Collaborative routes to clarifying the murky waters of aqueous supramolecular chemistry

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On planet Earth, water is everywhere: the majority of the surface is covered with it; it is a key component of all life; its vapour and droplets fill the lower atmosphere; and even rocks contain it and undergo geomorphological changes because of it. A community of physical scientists largely drives studies of the chemistry of water and aqueous solutions, with expertise in biochemistry, spectroscopy and computer modelling. More recently, however, supramolecular chemists — with their expertise in macrocyclic synthesis and measuring supramolecular interactions — have renewed their interest in water-mediated non-covalent interactions. These two groups offer complementary expertise that, if harnessed, offer to accelerate our understanding of aqueous supramolecular chemistry and water writ large. This Review summarizes the state-of-the-art of the two fields, and highlights where there is latent chemical space for collaborative exploration by the two groups.

Water is ubiquitous and essential to life on planet Earth. A full understanding of aqueous solutions is therefore of significance to a wide range of fields within the atmospheric, environmental, biological and geological sciences. Within the chemical sciences, a deeper appreciation of how non-covalent interactions and chemical transformations are influenced by water would benefit a variety of fields. However, as we highlight here, there are many open questions regarding the chemical and physical properties of aqueous solutions.

The aqueous realm is frequently bifurcated into the Hofmeister and hydrophobic effects, phenomena that respectively deal with the properties of solutions of salts and relatively non-polar molecules. However, it is becoming increasingly evident that these areas do in fact frequently overlap; two prime examples being the accumulation of large polarizable anions at the air–water interface[1-5], and the favourable interactions of polarizable anions with non-polar surfaces[6-15]. Thus the hydrophobic and Hofmeister effects are but part of a greater continuum of aqueous supramolecular chemistry, with many important and outstanding questions regarding the influence of the different kinds of non-covalent interactions involved.

Studies of water and aqueous solutions have mostly been driven by the physical sciences community, a broad range of scientists whose expertise in spectroscopy, computer modelling and biochemistry (to name just three areas) has generally not involved macrocycles or host molecules. However, for some time now, supramolecular chemists — with their expertise in macrocyclic synthesis and measuring weak non-covalent interactions — have been travelling a parallel course of exploration. This Review, inspired by discussions between sixteen members of each community at a recent workshop[16], is an attempt to summarize what we know about aqueous solutions, what we do not know about them, and where the two communities might find common ground for productive collaboration. In writing this Review the authors acknowledge that, as a distillation and translation of the thoughts of 32 minds, it cannot cover all of the salient literature.

A word about water

Water is polar (1.85 D, ε = 78) and, according to its Kamlet–Taft solvent parameters, a strong hydrogen bond (HB) donor (α = 1.17) and a good HB acceptor (β = 0.47). Through the combination of its permanent dipole, electron-deficient hydrogens and lone pairs, water forms strong electrostatic interactions with other waters and ions, but weaker interactions with less polar solutes.

In the crystalline solid state, each water molecule makes four HBs with its neighbours; however, in the liquid state near room temperature there are only ~3.6 HBs on average; the actual number varying according to exogenous factors and the specific diagnostic technique. Both theory and experiment have established that in the gas phase these HBs enable minimum-energy water clusters composed of 2–10 waters[17-20]. However, it is clear from many simulations that these structures do not persist in room temperature liquid water. The prevailing picture is that in the liquid state water possesses primarily tetrahedral structure[21,22]; however, a more controversial interpretation from X-ray absorption spectroscopy (XAS) and non-resonant X-ray Raman scattering (XRS) is that chains and rings are more dominant than cages[23]. Hence, the vague but enduring term of 'flickering clusters' is frequently used to describe water structure.

The hydrophobic effect

The hydrophobic effect[24-27] is a water-mediated phenomenon that is probably best not thought of as a force, despite the fact that it does result in effective attractions between molecules and between macroscopic objects. Rather, the hydrophobic effect is a self-sorting phenomenon; when water is unable to make sufficiently strong interactions with a solute, it prefers to associate with other water molecules (and non-polar solutes consequently also self-associate). Ultimately, this effect can be driven by entropy, enthalpy or both, and is dependent on the nature of the solute (size, shape, polarity) and the relative strengths of the resulting solute–solvent, solute–solute and solvent–solvent interactions. Thus the term 'hydrophobic effect' covers a wide variety of phenomena. The ubiquity, variety and complexity of the hydrophobic effect lies at the root of many misused terms and inappropriate nomenclature (Box 1)[28].

A partial list of factors affecting the hydrophobic effect

Factors influencing the hydrophobic effect include: (1) temperature — depending on how this affects enthalpy and entropy, binding and

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assembly can be enhanced, diminished or unaffected. The deeper reasons for these dependencies are still unclear; (2) ionic strength — it is becoming increasingly apparent that relatively polarizable anions have an affinity for non-polar surfaces[15]. Thus, both the ionic strength and the nature of the electrolyte are important in shaping the hydrophobic effect; (3) solute shape — from computational and experimental studies, it is evident that solvation of convex, flat and concave surfaces are different[29–32]. For example, within a concave surface, waters cannot make as many contacts with other waters as they can at more open surfaces (Fig. 1); (4) solute composition — even when focusing on simple non-polar solutes like hexane and benzene, there are a wide variety of polarizabilities and propensities to form van der Waals interactions. What lies behind the stronger K_{dimer} for cyclohexane versus benzene? Polarizability? Water–π hydrogen bonding? The answer is again unclear.

