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PERSPECTIVES

OPINION

Do we underestimate the importance of water in cell biology?

Martin Chaplin

Abstract | Liquid water is a highly versatile material. Although it is formed from the tiniest of molecules, it can shape and control biomolecules. The hydrogen-bonding properties of water are crucial to this versatility, as they allow water to execute an intricate three-dimensional 'ballet', exchanging partners while retaining complex order and enduring effects. Water can generate small active clusters and macroscopic assemblies, which can both transmit information on different scales.

It is surely time for water to take up its rightful position as the most important and active of all biological molecules, and leave behind the perception that its influence is inconsequential and its presence can be safely disregarded. Liquid water is not a 'bit player' in the theatre of life — it's the headline act. Although reminders of its central importance have been published regularly, although sparsely, over the past century (TIMELINE), the surges in interest that they generate have always fallen back, making way for the 'glitzier', and less ephemeral, structures and properties of other biomolecules such as proteins and nucleic acids. Even today, much of the published material that concerns biological molecules ignores the central role of water in life's machinery. Recently, however, its place as the principal and most versatile player in cell and molecular biology has become clearer.

In bulk liquid water, each water molecule not only forms up to four tetrahedrally directed hydrogen bonds with neighbouring water molecules, each molecule can also establish dipole and induced dipole interactions with other molecules (FIG. 1). Such water clusters give liquid water a heterogeneous character that can change with different physical and environmental conditions. The atoms of biomolecules can replace any or all of the links around each water molecule, which affects the structuring of adjacent water molecules and biomolecular groups as well as secondarily linked molecules and distant parts of the biomolecules. Such water molecules can be bound to the surfaces of biomolecules by stronger or weaker forces than their average bulk-phase interaction forces. Either way, they exchange places with other water molecules on a timescale of about 1–100 picoseconds[1], and this exchange depends more on the local topography and exposure to competing water molecules than on the strength of their binding. This rapid exchange of partners allows the surface shape of a biomolecule to change but does not significantly change other effects of hydration such as the net direction and strength of the hydrogen bonding.

Through the high density of liquid water's compliant, yet strong, hydrogen bonds, it controls protein folding, structure and activity[2]. It determines the twisting of the DNA double helix[3] and the recognition of the DNA sequence[4]. As the medium for biological processes, it can change and affect them in an instant, slowing them down at one moment, speeding them up at another, while constantly and rapidly transferring information from one place to another, both near and far. Water can function as individual isolated molecules, small clusters, much larger networks or as liquid phases that can have different 'personalities'. Water clusters show great diversity both in their structure and function, with their localized structures being determined by the free energy of bonding and non-bonding contacts[5]. Despite a clear, consistent and growing message that has been drawn from wide-ranging phenomena, unambiguous confirmation of the presence of particular water clusters remains elusive. This is because most experimental and theoretical techniques cannot observe large-scale clustering and only describe the fleeting environment around individual molecules[6,7]. In addition, simulations can give results that are based on incomplete sampling, and the resolving power of many experiments is limited by time and space averaging. Indeed, neither diffraction[8] nor NMR studies on pure water show any evidence of organized clustering, due either to methodological shortcomings or to the real absence of such clusters.

This article presents evidence for only some of the wide-ranging and complex issues that surround the biological activity of water, and it offers only a glimpse of reality. It covers water's controlling influence over protein and nucleic-acid structure and function, as well as over cellular activity. For deeper analyses, the reader is directed to the reference list and the Further information.

The effects of water on proteins

Water is an integral part of many biomolecules. In particular, protein–water interactions establish and mould the free-energy landscape that governs the folding, structure, stability and activity of proteins[9], as is discussed in the following subsections.

Protein folding. Proteins fold rapidly into well defined three-dimensional shapes that depend on the primary sequence of their amino acids. For this to be possible, the folding-energy landscape must consist of a multidimensional funnel with only small energy barriers that can be easily overcome by the available thermal energy (FIG. 2). This requires a mechanism that involves flexible, exchangeable and extensible linkages, and water-mediated hydrogen bonding is ideally suited for this purpose. It is therefore surprising that it has been proposed only recently that protein folding is mediated and guided by aqueous solvation[10]. Most protein folding occurs with water hydrating the peptide backbone and precisely manoeuvring the backbone through its secondary and supersecondary structural assembly towards

Timeline | **A brief history of biological water science**

~560 BC: Thales of Miletus declares that water is the primary essence of nature.

