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Water structure and chaotropicity: their uses, abuses and biological implications

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The concept of "water structure" has been invoked to explain all manner of aqueous phenomena. Here we look at the origins of this tendency to understand solute hydration in terms of structural changes in bulk water, and consider the validity of one particular example: the classification of small solutes as chaotropic or kosmotropic, and the putative relation of this terminology to notions of structure-making and structure-breaking in the solvent. We doubt whether complex phenomena such as Hofmeister and osmolyte effects on macromolecules can be understood simply on the basis of a change in solvent structure. Rather, we argue that chaotropicity, if understood in the original sense, arises from the activities that solutes exert on macromolecular systems, as well as from deviations of solvation water from bulk-like behaviour. If applied judiciously, chaotropicity remains a potent, biologically pertinent parameter useful for classifying and understanding solution phenomena in all types of living system.

Introduction

Water is a strongly mythologized substance in cultures throughout the world.[1,2] It is often regarded as an almost magical substance, with sacred, purifying, healing and life-giving characteristics. It seems likely that some of these ancient associations leak into scientific – or at least quasi-scientific – discussions of water,[3] where they express notions of what we might *like* water to be and to do.

Science *sensu stricto* is far from immune to preconceptions that colour the interpretation of experimental results and the framing of theories. Sometimes the intuitive appeal of a simple conceptual picture can take on the appearance of a kind of *prima facie* evidence, so that a particular interpretation persists more because it is readily grasped than because the empirical data support it. Or an interpretation might mutate even as it becomes part of the "received wisdom", becoming distorted into a form that seems better to fit intuition but corresponds rather little with the original meaning. One can see this happening, for example, with the Central Dogma of molecular biology, with Neodarwinism and with the concept of mind.[4]

Given the mythical status of water, our understanding of this substance might be particularly prone to this sort of "random drift". It is not hard, for instance, to find examples of a rather casual acceptance in the biochemical literature of Kauzmann's 1959 explanation for the attraction of hydrophobic solutes in water[5] – an interaction that is well attested experimentally and seems to be a key driving force in protein folding and aggregation. Kauzmann's argument that water hydrating a hydrophobic entity is more ordered and rigid in order to preserve hydrogen bonding – the classic "iceberg" picture of Frank and Evans[6] – leads to the notion that hydrophobic attraction is entropic, driven by the release of some of this "ordered water" as two hydrophobic surfaces come into proximity. Yet there is rather little direct experimental evidence for this picture of hydrophobic hydration.[7,8] The question is complex and still debated,[9] in part because the issues are as much a matter of dynamics as of structure; but it seems likely that Kauzmann's model is a simplification at best, and that despite its conceptual elegance and transparency it needs to be replaced with a more nuanced, less tidy view that acknowledges a whole variety of influences concerned with specific intermolecular interactions, interfacial dynamics, entropic and enthalpic compensation, geometry and scale – in other words, with the recognition that there may be a multiplicity of hydrophobic effects.[10]

At the root of this issue is an even more alluring notion: that of "water structure". There is a long-standing tradition of explaining the

complicated and often perplexing properties of water, hydration and aqueous solutions by appealing to the rather vague idea that water molecules can form more or less orderly arrangements in the liquid state when perturbed by solutes or surfaces. In some extreme forms, "structured water" has offered a convenient rationale for alleged water properties that are now see to be examples of pathological science – polywater[11,12] and the "memory of water".[13] But it is also still routinely evoked to account for more robust empirical phenomena. As Israelachvili and Wennerström have attested,[14]

> When confronted with unexpected experimental results water structure has commonly been used as a *deus ex machine* for explaining the observations.

For example, those authors considered how this picture offered a convenient explanation for the long-ranged repulsive force observed experimentally between two hydrophilic surfaces:[14]

> A picture emerged of hydrophilic surfaces bounded by a coat of structured water that opposed being disrupted… The range of this interaction was variously suggested to extend from nanometres to many micrometres, and various theories based on water structure were proposed.

