost of dissolving small nonpolar organics in water arose because they modify "the water structure in the direction of greater crystallinity – the water, so to speak, builds a microscopic iceberg around it." This idea that the solvation shell of dissolved nonpolar molecules is highly structured was subsequently used by Kauzmann in a model for the thermodynamics of protein folding [42]. In large part thanks to this paper that this general idea can still be found within undergraduate biochemistry textbooks. However, as we will expand upon below, the extent to which this is true is unclear.

1.4 Solvation Thermodynamics

While the customary viewpoint of supramolecular chemists is from the perspective of a molecular host, water scientists consider water to be the host of interest. Thus, rather than considering host and guest desolvation and host–guest complex formation, water scientists consider how water (the host) responds to the addition of a solute (the guest). In this section, we mostly opt to take this "reverse" perspective of the water scientist and discuss what happens when a solute is taken from the gas phase into the aqueous phase.

When applied to solvation processes, Gibbs (Bogoliubov and/or Feynman) inequalities rigorously describe and set upper and lower bounds for the thermodynamics of transfer from the gas to solution phase [43]. As we describe below, Gibbs inequalities, combined with Linear Response Theory (that relates and defines solute–solvent interaction energies), lead to some remarkable thermodynamic relationships. For example, both van der Waals and electrostatic solute–water interactions are well described by the Linear Response Theory, and one fascinating conclusion is that for the latter, there is nearly perfect cancellation of ion–water and water reorganization contributions to experimental hydration entropies. Thus, ion–water interactions formed upon dissolution invariably produce a decrease in entropy, but the resulting release of heat dissipated to the surrounding solution leads to an entropy increase of nearly equal and opposite magnitude. It is this entropy cancellation that is evidently responsible for the near equivalence of the experimental hydration entropies of noble gases and isosteric halide ions, despite the initial conclusion that the latter would strongly coopt and organize water molecules into a highly defined solvation shell. More generally, it is this negative ion–water interaction entropy and countering positive water reorganization entropy that is responsible for near equivalence of the experimental hydration entropies of ionic, polar, and nonpolar solutes. This suggests that in the binding of a charged amphiphile or ion to a nonpolar pocket of a host, the changes in solvation of the charge group are not reflected in the experimental ΔS for complexation [44, 45].

To fully understand solvation, it is important to consider all the solute–solvent and solvent–solvent interactions involved, and a convenient way to do so is to break the process down into three steps: the formation of a cavity the size and shape of the solute in water (a hard sphere is ideal), the "switching on" of van der Waals forces in the solute, and lastly the inclusion of electrostatic forces. Figure 1.1 illustrates this thought process, showing the change in water structure and solute–solvent (uv) interaction energy before (E^0_{uv}) and after "equilibration" (E_{uv}) of each step. As has been summarized elsewhere [43], the Linear Response Theory links these two energies (E^0_{uv} and E_{uv}) by a linear function, with dispersive and electrostatic interactions falling into very different linear response regimes.

The abrupt insertion of a hard-sphere potential into pure water results in (by definition) an initial, infinite solute–solvent interaction energy ($E^0_{\mathrm{uv(cav)}} \approx \infty$), which decreases to approximately zero once equilibrated. Gibbs inequality dictates that the free energy of cavity formation is invariably positive ($0 \leq \Delta G_{(\mathrm{cav})} \leq \infty$). Furthermore, a combination of experimental and simulation data reveals that the thermodynamic functions of cavity formation ($\Delta G_{(\mathrm{cav})}$, $\Delta H_{(\mathrm{cav})}$, and $\Delta S_{(\mathrm{cav})}$) are

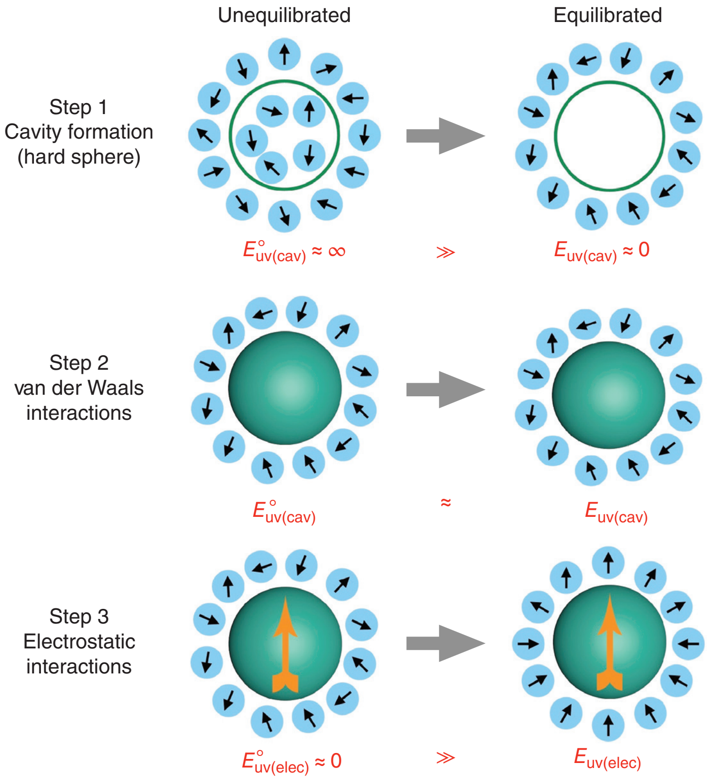

Figure 1.1 Three-step visualization of a solvation process for a solute (green circles) and its surrounding hydration shell of water molecules (blue circles with arrows depicting the dipole of each water). Step 1: cavity formation. Steps 2 and 3: introduction of van der Waals and electrostatic interactions, respectively. Each step consists of unequilibrated and equilibrated states, where the former depicts the system after the introduction or change to the solute but before the response of the solvation shell. The equilibrated state corresponds to the system after the hydration shell has responded to the change. Solute–water interaction energy descriptions for each state are derived from the Linear Response Theory and Gibbs inequalities [43].

nonlinear [46]. In addition, the cavity formation enthalpy in water is markedly temperature-dependent, a consequence of the fragility of the water H-bond network surrounding a nonpolar (hard-sphere) solute. It is this temperature dependence that dictates the large heat capacity changes seen in the solvation of nonpolar guests [47]. As we will see in the next section, host molecules greatly attenuate the free energy requirements of cavity formation.

In step 2 the van der Waals forces between solute and solvent are “switched on.” van der Waals forces are largely insensitive to the orientations of the water in the solute solvation shell, and Gibbs inequalities lead to an extremely restrictive constraint on dispersive contributions to solvation, namely, that $E_{\mathrm{uv(disp)}} \approx E^{0}_{\mathrm{uv(disp)}} \approx \Delta G_{\mathrm{(disp)}}$, and $S_{\mathrm{uv(disp)}} \approx 0$. In other words, dispersion interactions are expected to contribute enthalpically but not entropically to the solvation of nonpolar molecules.