Host molecules as tools for probing aqueous solutions

Supramolecular chemists have generated many families of hosts that possess negative curvature (concavity) and theoretically could provide an entirely new perspective on aqueous solutions. The list of available water-soluble hosts includes (but is not limited to): cyclodextrins[33], cucurbiturils[34] and bambusurils[35]; cyclophanes[36,37] such as pillarenes[38] and cavitands; the related calixarenes; and cyclotriveratrylenes (Fig. 2)[39]. In addition, there are several classes of host that utilize self-assembly to form water-soluble hosts[40–42], as well as 'foldamers' that, like proteins, are predisposed to fold into a conformation possessing a site for guest recognition[43–45]. These hosts provide a great opportunity for the physical community. In addition to providing these, supramolecular chemists can also contribute by designing new families of hosts. This includes the development of new macrocyclization processes — syntheses that take advantage of a wider diversity of building blocks for greater control of functionality, polarity, polarizability and symmetry. The physical community should be mindful though of the difficulties supramolecular chemists face. So far it has proven difficult to synthesize low dielectric binding sites possessing inward-pointing functionality for specific non-covalent interactions with a guest[46], so supramolecular chemists are some way from tailored pockets that resemble enzyme active sites. In part this can be attributed to the relatively small number of macrocyclizations that efficiently form hosts possessing sizable cavities, and a reliance on building blocks possessing rings that engender pre-organized structures that enable clearer connections between structure and function. Foldamers are one potential solution, and progress has been made in a few instances towards protein-like functionality[47,48]. Regarding host design, three points that the workshop frequently returned to are detailed below.

The importance of curvature. For the physical community there is a specific need for systematic experimental studies testing how pocket wettability (see below) is controlled by shape[28,30,31,49]. This issue has been studied computationally to a reasonable extent, but very few systems — primarily cyclodextrins, cucurbiturils and deep-cavity cavitands — have been probed experimentally, and only cyclodextrins in depth[50,51]. These studies reveal a tantalizing picture of how small structural changes in a host can lead to large changes in the thermodynamics of ligand/guest binding. However, much more work is needed in this area[52].

How important is pre-organization? Enzymes are both pre-organized and flexible. The first is key for high affinity, the second for selectivity and catalysis. Both may be key to controlling the solvation of a pocket. By contrast, synthetic hosts are generally pre-organized and not very flexible. A lack of flexibility means that a system cannot account for the distance and angle sensitivity of non-covalent forces. Thus, they cannot orchestrate these forces in the way

- The terms 'hydrophobic interactions' and 'hydrophobic forces' should ideally be eliminated from our vocabulary. The interactions underlying the hydrophobic effect, such as hydrogen bonding and dispersion interactions, are not unique to it. Describing it as a unique force suggests, incorrectly, that it is distinct from these (or other) interactions. The hydrophobic effect may drive association, but it should be called by that name, rather than labelled as a force.

- Labelling particular instances of the hydrophobic effect as classical (entropy favoured and dominated) or non-classical (enthalpy favoured and dominated) is not helpful. These obfuscating terms ignore the possibility of an exergonic association being driven by both factors. Additionally, this classical/non-classical bifurcation ignores the complex dependence of the thermodynamics on exogenous factors such as temperature. It is therefore best for the observed thermodynamic profiles to be stated specifically. For example: 'host and guest associate as a consequence of the hydrophobic effect with an enthalpy dominated profile'.

- The suggestion of a unique 'signature' of the hydrophobic effect is also not helpful. The two commonly discussed signatures; that dehydration of non-polar surfaces leads to: (1) an increase in entropy in the system, or; (2) a large and positive change in heat capacity, are not always true and are not unique to water. With no unique thermodynamic signature, it is recommended that the evidence in support of the hydrophobic effect be presented without resting on the weight of any single specific metric. Ultimately, it may be possible to designate a series of experimental and/or theoretical signatures to qualify and quantify the hydrophobic effect; but this is not possible at this juncture.

- The use of the term 'hydrophobic group' to describe an alkyl group is inappropriate. In chloroform, a methyl group is not 'hydrophobic', it is non-polar or apolar. The hydrophobic effect by contrast requires water to be present. For this reason, it is recommended simply that a moiety or functional group be described as non-polar.

enzymes do to optimize binding, minimize solvation or modulate a reaction coordinate. Foldamers[43–45] offer a bridge between pre-organized hosts and proteins, and the latent chemical space here is ripe for development by the two communities.

