Role of hydration and water structure in biological and colloidal interactions

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The conventional explanation of why hydrophilic surfaces and macromolecules remain well separated in water is that they experience a monotonically repulsive hydration force owing to structuring of water molecules at the surfaces. A consideration of recent experimental and theoretical results suggests an alternative interpretation in which hydration forces are either attractive or oscillatory, and where repulsions have a totally different origin. Further experiments are needed to distinguish between these possibilities.

WATER is the natural solvent. The vast majority of molecular interactions in living systems occur in an aqueous environment, and geological structures are constantly exposed to water. Because of increasing concerns about the environment, water is gradually replacing organic solvents for technological and household products such as water-based fuels and lubricating fluids. The factors that determine why water is often, but not always, a good solvent or suspending medium for colloidal and biological interactions is the focus of this Review.

It has long been appreciated that both as a pure liquid and as a solvent, water is complex and has some unusual if not unique properties[1]. The complexity of liquid water is due to a combination of the small size and distinct polar charge distribution of the water molecule[2]. The charge distribution can be modelled by four charges located along the four arms of a tetrahedron[2,3] which allows each water molecule to participate in strong polar (electrostatic charge-dipole or hydrogen-bonding) interactions[4] with a high degree of spatial directionality. The strong hydrogen-bonding water–water interaction results in a large cohesive energy or latent heat, a high boiling point, a high surface tension, and a reluctance to dissolve inert (nonpolar or hydrophobic) solutes with which it cannot interact through similarly strong polar forces[5].

But water can also strongly bind to and dissolve polar and hydrophilic compounds, and our main concern here will be to investigate those properties of water that make it such an excellent solvent for a wide variety of solute molecules and ions, and for suspending colloidal particles and biological structures such as proteins, DNA, viruses and cells. To explain the powerful solvent properties of water, scientists naturally turned to consider the intermolecular forces acting between the dissolved species in water and aqueous electrolyte (salt) solutions. The two major forces operating between two macromolecules or surfaces in liquids are the attractive van der Waals and repulsive electric double-layer forces[6]. The former is always present; the latter depends on the existence of charged surface groups. But even uncharged molecules and particles are often miscible with water—an observation that led Langmuir[7] and Derjaguin[8] to postulate the existence of an additional repulsive force that seemed to be unique to water. This hydration or structural force is believed to arise from the strongly bound and oriented first layer of water molecules on surfaces (Fig. 1 *top*) which may prevent two surfaces or macromolecules from approaching any closer than 5–6 Å—the thickness of two water molecules. There was good experimental and theoretical evidence for the existence of such a primary hydration shell or layer. But to explain a repulsion that exceeded the van der Waals attraction in both magnitude and range, this force had to propagate further than one or two molecular layers. It seemed natural that the first oriented layer could induce a second layer to orient, the second would likewise influence the third, and so on. A picture emerged of hydrophilic surfaces bounded by a coat of structured water that opposed being disrupted and thus gave rise to a monotonically repulsive force between two approaching surfaces in water. The range of this interaction was variously suggested to extend from nanometres[9–12] to many micrometres[13], and various theories based on water structure were proposed[14–17]. Interestingly, until the early 1980s, with reports that the hydrophobic interaction may be long-ranged[18,19], explaining unexpected *attractive* interactions does not appear to have been a serious problem, this is because at least one type of attractive force—the van der Waals force—is always expected to be present.

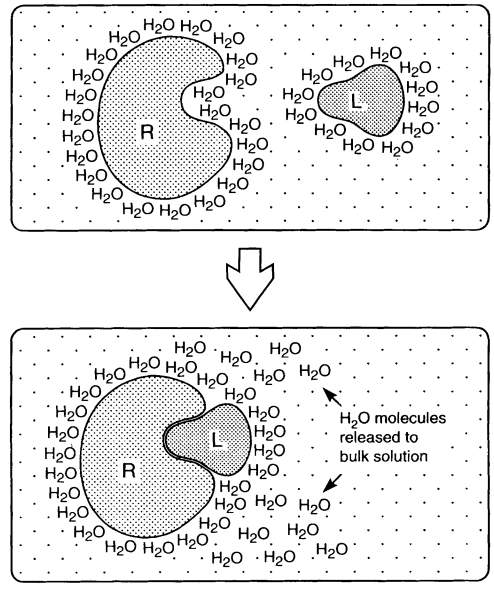

FIG. 1 Schematic illustration of a receptor molecule (R) binding to a ligand group (L) in an aqueous medium.