In contrast, “switching on” electrostatic interactions reorientates the solvation shell. Before equilibration the solute–water interaction energy is expected to be small ($E_{\mathrm{uv(elec)}} \approx 0$) because of the random orientation of the solvation shell water molecules and the weak van der Waals forces. However, once “switched on,” the interaction energy is expected to be strongly negative. In this case, Gibbs inequalities and Linear Response Theory lead to two important conclusions. First, that solute–solvent interaction energy is negative but less negative than the overall change in free energy ($E_{\mathrm{uv(elec)}} \leq \Delta G_{\mathrm{(elec)}} \leq 0$). Second, that electrostatic interactions are not expected to significantly contribute to experimental hydration entropies since $\Delta S_{\mathrm{(elec)}} \approx S_{\mathrm{uv(elec)}} + \Delta S_{\mathrm{vv(elec)}} \approx 0$. This is why the hydration entropies of halide ions are essentially indistinguishable from that of noble gases [48]. However, the large free energy of halide ion hydration directly reveals the very large, canceling solute–solvent and solvent–solvent reorganizing entropies ($\Delta G_{\mathrm{(elec)}} \approx TS_{\mathrm{uv(elec)}} \approx -T\Delta S_{\mathrm{vv(elec)}}$). Similar though less dramatic effects can be seen with neutral polar molecules [46].

These important points noted that what supramolecular chemists are adept at gathering are experimental thermodynamic data for guest complexations. How does such data relate to the individual solute–solvent and solvent–solvent enthalpy and entropy changes? As Eq. (1.1) shows, the free energy of solvation (ΔG) can be expressed solely by solute–solvent terms, specifically the solute–solvent interaction energy (E_{uv}) and entropy (TS_{uv}):

$$\Delta G = E_{\mathrm{uv}} - TS_{\mathrm{uv}} \tag{1.1}$$

In contrast, it can be shown that the corresponding enthalpy (ΔH) and entropy ($T\Delta S$) changes contain additional solvent reorganization contributions (Eqs. (1.2) and (1.3)):

$$\Delta H = E_{\mathrm{uv}} + \Delta E_{\mathrm{vv}} + P\bar{v} \tag{1.2}$$

$$T\Delta S = TS_{\mathrm{uv}} + T\Delta S_{\mathrm{vv}} \tag{1.3}$$

where E_{uv} is the overall solute–solvent interaction energy, ΔE_{vv} is the overall change in solvent–solvent interaction energy upon dissolution, P is the pressure, $\bar{v}$ is the solute partial molar volume, TS_{uv} is the overall solute–solvent entropy contribution, and $T\Delta S_{\mathrm{vv}}$ is the overall change in solvent–solvent entropy contribution upon dissolution. Note that at ambient pressure, the pressure–volume work ($P\bar{v}$) associated with increasing the volume of the system by an amount equivalent to the change in the partial molar volume of the host–guest complex is negligibly small, so $\Delta H \approx \Delta U = E_{\mathrm{uv}} + \Delta E_{\mathrm{vv}}$. Thus, the familiar $\Delta G = \Delta H - TS$ clearly implies that the solvent reorganization enthalpy and entropy must precisely compensate (as $\Delta E_{\mathrm{vv}} = T\Delta S_{\mathrm{vv}}$).

It is also possible to calculate the intrinsic (gas phase) free energy of host–guest complexation, given realistic interaction potentials and structures for the host, guest, and complex. Additionally, for rigid hosts and guests, the corresponding enthalpy and entropy of binding contain additional trivial ideal gas terms (for flexible systems there are also complicating terms arising from the binding-induced conformational change of the host and/or guest). Such calculations combined with solution thermodynamic data and Eqs. (1.1)–(1.3) mean that it is theoretically possible to parse out all the different contributions to host–guest binding thermodynamics.

In summary, because of weak entropic effects associated with van der Waals forces between a nonpolar group and water, and because of strong but compensation entropic effects with ions, the influence of water on host–guest binding free energies may be attributed entirely to binding-induced changes in solute–water interactions. It is important to note, however, that the corresponding binding enthalpy and entropy may also be significantly influenced by changes to water–water interactions (such as the solute-induced breaking of water–water H-bonds) upon host–guest binding.

1.5 The Three Effects

In aqueous solutions chemistry, there are two very familiar phenomena: the hydrophobic effect and the Hofmeister effect. We will, however, add a third here: the reverse (or inverse) Hofmeister effect. The hydrophobic effect is the observed tendency of nonpolar substances to aggregate in an aqueous solution and exclude water molecules; to reiterate the perfunctory adage, oil and water do not mix. We will discuss the inaccuracy of this adage and the details of the hydrophobic effect in Section 1.5.1. For biochemists and organic chemists concerned with organic solutes in water, the Hofmeister effect is most commonly and simply expressed as how salts modulate the hydrophobic effect. As we will see, some salts can increase the solubility of (macro)molecules, while others can decrease it, i.e. apparently by making the solute more hydrophobic. Finally, to make matters more complex, there is also the reverse Hofmeister effect. This is the phenomenon whereby salts that upon initial inspection would be expected to cause the solute under study to become more water soluble but instead induce the (macro)molecule to aggregate and precipitate out of solution. We will discuss the Hofmeister and reverse Hofmeister effects in Sections 1.5.2 and 1.5.3, respectively.

It is important to appreciate that while the hydrophobic effect and Hofmeister effects are usually discussed separately, current evidence suggests that the hydrophobic effect, the salting-in Hofmeister effect, and the reverse Hofmeister effect are rooted in common non-covalent interactions that occur between weakly hydrated solutes. This point is reinforced by considering the overview in Figure 1.2. In this simplified picture, molecules are considered to partake in only two kinds of non-covalent interactions: van der Waals and electrostatic interactions. This allows a general ordering of species going from left to right, from weakly to strongly solvated. All of the phenomena in question rely on relatively poor solvation to allow species-specific interaction between pairs of molecules. A pair of neopentanes will display the hydrophobic effect (although it is arguable that a bigger nonpolar surface is actually required for association in water to occur; *vide infra*). On the other hand, a neopentane and perchlorate (or tetramethylammonium) will display the salting-in Hofmeister effect; association of the two will increase the solubility of neopentane in water. The typical anionic Hofmeister series is shown in the upper "branch" of the figure; moving