The presence of polar and ionic groups. It remains unclear, at least in many details, how the properties of hosts are affected by ionic and polar functional groups. Water strongly interacts with dissolved polar molecules (osmolytes) and salts. In the extreme case of small inorganic ions, the waters in the first hydration sphere are profoundly affected in both a thermodynamic and kinetic sense[53]. Correspondingly, the presence of charged/ionizable groups within a host most certainly affects pocket solvation and hence guest binding. Work on cyclodextrins has shown how proximal charged groups can modulate guest association[54]. However, most synthetic hosts have not been probed to the same level. Perhaps unsurprisingly, considering the difficulties with synthesizing suitable binding sites and the dearth of kinetic studies on host-guest systems, to our knowledge, no research has been carried out examining how charged groups affect the kinetics of guest binding.
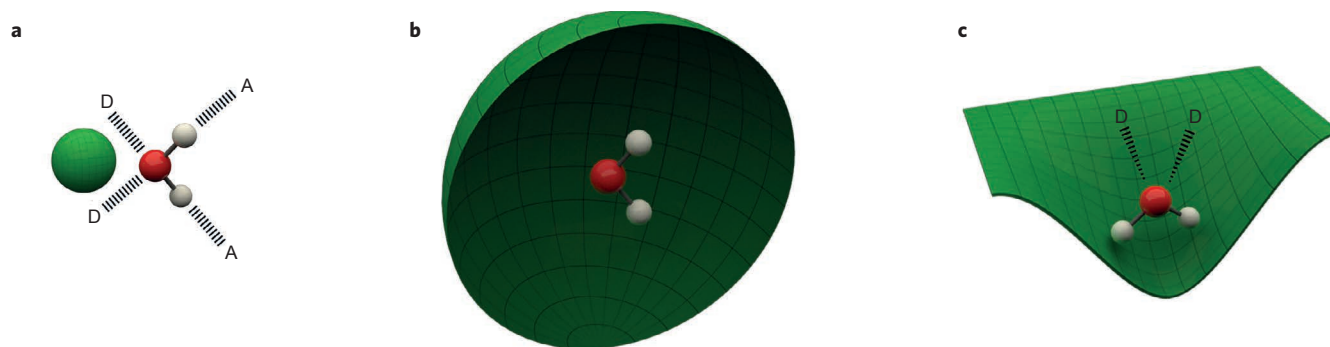
Figure 1 | Illustration of how gross shape influences solute solvation. a,b, The two extremes of solvation: small convex solutes barely disrupt hydrogen bonding between waters and a solvation shell water can accept (A) and donate (D) four hydrogen bonds (**a**); by contrast, an isolated molecule of water (**b**) cannot form any HBs with other waters. **c**, The intermediate case of solvating concavity, where any bound water only forms a limited number of HBs.

The special role of computational chemistry

The increasing role of computational chemistry in the study of aqueous solutions cannot be overstated. This is one area where the supramolecular and physical communities are beginning to overlap fruitfully. Indeed, host–guest systems are playing an important role in improving computation[55–57]; for example, through the Statistical Assessment of the Modelling of Proteins and Ligands (SAMPL) blind challenge (run in part by the Drug Design Data Resource (D3R)), which has provided an opportunity for modellers to predict the affinity of guests for proteins and synthetic hosts[52,56,57]. These challenges are extremely valuable not just to improve modelling, but also to learn how to actually gain insight; if a simulation model yields correct affinities, how is it determined which of the observed interactions are key to controlling affinity and which are incidental? Predictive modelling is helping answer this difficult question. This stated, the SAMPL exercise has only just begun to tap into the list of available hosts. Moreover, simulations directed towards kinetic studies remain uncharted, in part because of a lack of information from the supramolecular community, but also because of the computing requirements of long timescale calculations.

Other forms of collaboration between the two communities would be useful. For example, supramolecular chemists typically rely on off-the-shelf packages for modelling. There are significant issues with this, as force-fields in these packages are almost exclusively biased towards biological systems. This can mean that many non-covalent interactions may not be particularly well described. Collaborations can help here by aiding the movement of the supramolecular community away from highly detailed modelling (that is, quantum mechanics) in the gas phase to simulations in aqueous solution. Computationalists use many different water models, and which ones are best for probing supramolecular systems depends on what property is being sought. To the supramolecular community it is clear that polarization and charge transfer need to be taken into account in many cases, suggesting water models such as TIP4P-flucQ, AMOEBA, and other polarizable/charge transfer models will be needed, though it remains an open question exactly how much these effects contribute to guest binding.

The question of 'high-energy' water. Relative to the gas-phase, complexation events in water are attenuated. A solvating water molecule in a pocket is energetically (and literally) in the way of an incoming guest. In other words the hydrophobic effect is relative to other solvents, not the gas phase[24].

The extent to which water inhibits supramolecular interactions is a function of solute shape, and one important case in point is the solvation of a concavity, where the structure of the host prevents any water within it from forming its full complement of hydrogen bonds[30] (Fig. 1). Within the physical community such a concavity is usually described as being dewetted, or as possessing drying transitions; that

is, in a temporal sense the pocket fluctuates between fully hydrated and dry. There is currently considerable debate within the computational community as to what size or shape of pocket leads to a propensity to remain desolvated[31,58,59]. By contrast, supramolecular chemists have described such sites as being occupied with 'high-energy' water[60]. Care is needed here for three reasons. First, there seems to be some confusion as to whether the term refers to free energy or enthalpy. Regarding the former, the chemical potentials or partial molar Gibbs free energy of all water molecules in an equilibrated system are necessarily the same, so by this definition there is no such thing as high-energy water (although the Ben-Naim standard state Gibbs energy[13] of bound and bulk water need not be the same). Second, it is not yet possible to parse the overall enthalpy and entropy changes associated with guest binding into the many contributing factors[24,61]; the root-causes of observed guest-complexation thermodynamics are undoubtedly very complex. Third — going back to the idea of drying transitions — if water in a pocket has high energy it will only exist there transiently. How much does a high-energy water molecule that only resides in a cavity a small fraction of the time contribute to guest thermodynamics? There is much to learn here, and collaboration between the two communities is key. To pick just one possibility, some modelling tools such as GIST[62], WaterMap[63], JAWS[64] or SZMAP[63] may be useful in predicting water molecules which might be particularly easy to displace; it would be helpful to test these on established host-guest complexes to determine whether they can be predictive.

