Water: life's matrix

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Water is the archetypal liquid: in the Aristotelian scheme of elements it represents all things that flow. Yet we now know that water is profoundly unusual, and defies many of the expectations of liquid-state theory. These peculiarities may make liquid water uniquely suited to act as the matrix of life. At the same time, they make it hard to discern exactly how water acts in this capacity. The nature of water in the cell, and in particular the question of whether or not it resembles bulk water, has been hotly debated by biophysicists for decades, still with no consensus in sight. Nonetheless, it has become increasingly clear in recent years that water should be regarded as a biomolecule: not the passive backdrop on which life's chemistry is enacted, but an active participant. I shall look at the question of what we do and do not know about cell water and its interactions with other biomolecules. I shall also consider whether water's uniqueness justifies the decision by NASA to 'follow the water' in its search for extraterrestrial life, and shall briefly review the status of attempts to locate liquid water in the past or present environments of other planets and their satellites.

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Some time ago I was asked to contribute an article to a special issue of the journal *Cellular and Molecular Biology* [July 2001, **47(5)**], devoted to the topic of water in the cell. I decided that might be an interesting thing to do, but the guest editor of the volume seemed concerned that I should be aware of what I was letting myself in for. She implied that any statement about this topic entailed an element of risk and was likely to offend one party or another.

This was not entirely news to me. Some of the most empassioned exchanges that I'd encountered during my years as an editor at Nature have involved the structure and behaviour of water. Indeed, I had some kind of baptism of fire when I joined the journal in 1988, just weeks after it had published the now infamous paper by a group of French biologists on the 'memory of water'. As I was coming from a research group that specialized in the physics of liquids, that was a matter of not inconsiderable embarrassment. A year later water was back, in the form of cold fusion. I shall not say

any more about either of these notorious episodes here; there is plenty in my book. And even now, the polywater scandal of the late 1960s is spoken about in hushed tones and with embarrassed glances.

These disputes about what water is and what it does are by no means limited to academic science. I have delved a little into the extraordinary story of the Austrian forest-warden Viktor Schauberger and his ideas about 'living water', expanded later by Theodor Schwenk. This is an area where New Age mysticism blends into technological reality: I've heard other scientists swear that magnets really can descale kettles, and that specially treated metal rods can alter the taste and viscosity of water with a little gentle stirring. Recently a non-scientist friend told me about the work of Masaru Emoto, who changes the shape of snowflakes with prayer:

The photo at the top is of a frozen water sample from the lake at Fujiwara Dam, in Japan. The sample is described as "dark and amorphous, with no crystalline formations." But then the Reverend Kato Hoki, chief priest of the Jyuhouin Temple, prayed for one hour prayer beside the dam, new samples were withdrawn, and the crystals at the bottom were grown from this water.

Apparently music makes its influence felt too: when water was crystallized after being exposed to Elvis Presley's *Heartbreak Hotel*, the crystals are reported to have broken spontaneously into two.

Now, why am I telling you this? Certainly not to imply that this could be a good research field for your new institute. No, it's to remind you that studying water is not like studying any other substance. It is not a neutral subject. All of us bring to it a wealth of cultural associations that are probably impossible to put aside. Water is an elements—not, of course, in the chemical sense, but in the mythical and poetic sense, and that is not irrelevant. I'd recommend to you the book by the French philosopher Gaston Bachelard called *Water and Dreams*, which explores the various poetic incarnations of water. I believe that some such ideas—of water as purifier, water as saviour—lay unconsciously behind some of the pathological episodes of water science I've just mentioned.

Most prominent of all is the ancient and profound association of water with life. Albert Szent-Gyorgi, the Nobel laureate biologist, famously summed it up this way: "Water is the matter and matrix of life".

In one sense, this could be taken to mean simply that water is essential for life—a notion that is not proven but which I think is very hard to imagine as anything but true, and which—as I'll discuss later—conditions all of NASA's current thinking for its program in astrobiology.

In another sense, it means something more than that. Theodor Schwenk's idea of 'living water' seems to be essentially a poetic notion unconnected to any scientific criteria, although his supporters would no doubt disagree. But there is a very real manifestation of 'living water' in the form of the cytoplasm—water in the cell. Many biologists are

increasingly starting to think that there is something special about this water that sets it apart from ordinary, bulk water.

First, some basic things about bulk water. I doubt that to this audience I have to say very much at all about that, but it might nevertheless be just as well to establish a few points about water as a liquid. As I say, many years ago I worked for a short time on liquid-state physics, but I studied nothing at all about water. Why? Because a lot of conventional liquid-state theory breaks down when it comes to water. We were warned away from it; water was too difficult, so we stayed with liquid argon.