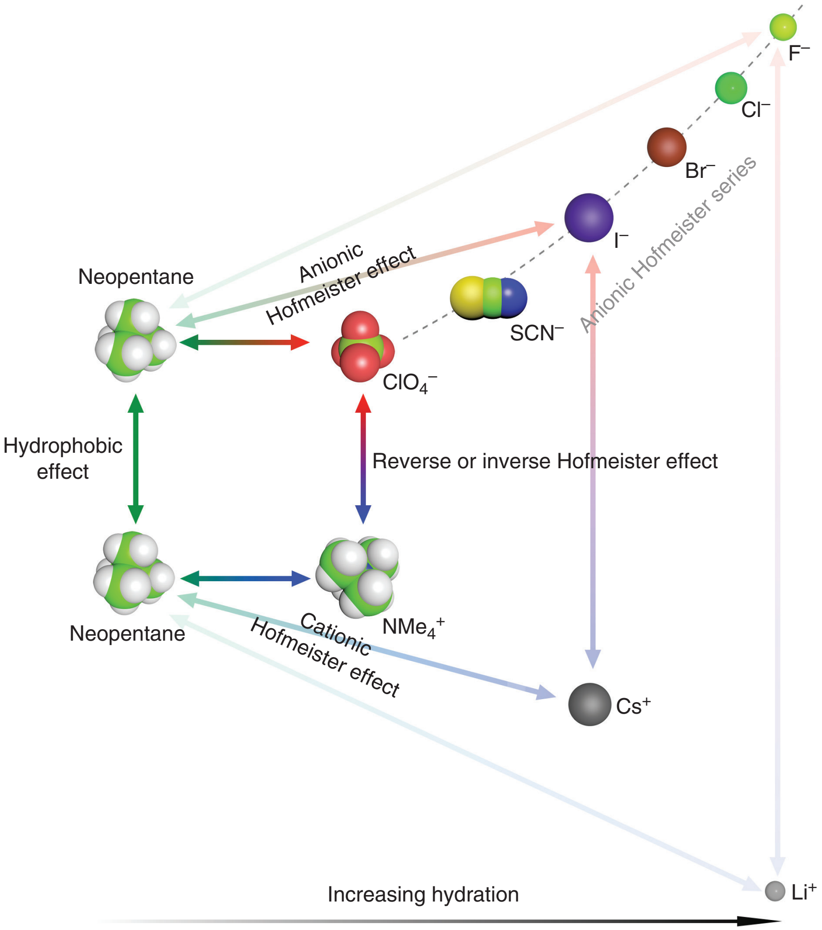

Figure 1.2 A unified view of the relationship between the hydrophobic effect, the salting-in Hofmeister effect, and the reverse Hofmeister effect. In this scheme, solutes are ordered in a continuum from nonpolar (i.e. in water, hydrophobic) on the extreme left to hard highly hydrated ions on the far right. In this simplified model, solutes such as neopentane are considered to form only van der Waals interactions with water and other solutes, while solutes such as fluoride form only electrostatic interactions with water and other species. Between these two extremes, from left to right, van der Waals interactions are slowly decreasing, while electrostatic interactions are increasing. The combination of these two interaction types lead to the salting-in Hofmeister effect between neutrals and anions (upper "branch," typical series of anions shown), with the strongest interactions involving the soft, polarizable anions depicted by the more intense arrows. The near-transparent arrow between neopentane and fluoride is for illustrative purposes only; anionic Hofmeister effects between such species are essentially nonexistent except at exceedingly high concentrations. Analogous interactions between neutrals and cations can lead to the cationic Hofmeister effect (lower "branch"), with the same gradation in arrow suggestive of the intensity of the effect. As depicted in the vertical, interactions between nonpolar molecules lead to the hydrophobic effect, while interactions between cations and anions can lead to the reverse Hofmeister effect between weakly solvated ions. As with the salting-in Hofmeister effect, reverse Hofmeister effects existing between hard ions such as fluoride and lithium are essentially nonexistent at moderate concentrations. Note that other interactions, such as reverse Hofmeister effects involving tetramethylammonium and iodide, are not shown for clarity.

from left to right, the anions are increasingly more strongly solvated and decreasingly capable of interacting with, for example, a neopentane solute. Between the anionic and cationic “branches,” the reverse Hofmeister effect is manifested; if a solute is cationic and poorly solvated, then exchange of its anion with a more weakly solvated anion can lead to aggregation and precipitation via charge neutralization. Again, the more weakly hydrated the two species, the stronger the effect.

1.5.1 The Hydrophobic Effect

Understanding the hydrophobic effect is key to understanding how organic molecules behave in water, their solubility, how they associate, and how they fold. For lucid discussions on water and the hydrophobic effect, readers are directed to reviews by Ball [20], Ben-Amotz [49], Blokzijl and Engberts [50], Sharp and Vanderkooi [51], Dill and coworkers [52], and Chandler [53].

Supramolecular chemists beware. Ever since the paper by Frank and Evans [41] suggesting “icebergs” around nonpolar solutes, there has been a continuous debate about the existence of highly structured water around nonpolar solvents. Yet, as has been stated previously, to date there is no good reason to suppose that water does organize around such solutes, and some evidence to indicate that it does not [20]. In short, the jury is still out. This uncertainty has unfortunately meant that the hydrophobic effect is frequently rolled out to explain a whole range of results and phenomena, *deus ex machina*.

Science is bringing an increasing number of tools to bear on the topic of the hydrophobic effect; however a note of caution is needed regarding one of the most powerful tools for molecular-level detailed analysis of water and solvation: computational chemistry. Specifically, the prevalence of off-the-shelf computational packages can lead to the assumption that *in aquo* atomistic molecular dynamics (MD) simulations of contemporary host molecules are routine. This is not the case. Consider first the limitations of water models. One of the best indicators of this is that the melting points of commonly used water models range from −127.55 to 0.75 °C [54]. That water models are approximations is also evident in the fact that there is no readily usable (for MD simulations) models that can faithfully replicate the phase diagram of water; the closer to perfect a water model is made, the more computationally costly it becomes. This is not to say that water models are not to be trusted; most function exceedingly well when measured against one or a small set of metrics. So, if an accurate potential of mean force or free energy of binding is required, TIP4P-Ew is an excellent choice [30]. However, water contemporary models are not sufficiently accurate that they easily satisfy multiple metrics or, importantly, will accurately reveal serendipitous explanations of physical or chemical properties. Limits to computational power also mean that for MD simulations a “complex” system might consist of homogeneous surfaces or shapes such as parallel plates, tubes, or hemispherical cavities (*vide infra*). Relatedly, calculations involving the average contemporary host–guest system are exceedingly computationally demanding, even when polarizability is ignored; with current computer power, the inclusion of polarizability limits atomistic MD simulations to molecules much smaller than the average contemporary host. Finally, force fields for atoms are largely biased toward those commonly found in Nature. If a researcher wishes to study the binding of most polyatomic inorganic species, she or he may have to build the requisite force fields. In short, although computational chemistry has much to offer the study of aqueous solutions, its limitations are real.

These issues noted that even in their absence the hydrophobic effect is difficult to precisely define because it is intimately linked to the hydration of each individual nonpolar solute; even if organic molecules were only composed of carbon and hydrogen, the hydrophobic effect would be exceedingly complex. Consider, for example, a dimerization process that is not possible to empirically determine: that of methane. Calculations are key here. The free energy of dimerization in water is equivalent to the sum of the interaction free energy in the gas phase and the corresponding water-mediated contribution. If the "methane" monomers are in fact hard spheres, their intrinsic free energy of interaction is zero. In such a case, it is possible to calculate a water-mediated interaction of $\approx \mathrm{R}T$. In contrast, methane dimerization shows a well depth of $1.2\,\mathrm{kJ\,mol^{-1}}$ ($\sim 1/2$ $\mathrm{R}T$ at 300 K) and a water-mediated contribution that can either be attractive or repulsive, depending on the calculation [55–60]. Indeed, the magnitude and sign of the water-mediated contribution is remarkably sensitive to the precise solute–water interactions [61]. Hence, the weak attraction between water and aliphatic groups means that they are on the knife edge between hydrophilic and hydrophobic regimes [49]; in water, alkyl chains are frequently pushed together, but sometimes they can be pulled apart; it all depends on the precise solute and if present, the precise nature of salts or buffers. One current rule of thumb is that $\sim 1\,\mathrm{nm}^2$ of hydrocarbon surface area must be desolvated in order for binding or assembly to out-compete thermal fluctuations (RT) and that below this value, smaller molecules are held apart by water [61].

This delicate balance arises because: (i) of the complex (and unknown) thermodynamics of non-spherical cavity formation, (ii) the van der Waals solute–solute interactions are generally weak, and (iii) with polar or charged molecules, there are almost compensating enthalpy and entropy contributions (see Section 1.4). This sets water apart from other solvents; it is as if that sometimes it is there and sometimes it is not. To put another way, binding to a host in organic solvents is commonly inhibited by the solvent. As a result, simple competition (guest vs. solvent) means that binding is weak relative to the gas phase. The weak interactions between solute and water mean, however, that water is not a good competitor and binding is enhanced relative to organic solvents [62]. Irrespective of the precise solvation details, it is paramount to recall this point: the hydrophobic effect is relative to other solvents, not the gas phase; there is no magical force that can "turbocharge" binding beyond the intrinsic gas phase affinity.