Spectroscopic/analytical techniques for aqueous solutions

The choice of analytical technique depends on many things, including the experimental assay, sensitivity, resolution, concentration requirements and time-scale. It is important to be mindful of these constraints when defining areas of mutual overlap between the two communities. The analytical expertise within the physical scientists' community covers a broad range, including: (1) spectroscopy; (2) chemometrics; (3) single molecule detection; (4) colloid and surface science; (5) mass spectrometry (MS); (6) microfluidics; and (7) electrochemistry. There are also many relatively new spectroscopies outside the supramolecular chemistry mainstream or under development by the physical community, some of which may not move from the specialized laboratory setting to becoming commercially available and/or routinely utilized. However, synthetic supramolecular systems are an excellent test-bed to assess the potential of each technique.

Two over-arching practical issues for collaboration are compound availability and solubility. A sample must be available in quantities compatible with the sensitivity of the analytical approach and the properties being probed. Very sensitive techniques including fluorescence and certain types of electrochemical measurements require little in the way of material, as do surface techniques such as

sum frequency generation spectroscopies. However, most techniques require larger sample sizes and higher concentrations, and as a rule-of-thumb, any technique that requires more sample than NMR (~500 μL at 10^{-3} M) is likely to be less appealing to the supramolecular community. Within the supramolecular community itself, analyses have been shaped by availability. The most common technique, NMR, is responsible for the field looking exclusively at the solutes, not the solvent. Moreover, this domination by NMR has limited the window of kinetic analysis. The use of other spectroscopic techniques such as UV-vis and fluorescence has helped in this regard, but the community has not embraced many of the available techniques, especially spectroscopies that probe solvation shells. In essence, supramolecular chemists have utilized the available tools (those used by synthetic chemists for characterization), not the techniques best suited for a detailed analysis of aqueous supramolecular systems (Table 1)[65–68].

Spectroscopy involving hosts and guests. It is hard to beat NMR. In large part its popularity within the supramolecular community arises from its ability to provide high-resolution details of the structural changes accompanying binding. By contrast, techniques such as UV–vis only offer limited structural information. There is of course a tradeoff here between selectivity and sensitivity. Working in the UV–vis region can provide single-molecule sensitivity with impressive temporal resolution, whereas NMR experiments require ~1 mM analyte concentrations (although longer acquisition times can allow 10 μM concentrations, and techniques such as Chemical Exchange Saturation Transfer (CEST) offer the possibility of going lower still)[69,70].

Spectroscopy involving water. Historically, supramolecular chemists have paid little attention to the role of solvent in complexation events. This is justified in many instances, but is harder to do so in water; understanding water structure may be key to interpreting the thermodynamics and kinetics of association. By contrast, the physical community can probe water in many different ways (Table 1). In addition to these, other potentially useful techniques include the optical Kerr effect, and narrow/broad-band THz/far-IR spectroscopy[71], and more established techniques such as NMR relaxation measurements using $H_2^{17}O$, electron spin resonance (ESR) and electron nuclear double resonance (ENDOR). En masse, these techniques have emphasized or raised many questions about the nature and implications of the related topics of 'highly structured' water, high X-ray occupancy and water molecules of limited translational and rotational motion. To address these questions, the two communities need to identify and create hosts with customized water solvation, for such relatively simple systems would greatly enhance the information generated by physical scientists.

Chemometrics. Chemometrics[72] — using mathematical and statistical methods to design or select optimal procedures and experiments — has two major benefits: (1) it provides a way to search within large amounts of data for relationships that are otherwise unperceivable and/or unintuitive. For example, sensing chemometrics can probe patterns of responses in a pool of receptors to obviate the need for highly selective hosts; (2) it can potentially reduce bias by telling us what is important rather than what we think is important.

The extent to which chemometrics can be used to tease out physical and structural factors contributing to the hydrophobic and Hofmeister effects is unknown at this time. Towards determining this, one of the grand challenges for the two communities is to generate reliable data stored in a form that can be universally accessed and amalgamated with existing data. Once a database is established, a systematic chemometric analysis may help in identifying important parameters behind different phenomenon. However, a 'correct answer' from such an endeavour will only come about by collecting and compiling the right information.