1781: The composition of water (two parts hydrogen to one part oxygen) is discovered by Henry Cavendish (see A Brief Biography of Henry Cavendish in the Further information).

1806: Grotthuss describes the polarity of the water molecule and the mechanism of proton hopping[50].

1888: Hofmeister discusses the effects of aqueous salts on protein precipitation[51].

1892: Röntgen develops Whiting's earlier idea that water contains tiny ice crystals, the concentration of which changes depending on temperature and pressure[52].

1920: Hydrogen bonding is first suggested to occur in water by Latimer and Rodebush[53].

1933: Bernal and Fowler develop a water model that consists of an equilibrium of dense and less-dense water clusters[54].

1945: Frank and Evans describe the hydration around hydrophobic groups as being like an 'iceberg'[55] — that is, as being a structured patch of water.

1953: Watson and Crick state that hydration is crucially important for the conformation of nucleic acids[56].

1954: The structure of the hydrated proton ($H_9O_4^+$; known as the Eigen cation) is proposed[57].

1957: Szent-Gyorgyi extends the 'iceberg' theory to protein molecules in cells[58].

1959: Kauzmann introduces the concept of 'hydrophobicity' to explain how proteins fold[59].

1965: Ling describes virtually all water in living cells as being different from normal liquid water[60].

1967: Narten, Danford and Levy complete an X-ray diffraction study of liquid water and generate its radial distribution function[61].

1971: Rahman and Stillinger describe the first molecular dynamics study of liquid water[63].

Water is found bound to a protein using X-ray diffraction[62].

its ultimate and unique active structure. Only late in structural development is water squeezed out by cooperative peptide hydrogen-bonding interactions from the more hydrophobic areas towards the more hydrophilic ones. Although water is implicitly involved and implicit water models are used in protein-structure prediction to reduce the computational overhead, empirical methodologies for protein-structure prediction usually fail to include any explicit water molecules. However, it is clear that water needs to be explicitly involved in the more powerful structural prediction methods that realistically follow the biological mechanism of folding[11].

Protein structure. Water is not present simply to fill up the available space in and around biological proteins[1]. Water molecules occupy specific sites and form localized clusters with structures that are determined by their hydrogen-bonding capabilities and the structure of the protein. Water molecules occupy cavities in most globular proteins, where they are present in similar numbers to the numbers of individual amino acids that are present and conserved as an integral part of the protein[9]. Although these water molecules are exchangeable with external water molecules, many of them are as essential for function as the amino acids[9]. Surface water molecules are held in place most strongly by charged amino acids[12], but are also held in place by other polar groups. 'Slippery water clusters', which have greater freedom for two-dimensional translation along a surface but have restricted molecular rotational mobility, form around exposed hydrophobic areas[13]. The exchange of surface water, and therefore the persistence of local clustering and the overall flexibility of the system, is controlled by the exposure of the water molecules to the bulk solvent, with greater exposure correlating with greater flexibility and more liberated protein-chain movement[9].

Water molecules can bind between the areas of a protein that approach one another but that are not touching, and can transfer surface information between subunits and away towards the bulk (FIG. 3a). As water molecules and their clusters are extremely accommodating in terms of their orientation and spatial distribution, they can lower energy barriers between the energy minima of neighbouring systems. Water can therefore function as a lubricant in that it eases the necessary rearrangements of peptide amide–carbonyl hydrogen bonding during conformational changes, which gives proteins the flexibility they need for their functions[14]. The hydrogen bonds of water molecules and their clusters can also function