Unsurprisingly perhaps, this knife-edge solvation means that the thermodynamics of the hydrophobic effect are mostly dependent on temperature. Thus, while the slightly positive hydration free energies (ΔG) is only weakly dependent, the ΔH and $T\Delta S$ terms are highly temperature-dependent. Inevitably then, at some temperature $\Delta H = 0$ (i.e. $\Delta G = -T\Delta S$) and $T\Delta S = 0$ (i.e. $\Delta G = \Delta H$). Furthermore, as $\Delta S = -(\partial \Delta G/\partial T)_P$, ΔG must attain a maximum when $T\Delta S = 0$. This means we must take care when stating that a process is "driven" by enthalpy or entropy. In other words, it is not useful to define a binding event in water as being driven by either the "classical" (entropically driven) or "nonclassical" (enthalpically driven) hydrophobic effect. Entropy changes are not a good thermodynamic signature of this phenomenon. Relatedly, while hydration of an alkane inevitably results in an increase in the heat capacity ($\Delta C_P = \mathrm{d}H/\mathrm{d}T$) of the solution, this phenomenon is not unique to water. Hence this change in ΔC_P should not be used to identify the hydrophobic effect. Interestingly, it may, however, be the case that the sizable temperature dependence of ΔC_P itself may be a unique signature.

A simple view of how solvation is intimately tied to the shape of a solute is to consider how the number of dangling H-bonds of a solvation shell water – those that are H-bonded to a solute rather than another water – varies according to its local environment [63]. Little is known about dangling H-bonds, but the use of Raman-MCR spectroscopy, which allows the unique solvation shell water molecules around solutes to be probed, suggests that for aromatic surfaces at least, OH–π bonds are 20% weaker than H-bonds of bulk water but are more flexible. In other words, dangling H-bonds are entropically favored but less enthalpically favored than H-bonds to the bulk.

Figure 1.3 shows how the number of dangling H-bonds increases with increasing size of convex solutes and how it increases further with concave surfaces. In other words, strongly positive curvature allows water to form its full complement of water–water H-bonds, whereas the ultimate in negative curvature – the fully encapsulating sphere – forces a water to form four dangling H-bonds. The small size of water means that it is capable of finding itself in all of these situations [64].

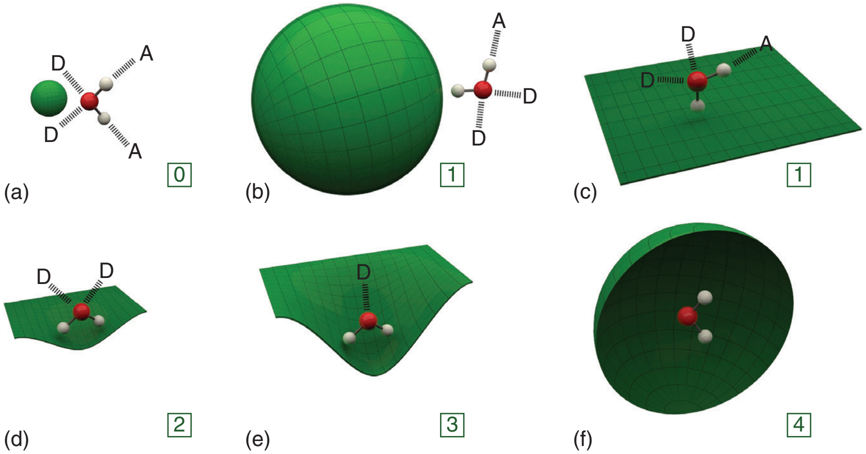

Figure 1.3 Idealized representations of the solvation of different shape solutes: small convex (a), large convex (b), flat (c), shallow concave (d), deep concave (e), and fully encapsulating (f) (half of surface removed for visualization). In each example, a water molecule is shown along with an idealized number of strong H-bonds (donor, D; acceptor, A) to other water molecules in the solvation shell of the solute. The corresponding numbers of dangling H-bonds, i.e. H-bonds to the solute, are shown in the green boxes.

To a first approximation, guest molecules are convex, and it was this shape that was the first utilized by Frank and Evans to probe the hydrophobic effect [41]. Building on Kauzmann's idea that the hydrophobic effect is dependent on the size of the solute [42], Stillinger was the first to demonstrate that small solutes are solvated differently than large ones [65]. The most successful model for describing how the hydrophobic effect changes with size is the Lum–Chandler–Weeks theory [53, 66]. This unified and general theory of solvation of small and large apolar species in water reveals that in the case of small solutes, the H-bonding network of water molecules around the solute is distorted yet complete. The result is a hydration layer denser than that seen in the bulk; the solute is said to be wetted. In these cases, it is found that ΔG_{solv} scales with the volume of the solute. With solutes of diameter ~1 nm or larger, the distortion of the H-bond network around the solvents reaches a critical point, and the network breaks. Dangling H-bonds to the solute begin to form, and as a result the cohesive forces of the water molecules are depleted. This leads to a dewetting of the surface of the solute, where the distance between the solute surface and the average water in the first solvation shell increases relative to the O⋯O distance in bulk water [53]. In such cases, ΔG_{solv} is dominated by interfacial free energetics and scales with the surface area. This wetting/dewetting transition around 1 nm in diameter leads to an entropy–enthalpy crossover. ΔG_{solv} is dominated by entropy at small solute size, but enthalpy at sizes greater that ~1 nm in diameter.

This solvation crossover has been investigated in depth computationally [67–72], but it has been hard to verify experimentally because of the low solubility of alkanes. Nevertheless, evidence of dewetting has been obtained by high-energy X-ray reflectivity measurements of the interface between water and octadecylsilane monolayers [73, 74], and evidence of a thermodynamic crossover has been obtained from single-molecule force spectroscopic studies of hydrophobic polymers [75, 76]. Furthermore, a combination of femtosecond 2D-IR spectroscopy and femtosecond polarization-resolved vibrational pump–probe spectroscopy has shown a size-dependent correlated slowing of the vibrational frequency dynamics and orientational mobility of solvation shell water molecules around nonpolar groups [77]. Relatedly, Raman-MCR spectroscopy of simple alcohols has also provided evidence of a transition, with size-dependent (and temperature-dependent) changes in solvation shells – from those with greater tetrahedral structure than bulk water to those with dangling H-bonds [78]. Similar results have also been observed with carboxylic acids and tetraalkylammoniums [79]. Intriguingly, the degree of water structure or the change in the nature of the solvation shell is dependent on whether the solubilizing group is a neutral alcohol, a negatively charged carboxylate, or a positively charged ammonium. Evidently, there is much to learn regarding how functional groups influence nonpolar group hydration, and the complications associated with introducing a charge into a guest solute are exquisitely illustrated by computational work examining the introduction of positive charge into helium-like particles with diameters ranging from 0 to 30 Å [80]. As anticipated, for the uncharged particle, ΔG_{solv} became more positive with increasing size, and this was attenuated by the introduction of positive charge. The hydrophobic to hydrophilic crossover was found to be 0.4*e*, where, interestingly, ΔG_{solv} was found to be ~0 at all diameters.

In other words, unlike hydrophobic solutes, the 0.4*e* charged solute showed no entropy–enthalpy crossover.