Single-molecule detection. Observing the statistical variation for the binding of a single molecule (versus ~10^{14} or more molecules simultaneously) has considerable power. For example, fluorescence techniques can identify clear steps in a 'simple' binding event, and can reveal details about multi-step processes. The caveats associated with fluorescence methods are that any single molecule only fluoresces some of the time (blinking) — for example, when it is bound in a particular orientation — and that it is difficult to separate the free state versus the 'bound-but-not-fluorescent' state. Moreover, excitation at suitable UV wavelengths may be challenging both from a technical perspective and in terms of photobleaching.
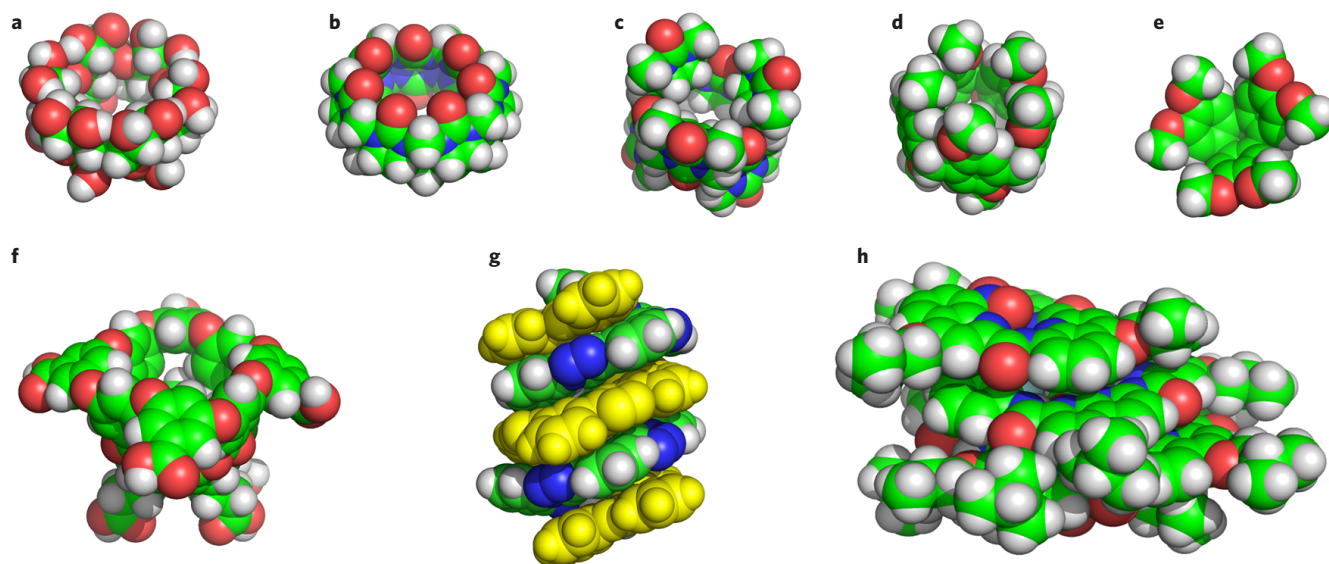

Figure 2 | Illustrative water-soluble hosts (not all shown with water-solubilizing groups) and examples of foldamers. a–h, A cyclodextrin (**a**); a cucurbituril (**b**); a bambusuril (**c**); a pillarene (**d**); a cyclotriveratrylene (**e**); a deep-cavity cavitand, and foldamers forming (**f**); double around a chloride anion (not shown) (**g**); a single helix around a bound sugar (not shown) (**h**). Colour key: green, C; white, H; red, O; blue, N. In structure **g** one of the two (identical) helices is shown in yellow for clarity.

Although the biochemistry community has embraced these types of experiments, the supramolecular community has just barely done so; for example, in studying the design of single molecule probes for probing RNA–protein complexation[73]. There are many options for the two communities to consider, including: nanoprinting of host-guest arrays for rapid analysis of multiple, related complexation events such as on the surface of a protein, and; probing multi-step processes such as those of artificial molecular machines[74,75].

Thermodynamics of association

Measuring the changes in thermodynamics upon complexation yields information that — at least in theory — can be used to guide design; it can direct modellers by constraining experimental and mechanistic explanations.

A large amount of isobaric thermodynamic data has been gathered in the literature using isothermal titration calorimetry (ITC), UV–vis, NMR and surface plasmon resonance (SPR). However, there are issues with the accuracy and hence utility of large portions of it[76,77]. In terms of the sheer quantity, $\Delta G(K_a)$ data dominates: $\Delta G(K_a) > \Delta H \approx \Delta S \gg \Delta C_p \gg \Delta V$. This situation is a reflection of the dominance of spectroscopy and the ease by which it yields K_a. This data is relatively reliable; although Thordarson has highlighted that, despite the availability of superior non-linear regression methods, less accurate linear regression methods for K_a determinations are still utilized by portions of the supramolecular community[78].

The ready access to ΔG data has meant that it is not just used to probe the hydrophobic effect, but also to judge the success of simulations. D3R is a case in point, as is the related SAMPL series of challenges[55–57,63]. Irrespective of the rationale for gathering the data, ΔG calibration data standards (compared to MS calibrants) for host-guest chemistry may be very useful. Alkali metal ion binding to 18-crown-6 is already an unofficial (weak-binding) standard; suitably pure cucurbiturils could function as standards at the other end of the K_a continuum.

In spectroscopic determinations, ΔH and ΔS values are derivatives obtained by van't Hoff plots (ln K_a versus $1/T$). The limited reliability of this data has been well documented: (1) an insufficient number of K_a determinations for determining the gradient ($m = -\Delta H°/R$, seven is excellent but four and even three are common), and; (2) the narrow T range for recording K_a (to avoid line curvature) relative to the extrapolation to $x = 0$ ($c = \Delta S°/R$). Gratifyingly, ITC has increased in popularity within the supramolecular community, and has allowed direct measurements of ΔH, and measurements of the first derivatives ΔG, ΔS and ΔC_p with (ideally) the same accuracy that spectroscopy provides for ΔG (ref. 77). However, there has been little expansion of differential scanning calorimetry (DSC) outside of the physical community, even though this directly measures ΔC_p.