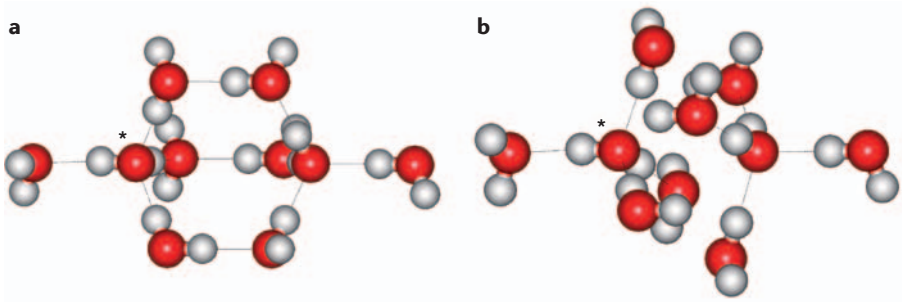
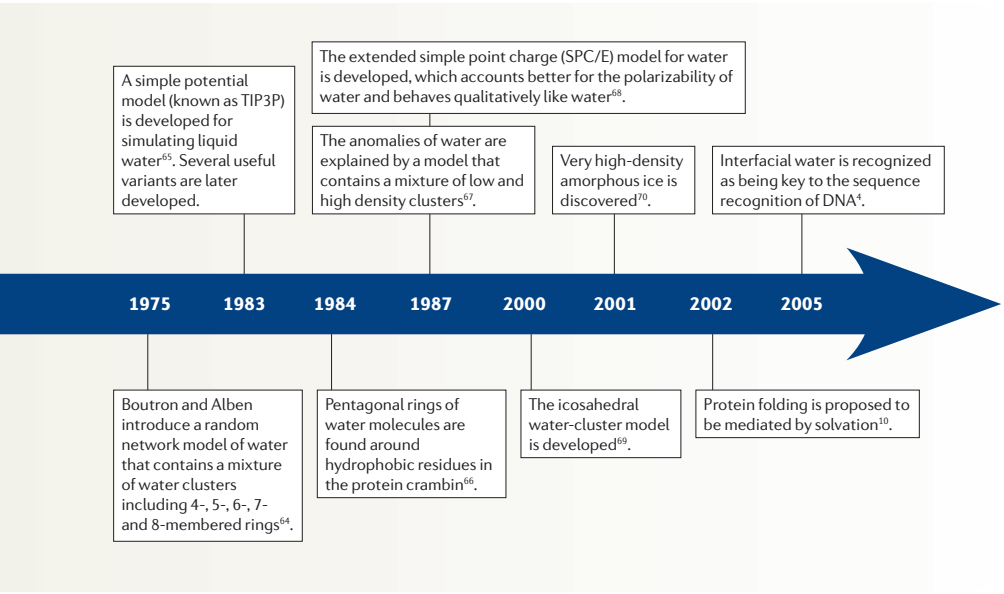


Figure 1 | **The dependence of water clustering on hydrogen-bond density.** Representative structures of water clusters in liquid water are shown. **a** | A tetrahedrally coordinated water molecule (marked with an asterisk) in an open-hydrogen-bonded, low-density structure. All of the hydrogen bonds are shown as being linear. However, although linear hydrogen bonds are the lowest energy structures, there will be considerable variation. Open structures such as this one can be extended to form extensive, almost isotropic networks. **b** | A water molecule (marked with an asterisk) is tetrahedrally coordinated to four other water molecules by means of hydrogen bonds, but is also equally close to three non-bonded water molecules that belong to another hydrogen-bonded cluster. These non-bonded interactions are formed from multiple dipoles and induced dipoles. Such clusters have greater entropy and raised enthalpy, and result in high-density local structuring. Actual structural changes in liquid water that are approximated by the conversion between these structural forms have been shown to occur at low temperatures[42] and high pressures[43], and are thought to be responsible for the extraordinarily anomalous changes that occur in the physical properties of water upon temperature change (see Sixty-three Anomalies of Water in the Further information). However, the question of whether water functions more as a one-state or a two-state system still has to be definitively answered. The atom colouring is: hydrogen, white; and oxygen, red.



as 'mechanical clutches' that transmit relative movement between subunits and domains, depending on the circumstances.

Protein activity and dynamics. A certain amount of water is necessary for the biological activity of all proteins. Even apparently 'dry' enzymes in the presence of gas-phase substrates show no activity without some water present[15]. Most soluble enzymes require a substantial number of water molecules to function. Recent work has indicated that, at the least, a spanning network of hydrogen-bonded water molecules that covers most of the surface of an enzyme is required for enzyme activity[16]. Such networks connect all of the hydrogen-bonded water clusters on the surface of a protein and control the dynamics of a protein, such as its domain motions. Reciprocated structural interactions between water molecules and a protein thereby transmit information around a protein[13]. Such dynamics, in turn, underlie the biological function of a protein[17]. Notably and significantly, the spanning water network has power over protein dynamics. Support for this view comes from experiments that have disrupted the spanning network, for example, by changing the temperature to increase hydrogen-bond breakage. Such disturbances tend to cause a protein to denature and lose its biological activity[18], but only in the presence of water.