As Israelachvili and Wennerström pointed out, this tendency is not a recent one. Even in 1936, Hartley lamented the "widespread tendency to use 'hydration' in colloid chemistry as a sort of universal explanation of puzzling phenomena" – a tendency fortified, he said, by its "inaccessibility to direct experimental determination."[15]

Here we wish to comment on another common example of this tendency. Closely related to the views of Frank and Evans and Kauzmann is the idea that the behaviour of small solutes in water, such as ions and osmolyte molecules like urea and guanidinium chloride, can be rationalized on the basis of their ability to either disrupt or enhance the "water structure" existing in the bulk liquid. This idea is sometimes described as structure-making or structure-breaking,[16] or alternatively as a tendency for the solute to be either chaotropic (inducing disorder) or kosmotropic (inducing order). Delving into the historical origins of this picture is illuminating, for it reveals the same process mentioned above of mutation towards an existing preconception. We ask whether these ideas about water structure and chaotropicity have any validity and – a related but not identical matter – any utility.

Water as defective crystal

Like many flawed but persistent preconceptions in science, the concept of water structure holds some truth. There's no doubt that water is a "structured liquid" – that is to say, its molecules are distinct from the pseudo-spheres of simple liquids by virtue of their hydrogen bonds, which introduce directional bonding preferences. There are certainly situations in which this characteristic can result in strong orientation of solvent molecules, for example in the formation of an oriented monolayer of water on metals[17,18] or the crystallographically well defined positions and orientations of some water molecules hydrating proteins, DNA and other macromolecules.[19] But the old "iceberg" picture of hydrophobic hydration,[6] along with modifications such as Pauling's clathrate description,[20] posit something altogether more crystalline, or at least liquid-crystalline. Those ideas cannot be separated from the historical context in which they were developed.

The fact is that a consideration of the structure of liquid water was tightly bound up with the notion of liquid-state structure as a whole. After all, when X-ray crystallography was just beginning, there was no reason to suppose that water was not the archetypal liquid, rather than being a highly unusual one.

The liquid bridges states that can, at least in ideal terms, be considered as perfectly ordered (the crystal) and perfectly disordered (the gas). The question seemed to be which of these is the best starting point from which we can access the liquid: is it more like a dense gas or an imperfect solid? It has become clear today, of course, that neither does full justice to the issue, but it's understandable that this should have seemed a fair place to start.

The discoverer of X-rays Wilhelm Röntgen tried to explain water as something like a literal mixture of the two[21] – basically as ice-like clusters dispersed in an almost gas-like fluid. The long legacy of that view extends not only to some of the more contemporary mixture models[22,23] but also to the idea that water may have two distinct supercooled fluid states[24,25], which, in displaying greater or lesser degrees of structure, remain at least notionally akin to rather ice-like and gas-like states. The merits of that picture are currently contested,[26-28] which in itself testifies to the tension inherent in a view that regards liquid water as poised between a tendency to become organized and a counteracting entropic impetus towards disorder.

As early as 1916, X-ray scattering was applied to liquids such as water by Debye and Scherrer,[29] who showed that the diffraction pattern was not entirely featureless but consisted of diffuse rings that Debye interpreted coming from both intra- and intermolecular interference. Since the early work on water structure was done largely by people schooled in crystallography, it is scarcely surprising that they should have tended to side with the "defective crystal" view of liquid structure. That was a notable feature of the highly influential model proposed in 1933 by Bernal and Fowler.[30] They argued from quantum-chemical considerations that the water molecule can be represented using a simple point-charge model with tetrahedral geometry, and noting the similarity here with the tetrahedral structure of silicates, they developed a model in which water was regarded as akin to distorted quartz.

This set the scene for other models that started from a crystalline viewpoint. Notably, in the 1950s Eyring devised a general picture of the liquid state consisting of an essentially crystalline close-packing threaded with many dislocations.[31] Molecules that escape from this close-packing could, in Eyring's picture, wander almost gas-like between the dense clusters, making it a descendent of Röntgen's solid/gas mixture model.

The 1950s was a particularly formative time for theories of liquid-state structure in general, and of water structure in particular. It was then that Pauling outlined his own view of water as a kind of disorderly crystal based on the clathrate.[20] Bernal, meanwhile, modified his more crystalline view of liquids by exploring random packings of spheres,[32] and he constructed a model of water that respected the local tetrahedral arrangement while producing no long- or medium-range order among molecules: a random hydrogen-bonded network in which the molecules are connected in rings with between four and seven members.[33] Static structure was still the prevailing paradigm for ideas about the liquid state.