What is the most useful thermodynamic data to probe the hydrophobic and Hofmeister effects or to optimize binding? ΔG alone is certainly not useful all of the time, there have been examples where dramatic changes in ΔH and ΔS flag an underlying chemical phenomenon that would not have been apparent measuring (an insensitive) ΔG. So perhaps ΔH, ΔS and ΔC_p are more important? Care is needed here; for example, it is common to think ΔH and ΔS can give information on how to improve binding. If binding is enthalpically dominated, it is assumed this can be improved upon by adding strong non-covalent interactions. But this approach frequently fails. Moreover, what of entropically dominated processes? How does one improve affinity in those cases? The goal of understanding the root cause or physical meaning of ΔH and ΔS in the context of aqueous supramolecular chemistry is compounded by a frequently overlooked fact: the observed ΔH and ΔS contributions to binding have different roots depending on the nature of the guest undergoing desolvation[61]; what we know (and what we need to know) about ΔH and ΔS is context dependent.

Relatedly, there is considerable debate about enthalpy–entropy compensation (EEC) and about the relevance of the slope of linear regression of ΔS–ΔH plots. As has been recently reviewed[76], EEC may be due to handling and correlated errors, window effects (instrumentation limitations, data selection bias and publication bias), or indeed may have a physical basis such as solvent reorganization and conformational restriction. There is no clear understanding of the root cause(s) of EEC, and there is considerable latent chemical space here for the two communities to collaboratively address this void. Possible strategies include chemometric analysis of the data in the literature, combined with high through-put strategies such as high-performance liquid chromatography[79] for gathering new data. Whatever the approach, with ΔC_p becoming more accessible (a situation that would further improve with greater utilization of DSC), the number of ways to probe the complex relationship between thermodynamics and structure is increasing.

Ion binding and the Hofmeister effect

Buffers and salts are important components of all living systems. However there is a vast swath of chemical space concerning ions in water (for example, their affinities for interfaces) that is poorly understood. Consider pure water. There is still debate concerning whether the air–water interface has a surplus of protons or hydroxide ions: some spectroscopic studies support the view that the surface of water is acidic[80], whereas electrophoretic mobility experiments of air and oil droplets in water suggest that it is negatively charged

Table 1 | Useful spectroscopic techniques

Spectroscopy	Sensitivity	Comments
Raman	Moderate	Vibrational information on hosts and guests
Raman multivariate curve	Moderate	Raman variant for probing solvation shell water molecules around hosts and guests
IR	Moderate	Vibrational information on hosts and guests. Pump–probe experiments can provide fast time resolution
2D IR	Moderate	Can correlate coupled vibrational modes in host guest systems Provides time resolved information on water dynamics
Sum-frequency-generation	Sub-monolayer	Interface specific technique; provides information on interfacial water structure and molecular orientation
UV–vis	Excellent	Commonly used to measure thermodynamics of relatively strong ($K_a < 10^8 \, M^{-1}$) hosts-guests binding
Fluorescence	Excellent	Used to measure thermodynamics of complexation ($K_a < 10^9 \, M^{-1}$) Can provide time resolved information on bindings and dynamics at the single molecule level
NMR	Low	Provides excellent chemical specificity Commonly used to measure the free energy of binding of hosts and guests ($K_a < 10^4$) Dynamic Nuclear Polarization for water dynamics
X-ray adsorption	Low	Provides information on binding and ion interactions

and, hence, basic[81]. In dealing with salt solutions, Debye–Hückel theory (DHT) is commonly applied[82]. However, salt concentrations in living systems are often beyond the boundaries of this theory. Moreover, DHT cannot account for ion–ion association (the Bjerrum length is context dependent), assumes that ions are point charges, and ignores ion–solvent interactions. On this last point, there is an ongoing debate about the magnitude and significance of non-covalent forces between even simple ions and water. Consider for example, that many cations do not undergo charge transfer, whereas quasichemical calculations suggest that halide ions may undergo 20% charge transfer to their solvation shell[83]. The consequences of these differences remain unexplored.

How salts affect solutions comes under the rubric of the Hofmeister effect, a phenomenon that has been observed in over forty physicochemical measurements, the classic exemplar of which is the ability of some salts to increase (or decrease)[84] the solubility of (macro)molecules in water. In other words, some so-called 'salting-in' salts decrease the apparent strength of the hydrophobic effect, whereas 'salting-out' salts do the opposite. Countless studies have revealed that the Hofmeister effect is most evident and reproducible with anions, and the existence of a Hofmeister series (Fig. 3) irrespective of the metric (dependent variable) investigated. In the last fifteen years there has been a renaissance in this field driven by new spectroscopic techniques, improved computational power and an increase in the sophistication of the systems under study[85], the outcome of which has been a switch away from the idea that the Hofmeister effect arises through the effects of salts on water structure indirectly perturbing the properties of the co-solute, to rather direct interactions between salts and co-solute. However, these are poorly understood and the interpretations are still being developed.

Anions. The Hofmeister effect is most evident with anions, and as the tailoring of solute structure, instrumentation and computing power have improved, so the Hofmeister effect has become evident at lower and lower concentrations; manifestations of the phenomena can be observed between 1–100 mM, well within the solubility limits of water-soluble hosts[86]. The low-hanging fruit here are relatively weakly solvated salting-in anions such as SCN^- that have an affinity for non-polar binding sites. Can macrocycles show these types of effects with more strongly solvated anions such as Cl^-? Recent results suggest that this may be the case[35]. Such studies may address the open question of the role of non-polar binding sites in protein stabilization and solubilization relative to HB donor Nest and CαNN binding sites[87,88]. There are many open questions here, and new hosts are required for this still developing field[89].