The strength of the water network manages protein dynamics, with greater protein movements occurring in regions that contain many weak, bent or broken hydrogen bonds, because of the presence of more reactive water. Conversely, restricted movement results from the presence of many strong intramolecular aqueous hydrogen bonds. The structural elements that are most exposed to the bulk aqueous media are the least restricted[12]. The water network links secondary structural elements in the protein and therefore determines not only the fine detail of the structure of a protein, but also which particular molecular vibrations are preferred[16].

Protein–ligand interactions. Ordered water molecules at the surfaces of and between biomolecules frequently mediate binding interactions. Water-induced effects can be large and decisive in the selection of the best binding site[19]. The displacement of bound water molecules from their surface hydration sites often compensates energetically for the formation of direct interactions between biomolecules. There is normally a close relationship between the enthalpic loss and entropic gain of the water molecules that are being displaced and the enthalpic gain and entropic loss of the protein–ligand interaction that is forming, which eases both binding and release[20]. However, water molecules can remain in place when a new interaction is formed (FIG. 3b), and they, together with peripheral hydrating linkages, can affect the specificity, stereochemistry and thermodynamics of the binding process[19]. The energetic optimization of hydrogen-bonded networks (including their entropic consequences) that involve a protein, water and a ligand is an intrinsic part of all molecular recognition processes that involve binding proteins and enzymes.

Water in proton and electron transfer

Ordered water molecules that connect donor and acceptor sites can facilitate both proton-transfer processes and electron-transfer reactions. In both cases, the transfer is faster when the linking water molecules are more strongly hydrogen bonded.

Water in proton transfer. There is an extraordinary process, commonly referred to as the Grotthuss mechanism, whereby protons can move speedily in an aqueous environment[21]. Because transfer occurs between

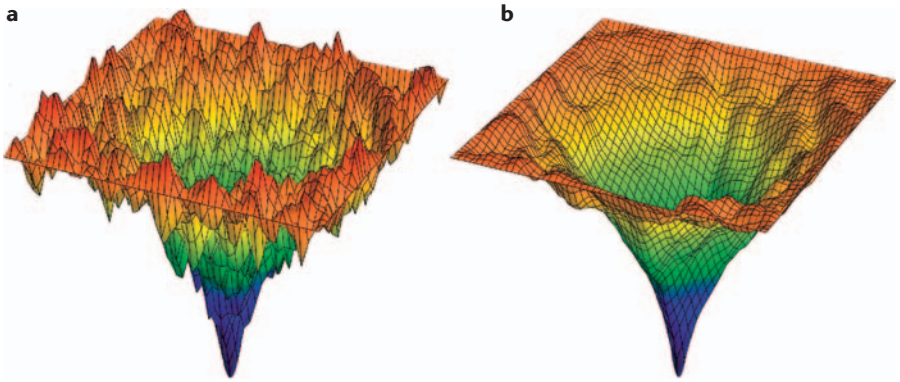


Figure 2 | **The importance of water in protein folding.** Schematic potential-energy funnels for the folding of proteins. The rim (red–orange) represents the high energy of the unfolded protein, with folding lowering the energy towards a minimum-energy structure that is at the bottom of the funnel (dark blue). It should be noted that these funnels represent three-dimensional landscapes, whereas the actual energy landscapes are multidimensional. **a** | The folding-energy landscape in the presence of low hydration highlights the numerous barriers to the preferred minimum-energy structure on the folding pathway. There are many local minima that might trap the protein in an inactive three-dimensional molecular conformation. **b** | When a protein is sufficiently hydrated, a smoothed potential-energy landscape is evident. This allows proteins to attain their active minimum-energy conformation in a straightforward and rapid manner.