Ambiguities of structure-making and breaking

This crystalline view is also reflected in Gurney's influential 1953 book *Ionic Processes in Solution*,[34] where one can detect a constant pull towards regularity in the descriptions of water structure:

> At any moment we may expect to find that the water consists largely of ordered groups of molecules… but there is no reason why the molecular planes in one such group should bear any simple relation to the planes in an adjacent group…. In water we do not know what is the average size of these approximately regular groups; but we must suppose that the thermal agitation is continually breaking up the larger groups, while elsewhere, at the same time, molecules are falling into an ordered arrangement, so that a balance between order and disorder is maintained. [p49]

Gurney remarks on "to what a slight degree the thermal agitation succeeds in breaking up the almost crystalline order".

Gurney's book is often attributed as the origin of the idea that ions can exert structure-making and structure-breaking effects on water structure. However, that the hydrogen-bonded network might be perturbed by salts was an older idea, having been suggested only a year after Bernal and Fowler presented their crystal-oriented view of water structure.[35] Gurney does not actually use the terms structure-making and breaking, but he does suggest that some ions can induce "a local breaking up or loosening of the water structure in the ionic co-sphere". The image conjured up here is of ions as defects in an almost crystalline lattice.

Structure-making and breaking can still now be regarded as a conventional view – not in the sense that it represents a consensus, but because one can still use these terms to discuss hydration without any obligation to suggest that they are controversial. In a recent review of the field,[16] Marcus says that

> Since the publication of Gurney's book, the concepts of "structure making" and "structure breaking" by ions as their effects on the water structure have been generally accepted and applied to the explanation of a variety of phenomena exhibited by electrolyte solutions. In recent years, however, these notions have been challenged… but mainly concerning rather concentrated solutions. Their validity for dilute solutions was reaffirmed by other authors.

Yet not only has this view been highly contested[36] (not least by Mancinelli et al.[37], who Marcus cites among those who have "reaffirmed" the idea), but there is not even any clear or consensus picture of what "structure" means in this context. For example, some discussions seem to interpret an enhanced water density in a hydration shell as a case of structure-making, which is by no means obvious when ordering in bulk water leads to the less dense ice-I phase. Franks[38] states that, if small and/or highly charged ions have an enhanced degree of order in their hydration shells, it is an ordering that is "quite incompatible with the tetrahedrally hydrogen-bonded network in unperturbed water", and must therefore to some degree perturb that bulk structure. In other words, it would seem that *all* ions would by this measure perhaps be better considered as "structure-breakers", or perhaps one should rather say "perturbers of bulk structure". That is in fact the view attested by Mancinelli *et al*.[37], who reported that the radial distribution functions of water measured by neutron diffraction are perturbed for *all* the ions they studied and concluded that there seems to be no good reason to think that ions can be conveniently partitioned into structure-makers and breakers.

But might "structure" be interpreted another way – for example, as an enhancement of hydrogen-bonding strength, or greater tetrahedrality, or a bulk entropy deficit, or a slower orientational relaxation, or something else? The finding by Omta *et al.* that there is no apparent perturbation of the reorientation dynamics of water molecules outside the ionic first hydration shell[36] was presented as evidence against structure-making and breaking; but one might equally regard it as addressing just one aspect of a complex and probably ill-posed question.

If we accept that the jury is still out on whether structure-making and breaking is a useful umbrella concept, we must also consider how to think about the closely related concepts of chaotropicity and kosmotropicity. Not least, what exactly is this relation? Chaplin[39] has this to say:

> The terms 'kosmotrope' (order-maker) and 'chaotrope' (disorder-maker) originally denoted solutes that stabilized, or destabilized respectively, proteins and membranes; thus chaotropes unfold proteins, destabilize hydrophobic aggregates and increase the solubility of hydrophobes whereas kosmotropes stabilize proteins and hydrophobic aggregates in solution and reduce the solubility of hydrophobes. Later these terms referred to the apparently correlating property of increasing, or decreasing respectively, the structuring of water.