The aforementioned analytical techniques need to be brought to bear on this topic. Such studies also have to be expanded across a wide pH range. Hydroxide has an apparent dangling ('non-polar') OH that is not hydrogen bonded (and is blue-shifted relative to hydroxide in the gas phase)[90]. Where it (and H_3O^+) lie in the Hofmeister continuum[91,92], and how pH affects the Hofmeister effect, have not been agreed upon.

Computationalists can help here to study the blurred boundary between the anionic and lipophilic realms occupied by I^- or SCN^-, but one important caveat is that adequate potentials have only been devised for the most common anions (at infinite dilution). There is a strong need to address this. These studies may aid the decomposition of anion interactions into more fundamental forces — compare with hydrogen bonding decomposition by Morokuma[93], or π–π decomposition stacking by Sherrill[94] — such that it may be possible to determine the effects of 'turning down' Coulombic interactions and 'turning up' van der Waals interactions for a set anion geometry. This information would be key to understanding the interplay between receptor lipophilicity and anion binding in through-membrane transporters and channels[95].

Salting-out   Salting-in

$$F^- \sim SO_4^{2-} > HPO_4^{2-} > MeCO_2^- > Cl^- > NO_3^- > Br^- > ClO_3^- > I^- > ClO_4^- > SCN^-$$
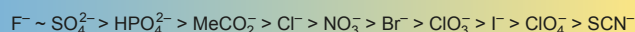
Figure 3 | The Hofmeister series for anions. The Hofmeister effect pertains to how salts affect the properties of solutions, such as their ability to alter the solubility of (macro)molecules in water. Some ions are capable of salting-in; that is, increasing macromolecular solubility and decreasing the apparent strength of the hydrophobic effect, whereas salting-out salts do the opposite.

Cations. Cations display weaker Hofmeister phenomena[96], and this may hinder a thorough understanding of their solvation and properties. In supramolecular chemistry, cation recognition has a long history dating back to Pedersen's crown ethers; a family of hosts that were themselves based on the earlier macrocyclic Schiff bases designed for binding transition metal cations[97]. Over the years, crown ethers that are selective for a vast range of organic and inorganic cations have been devised[98]. However, the supramolecular community has not investigated Hofmeister phenomena using these macrocycles. Indeed, there is much to learn here. Gas-phase spectroscopy of (hydrated) host–metal-ion complexes could inform knowledge of cation solvation. An ideal starting point might be crown-ether complexes; there is precedent for such studies[99,100], however the challenge is the spectroscopy. Another issue with this approach is the low temperatures required, but it may be fruitful for computational chemists to study the consequences of 'warming up' such clusters.

As with anions, synthetic cation channels and transporters are relatively rare. Lariat ethers[101] and their ilk were used to pioneer this area of supramolecular chemistry, but more recently the range of strategies has diversified considerably[102]. Simulations for discrimination of Na^+ and K^+ in membrane channels have been performed by the physical community[103], but there is considerable opportunity for collaborative efforts to understand cation selectivity as well as ion-pairing cooperativity (which can be exploited to transport uphill using co-transport or counter-transport strategies). For transporter design, there is some information available from modelling studies using 'U-tube' experiments[104].

As with anions, many simple biologically relevant cations are well modelled. However, beyond the traditional boundaries of the Hofmeister series there are difficulties with small, highly charged cations that show complex solvation dependencies on pH, temperature, and counter anion. Moreover, although best not thought of as Hofmeister cations, this includes biologically relevant transition metals, which because of the dominating effects of ligand donation and charge transfer to their *d*-orbitals, are poorly modelled.

Recognition, mimicry and interactions with biomolecules

There are many facets of proteins that are conceivably of interest to supramolecular chemists, but the focus has mostly been on understanding and controlling their structures, and recognizing their open surfaces. With a few important exceptions[105], supramolecular chemists have paid less attention to enzyme mimicry. With respect to understanding and controlling structure, the focus has been exclusively on secondary structure[106,107]. Understanding and controlling tertiary and quaternary structure is daunting, but synthetic modules that engender shape-programmable molecules may lead to artificial mimics of these structures[108], and the potential diversity of such systems is large[43].

In terms of the recognition of open protein surfaces, supramolecular chemists have been inspired by protein–protein recognition sites and the recognition of convex protein surfaces[109]. Interestingly, examples such as barnase–barstar demonstrate the existence and role of water molecules at specific points in the protein-protein interface; that is, it is not necessary to remove all interfacial

waters for selective, tight recognition[110]. This is an important point for supramolecular chemists designing systems for protein recognition, and raises the question as to whether supramolecular systems can be designed to probe the effects of interfacial water. Nature again offers inspiration. For example, studies of antifreeze proteins may prove informative[111]. It was originally thought that there might be specific recognition sites involved in inhibiting freezing, but fundamentally the key features are deep cavities and hydrogen bonding. Peptoid oligomers that bind to ice and mimic antifreeze proteins may also guide the design of new hosts[112].

Perhaps the ultimate challenge in protein recognition — the recognition of individual residues on an open surface — is difficult, but pioneering work has shown that rare residues such as those post-translationally modified in histone proteins can be successfully targeted[113].

