

Water — an enduring mystery

Yet another theory of liquid water structure raises questions about interdisciplinarity, drug design, astrobiology, molecular biology, geochemistry and more.

Philip Ball

No one really understands water. It's embarrassing to admit it, but the stuff that covers two-thirds of our planet is still a mystery. Worse, the more we look, the more the problems accumulate: new techniques probing deeper into the molecular architecture of liquid water are throwing up more puzzles.

This guilty secret has myriad ramifications. Water defines the terrestrial environment. It is central to Earth and atmospheric sciences, to biology and to many technologies. The common assumption that water is well characterized has led to explanatory edifices built on shaky ground. The situation is unsatisfactory intellectually and hazardous in practice.

Everyone is agreed that one aspect of water's molecular structure sets it apart from most other liquids: fleeting hydrogen bonds¹. These feeble bonds that link the molecules constantly break and form above water's melting point, yet still impose a degree of structure on the molecular jumble.

That's where the consensus ends. The standard picture of liquid water¹ posits that each molecule of H₂O is, on average, bonded to four others in a tetrahedral motif. This repeated, constantly reorganizing unit defines a three-dimensional network extending throughout the liquid. This prevailing view comes largely from neutron-scattering studies and computer simulations, and it makes good sense in the light of the unambiguously tetrahedral arrangement of molecules in ice crystals.

In 2004, the latest instalment in a long line of dissension emerged. Lars Pettersson and his colleagues based at Stockholm University in Sweden published a controversial paper in *Science* claiming that molecules in liquid water bind on average to just two others, forming chains and rings². It was a 'string theory of water', if you will. Pettersson's group used X-ray absorption spectroscopy to probe the local environment of individual oxygen atoms.

The interpretation was greeted with scepticism, but the 'string theory' won't go away. The Swedish researchers now claim, in work as yet unpublished, that the conventional tetrahedral structure is not the only way to interpret previous data on water structure from X-ray, neutron



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scattering and infrared spectroscopy. The 'string' model fits the results too, they say. With physicists and chemists at several institutes in Japan, they have refined their view through X-ray emission spectroscopy.

Water, they now suggest, is a muddle of two different structures. It is a random soup flecked with tiny 'icebergs', each comprising 100 or so loosely cohering molecules. The clusters, they argue, are relatively 'open' and strongly hydrogen-bonded, in keeping with the conventional tetrahedral model. The soup is made from the 'string' structure described in 2004 — denser and with fewer hydrogen bonds.

Such a two-state model would fundamentally change our picture of how dissolved substances behave. Non-polar solutes might be partitioned into the strongly hydrogen-bonded clusters; polar solutes such as ions would swim in the

disorderly soup. The consequences would be felt from geochemistry to industrial processing to colloid science. Biologists in particular would need to take heed, because liquid water, widely acknowledged as the 'matrix of life' on our planet at least, is not just a passive scaffold. It has many active roles in molecular biology³, minutely influenced by its structure.

Right now, many water researchers dismiss the Stockholm work as a storm in a teacup. They think that the aberrant results will turn out to have some mundane explanation, perhaps simply data misinterpretation. It demands considerable suspension of disbelief to accept that the conventional picture of water, assembled painstakingly over the best part of a century, is fundamentally wrong — although stranger about-turns have happened in science.

Regardless of its outcome, this debate is interesting as an illustration of just how difficult it is to understand water, and how widely the uncertainties ripple out. And the



dispute is just one of many. For example, does water form two different liquid phases under extremes of temperature and pressure? How does it rearrange its molecules next to a surface or to accommodate solutes? Is most of the water in cells structurally akin to the pure liquid at all? If or when these spats dissolve, history leads us to expect others to bubble up in their place.

Too anomalous, too strange

The constituency pondering such issues should be far wider than at present. One problem is that even many of those who work on general theories of the liquid state of matter won't go near water: it is too anomalous, too strange. It does not do what liquids are 'supposed' to: it expands on freezing, it is densest in the liquid state at 4 °C rather than becoming steadily denser as it cools; it has an abnormally high heat capacity, odd viscosity, and more. Most of these anomalies are rationalized by the standard tetrahedral hydrogen-bonded network. But it's still not clear how this delicate molecular interlinking translates to bulk-scale behaviour. Computer simulations are often used to explore matter's molecular-scale character, but for water these are notoriously sensitive to how the forces between molecules are modelled.

There's nothing new in a two-state picture of water. In 1892, well before hydrogen bonding was recognized, Wilhelm Röntgen proposed that cold water contains microscopic 'icebergs' in a fluid 'sea'. In the 1920s Henry Armstrong's 'hydrone' theory propagated the idea of long-lived clusters of water molecules, as did Oleg Samoilov's 'interstitial' model in the 1940s. Most recently, the chemist Wilse Robinson in Texas tirelessly promoted the idea that water is a mixture of two forms until his death in 2000.