The reason why water is too difficult, of course, is its hydrogen bonds. From the point of view of liquid-state theory, hydrogen bonds complicate matters because they mean that the short-ranged, local ordering around any one molecule is dominated not by hard-sphere repulsive interactions, as it is in simple liquids, but by orientation-specific attractive interactions. These link water molecules into the well-known random hydrogen-bonded network, for which Gene Stanley and others showed in the 1980s that a description in terms of percolation theory could be appropriate.

What is more, the hydrogen bonding sets up a tension between the usual tendency in liquids of close molecular packing and the requirement for molecules to be kept 'at arms' length', as it were, in order to form hydrogen bonds. This is the central feature of water's structure and behaviour, so it was essential that I showed it in my book. But…[Show slide & tell how I was discouraged from drawing real ball & stick molecules in my book.]

This arrangement gives water an unusually high degree of local structure, and also a propensity to leave lots of empty space between molecules—which, of course, results in the density drop on freezing. It is also possible to ascribe to this structure anomalies such as the 4 C density maximum and the possible existence of two different phases of deeply supercooled water with a critical point estimated at 220 K and 100 MPa. [Phase diagram]

For cell biologists interested in water, the key question is how this unusual degree of structuring is modified by the presence of biological macromolecules, membranes, dissolved ions and so forth. This is the issue on which there is no agreement.

Key questions:

First of all, let's have a look at the aqueous environment of the cell. Cell water represents 70-80 percent of the cell's mass and volume, and one can calculate just on the basis of averages that the macromolecules are separated from one another by typically no more than 3-4 layers of water—a distance of around 1 nm. In this sense, there is probably little if any bulk-like water in the cell. Just about all the cell water 'feels' the effect of the biomolecular constituents. The question is: what is the nature of that influence? In 1969, Rudolph Peters had this to say:

"I believe the cell to be organized not only in respect of its grosser parts such as the nucleus, but also in regard of the actual chemical molecules of which it consists. Owing

to the microheterogeneous nature of the system, surface effects take precedence over ordinary statistical, mass-action relationships and become in the ultimate limit responsible for the directing of the whole, and therefore, the direction of activities."
[Proc. Roy Soc. B, **173**, 11-19 (1969)]

The cytoplasm is widely regarded as a gel which seems able to maintain its integrity even if large ruptures are made in the cell membrane. On the other hand, many molecular biologists continue to use the paradigm of free diffusion to describe molecular motions in the cytoplasm, and this picture often seems to be remarkably satisfactory.

A typical example of a biologist's view of cell water looks something like this:
[Slide]
"Structured water' is a term common to the cell and molecular biology literature, but what, precisely, is meant by it?

The idea that cell water somehow has a different structure from bulk water goes back a long way. Szent-Gyorgyi talked about the structuring of cell water in the 1950s, and G. N. Ling proposed in the 1960s that cell water forms organized, layered structures on the surface of proteins. This sort of notion owes a great deal to the idea of Henry Frank and Marjorie Evans in 1945 that hydrophobic solutes increase the ordering of water.

Proteins contain both hydrophobic and hydrophilic regions, and one needs to ask how water responds to both. There is still no consensus in either case. Once Bernal, Pauling and others had clarified the basic tetrahedral local ordering of liquid water in the 1930s, discussions of water structure began to be couched in terms of an essentially crystalline paradigm, rather than the gas-like starting point of conventional liquid-state theory. Frank and Evans supposed that hydrophobic solutes increase this ordering in order to minimize the disruption to the hydrogen-bonded network. They said "When a rare gas atom or nonpolar molecule dissolves in water... the water, so to speak, builds a microscopic iceberg around it."

In 1959 Walter Kauzmann realised how this sort of picture could lead to the observed attraction between hydrophobic species in water. As two hydrophobes come together and their ordered hydration spheres overlap, some 'structured' water is freed and there is a gain in entropy. According to this explanation, the hydrophobic force has an entropic origin.

This force is of crucial importance to cell biology. It accounts for the aggregation of proteins and is one of the main driving forces of protein folding—the idea being that the protein chain 'hides' its hydrophobic regions by bundling them up together in the protein's interior.

Kauzmann's model is still popular, but there seems to be no good evidence that it is right. It is hard to measure water structure around hydrophobic solutes, because of their very insolubility. But recent neutron scattering and EXAFS measurements for solutes like methane and krypton show no clear sign of increased water ordering in these hydration

shells. There is some indication that water molecules tend to align their O-H bonds tangential to hydrophobes, and that they may lose some rotational freedom as a result; but there is no evidence for greater positional ordering in clathrate-like structures.