What about enzyme mimicry? The important difference between synthetic hosts and biological ones is one of size, and this leads to other important differences including: polarity, flexibility and intricacy of the binding motif. Macromolecules such as proteins evolved to be rather large, in part because this quality allows for: multiple functions (to act as network nodes), sufficiently large binding pockets with introverted functionality, and the benefits of allosterism (cooperativity, transition state stabilization, product release and so on). Contemporary hosts are primitive by comparison. However they can be tuned to a fine structural level and occupy a wider functional group space than proteins. How do supramolecular chemists close this gap in complexity? Possible strategies include: (1) copying nature and using repetitive coupling chemistries to easily build different systems out of a family of building blocks (peptidomimetics)[43]; (2) embracing robotic/automation for targeted syntheses and/or accelerated serendipity approaches[114] to host design; (3) using fragment-based approaches and structure-based design[115].

Within the physical community, one ongoing debate involves the solvation of proteins and how this influences their properties. There is good evidence that the large-amplitude motions of proteins are mediated by the translational diffusion of water[116]. However, the details are far from clear. Overall, the field does not yet seem to have determined whether dynamic water is important to the thermodynamics or kinetics of protein folding, ligand binding, or enzyme catalysis. The issue is complicated by the fact that some proteins can function, albeit more slowly, in organic media[117].

Replacing the hydration shell water molecules of proteins with species such as glycerol and trehalose suppress their longer-range conformational dynamics[118]. There is therefore considerable opportunity to expand on this phenomenon and systematically investigate the effects of poly-ols and multi-valent carbohydrate conjugates involving scaffolds such as dendrimers. Supramolecular chemists have not ventured deeply into this topic, and studies with how poly-ols influence foldamers may provide information pertinent to protein hydration in general.

Nucleic acids have not been targeted to the same level as proteins. The specific recognition of duplex DNA/RNA; that is, targeting the major or minor groove, is difficult without relatively large synthetic receptors[119], but low-hanging fruit may be found in highly specific DNA structures that offer relatively unique grooves or phosphate group patterns that can be selectively recognized. Such examples can be found in the structures built from DNA origami[120]. The supramolecular community has not yet addressed the selective recognition of such structures to any great extent[121], and communication between the supramolecular, physical and DNA origami communities may be useful.

If there is still considerable latent chemical space for supramolecular chemists to investigate in the area of nucleic acids, there is even more room for exploration in the third class of bio(macro)molecules, the carbohydrates. Carbohydrate recognition is very different from selective recognition of proteins or nucleic acids, and in many ways it is the opposite of binding to non-polar groups; strategies such as good surface complementarity may not apply. As a result, supramolecular chemists have carried out only limited work in this area[122]. Physical scientists can potentially help here by studying the stereo-electronic effects and conformations of carbohydrates and their derivatives.

Carbohydrate and protein recognition overlap with the lectins, and there is considerable opportunity to improve our understanding of how lectins bind carbohydrates, and as discussed above, our understanding of small polyol association with proteins in general. Beyond the non-specific binding of glycerol, does trehalose stabilization of proteins and its role in anhydrobiosis (a dormant state induced by drought whereby an organism becomes almost completely dehydrated) involve any degree of specificity?[123] Can small polyol additives be used to mimic bound waters at specific sites on a protein surface? And if so, which residues are key for selective recognition?[124]

Supramolecular research into membranes has focused on ion transport, and it is well understood that many hosts can pass through membranes. By contrast, less work has been carried out in specific membrane recognition[125], or simply examining host-guest complexations/assemblies at interfaces[126] — there is much to learn about supramolecular chemistry at membrane surfaces. A membrane has directionality (and curvature), can have large dipoles and its interfacial water has relatively high concentrations of ions. Moreover, the potential at the surface impacts water and ion mobility at or near the Stern layer. There are great opportunities for both communities to identify simple, telling supramolecular systems at artificial interfaces. Analogous studies at complex phospholipid membranes are a long way off, but it appears that there are ample opportunities for probing lipid bilayers by the use of covalent or non-covalent probes.

Conclusion

By its very nature, this Review is constrained in scope. The focus has been on organic and biochemical systems, which covers just one small fraction of the role water plays on Earth. Nevertheless, it is evident that there exists vast tracts of empty or near-empty latent chemical space that are ripe for exploration, if the supramolecular and physical sciences communities combine their expertise. Grand challenges abound at a fundamental level: understanding the solvation of non-polar surfaces and ions, and how these solvation types combine to affect each other, will ultimately bring mastery of the hydrophobic and Hofmeister effects, and likely push aside grand tenets such as DHT; similarly, understanding the root causes of EEC will have major ramifications to the way we look at thermodynamic data. Towards this, and at a more practical level, 'folding in' to these collaborative studies technologies such as -printing/lithography, chemometrics and improved computational modelling, has the potential to accelerate our understanding of aqueous solutions, as do testing spectroscopies with small molecule models to accelerate spectroscopic development. The combination of small-molecule models and the expertise of the physical community will allow new insight into how water controls the properties of proteins, nucleic acids, carbohydrates and lipid membranes. And what will come from all this fundamental work? One can only speculate. However one thing is clear: collaboration between the two groups can help clarify what are still very murky waters.

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Author contributions

All authors contributed to the initial grant application, the running of the workshop, and writing the manuscript.