Zero curvature, i.e. a flat surface, represents the transition between the convexity of a guest and the concavity of a host, and not surprisingly their hydration is very similar to that of large convex solutes. For example, X-ray reflectivity measurements and MD simulations revealed significant dewetting of the surface of a crystalline monolayer of n-$C_{36}H_{74}$ and the creation of a 1.0 Å wide vacuum layer at the surface [81]. Furthermore, vibrational sum-frequency generation spectroscopy has demonstrated that D_2O near a hydrophobic surface has enhanced orientation and structure and stronger H-bond interactions relative to the D_2O/air interface consistent with dangling H-bonds and water dipoles perpendicular to the hydrophobic surface [82].

The hydration of a singular flat surface has however only tangential relation to the solvation of either a host or a guest. Of greater interest to the supramolecular community is the substantial body of computational work examining the solvation of parallel plates, which gives an inkling to the solvation of hosts. Sufficiently separated, each plate is hydrated as if in total isolation. However, as separation of the two hydrated plates decreases, so full solvation of the intervening space becomes increasingly difficult. At a critical distance, a drying transition emerges. In other words, the average density of water is found to be lower than the bulk. If water molecules are counted as a function of time, the intervening space is found to oscillate between empty and fully solvated and/or be occupied by an intermediate state of partial solvation. Finally, at a critical distance, the space between the plates fully dries, and hydrophobic collapse occurs. It has been found that this critical separation distance between two nonpolar plates is linearly proportional to their interfacial area [83]. For plates of 2 nm in diameter, the critical distance is ~1 nm. Furthermore, in work supporting this general finding, it was found that plates of area of $1\,\text{nm}^2$ only lead to drying transitions at a distance of less than 0.9 nm [84].

Phase transitions in intervening water between plates have also been observed computationally [85]. For example, changing the distance between nonpolar surfaces revealed a liquid–solid phase transition in which the solid phase has a bilayer amorphous structure and a fully connected H-bond network. This freezing is observed at a separation distance of ~1 nm and was found to be entropically unfavorable but enthalpically favored. Similarly, MD simulations of water confined between pairs of nonpolar or polar nanoscale plates revealed the effects of pressure on the hydration of the intervening space [86]. When water was confined between the nonpolar plates, capillary evaporation occurs between the plates at low pressure, with the smaller separations between plates eliciting drying at higher pressures. Furthermore, at select distances and pressure, the intervening water crystallized into a bilayer ice structure. In contrast, water confined by hydrophilic plates remained in the liquid phase at all pressures and distances studied. Interestingly, it has also been observed that nonpolar/polar patterning on plates plays a fundamental role on inert-plate hydration [87]. For example, an analysis of five pairs of plates containing equal amounts of nonpolar and polar groups arranged in different patterns revealed both qualitative and quantitative differences between them. Thus, if all the nonpolar area on each plate was grouped in the center, dewetting between the plates was observed. However, if the same area of nonpolarity was spaced out evenly on the surface, no dewetting at the same plate separation was seen. In pairing plates that were polar and nonpolar, respectively, it was the latter that was found to dominate wetting/dewetting. Overall there is some ambiguity with the study of parallel plates, in large part because of the water models and precise protocols used. However, it is evident that the larger the surface areas of the plates, the larger the critical distance before drying transitions and/or water structure changes are observed and that in all cases at some minimal critical distance, complete dewetting occurs.

The hydration of concave (negatively curved) surfaces (Figure 1.3) is where the exceedingly small size of water and its ability to solvate the tightest of surfaces comes to the fore. This is the domain of water-soluble hosts; however the most detailed molecular-level understanding of such surfaces arguably comes from *in silico* studies of carbon nanotubes. Solvation of the inner bore of nanotubes is similar to that seen with parallel plates, but their negative curvature often results in more extreme examples of structured water or dewetting. For example, with narrow carbon nanotubes of ~8 Å diameter, water wires are observed in which each water molecule has ~2 dangling H-bonds [88, 89]. As a result of the limited H-bonding between these molecules, only a small reduction in the van der Waals attraction between water and tube is sufficient to induce complete dewetting. Water flow through such nanotubes has been shown to occur in bursts and is limited only by the barriers of entry and egress. In other words, the tube itself is essentially frictionless, and the flow rate is nearly independent of its length [90]. Counterintuitively perhaps, it has been determined that as the diameter of the bore is increased, water transport rates decrease. This has been attributed to increased H-bonding between water molecules [91].

In general, with slightly wider nanotubes, phase transitions in the occupying water are observed [92, 93]. Thus, widening a tube can convert bore water from a gas-like state to an ice-like state or even to stacked layers of pentagons or hexagons [94, 95]. It is thus perhaps not surprising that there is evidently a nonmonotonic relationship between diameter and the relative ΔG of filling. Thus, whether or not this filling is dominated by enthalpy or entropy depends on the bore diameter; solvation of 1.1–1.2 nm tubes was observed to be dominated by enthalpy, while solvation of smaller or larger bores was slightly dominated by entropy. Furthermore, with the exception of 1.1–1.2 nm nanotubes, confinement within the carbon nanotubes leads to an increase in the translational entropy of bound water. Again, however, these sorts of effects are model dependent.

Relatedly, *in silico* studies have also been carried out on wholly artificial hosts composed of homogeneous nonpolar surfaces. Thus, in early work Monte Carlo simulations of the thermodynamic stability of water clusters inside smooth graphene-like spherical cavities and the fullerenes C_{140} and C_{180} revealed thermodynamically stable water clusters composed of 3–9 water molecules [96]. The smallest stable water cluster, observed in a 1 nm diameter spherical cavity, was a trimer held together by three H-bonds. With a slightly smaller diameter of 0.9 nm, a thermodynamically unstable dimer was observed.

By way of another example, 0.8 nm diameter hemispherical pockets "carved" out of neutral particles aligned in a hexagonal close-packed grid have also been studied. These pockets were shown to have average water densities lower than the bulk as a result of fluctuations between empty and filled states [97]. The 0.8 nm diameter pocket was deemed close to a critical size for promoting transitions between gas-like and liquid-like phases. Further work comparing this cavity size with a smaller 0.5 nm diameter pocket revealed that although both possessed vapor-like regions of reduced water density, the smaller pocket possessed a substantial desolvation barrier, whereas the 0.8 nm cavity did not [98, 99]. A more detailed study estimated ΔG°, ΔH°, and $T\Delta S^\circ$ along a concavity guest binding trajectory as a function of the charge of the host and guest [100, 101]. This revealed a range of thermodynamic signatures in which water enthalpic or entropic contributions drove cavity guest binding or rejection. For example, the binding of a neutral guest to a neutral host was driven by enthalpy but was entropically penalized. Overall, bound water molecules with dangling H-bonds were attributed to complexation. The same groups calculated changes in the 1D- and 2D-IR spectra during guest binding, suggesting a strategic route to correlating changes in water structure with identifying signature vibrational spectra. Collaborating earlier work from the Rick group with a cavitand host [102], the wholly artificial pockets exhibited solvated–desolvated oscillations, the magnitude and timescale of which were modulated by an approaching guest [103]. These results suggest that nonpolar guest binding to concavity does not necessarily require a dissociative (S_N1-like) mechanism, but rather can follow a triggered dissociative mechanism in which the pocket spontaneously evacuates on the approach of the guest.