The interpretation of many phenomena in terms of an extensive protective layer of structured water around molecules and surfaces leads naturally to certain implications and consequences. Thus, to change the hydration forces in water, emphasis was placed on modifying not the structure of the hydrated surfaces, but that of the water itself, for example, by adding 'structure makers' or 'structure breakers' (such as salts) to the solution[20]. In colloidal and biological systems, the idea that the hydration layer must be overcome before two molecules, colloidal particles or

membranes can come into contact and react, adhere or fuse is prevalent, again with the implications that factors that alter the water structure—for example, by dehydrating the surfaces—are required to initiate these phenomena[9].

An alternative possibility is that the origin of short-range stabilizing repulsions do not arise from water structuring effects associated with some peculiar property of water–water interactions intrinsic to water, but are more to do with the chemical and physical nature of the surfaces. With this new interpretation of the origin of these forces, many colloidal and biological interactions in water are seen in a completely different light, with different mechanisms suggesting themselves for controlling phenomena such as particle adhesion, membrane recognition and fusion, biochemical reactivity and the rates of complementary associations. Here we review the experimental evidence that leads to this suggestion, and compare its explanatory power with that of the standard picture of hydration in terms of water structure. We suggest that any interaction that may arise from water structuring effects is expected to be monotonically attractive or oscillatory, not repulsive. The alternative model implies that water has more in common with other molecular liquids than is often supposed. But a firm resolution of the question of which model is to be preferred will have to await further experiments and theoretical modelling, some of which are suggested at the end of this Review.

Molecules and surfaces that associate in water

The total interaction between any two solute molecules or particle surfaces in water involves, in addition to the direct interaction, an additional interaction arising from the displacement of the solvent (water) molecules as the two suspended molecules come into contact (Fig. 1). Thus, the overall energetics of any association or dissociation process in water also involves what is commonly referred to as dehydration or hydration of the interacting surface groups. But it has never been an easy matter to establish the mechanism behind this interaction, or even whether it is the major contributor. For example, it is widely believed that the DNA double helix owes its stability to the hydrogen bonds holding the two strands together. However, in the dissociated state, the individual strands can form even stronger hydrogen bonds with the solvent water molecules[21], so the hydrogen bonds can be only part of the picture. The overall energy balance that favours the association of two DNA strands and other macromolecular associations in water depends on a competition between solute–solute, solute–solvent and solvent–solvent bonds (including hydrogen bonds) the outcome of which is generally not obvious or simple to analyse.

Similarly, the macroscopic surfaces of certain colloidal and biological surfaces that expose hydrogen-bonding sites (such as bilayers of fatty acids[22]) or a surface layer of ordered water molecules (such as brushite crystals, $CaHPO_4 \cdot 2H_2O$; refs 23,24) associate spontaneously in water, expelling any bulk liquid water into the solvent reservoir as they do so. In all of the above cases, we would say that the dehydration of the surfaces or exposed molecular groups is energetically favourable, and that it results in an overall attraction between these groups in water.

The full picture is often more complex because additional attractive nonpolar van der Waals and hydrophobic interactions between surface nonpolar groups will also contribute towards, or even dominate, the overall attraction. For example, the attraction between the nonpolar nucleotide rings in DNA is stronger than the hydrogen-bonding interaction between the polar groups[25,26], so that the hydrogen bonds should be seen as providing the structural specificity of the DNA double helix but not the major driving force of the association[21].

Molecules and surfaces that are stable in water

In other cases, strongly hydrophilic molecules (certain proteins, viruses and DNA) or surface groups (such as the polar head groups of surfactant and lipid molecules) are found to effectively repel each other in water; that is, the structures (micelles, vesicles) remain dispersed in water. In this case we would say that the dissociation or repulsion arises because their hydrogen-bonding interaction with water is stronger than the solute–solute interaction, that is, it is due to their favourable hydration interaction. But here too, additional repulsive forces may be involved that must be taken into account. These include the electric double-layer forces between any charged surface groups[6,19], and the entropic or thermal fluctuation forces between any mobile surface molecular groups[27,28]. Either of these forces could dominate over the other interactions (both attractive and repulsive) and govern the overall repulsion.