Marcus too speaks of "kosmotrope" and "chaotrope" being "more or less equivalent terms" to structure-making and breaking.[16] So it is instructive to discover that chaotropicity originally had *nothing to do with* the question of "water structure". Rather, it was a term denoting the degree of structure in a macromolecular solute. It first appears in the report of Hamaguchi and Geiduschek of Hofmeister-type specific-ion effects on aggregation of biological macromolecules.[40] These effects (of which more below) were originally identified by Hofmeister[41] in experiments on the "salting in" (enhancement of

solubility) and "salting-out" (aggregation and precipitation) of proteins in electrolyte solutions, although Hamaguchi and Geiduschek were in contrast looking at salt effects on the secondary structure of DNA. They ranked anions into what they call a "chaotropic" series, which they explain refers to the ions' tendency to induce disorder – but that is, disorder in the *nucleic acid*, not the *water*.

This usage of chaotropicity as a measure of an ion's ability to disrupt macromolecular structure apparently persisted for some time among biophysicists and biochemists. But by the time the complementary term "kosmotrope" was added, apparently in the 1980s,[42] the blurring of meaning that Chaplin and Marcus mention was well underway. Hamaguchi and Geiduschek themselves discuss what they call the "venerable" idea that the action of electrolytes on proteins and other macromolecules is due simply to "dehydration", that is, to competition for hydration water. But they say that this cannot fully explain their results, and, citing Frank and Evans, they talk about the anions as "structure formers" and "structure breakers". They say:

> Insofar as the denaturing anions exert their effect on DNA through their modification of the structure can be classed as *hydrophobic bond breaking agents.* The structural details of these effects are not, at present, established.[40]

So from the outset there was potential confusion about what is being ordered or disordered: the solute or the solvent.

Explaining Hofmeister effects

Together, structure-making and breaking have been particularly tenacious as a putative mechanism for explaining Hofmeister effects.[42] The basic idea here is that large, low-charge ions such as I^- and NH_4^+ disrupt water structure – they are structure-breakers – while small or highly charged ions such as F^- and Mg^{2+} are structure-makers, imposing order on the hydrogen-bonded network. Then salting-out and salting-in of proteins are explained on the basis of entropic changes induced in their hydration shells by the addition of ions, or alternatively, of a reduction in the strength of hydrogen bonding of water molecules complexed to dissolved ions. The classical hypothesis is that salting-out arises from a competition for solvation between the salt and the protein, in which an ion's ability to sequester waters of solvation is somehow connected to its effect on water structure. Thus the structure-making effect of small or highly charged ions depletes proteins of hydration water and causes precipitation. These ideas have recently been extended to the case of hydration of ionic liquids ("green" solvents consisting of molten salts of complex ions), which have been classified as chaotropes and kosmotropes in water on the basis of the thermodynamic parameters of hydration.[43]

Such proposals have the attraction of offering a unifying scheme for Hofmeister effects. But does an interpretation of aggregation phenomena in terms of the "ordering" or "disordering" effects of ions on water structure have any validity? Zangi has addressed this question using molecular dynamics simulations to look at the correlation between the propensity of various ions to alter the hydrophobic interaction (and thus to salt in/salt out) and changes in structural and dynamical properties they induce in water.[44] While there is a monotonic relationship between the reduction in hydrophobic interaction and the increase in water structure as measured by the partial radial distribution factors, Zangi was unable to identify one single property that could predict the change in the strength of the hydrophobic interactions. Nor could any such property predict the transition from salting-in to salting-out behaviour. Changes in water dynamics, meanwhile, were induced by changes in the ion-water interaction, and not changes that the ions introduce to the 'structural ordering' of the water itself. As a result of all this, it seems that predicting whether a particular ion will induce salting-in or salting-out cannot be done on the basis of the properties of the salt solution alone, in the absence of the macromolecule itself.