As will be discussed in Chapter 2, the supramolecular community has generated a multitude of host families possessing concavity (negative curvature) for guest binding. The diversity within this family, combined with the synthetic prowess of supramolecular chemists, offers countless opportunities to probe the hydrophobic effect (and the Hofmeister effect; *vide infra*). As should be apparent, there are still many open questions pertaining to the driving forces behind guest complexation in water. However, many fundamental points are well understood. First, although small, water is not a good competitor for a host pocket, and hence guest binding is enhanced relative to organic solvent [62] (recall the hydrophobic effect is relative to other solvents, not the gas phase). It is also well established what the key role of a water-soluble host is to template cavitation in water. Consider, for example, how difficult it is to make a cavity in water, say, for a 10 (non-hydrogen)-atom molecule of volume ~300 Å^3. Revised scaled particle theory calculations [104, 105], combined with an analytical equation of state for cavity formation derived from experimental and simulation results [46], reveal that the free energy of formation of a ~300 Å^3 cavity is ~100 kJ mol^{-1}. This is a virtually unsurmountable energy requirement for the non-covalent interactions formed in a host–guest complex to counter. However, as Rick has shown, the free energy of desolvation of such a cavity in a host is only ~20 kJ mol^{-1} [102]. This idea was suggested many years ago by Bender and coworkers [106] and has also been recently observed in cucurbiturils [107–109]. Thus, to paraphrase Cram, the concave structure of water-soluble hosts prepays the free energy costs to promote water cavitation. In other words, the primary role of a water-soluble host is the energetically reasonable formation of nothingness in water. Hosts template water cavitation.

How do enthalpy and entropy factor in to this templation of a cavity? Figure 1.3 tacitly states a partial answer to this: while the hydration of small convex solutes is entropically costly, the solvation of concavity is enthalpically expensive. As just alluded to, the idea that it is enthalpically costly to solvate concavity was first expressed by Bender in 1967, who stated that "water molecules in the cavity cannot form their full complement of H-bonds as a result of steric restrictions" [106]. Emerging from this has been the idea of "high-energy" water inside the hosts [107–109] and that this is responsible for what has been called the "nonclassical hydrophobic effect." However, caution is warranted here. First is with regard to the two points of nomenclature. As previously mentioned, the extreme temperature dependence of ΔH and $T\Delta S$ means that the terms "classical" and "nonclassical" hydrophobic effects are rather arbitrary; the knife-edge hydration of nonpolar molecules means that a spontaneous complexation event can switch from enthalpically promoted to entropically promoted with only a small change in temperature (or even within replications of a single experiment). Parenthetically, if associations dominated by enthalpy are termed "nonclassical" and those dominated by entropy "classical," what are complexation events driven by both to be called? The second nomenclature point is that the term "high energy" means different things to different chemists. Unsurprisingly, there are examples in the literature where "high energy" is used in a free energetic sense and an enthalpic sense. Hence, attributing the "nonclassical hydrophobic effect" to "high-energy water" is imprecise and unhelpful.

There are also fundamental problems with the concept of "high-energy water." For example, since the chemical potentials or partial molar Gibbs free energy of all water molecules in an equilibrated system are necessarily the same, using the term in the context of the standard definition of free energy is misleading. Furthermore, there are issues that the field has yet to deal with even if "high energy" is strictly used to mean "high enthalpy." Thus, while in the most general terms the solvation of a convex guest is entropically penalized and the solvation of a concave host is enthalpically penalized, a key open question is how these two factors combine when a concave host and convex guest are desolvated to form a complex. At first glance it would seem that as host–guest complexations are mostly exothermic, that concavity desolvation dominates over convexity desolvation. However, this has yet to be determined unequivocally.

Furthermore, what of entropy? The term "high-energy water" sidesteps how entropy factors in. Do hosts also promote cavity formation in water by organizing bound water exceptionally well? Water binding to a pocket reduces its entropy of translation, but, being unable to form a full complement of H-bonds, a bound water also possesses a countering increase in its entropy of rotation. How these factors contribute to the overall entropy of binding is still unclear.

For a simple cavity in water, enthalpy and entropy contribute to the overall free energy cost in a size- and temperature-dependent manner [46, 104, 105].

Unfortunately, what we know about cavity formation in water is not likely to directly pertain to what happens in the cavity of a host. All it does is serve as a warning as to how complex host pocket desolvation is likely to be. There is much to learn about how enthalpy and entropy combine to affect cavity solvation and how this changes as a function of cavity size.

The complexity of thermodynamic analyses aside, hosts do promote cavitation in water. Thus, until a clearer thermodynamic picture of the solvation of concave hosts is forthcoming, discussions of cavitation are arguably best described in the context of drying transitions. Along these lines, mapping the water occupancy of different cavities, for example, with software such as WaterMap [110], which uses explicit water MD simulations and inhomogeneous solvation theory [111] to calculate the enthalpy, entropy, and free energy of water molecules within a solvated binding site relative to bulk water [112], may be helpful to begin to understand how cavity shape affects solvation and hence guest binding.

In summary, the fundamentals of the hydrophobic effect are exceedingly complex. There is much to learn about the factors that control the solvation thermodynamics of both hosts and guests and the spectroscopic properties of their solvation shells. But there are also significant opportunities to take what is currently known about water and the hydration of nonpolar (and polar) molecules and apply it to understanding and engineering host–guest complexation and assembly processes.

1.5.2 The Hofmeister Effect

In reference to Figure 1.2, the second prominent phenomenon observed in aqueous solution is the Hofmeister effect [113–116]. All life as we know it involves salty solutions, and therefore understanding how salts interact with water and how they interact with other solutes is key to a myriad of different sciences. For example, a general understanding of the supramolecular properties of ions would improve computational modeling and hence reduce costs and speed up ligand identification in computer-aided drug discovery [117]. Similarly, an improved understanding of the supramolecular properties of chloride ions would improve our understanding of ion transport and diseases such as cystic fibrosis [118].

The Hofmeister effect was first reported between 1887 and 1898 in a series of papers published by Franz Hofmeister, the most important one of which was titled *Concerning regularities in the protein-precipitating effects of salts and the relationship of these effects to the physiological behavior of salts* [119, 120]. This paper described the ordering of various salts according to their ability to precipitate egg white proteins from aqueous solutions. The salts used had either a common cation or anion and therefore allowed for the ready separation of cationic or anionic effects. This series of ions is now known as the lyotropic or Hofmeister series, but in truth there is no reason to limit studies to salts from Hofmeister's original selection; Hofmeister's choice of salts was based on availability, not on what can and what cannot induce the Hofmeister effect.

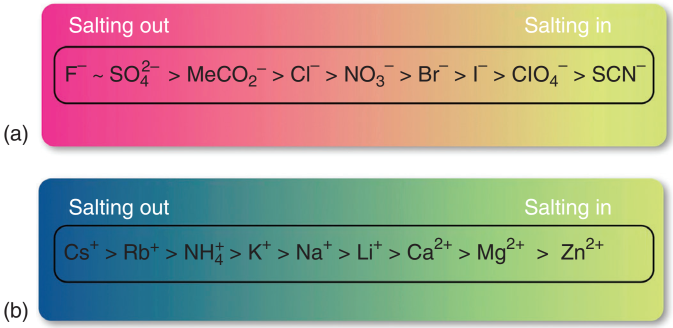

Figure 1.4 Typical ordering of anions (a) and cation (b) in the Hofmeister series.