The repulsive electric double-layer force between similarly charged surfaces in electrolyte solution is generally attributed to the electrostatic repulsion between the surface charges. But as has been stressed in the literature[29], the purely electrostatic contribution to the net interaction is actually attractive—the net repulsion arising from the entropy or osmotic pressure of the counterions between the surfaces. This important conceptual point can be explained simply as follows: the diffuse atmosphere of counterions surrounding a charged surface (Fig. 1 *top*) arises, not because of the electrostatic interaction between the surface co-ions and oppositely charged diffuse-layer counterions, which is attractive and therefore opposes dissociation, but because of the overriding effect of the increased entropy of the counterions when they leave the surface. When two such surfaces approach each other, the counterions are pushed back onto the surfaces, and work has to be done against this entropic (osmotic) pressure; but as far as the electrostatic processes are concerned, the pushing of the counterions back onto the oppositely charged surfaces is actually favourable. The entropic, rather than electrostatic, origin of the electric double-layer repulsion is significant when considered with the other types of interactions that give rise to an effective repulsion between molecular groups or surfaces in water, and we return to this matter and its implications in the final section.

Measurements of forces between surfaces

In recent years, experimental techniques for directly measuring the forces between surfaces in liquids have matured. We can now

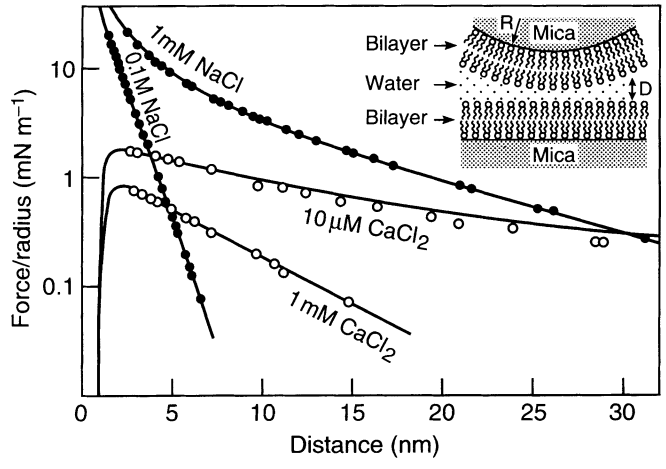

FIG. 2 Typical DLVO force profile measured between two smooth charged surfaces in aqueous electrolyte solutions. The force, *F*, normalized by the surface radius, *R*, is plotted against surface separation, *D*. The data shown here are for two negatively charged bilayers composed of phosphatidylglycerol lipids[83], which are common constituents of biological membranes. The agreement between experiment (circles) and the DLVO theory (lines) is very good, and shows that the overall interaction is dominated by repulsive electric double-layer forces at large separations and attractive van der Waals and ion-correlation forces at small separations[3,19,84]. The maximum at B is known as the force or energy barrier. For many systems, the continuum DLVO theory has been found to describe satisfactorily the surface interactions down to separations of 1–2 nm (refs 19, 27, 30, 73).

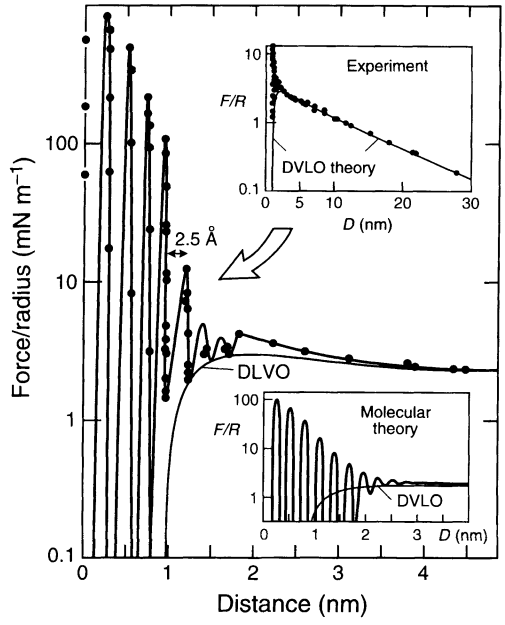

FIG. 3 Short-range deviations from continuum DLVO theory measured between mica surfaces in 1 mM KCl solutions[36,37]. Below 20 Å (about eight water-molecule diameters) the force becomes increasingly oscillatory, with a periodicity equal to the diameter of water molecules (2.5 Å), indicative of the existence of diffuse water layers between the surfaces. Inset, theoretically predicted force for this system at small separations, based on a non-continuum molecular theory[81].