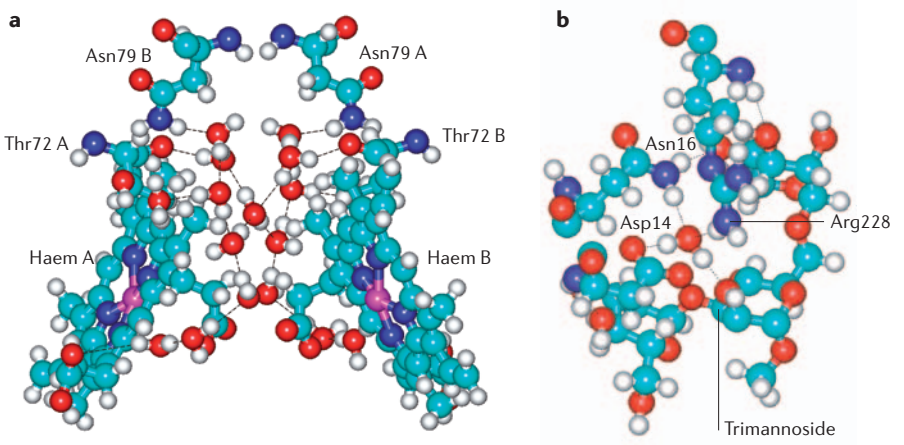


Figure 3 | **The importance of water in protein structure. a** | A cluster of water molecules (red and white molecules) can be seen between, and connecting, the haem groups and protein residues of the two identical subunits (labelled subunit A and subunit B) of *Scapharca inaequivalvis* haemoglobin. Note the symmetry of the well ordered water cluster that has formed two pentameric rings[44] (centre left and right). Following oxygen binding, the water molecules transfer information between the subunits before the water cluster is disrupted by the loss of six water molecules. This structure was created using the Protein Data Bank (PDB) accession file 4SDH[45]. **b** | A single water molecule in the ligand-binding site of concanavalin A functions as a link between Asp14, Asn16 and Arg228 of the protein and the 2′-OH hydroxyl group of the trimannoside ligand[20]. This structure was created using the PDB accession file 1ONA[46]. For clarity, only the relevant parts of the proteins are shown in both parts of the figure. The atom colouring is: carbon, light blue; hydrogen, white; nitrogen, dark blue; oxygen, red; and iron, pink.

pools that contain low concentrations (~10^{-7} M) of protons, conventional diffusion would be extremely slow, because it depends on concentration gradients. By contrast, the Grotthuss mechanism is most efficient in neutral solutions[21]. Following the addition of a proton to one end of a hydrogen-bonded chain (often referred to as a 'water wire'), hydrogen-bond flipping down the chain releases a different but identical proton at the other end of the water wire. Protons therefore move fastest when an ordered one-dimensional hydrogen-bonded chain facilitates their movement[21]. A common structural theme among proteins that are involved in proton translocation is a string of water molecules in a hydrophilic cavity that is lined with polar amino acids. Thermal fluctuations that involve these polar amino acids modulate proton transfer by rocking the water molecules. So, the polarization of the hydrogen bonding varies with the closing and opening of the hydrogen-bonded distances, which facilitates proton transfer (FIG. 4a). Water wires are so efficient at transferring protons that they are deliberately disrupted in aquaporin molecules, which allow the single-file translocation of water molecules through membranes. Halfway through these water-transfer channels, water molecules are intentionally reorientated to prevent sequential hydrogen bonding and the resulting facilitated proton transfer[22].

Water in electron transfer. Electron transfer between biomolecular redox sites involves electron tunnelling through the intervening space, which can be facilitated in part by proton movement in the reverse direction. The rate of transfer depends on both the extent and the structure of the intervening space. Structured water clusters near redox cofactors accelerate electron transfer by producing strongly coupled tunnelling pathways between the electron donor and acceptor[23] (FIG. 4b). More generally, water molecules can facilitate or disrupt this process through the degree of alignment of their dipoles and their capability to form water wires.

Water and nucleic-acid structure

The average strength of aqueous interactions with nucleic acids is far greater than that with proteins due to the highly ionic character of nucleic acids[24]. Although the importance of water is often overlooked, both DNA structure and the crucial recognition of its sequence are reliant on aqueous interactions, with the DNA helix expanding and contracting depending on its hydration status[3]. Water hydrates both the major and minor grooves of DNA by forming links with the polar atoms at the edges of base pairs, and the orientation of the water molecules depends on the bases and their sequence. The hydration in the minor groove has a complex pattern that includes water hexagons in the initial spine of hydration, secondary hydration, and hydration out as far as the fourth aqueous shell[25]. This sequence-dependent hydration, which extends to the rim of the grooves, can function as a 'hydration fingerprint' for a given DNA sequence. This enables proteins to sense the base sequence from the outside of the grooves, and allows the rapid and lubricated sensing of the DNA sequence[4]. The weak binding of proteins to DNA involves mainly the secondary hydration, which allows proteins to slide along the DNA in a manner that is facilitated by the remaining primary hydration water molecules. On encountering specific sites, specialized proteins bind strongly to the DNA, and this is necessary for the processing of genetic information. Proteins do this by releasing and replacing some of the bound water molecules, which compensates for the entropic cost of protein binding.