So the origin of the hydrophobic force is still unclear. An alternative explanation is that the small size of the water molecule means that it is hard to 'make space' for a hydrophobic molecule—that is, there is a high free-energy cost, in both enthalpic and entropic terms, of creating a cavity in water. This too seems insufficient as a complete explanation. There is plenty more to be said about hydrophobic forces and the way they depend on factors like temperature and pressure, and I will simply refer you to two good discussions:

W. Blokzijl & J. B. F. N. Engberts, *Angew. Chem. Int. Ed.* **32**, 1545-1579 (1993)
N. T. Southall, K. A. Dill & A. D. J. Haymet, *J. Phys. Chem. B* **106**, 521-533 (2002)

What about hydrophilic solutes? Here the situation is no less confused. Do ions increase 'water structure', or decrease it? The classic studies of Franz Hofmeister in the 1880s on the effect of different salts on solubility of proteins has given rise to the classification of ions as 'structure-making' and 'structure-breaking'—or in the modern terminology, kosmotropic and chaotropic. Small or highly charged ions, like fluoride and sulfate, are considered structure-making. They reduce protein solubility (this is the process known as salting-out), which is explained in a hand-waving way as being due to the greater resistance a highly structured solvent puts up to disruption by macromolecular solutes. Big, low-charge ions like iodide and perchlorate are structure-breaking, ostensibly reducing water structure and promoting solubility. Chaotropic or structure-breaking ions also promote protein denaturation and destabilize membranes.

Philippa Wiggins has constructed a theory of cell water based around the idea that structure-makers create hydration shells of low-density, ice-like water, while structure-breakers create high-density water in which many hydrogen bonds are broken. [Slide] Wiggins argues that these two types of water are entirely different solvents, and that they set up osmotic and viscosity gradients in the cell which have important consequences for cell function.

The problem is that direct probes of water structure show no support for this kind of two-state model, nor any clear signature of structure-making or structure-breaking by ions. Felix Franks says "Much has been written about this concept, much of it misleading."

Yet it does seem that water's hydrogen-bonded structure displays some sensitivity to the influence of solutes. The hydrophobicity of D-talose, for example, is greater than that of its isomer D-galactose, which seems to be related to the relative comfort of fitting the OH groups into the three-dimensional jigsaw of the hydrogen-bonded network. [Slide]

All this concerns small solutes; but macromolecules can in some ways be regarded as mesoscopic surfaces. How water responds to the presence of a surface opens up another can of worms. Some biologists get very exercised over the high degree of structuring seen

in water next to a hydrophilic surface, as for example in these results measured by Roger Horn and Jacob Israelachvili using the SFA:
[Slide]

But there is in fact nothing very remarkable about this kind of layering in a liquid next to a wall. It is predicted even for simple liquids, and says nothing about water's status as a 'structured liquid'. The layering is due to packing effects—essentially the same thing that gives rise to oscillatory radial distribution functions. Indeed, one can ask whether water might be *less* inclined to form these two-dimensional layers since that will disrupt the three-dimensional hydrogen-bonded network. Israelachvili and Hakan Wennerstrom created some controversy recently when they claimed that water between hydrophilic surfaces behaves just like a normal liquid, and that the relatively long-ranged repulsions that have been observed between such surface, and explained by water's unusual degree of structure, are just experimental artefects.

Hydrophobic surfaces create another controversy. Some very recent neutron-reflectivity results by Bob Thomas's group at Oxford suggest that liquid water is separated from such surfaces by a very thin (1-nm or so) layer of gas—there is drying at the surface. This is expected theoretically. But it's been known for a long time that two hydrophobic surfaces separated by a thin film of water seem to attract one another over very large distances—300 nm or so, corresponding to about 1000 molecular diameters. The origin of this long-ranged hydrophobic attraction is still disputed. For a long time it too was sometimes attributed to 'water structuring' effects, although now there seems to be good evidence that it is caused by the nucleation of sub-microscopic bubbles of dissolved gas, which create menisci that pull the surfaces together. (See Carambassis et al., *Phys. Rev. Lett.* **80**, 5357-5360 (1998).)

A paper has just appeared in *Science* (25 Jan: **295**, 663) by Steve Granick's group which looks at the unusual consequences of bring together a hydrophilic and a hydrophobic surface in water. This sounds like a rather contrived situation, but it could certainly happen in the cell.

What is bound water?