accurately measure the forces between smooth solid surfaces, lipid bilayers or model biomembrane surfaces containing proteins and other biological molecules using the surface forces apparatus[3,19,30] or osmotic pressure technique[9,31,32] and between microscopic colloidal particle surfaces using the atomic force microscope[33]. In the osmotic pressure technique[9,31,32] inter-bilayer forces are measured by osmotically withdrawing water from a dispersed stack of bilayers in solution while measuring the change in the lamellar repeat spacing with X-rays. In the surface forces apparatus[3,19,30], the forces between two surfaces immersed in a liquid are measured from the deflection of a spring supporting one of the surfaces, while the distance between the surfaces is measured using an optical interference technique. As an example, Fig. 2 shows the measured forces between two charged lipid bilayers in aqueous NaCl and $CaCl_2$ solutions. At long range, there is a repulsion that increases exponentially with decreasing separation. At a separation of $D \approx 2.5$ nm, the repulsion turns into an attraction. This behaviour of the forces was predicted fifty years ago by Derjaguin and Landau[34] and Verwey and Overbeek[35], and the so-called DLVO theory has since formed the basis for understanding colloidal interactions in liquids, especially aqueous solutions. The salient features of this theory are that the long-range repulsion is due to the electric double-layer force, whereas at shorter range the attractive van der Waals force wins out.

When we consider the approach of two real surfaces or macromolecules that interact with an equilibrium interaction potential as shown in Fig. 2 (the interacting species could be two clay particles, a ligand–receptor pair or a DNA–repressor pair), it is clear that the dynamics of the association process will be strongly influenced by how the interaction energy or force varies with distance. Thus, even if it is energetically favourable for the two species to come into contact (high binding energy at $D = 0$), the association may be kinetically inhibited due to the existence of the repulsive force or energy barrier at some finite separation (compare Fig. 2). This can give rise to slow aggregation even when the bound state is highly favourable. Conversely, an association process of low binding energy can be anomalously rapid if there is a long-range attractive force that steers the two partners together.

As the experimental techniques have improved and as more systems are studied, it has become evident that, although very useful as the reference interaction, the DLVO theory leaves many observations unexplained[19,30]. Figure 3 shows the measured force between two mica surfaces in aqueous KCl solutions[36,37]. The long-range force, beyond about 2 nm, is well described by the DLVO theory, but careful measurements reveal oscillations in the force at short range. The period of these oscillations is $\backsim 0.25$ nm which corresponds to the size of a water molecule. The water molecules appear to be partially arranged in layers, and as two surfaces approach these layers are squeezed out sequentially. Such short-range ordering occurs for all simple liquids between smooth, rigid surfaces and it is not exceptional for water or smooth mica surfaces[19]. The oscillatory force is always expected to be attractive at contact ($D = 0$)[38,39].

Water structure effects

Theoretically, for two smooth surfaces interacting in an aqueous solution by a combination of DLVO and oscillatory forces, the contact interaction in the limit of $D \rightarrow 0$ is always expected to be attractive[38,39]. However, the most detailed measurements of the forces between some surfaces, notably between silica surfaces and uncharged lipid bilayers, show a monotonically increasing repulsive force in this range (Fig. 4). What is the source of this repulsion?

When confronted with such experimental findings investigators have taken refuge in the old idea of water structure, envisaging a layer of structured water molecules, extending some 1–3 nm or more from each surface, which provides a protective force barrier against the approach of two 'hydrophilic' surfaces or groups. This interpretation seems reasonable for a number of reasons. Water is known to have strong and directional intermolecular interactions and an unusually high degree of structure in the liquid phase at ambient temperatures. It thus appears conceivable that the extra monotonically repulsive force between two particles in water could arise from a particular structure or order that is propagated through the solvent. Through the years this idea has had a remarkable appeal. When confronted with unexpected experimental results water structure has commonly been used as a *deus ex machina* for explaining the observations[40–43]. The temptation is not new: both long-range and short-range forces arising from water structure were controversial issues already in the 1920s and 1930s (compare Porter forces[44]) as the following quotation from Hartley's 1936 classical book[45] on surfactants illustrates: "There is a widespread tendency to use 'hydration' in colloid chemistry as a sort of universal explanation of puzzling phenomena. Its inaccessibility to direct experimental determination fortifies this tendency."