Such ambiguities allow wilder ideas to insinuate themselves. The polywater affair of the late 1960s stemmed from a claim by Russian chemist Boris Deryaguin and his colleagues to have observed a gel-like form of water in small capillary tubes. Even more outrageous were the experiments on high-dilution biological solutions, conducted by the late Jacques Benveniste and his collaborators in France in the 1980s. These created the notion of the 'memory of water', whereby the liquid can allegedly become imprinted with biomolecular information. Still wheeled out in justification of homeopathy, this improbable idea continues to trade on genuine uncertainties about water structure.

Do these disputes matter to anyone but those involved? One of the strengths of science is that it can operate in a modular manner. We can make a lot of progress in one direction while deeper questions about more fundamental issues remain unre-

solved. Were that not so, every field would halt until we had a theory of quantum gravity. Chemists can formulate effective models of atomic bonding and molecular structure without knowing nuclear physics; evolutionary biologists need not grasp the chemistry of genetics.

But molecular biology depends inextricably on what water is like at the molecular scale. The iconic view of DNA's double helix, for example, is disingenuous: it is only the molecule's structure in water. In the gas phase, the helix looks as though a child has stamped on it. Hydration changes, such as removing water from the surface of the molecule, can induce switches in DNA conformation. Recent experiments show that the double helix spontaneously unzips when dragged into a non-aqueous solvent, suggesting that the same might happen in a low-water environment. It is likely that nature exploits these properties to manipulate DNA; maybe, for example, hydrophobic cavities in enzymes assist the unzipping that precedes replication.

When proteins bind their substrates, an intervening sliver of water must shift out of the way. This process depends on the structure of the confined water. Moreover, many enzyme binding sites have water molecules attached to their hydrophilic regions. Some of these molecules cede their position to the incoming substrate, others stay in the binding site and supply hydrogen-bonding bridges to the docking entity.

Chaos and order

All this entails a subtle balance of energy costs: that of bond making and breaking (enthalpy) and that of disorder changes in the molecular components (entropy). The enthalpy cost depends in part on how many hydrogen bonds the expelled water molecules make in the bulk liquid; the entropy change is also contingent on the degree of ordering there. Estimates imply that, on average, the various costs and benefits of releasing a water molecule cancel out, so small factors specific to each case could tip the scales either way, making binding more or less favourable. A similar fine balance probably governs the important interactions of proteins with carbohydrates.

These delicately poised energetics are crucial in drug design. Here the aim is to engineer good binding between a drug and its target — a small molecule, perhaps, that slots into an enzyme binding site to block it. Some inhibitors of HIV-1 protease, a key target in AIDS therapies, bind via a bridging water molecule. Others have been designed to exclude it. But docking design has so far capitalized little on water molecules because their role is not

sufficiently understood or quantified.

The role of water structure in molecular biology is perhaps most apparent in the attractive force that operates between two hydrophobic entities in water. Hydrophobic attraction drives the correct folding of protein chains, the binding of some proteins to substrates, and the aggregation of proteins into functional units and dangerous clumps such as amyloid fibrils. It is one of the key forces in molecular biology. And no one understands it.

Several biochemistry textbooks will tell you that it was explained by Walter Kauzmann in 1959. Kauzmann argued that water becomes more 'highly structured' around hydrophobic solutes, and that the release of some of this water into the relative chaos of the bulk liquid when two such

solutes stick together produces a favourable increase in entropy⁴.

This is almost certainly wrong. For one thing, the hydrophobic interaction may operate in different ways for small solutes such

as methane and for the kind of extended nanoscale surfaces that proteins have⁵. Such surfaces may stick together via a sudden, coordinated expulsion of many water molecules, although whether and how much this happens in real protein folding and association remains unclear. The point is that all such mechanisms depend fundamentally on the structure of bulk water and how that alters near a hydrophobic entity.

Must water's enduring mystery merely induce despair in those who want to know how proteins fold and function, say, or how minerals dissolve or suspended particles clump together? No. Sometimes the details won't matter much, sometimes empirical knowledge and heuristics will suffice. Think of this puzzle more as an exercise in humility. Water reminds us of the dangers of doing science in silos, the risks of leaving apparently tidy explanations unexamined, the importance of not letting ubiquity lead to invisibility, and the recognition that new ways of studying the world can exacerbate as well as dispel confusion. ■

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For further reading visit <http://tinyurl.com/2hfh8s>.

For more on water see www.nature.com/news/specials/water/index.html.