Another challenge to the picture of structure-making and breaking comes from Corridoni *et al.*,[45] who looked at how changes in ionic concentration affect the viscosity of the electrolyte solution. Classically, the viscosity is found to be (almost) linearly related to the concentration, and it has been asserted that the magnitude of the coefficient of proportionality depends on how the ions perturb the water structure. Corridoni *et al.* used neutron scattering and simulations to look for some structural parameter that can be correlated with this coefficient. They found that the change in viscosity seems to be unrelated to any structural changes in the bulk liquid, but instead pertains to changes in the local hydration shells of the ions. As a result, they say this: "the particular effect of solutes ranked in the Hofmeister series must be looked at in terms of specific ion interactions with hydrophilic or hydrophobic surfaces".[45]

Zangi *et al.* have shown how, even when the direct interactions of ions and solutes is taken into account, it can be hard to tell a simple story about ion solvation effects.[46,47] They considered how ions interact with small hydrophobic particles 0.5 nm across (Figure 1).[46] They found that ions with high charge density (q) induce salting out by promoting stronger hydrophobic interactions that cause particle aggregation. But low-q ions could have either a salting-out or a salting-in effect, depending on their concentration. These effects are related to preferential absorption or exclusion of the ions at the particle surfaces, but not in any simple, monotonic fashion. High-q ions tended to be depleted at the surface of the hydrophobic particle clusters, but are tightly bound to water elsewhere, thereby decreasing the number of water molecules available for solvating the particles. Low-q ions are absorbed preferentially at the particle surfaces, and at high ionic concentrations this can lead to salting-in because the hydrophobic particles form clusters surrounded by ions in a micelle-like arrangement. At lower concentrations, the ions are unable to solubilize aggregates in this way, and salting-out occurs.

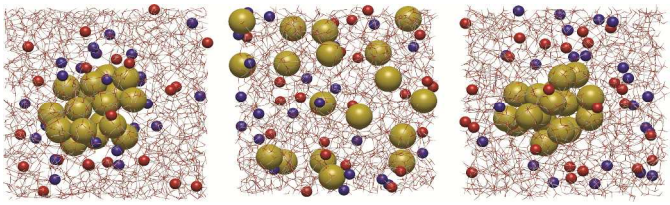

Figure 1 The influence of ions (red and blue, representing anions and cations) on the aggregation of hydrophobic particles (yellow), for ions with small (left), intermediate (middle) and high (right) charge density *q*. From ref. 46, © American Chemical Society.

Analogous partitioning of ions at the surfaces of nanoscale hydrophobic plates should alter the hydrophobic interaction between them[47] (Figure 2). But it seems that Hofmeister effects may have a different origin for small and large hydrophobic particles. Whereas in the former case there is an increase in hydrophobic aggregation for both high-*q* and low-*q* but not medium-*q* ions (except at high concentrations), for hydrophobic plates the relationship is monotonic, with an increasing tendency towards salting-in as the ion charge density decreases.

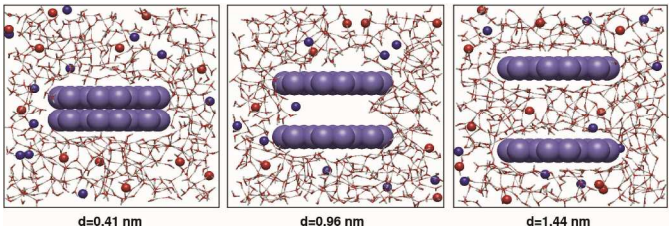

Figure 2 The distribution of ions around and between two hydrophobic plates as a function of the plate separation. From ref. 47, © American Chemical Society.

This need to explain Hofmeister effects in terms of direct ion-solute-solvent interactions, rather than structure-making or breaking, now seems to be an emerging consensus.[48] If one compares a widely cited paper on Hofmeister effects from the 1980s[42] with a relatively recent one from 2004[49], the first makes frequent reference to structure-making and breaking, the second does not mention them. Moreover, there are reasons to believe that hydration of simple anions might be quite different, both structurally and dynamically, from hydration of cations, so that a single concept of water structure will not suffice to characterize it.[50]

Hofmeister effects seem to be now acknowledged as a subset of phenomena in which biomolecules are influenced by other species in solution, which could be regarded, depending on the concentration, as either cosolutes or cosolvents. Some of the same considerations that apply to ions apply also to osmolytes, cryoprotectants, denaturants, protein-stabilizing agents, polysaccharides and polyelectrolytes. There is now a growing view that all types of protein denaturation are intimately connected to changes in the way the macromolecule is hydrated,[51,52] and that cosolutes that influence denaturation and aggregation are not simply altering the structure of water in some global sense, nor simply competing for hydration water. Rather, to understand all these effects one probably needs to focus to a large extent on the direct interactions between the ions or cosolutes, the biomolecule, and its first and perhaps second hydration shell – all of which may of course depend in different ways on concentration and temperature.