Subsequent to Hofmeister's initial discovery, an extensive body of work has been built up. What has intrigued so many scientists is that irrespective of the dependent variable being probed in an experiment, an ordinal sequence such as that shown in Figure 1.4 is observed. Every conceivable bulk property of water or aqueous solution has been investigated at some time, but arguably the most important (or prominent) experiments concerned how salts affected proteins or other biomacromolecules. Hence salting-out salts usually induce the precipitation of a protein, while salting-in salts will typically increase its solubility. In other words, species such as F^- appears to increase the hydrophobic effect, while I^- appears to weaken it.

Studies probing the effects of cations vs. anions reveal that the anionic Hofmeister effect is more prominent. As a result, the reproducibility of the anionic Hofmeister series in Figure 1.4 is much stronger. There are three important reasons as to why this is so. First, there are far more inorganic anions than metal cations. Second, in general anions are larger than cations; the second largest monovalent metal cation, Cs^+, is approximately the same size as the second smallest halide Cl^-. On average, this larger size leads to a greater charge diffusivity, a weaker hydration shell (though importantly, not a lower free energy of hydration), and the possibility of closer non-covalent interactions with other solutes. These points mean that not only are the Hofmeister effects stronger with anions, but also by virtue of sheer numbers, they are more prevalent. A third factor behind the prominence of anionic Hofmeister effects is that biomacromolecules are by far the number one target in Hofmeister studies, and in these the negatively charged groups (carboxylates, phosphates, and sulfates) tend to be more strongly solvated than their complementary cationic groups (primarily ammonium and guanidinium). As a result, when a salt is added to the solution of a solute, it is the anion that is more likely to affect it.

The model Hofmeister formulated to account for these observations was based on the theory of electrolytic dissociation developed by Arrhenius and Ostwald, and tried to link the observed ordering of the ions with their strength of hydration or, as it was known at the time, their water-absorbing effects. The implication was that a salt either "pulled" water from the hydration shell of a protein or it did not. Over time, this idea morphed into the theory of salts being either water structure makers or water structure breakers, and key to this development was that, due to technological restraints at the time, researchers were limited to studying bulk phenomena. Thus, the seminal work of Jones and Dole measuring the viscosity of solutions of strong electrolytes culminated in what is now called the Dole–Jones equation, an expression describing the relationship between the viscosity of a solution and the concentration of solute [121]. Extending this work (and that of Bernal and Fowler describing water structure [26]), Cox and Wolfenden subsequently observed that the sign of the coefficient characterizing solute–solvent interactions in the Dole–Jones equation (the β-coefficient) is correlated with the temperature coefficient of the electrical mobility of an ion [122]. Furthermore, they logically tied the mobility of an ion to the local viscosity of the water molecules in its solvation shell and formulated the idea that ions with positive (negative) β-coefficients "polymerized" ("depolymerized") the water. This idea was expanded upon by Gurney, who discussed ions as bringing local order or disorder to water [123]. Interestingly, Gurney mostly used the wording "local order or disorder" in the early pages of his text but in the latter sections tended to drop "local" and just discuss the effects of ions as inducing order or disorder. This was perhaps an omen. For many subsequent years, the idea that ions are either water structure makers or breakers became dogma, and it has only been in the last three decades or so that new developments in spectroscopic, computational, and macromolecular design strategies have downgraded the role of water-mediated ion effects on proteins (*vide infra*) [114].

But before going into the Hofmeister effect further, it is important to highlight two heavily utilized terms in the field: "kosmotrope" [124] and "chaotrope" [125]. Originally these terms were coined to describe the effect ions had on biomacromolecular structure. Thus, salting-out salts were called kosmotropes (from the Greek noun *kosmos* or order) because they were noted to stabilize the fold of proteins. In contrast, salting-in salts, or chaotropes, were noted to destabilize the fold of a protein and hence bring chaos. However, these terms have gone through a linguistic shift in their use and meaning and have subsequently become synonymous with the idea of water structure making and breaking [113]. Like the hydrophobic effect these terms have come to be used *deus ex machina* to describe the effects of salts. This is an unfortunate state of affairs for, as we will discuss, there is increasing evidence that at moderate concentration salts do no greatly modify the structure of water. Hence, describing salts as kosmotropes/water structure makers or chaotropes/water structure breakers is ambiguous at best and quite possibly wholly incorrect. Avoiding these issues, terms such as "salting in" and "salting out" have become more popular (although they too have issues; *vide infra*).

So, what are the roots of the Hofmeister effect? Our current understanding of salting-out salts is relatively straightforward. Thus a 5 M solution of $NaSO_4$ requires so many water molecules of solvation that there is insufficient water left to solubilize the protein. Hence it is precipitated from solution. On the other hand, salting-in salts are now thought to directly interact with a protein to weaken its fold and apparently attenuate the hydrophobic effect [114] and can do so at much lower concentrations, e.g. 2 mM in the case of a synthetic host–guest pair [126]. There are two reasons why direct ion–solute interactions are now favored over water-mediated interactions. First, with improvements in spectroscopy and calorimetry, it is now evident that salts do not influence the structure of bulk water. For example, dielectric relaxation data show that there are six slow water molecules hydrating the Mg^{2+} ion in $Mg(ClO_4)_2$, one slow water molecule associated with hydrating SO_4^{2-} in Cs_2SO_4, but eighteen slow water molecules hydrating the ions of $MgSO_4$; there is synergy in ion pairing of divalent ions. Importantly, however, there is no evidence that this synergy extends beyond the solvation shell [127–129]. Similarly, Raman spectroscopy and Monte Carlo simulations have determined that an ion exerts little influence beyond this solvation shell [130], while a combination of vibrational sum-frequency spectroscopy and surface potential measurements upon a monolayer has done likewise [131]. Finally, pressure perturbation calorimetry, which measures the heat transfer resulting from a pressure change above the sample solution, has failed to reveal bulk changes to water structure upon the addition of salts [132]. Briefly, if bulk water structure is changed by a salt, the sign of how the heat capacity (C_P) changes as a function of pressure (P) at constant temperature $(\partial C_P/\partial P)_T$ should correlate with whether a salt is a water structure maker (negative) or a water structure breaker (positive). However, no such correlation has been found. Taken together, these results suggest that the salting-in Hofmeister effect does not involved significant water-mediated effects and therefore must involve direct ion–macromolecule interactions [133].

So, if anions do not influence solutes through water, where do anions bind in proteins? NMR, thermodynamic, and MD studies of an uncharged 600-residue elastin-like polypeptide, $(VPGVG)_{120}$, found that weakly hydrated anions complex primarily to binding sites composed of an amide nitrogen atom and an adjacent α-carbon atom [134]. In addition, weaker anion association to the nonpolar side chains of the polypeptide was also observed. Similar affinities of weakly hydrated anions have also been seen at the liquid water surface, frequently used as a surrogate for nonpolar surfaces [135]. Furthermore, such nonpolar to anion interactions have been quantified using NMR and ITC in a host–guest system [44, 136]. These studies revealed significant affinity of these types of anions for nonpolar concavity, as well as a salting-out-type effect induced by cation complexation to solubilizing carboxylate groups.

All of this data points to the salting-in phenomenon of the Hofmeister effect being engendered by the weak solvation of anions allowing them to interact with nonpolar surfaces. There are likely equivalent (but weaker) interactions involving cations, but these have not been explored to nearly the same extent. Key to progress here is most certainly a thorough understanding of the hydration of ions. To highlight just one example, even if the free energy of hydration data available in the literature was comprehensive (it is not), it would not be sufficient. What is needed is a thorough understanding of the “plasticity” of a solvation shell, i.e. how energetically feasible is it to push some water molecules in the hydration shell of an ion aside and allow non-covalent interactions with other solutes. Only once this has been determined can the supramolecular properties of an ion be more thoroughly explained.