Cellular water and cell activity

Cellular water. Although some NMR[26] and most, if not all, molecular dynamics studies[27] show that water outside the inner hydration layer around biomolecules is predominantly unaffected by the biomolecules, other work indicates that water inside cells behaves differently from water outside[28]. For example, the viscosity of intracellular water is higher and its capability to diffuse is lower, as has been noted by NMR[29]. This is not simply due to the presence of different solutes or the existence of extended surfaces, but seems to be a more complex consequence of a combination of factors. Indeed, convincing arguments have been put forward that propose that the intracellular milieu is a gel[30], a liquid crystal[31] or consists of extensively polarized aqueous structuring[32]. Cells are crowded places, and a high proportion of water is associated with biomolecular interactions and is osmotically unresponsive[33]. In this environment, small changes in protein conformations can tie up more water, which further reduces diffusive processes, or can release significant amounts of water, which increases the fluidity and activity of the intracellular environment[34]. Aqueous solutions can form separate, but still fully aqueous, phases depending on the solutes that are present. Such phases consist of low-density water with more extensive hydrogen bonding or high-density water that has fewer and more-bent hydrogen bonds[35]. As such phases have differing hydration characteristics, reactivities and metabolic capabilities, it is clear that such macroscopic structuring can

have an important role in cellular activity. For example, coherent phase microscopy images of cyanobacterial cells and spores, which are responsive to refractive index and size changes, are sensitive to variations of their metabolic states. This might indicate changes in the hydration status, although the effect might be entirely due to membrane effects[36].

Cancer cells contain more free water than normal cells, and the degree of malignancy increases with the degree of cell hydration. It follows that intracellular hydration might be a primary factor in carcinogenesis[37]. This could be because increased hydration facilitates the acceleration of intracellular processes, including respiration, which enhances the competitive edge of a cancer cell for using nutrients.

Communication at a distance. Cells contain several molecules, such as cyclic AMP, that function as messengers between biomolecules and turn processes on and off. Although metabolite diffusion is not generally thought to be limiting over small distances[38], the rapid response of cells to changing circumstances seems unlikely to be due only to concentration changes that are sensed following simple diffusive processes. Instead, cells possibly benefit from more speedy information transfer. Water is ideally placed and perfectly suited to this function. A simple example of this is the release of protons to water by one biomolecule that influences the behaviour of other non-touching biomolecules[39]. However, more complex aqueous effects can also be observed. Changes in protein conformations alter the active water content inside cells, which causes water influx or efflux[40] and therefore alters the activity of the hydrating water around other biomolecules. In addition, shifts in the equilibria that involve the high concentration of actin molecules and their polymerized chains necessarily alter the compartmentalization of the cells through changes to the cytoskeleton. The extent of the static charged surfaces of actin, tubulin and intermediate filaments might well affect the amount of free water and therefore the metabolic activity of these water molecules in the cell[41].