For instance, it was long supposed that denaturants such as urea or guanidinium chloride (GdmCl) somehow perturb water's bulk structure in a way that destabilizes the folded protein, perhaps by altering the hydrophobic interactions that keep insoluble residues buried.[53] But a better explanation seems now to come from a consideration of how denaturants like this affect the protein's hydration shell.[54] For lysozyme, urea at high concentration displaces water from the hydration shell and penetrates into the hydrophobic core, suppressing the native fold in favour of a swollen, rather disordered 'molten globule'.[55] GdmCl appears to have a different denaturing mechanism: the molecules stick to the hydrophobic surfaces and solubilize them in a manner akin to a surfactant.[56] This effect may in fact be important for urea too, which can accumulate at hydrophobic surfaces because of the favourable dispersion forces. Furthermore, urea not only alters hydrophobic interactions but also disrupts the hydration of hydrophilic parts of a protein, in effect usurping hydrogen bonds that otherwise help to bind the native state together.[57] While these ideas are still being debated, the emerging picture is one in which denaturants exert their influence through direct interactions with the solute, not by restructuring the bulk solvent.

Some other small molecules, such as trehalose, glycine betaine and trimethylamine-*N*-oxide (TMAO), have the opposite effect, stabilizing a native protein against denaturation. This mechanism is also still under discussion, but the idea that the cosolutes somehow alter water's global hydrogen-bonding arrangement has been increasingly challenged;[58,59] the stabilization of proteins again more probably results from direct interactions. This latter interpretation is consistent with the suggestion of Rösgen *et al.*[60], deduced from thermodynamic data, that "neither bulk water nor protein hydration is the main player in osmolyte concentration-dependent effects on protein stability."

So while the principles of how small molecules are hydrated and how they interact with macromolecules to influence solubility, conformation and aggregation are clear enough, the way they play out in practice apparently involves a complicated interplay of effects that offers little purchase for intuition. This seems to be a rather ugly view of the matter, lacking any of the elegance that an idea such as structure-making and structure-breaking can afford. At the very least, there may be a case for broadening any classification of hydration from the simple dichotomy of order and disorder so as to encompass a wider variety of classes and influences – a view recently advocated by Morita *et al.*[61]

Not only does this seem to go against the grain of science's quest for unifying mechanisms, but it forces us to question whether a complex problem such as how two macromolecules interact in the cytoplasm can really be compartmentalized into issues that can be individually addressed by the physical chemist, the biochemist, the biophysicist, and the cell biologist. That is a daunting thought – that perhaps there is no convenient renormalization that works for understanding the chemistry and physics of cells at different scales – but it is one that might need to be entertained.

The case for chaotropicity

One might characterize a great deal of the discourse on molecular-

scale aqueous phenomena over the past several decades as a struggle to escape the influence of the powerful attractor represented by the idea of "water as defective crystal". We can see the effects of this attractor in the way that the concept of chaotropicity (and later its complementary term kosmotropicity), which began as a description of what happens to dissolved macromolecules, became mutated into perceived description of the liquid state of water itself.