1.5.3 The Reverse Hofmeister Effect

Weakly solvated anions can also induce the reverse Hofmeister effect, and, not surprisingly, this phenomenon can also teach us much about living systems. For example, the reverse Hofmeister effect has been linked to protein deposition in the cardiovascular system and thrombosis [137], as well as Alzheimer's [138] and Parkinson's disease [139].

As depicted in Figure 1.2, whereas weakly hydrated ion interactions with nonpolar groups induce the salting-in phenomena, the same anions can induce the reverse Hofmeister effect by ion pairing. There is an important ramification associated with this point. Namely, the existence of the reverse Hofmeister effect means that the Hofmeister effect in general can *only* readily be explained by direct ion–solute interactions; it is hard to reconcile how a salt might be a water structure breaker in the presence of one protein, but a water structure maker in the presence of another. The reverse Hofmeister effect also emphasizes a problem with the salt-in/salt-out nomenclature, viz that salts such as NaI can be both.

The most prominent example of the reverse Hofmeister effect is the enzyme lysozyme [140–142]. The precipitation of lysozyme follows the Hofmeister series at high pH or high ionic strength, but under neutral and acidic conditions, when most/all acidic and basic groups are protonated, there is an apparent reversal of the Hofmeister series [143, 144]. In other words, weakly hydrated anions salt out lysozyme better than strongly hydrated ones. NMR and MD studies with tripeptide models have shown that the reverse Hofmeister effect in proteins such as lysozyme is rooted in charge neutralization [145]. In other words, weakly solvated anions can closely associate with positive charges on a protein and induce precipitation. This has ramifications for the one third of the proteome consisting of proteins with a $pI > 7$ [146].

It is useful to note here the relationship between the reverse Hofmeister effect and the solubility of small ammonium compounds in water. Thus, it is well known that to make an ammonium salt more water soluble, the chloride (Cl^-) salt might be formed, whereas if organic solvent solubility is required, the perchlorate (ClO_4^-) salt might be targeted. Organic chemists have been content with this rule of thumb and have therefore not investigated this phenomenon further. However, it seems that the study of the solubility of small ammonium ions in water could lead to a new level of understanding of the reverse Hofmeister effect in biomacromolecules.

The latency of the reverse Hofmeister effect is apparent even with simple salts [127]. Thus, dielectric relaxation spectroscopy, far-infrared (terahertz) absorption spectroscopy, femtosecond mid-infrared spectroscopy, and X-ray spectroscopy and scattering, as well as MD simulations, have all revealed that it is inappropriate to think of salts forming statistical mixtures of fully hydrated ions [127]. Rather, ion pairing and ion clustering are in fact common. For example, even monovalent salts such as alkali halides or carboxylates form solvent-separated ion pairs ($M^+\cdots H_2O\cdots H_2O\cdots A^-$), solvent-shared ion pairs ($M^+\cdots H_2O\cdots A^-$), and, to a small extent, contact ion pairs ($M^+\cdots A^-$). Population distributions are shifted toward contact ion pairs in the case of higher valency ions, but unfortunately, we have not yet formed a thorough understanding of how ion specific these distribution profiles are, nor the extent of specific ion-pairing effects.

More importantly, weakly hydrated anions such as thiocyanate (SCN^-) and *tetra-n*-alkylammoniums (R_4N^+) can form clusters or aggregates. For example, in 1 M solutions of KSCN, about 20–30% of anions are in clusters of an average size of two to three (with an approximately equal number of cations) [147]. It is therefore now apparent that aggregates involving CsSCN or Me_4NSCN, or those involving more polarizable anions, would likely demonstrate larger aggregates or aggregates at lower concentrations. Along these lines of thought, recent studies of a host–guest system revealed specific anion binding sites and affinities in the host and allowed anion binding to be traced to aggregation and ultimately (at high enough concentration) precipitation of the host and its complexes [126].

Some time ago, Collins formulated what he called the law of matching water affinities [148], a conceptual framework that states that the charge density of an ion is the important physical variable for specificity of ion pairing. Thus, Collins used hydration enthalpies [149] to build a rule of thumb that states the free energy of ion pairing tends to be more favorable if the anion and cation are of similar size, rather than if one ion is small and hard, while the other is large and polarizable. This rule of thumb has been difficult to unequivocally verify, and much work still needs to be carried out. Undoubtedly, an expanded and stronger framework would have to take into account findings from reverse Hofmeister effect studies. There is still much to learn here.

1.6 Conclusions and Future Work

The three effects, the hydrophobic effect, the Hofmeister effect, and the reverse Hofmeister effect, are intrinsically linked to each other by relatively weak solvation of solutes and the high cohesiveness of water. In studying the hydrophobic effect, scientists look to describe the attraction between nonpolar molecules in an aqueous environment. In contrast, investigating the salting-in Hofmeister effect turns the attention toward "greasy" polarizable ions interacting with similarly greasy neutrals. Evidence points to anions being more important, but "greasy" cations have not been investigated as thoroughly. Finally, the focus of the reverse Hofmeister effects concerns how the same "greasy" anions and cations associate with each other. Arguably, a significant handicap impeding progress on all three fronts has been the tendency to investigate structurally complex macromolecules such as proteins. It was the proteinaceous world that first identified the Hofmeister effects, and proteins have also been a staple of examining the hydrophobic effect, but as all three effects may or may not be present in one protein, and as these phenomena often counter one another, the big picture has been exceedingly difficult to visualize (let alone understand).

The supramolecular community has much to offer here. Reductionist philosophies have their role to play in science, and from the authors' perspective, it is the reductionist viewpoint of supramolecular chemists that is needed to move aqueous supramolecular chemistry forward. They cannot do so alone of course; the water community – a diverse group of researchers from many different branches of science – is the water expert. But it is the supramolecular chemistry community who has a broad set of tools to offer the water community, tools, it should be added, that are mostly brand new to majority of the latter. It is up to individual scientists, both within the water and supramolecular communities, to decide how they should reach out across the divide and bridge these two areas [28]. But build bridges they must.

There are obviously countless possibilities here, but one anecdotal example familiar to the authors may be illustrative. Approximately one quarter of the average ~\$2.6 billion needed to bring a new drug to market arises from preclinical studies to identify ligands with suitable drug-like properties and receptor affinities. Consequently, improving ligand identification is key. The Drug Design Data Resource (D3R; https://drugdesigndata.org/) aims to "advance the technology of computer-aided drug discovery through the interchange of high quality protein–ligand datasets and workflows, and by holding community-wide, blinded prediction challenges." These blind challenges with proteins are, to say the least, challenging. Hence, one relatively new component of the D3R is the Statistical Assessment of the Modelling of Proteins and Ligands (SAMPL) [150]. The main thrust of the annual SAMPL exercise consists of blind challenges in which a supramolecular chemistry group collects but does not disseminate thermodynamic data for host–guest complexation targets, while computational groups attempt to calculate the host–guest affinities *a priori*. The ultimate release of the empirical data allows computational chemists to evaluate the success of their latest strategies. By such endeavors, the D3R hopes to ultimately improve ligand affinity predictions such that rapid and cheap computer calculations can replace the majority of early lead screening and syntheses from the drug development pipeline. The annual SAMPL exercise is but one example of how the two communities can create new knowledge that would otherwise be hard to obtain. By such endeavors science gains much better control of the aqueous world while simultaneously pushing human knowledge further into unknown territories.

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