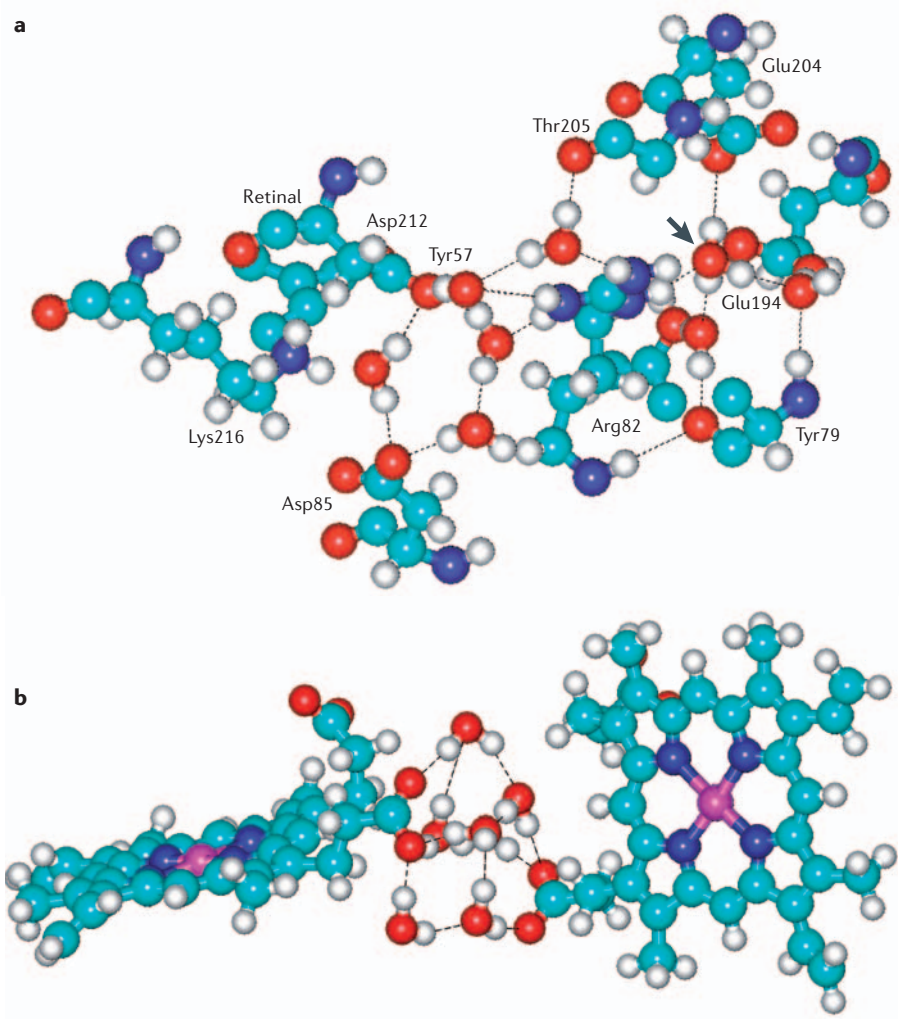


Figure 4 | **Water in the transfer of protons and electrons. a** | The arrangement of water molecules at the key proton-transfer site in bacteriorhodopsin[47]. Proton transport by bacteriorhodopsin is driven by the photoisomerization of all-*trans*-retinal (pK_a 13) to 13-*cis*-retinal (pK_a ~8.45), which transfers the excess retinal proton from its Schiff base with Lys216 to Asp85. The uncharged Asp85 then releases the pentagonal hydrogen-bonded ring. This causes the centrally placed Arg82 side chain to flip towards the protonated water molecule that is marked by an arrow, which results in the release of a proton from this water molecule through a hydrogen-bonded chain (also known as a water wire) to the extracellular space. The Schiff base is reprotonated from the cytoplasm through another associated water wire. This structure was created using the Protein Data Bank (PDB) accession file 1C3W[48]. For clarity, only the relevant parts of the protein are shown. **b** | The structured water cluster between two molecules of bovine liver cytochrome b_5 (as determined by molecular modelling), which allows rapid electron transfer[23]. The electrostatic interactions of the water molecules provide a large donor-to-acceptor coupling that produces a smooth distance dependency for the electron-transfer rate. For clarity, only the water cluster and the cytochromes are shown, and the protein residues are hidden. In this structure, which was created using a file kindly provided by I. Balabin, Duke University, Michigan, USA, explicit water molecules were added[23] to the protein structure given by the PDB accession file 1CYO[49]. The atom colouring is: carbon, light blue; hydrogen, white; nitrogen, dark blue; oxygen, red; and iron, pink.

Conclusions

Water is an integral part of biomolecular structural organization, and is central to the assembly and three-dimensional shape of proteins and nucleic acids. More importantly, water is essential for the function of these molecules, guiding their interactions and facilitating their biological activities. In particular, the capability of water clusters to reach out from the surface of biomolecules to allow interactions at non-contacting distances increases the likelihood and specificity of such interactions. At the extreme level, changes in water clustering and water activity in one part of a cell can be quickly sensed elsewhere without the need for diffusional processes.

In the future, the importance of nanoscopic pools of differing aqueous phases for intracellular activity should be clarified. It is also hoped that no discussion of biological processes will be thought to be complete without assessing the role of water.

Martin Chaplin is at the Department of Applied Science, London South Bank University, Borough Road, London SE1 0AA, UK.
e-mail: martin.chaplin@lsbu.ac.uk

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Competing interests statement
The author declares no competing financial interests.

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