Does this suggest that it is time to abandon these terms? Recent work by one of us (J.E.H.) suggests that, on the contrary, we might do well to retain at least the concept of chaotropicity *in its original sense*: referring to the effects of solutes not on water but on macromolecules, so that it labels the tendency of ions and other dissolved substances to aggregate, disperse or structurally disrupt lipid bilayers and proteins or other macromolecules. Used in this strict fashion, chaotropicity and kosmotropicity

- can precisely describe activities of substances on biomacromolecules
- are relevant to the structural interactions of macromolecular systems *in vitro* and *in vivo*
- can be empirically measured
- do not make speculative, untested assumptions about mechanisms
- do not make any assumption about substances affecting the bulk structure of liquid water
- give rise to specific stress responses in microbial cells
- predict the windows over which macromolecular systems retain sufficient stability and flexibility to function
- are consistent with, and explain, many hitherto unresolved phenomena in microbial cell biology and microbial behaviour within ecosystems
- can determine the windows over which microbial cell division occurs and therefore impact habitability for diverse environments
- enable biotechnological manipulations to optimize, moderate or prevent microbial growth and metabolism.

Perhaps the key underlying consideration here is that chaotropicity and kosmotropicity may function much as do terms such as hydrophobicity and Hofmeister effects, in that they measure a phenomenon without regard to the underlying molecular mechanisms. Cray *et al.* have devised an assay for a direct, empirical measure of chaotropicity and kosmotropicity, based on the effects of the solutes on the gel-point of agar.[62] This chaotropicity–kosmotropicity scale correlates with the ability of chemically diverse chaotropes to induce structural as well as functional changes in proteins and membranes.[62-64] A universal scale of this sort could be very useful for elucidating mechanisms of cellular stress and the corresponding stress-responses, and understanding the windows within which metabolism or cell division can proceed for specific microbes.

Approached in this spirit, chaotropicity can provide a useful category for classifying the effects of solutes on micro-organisms. Chemically diverse substances, including urea, phenol, benzyl alcohol, ethanol, butanol, $MgCl_2$, guanidinium chloride, and LiCl induce a chaotropicity-specific cellular stress-response, regardless of microbial species.[65,66] Hydrophobic stressors of cellular systems, including benzene, toluene and hexane, partition preferentially into the hydrophobic domain of macromolecular systems but also induce a chaotropicity-mediated (that is to say, operating via the loosening of macromolecular structure) stress and corresponding stress-response in microbial cells.[66] The stress responses of microbial cells to both chaotropic solutes ($\log P_{octanol–water} < 1.9$) and hydrophobic stressors ($\log P > 1.9$) include up-regulation of diverse proteins involved in protein stabilization, modification of membrane lipid composition to increase lipid order, and production of kosmotropic compatible solutes.[65,66] The remarkable similarity between cellular responses to soluble chaotropes, hydrophobic stressors that induce a chaotropicity-mediated stress, and high temperatures indicates that the underlying stress mechanism in each case involves enhanced flexibility of the cells' macromolecular systems.

For each type of macromolecular system, there is a finite window of flexibility–stability over which it can retain structural integrity and functionality.[62,67-69] The primary factors that determine the extent of the window are the prevailing temperature and the net chao-/kosmotropicity of substances present. This principle is seen also at the whole-cell level, where growth windows are determined according to the net effect of temperature and chao-/kosmotropicity on macromolecular function.[70-74] Not only can the chao-/kosmotropicity of solutes thus enhance or reduce the temperature tolerances of microbial cells and vice versa, but the limits of the functional biosphere are determined by these factors at specific locations on Earth.[70,73-76] At higher concentrations of chaotropic substances, environments become effectively sterile[75,76] because macromolecular systems are denatured and cells may lyse or become "mummified".[75,77]

Many cellular metabolites, including compatible solutes (stress metabolites) and hydrophilic polymers, can be regarded as kosmotropic within this schema, in the sense that they can oppose or reverse the effects of chaotropes.[62] Microbes can preferentially utilize or accumulate chaotropic or kosmotropic metabolites depending on the prevailing environmental conditions.[66, 72,74,78-80] They also utilize the inhibitory potency (and indeed lethality) of chaotropicity by producing an impressive arsenal of chaotropic and hydrophobic stressors as antimicrobials.[79,81] These include moderately chaotropic substances produced by some microbes in considerable quantities (such as ethanol, butanol and acetone), highly potent chaotropic solutes (such as ethyl acetate, dichloromethane, 2-phenylethanol), and hydrophobic substances (such as ethyl octanoate, hexane, octanol, isoamyl acetate)[79,81]. These substances are usually referred to collectively as volatile organic compounds. The production of these chaotropic antimicrobials enables some microbes to act as aggressively, as invasive microbial 'weeds',[79] while the chaotropic activities of such metabolites is widely used by clinicians, biotechnologists and food scientists as biocides and food preservatives (e.g. ethanol), as well as flavour substances.[83] Chaotropicity can also be responsible for product-induced inhibition of biofuel fermentations and other biotechnological processes.[70.82]