From the physicist's point of view, then, the behaviour of water around solutes and in confined geometries is full of complexity. Experience with simple liquids tells us that in such situations a liquid deviates from bulk-like behaviour; the question of what water's hydrogen-bonded structure adds to this picture is still open in many ways.

But biologists have tended to gloss over these details by invoking a simpler picture of cell water. For a long time they distinguished two quite different kinds of water molecule: 'bound' and 'free'. Free water was assumed to behave much as it does in the bulk. Bound water is hydrogen-bonded, or at least quite firmly attached, to proteins and other macromolecules, so that it remains in place when the macromolecule is crystallized. Bound water constitutes 30-50% of the mass of 'dry' protein powders, and up to 80% of the mass of crystalline DNA. These water molecules are like limpets attached to the

macromolecule, and their positions are generally related to the protein's own atomic structure, so that the waters can become visible in X-ray crystallography and neutron-scattering experiments on protein crystals.

[slide] Yellow, green and purple spheres show bound water in different hydration shells.

This bound water can thus be considered an integral part of the biomolecule itself. There are now many examples of this water playing an essential role in protein function, and I want to give you a few examples of this.

Water wires:
Water trapped as hydrogen-bonded chains in protein channels seems able to act as a kind of proton wire, transferring protons rapidly via the Grotthuss process of hydrogen-bond flipping.

[Slide]

This can facilitate the delivery of protons to a catalytically active site, for example in cytochromes and bacteriorhodopsin. There is a hydrogen-bonded network of bound water molecules inside the channel of bacteriorhodopsin (a bundle of seven transmembrane helices), stabilized by acidic and basic residues on the helices. This channel acts as a wire to conduct protons from a Schiff base group on the photosensitive retinal pigment, via a $H_5O_2^+$ group in which a proton is delocalized. [Rammelsberg et al., Biochemistry 37, 5001 (1998); Spassov et al., J. Mol. Biol. 312, 203 (2001) – Klaus Gerwert's group at Bochum.]

Transient proton wires have been implicated in the leakage of protons across cell membranes. Such chains may be very short-lived, but can nevertheless transport protins during this time because the Grotthuss mechanism can be so rapid. The very recent simulation studies of water molecues inside the hydrophobic channels of carbon nanotubes may provide a good model system for understanding hydrogen-bonded chains in proteins. (Another analogue system was reported by Terao et al., *JACS* **123**, 10468-10474 (2001).)

Selective binding:
Quiocho et al. at the Howard Hughes Medical Institute in Texas have shown how water in the binding pocket of bacterial arabinose-binding protein provides a part of the jigsaw that enables the protein to bind its ligand L-arabinose selectively. [*Nature* **340**, 404 (1989).] In a more recent study of carbohydrate binding by proteins, Geert-Jan Boons from the Complex Carbohydrate Research Center in Athens, Georgia, and coworkers have looked at the interaction between trisaccharides and the legume lectin protein concanavalin A. In the natural complex with this trimannoside, there is a crystallographically defined water molecule in the binding site which provides a hydrogen-bonding bridge with the ligand. Perhaps surprisingly, this complex turns out to have a higher binding constant than one with a trimannoside derivative in which there is an extra hydroxy group which displaces the bound water. This seems to be because the bound water supplies an enthalphic contribution to the binding energy that outweighs the favourable entropy gain when the water is displaced. In other words, the bound water is not just some accident of design, but is part of the fine tuning of the process – a fine-

tuning that involves a really quite delicate balance between the entropic and hydrogen-bonding contributions of bound water and water in the bulk-like liquid network. [JACS 123, 12238 (2001)]

Enzyme activity:
Bound water molecules can take part in chemical reactions in the active sites of proteins. For example, it seems that a water molecule can act as a nucleophile to initiate splitting of a lactam ring in the bacterial enzyme zinc lactamase, which is involved in antibiotic resistance (lactams are used as antibiotics). [M. Krauss et al., J. Phys. Chem. B 105, 8040 (2001). Krauss is at the Maryland Biotechnology Institute.]
And Minoru Sakurai and colleagues in Tokyo (Inst. Technol.) have shown that hydration of an enzyme (ribonuclease T1) tunes its electronic structure, relative to the gas phase, so as to make the frontier orbitals (HOMO and LUMO) those that are localized within the enzyme's active site. In other words, the enzyme is designed for use in water. [K. Ohno et al., JACS 123, 8161 (2001).]