Some microbial habitats are chaotropic, such as those containing hypersaline concentrations of $MgCl_2$ or $CaCl_2$, sugar-rich habitats that contain high concentrations of fructose or glycerol, and substrates with high levels of urea, ethanol, phenol or other chaotropes.[65,75,76,79,81,82,84] A number of research groups are currently seeking chaophilic microbes capable of optimal levels of metabolic activity and multiplication *only* under chaotropic conditions.[73,75,85,86] Chaophilic enzymes have also been sought by directed evolution of protease.[87] Whereas chaotropic substances are generally inhibitory for cellular systems, they are apparently able to increase macromolecular flexibility at temperatures of below 5-10°C, allowing metabolic activity and cell division to persist at temperatures that would otherwise not be permissive.[74] This observation can have implications for the habitability of cold extraterrestrial environments, as well as for planetary protection, since some extraterrestrial environments have sufficient water and a biologically permissive vapour pressure/water activity to enable microbial replication and multiplication.[88-91]

Collectively, these findings argue the case for chaotropicity in the proper sense being a useful category – provided that it can avoid the temptation to associate "chaos" with a disordering of "water structure". They also give rise to a number of intriguing questions. We know, for example, that microbial cells are sensitive to changes in water activity in the region of 0.001 a_w-units;[88,89] but how sensitive are they to minute changes in chaotropicity? More generally, while cells contain, and are usually surrounded by, complex solutions containing both chaotropes and kosmotropes, there has been relatively little work[73,76] on their net effect at the level of solute-water-macromolecule interactions or their implications at the level of cell biology.

Discussion

We have argued that, while there are very good reasons to examine the question of biomolecular hydration from a structural perspective that considers the configurations of water molecules, the vaguer notion of "water structure" as some kind of global phenomenon that can be modified by solutes or other forces is seldom of much explanatory value, not well motivated theoretically or experimentally, and most probably a construct that has become established for historical reasons. We have shown how such notions are contagious, so that for example the idea of chaotropicity as a qualitative or empirical characteristic related to the integrity of macromolecular structure in aqueous solution has morphed into an alleged property of the structure of water itself.

There is some reason to think that the powerful attractor of "water structure" as a catch-all default mechanism for aqueous phenomena might begin now to weaken. One reason for that is that the whole concept of structure in physical chemistry is now becoming more nuanced by the issue of dynamics. Now that there are well-established techniques for looking at molecular motions on many time and length scales and exploring their collective aspects,[92] drawing static pictures of ice-like hydrogen-bonded networks is becoming less relevant or meaningful. We can see a similar trend in molecular biology as a whole, which was also first pursued by Bernal, Pauling and others as a primarily structural science. It was initially about locating the positions of atoms in crystals, concerned with molecular shape and shape complementarity, locks and keys and the fitting together of surfaces. Now it is increasingly about fluctuations, dynamics, and the couplings of motions in biomolecules and their solvation environment.

One might argue that if the emerging view of hydration is (at least at present) a messy one, then where there is mess there needs to be compromise. Hoffmann[93] has suggested that chemistry is sufficiently complex a science that it needs fuzzy rules of thumb: ideas that might not be rigorous but are useful, like oxidation states, covalent bonds and electronegativity. Explorations of what we might still need to call "water structure" doubtless need such fuzzy concepts too, but perhaps we have simply not yet recognized the right ones. Properly applied, chaotropicity might function as one such empirically defined "black box" term that can help us to classify and organize our thinking while acknowledging that at a deeper, mechanistic level the story is more complex and not so easily compartmentalized. In any event, finding the right semi-truths to guide conceptual thinking in science is an art, an important part of which is to ensure that we do not get trapped into believing our convenient fictions.

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Notes and references

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