Allosteric effects:
William Royer et al. (Univ. Mass Medical Centrer) have reported evidence that water molecules bound at the interface of the dimeric haemoglobin of the mollusc *Scapharca* act as transmission units for allosteric effects that promote the cooperativity of oxygen binding. In other words, structural changes in one subunit on binding an oxygen molecule are communicated to the other subunit to increase its oxygen affinity via interfacial water molecules between the subunits.

DNA:
Four hydration shells around DNA are visible to X-ray crystallography. This water sheath becomes part of the structure, and it seems that these layers can act as a kind of switch that, by flipping hydrogen bonds, allows a ligand that binds in the major groove to release bound sodium ions from the minor groove. Changes in the hydration shell can also act allosterically to control ligand binding to DNA by affecting the stability of the double-helical secondary structure.

What are the consequences of these subtle sensitivities of cell components to water activity and structure? How is cell water tied up with the 'health' of the cell? There is some evidence that the nature of cell water differs in healthy and diseased cells. NMR measurements of water mobility can be hard to interpret because they can in some sense represent an average between 'free' and 'bound' water, which exchange rapidly on the NMR timescale. But there is some indication that the mobility of cell water is generally different from that of bulk water, and that these motions become more bulk-like in cancer cells. Thus it has been claimed that life 'tames' cell water, bringing it into a more highly structured state (whatever that might mean exactly) that is better able to facilitate cell molecular processes.

In any event, a clear picture emerges in which water is not life's matrix in the sense of being a passive backdrop on which the tapestry of life unfolds. Rather, it is a biomolecule in its own right, albeit an extremely complex one that can engage in many different

processes and form almost a kind of versatile 'loose macromolecule' that interacts with proteins, nucleic acids and membranes.

This image casts a new light on the question of whether water is essential for life. No other small solvent molecule has the right geometry and chemistry to form the kind of extended, three-dimensional hydrogen-bonded network we find in liquid water. If this structure is indeed crucial for enacting life's delicate molecular processes, that strengthens the case that life—all life—depends on water. If we assume that all chemical processes complex enough to qualify as life will require the same kind of characteristics we see in protein interactions—exquisitely crafted and self-assembling architectures, molecular recognition, allostery, a combination of flexibility and robustness—then it is hard to identify any other solvent that seems likely to support such things. (True, some enzymes can function in non-aqueous solvents, but only after folding in water.)

There is a very nice short article by Chris Chyba in *The Planetary Report* (May/June 1998 – slide) in which he asks the question: why water? Chyba points out that the habitable zone in solar systems is generally taken to be the range of distances from the central star that can support liquid water. We have to be careful about this. Strictly speaking, Europa lies outside our solar system's habitable zone but nevertheless seems to harbour a liquid ocean because of tidal heating from Jupiter. The same may be true of Jupiter's other moon Callisto. And John Brownlee and others have pointed out that there is more to a habitable zone than liquid water, so that habitability may be a more rare phenomenon in the universe than one might naively expect.

All the same, we tend to delimit habitability first and foremost by 'following the water', as NASA puts it:
('Water – Life's Elixir in the Solar System'; "the science strategy for Mars exploration is "to follow the water""—NASA web site.)
Chyba warns that we should 'beware the hydrocentric'—that we should be wary of some arguments for water's indispensability for life. It's hard to see how nonpolar solvents like the liquid hydrocarbons thought possibly to exist on Saturn's moon Titan could possibly support complex chemistry, but Chyba does not rule out ammonia as a potentially life-supporting solvent. I think, for the reasons given above, as well as others such as ammonia's much weaker amphoteric nature, it is unlikely that ammonia could replace water as another matrix of life. One interesting question is how, if life were to have evolved elsewhere, it has solved the problems that water poses, such as its tendency to hydrolyse nucleic acids and proteins.

Where do we stand in terms of getting answers to these questions?

Mars probes:

Mars Odyssey: was launched last April, is now in planetary orbit and will begin mapping in February. It will map the distribution of the elements and minerals on the planet's surface, especially water ice. It uses thermal emission imaging and gamma-ray spectroscopy to identify elements.

Mars Express: scheduled for launch in early 2003, to arrive December 2003. The orbiting spacecraft will search for signs of subsurface water, and the mission includes a lander—Beagle 2—which will conduct exobiological and geochemical studies of the surface.

Sample returns are scheduled for missions in 2014 and 2016.

Europa probes:

Europa Orbiter: scheduled for launch in 2008, to arrive in 2010. This will investigate the putative subsurface ocean in more detail than the Galileo mission, and will search for candidate sites for future lander missions.

I am placing my hopes on the Europa missions, for the simple reason that they can potentially answer the key question about liquid water: does its existence on another world simply make life possible, or inevitable?