The attribution of all short-range repulsions in water to surface hydration layers has fostered a whole family of concepts that by now have become well established—that other properties of water such as its diffusivity and dielectric constant must be very different near a surface; that other hydrophilic groups cannot contact each other or chemically interact when protected by a hydration layer, and that before certain adhesion and fusion events can occur, two colloidal particles or biological membranes must somehow overcome their hydration force barrier. The search for salting-out and dehydrating agents, such as water structure breakers, has followed naturally from this hypothesis, as does the current search for a satisfactory theoretical description of water structure at surfaces.

Marçelja and co-workers[14,15] made an ingenious attempt to construct a detailed molecular model of water-mediated structural interactions that accounted for some of the experimental results of the type shown in Fig. 4. The Marçelja theory, formulated in terms of a decaying water structure from each surface,

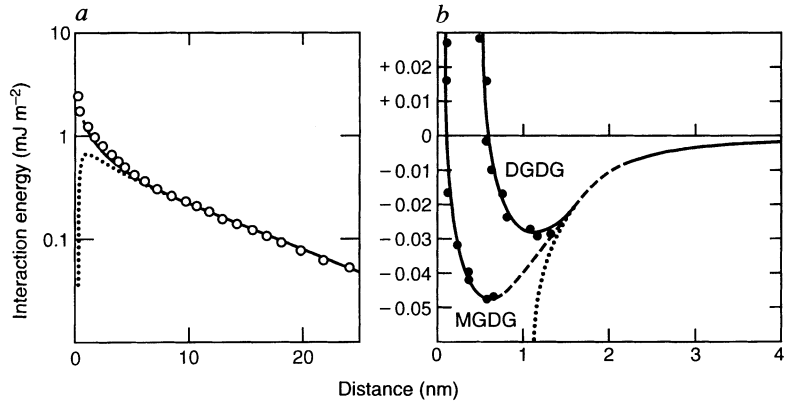

FIG. 4 Measured forces F (expressed as interaction energies, $E = F/2\pi R$) between surfaces in aqueous solutions that appear to show an additional monotonic repulsion at small separations, below ~20 Å, where the DLVO theory would predict an attraction down to contact (at $D = 0$). *a*, Two silica surfaces[62,68]. Dotted line, DLVO theory; solid line, modified DLVO interaction for the case where the planes of origin of the double-layer and van der Waals forces are shifted by 5 Å relative to each other[62], as illustrated in Fig. 5*a*. *b*, Two supported uncharged glycolipid bilayers of monogalactosyl diglycoride (MGDG) and digalactosyl diglyceride (DGDG) in water[85]. At distances beyond the adhesion minima the measured forces are well described by the DLVO theory, which consists of a pure van der Waals attraction in this case of two uncharged surfaces. Similar monotonic repulsions have been measured between other lipid bilayers[27,54].

predicts an exponentially decaying repulsive force of a magnitude that is determined by the degree of orientation or polarization of the first layer of water molecules, and with a decay length that is a characteristic property of the liquid (water). This theory is widely quoted whenever a short-range monotonic repulsion is found in water. However, it has been increasingly clear that the Marçelja model is unsatisfactory: computer simulations do not support the structural effects[28,46–48]; measured forces are too system-dependent in both magnitude and distance-dependence (often not exponential)[27,49]; fitted decay lengths in water vary by more than an order of magnitude[49–53], from 0.6 Å (ref. 50) to above 6 Å (refs 49,53); the absence of an adhesive minimum at contact is unexplained; the observed temperature effects[27,32,51,53–55] remain a mystery; bilayer surfaces have been found to be highly diffuse and disordered[48,56] and recent measurements show that these forces are not specific to water[57,58]. It is also fair to say that no theory, based on water or solvent structure, has been proposed that is predictive, theoretically sound and that accounts qualitatively for the observed effects.

Effects of surfaces on interactions in liquids

Recent experiments suggest that to understand how repulsive short-range forces of the type shown in Fig. 4 arise, it is necessary to focus on the detailed structure and properties of the interacting surfaces or interfaces rather than on the structure of the solvent between them. Although the forces in Fig. 4*a* and *b* show some similarities, careful measurements have revealed that they may have different molecular causes. Silica is a solid with uncharged silanol (Si–OH) and charged silicic acid ($Si–O^-$) groups at the surface[59]. Protons can dissociate from the surface silanol groups, generating a DLVO-type repulsive double-layer force at long range. Furthermore, the silica surface is generally amorphous and not molecularly smooth: a low density of molecularly thin silica 'hairs' stick out from them[60]. This pushes the double-layer repulsion farther out relative to the van der Waals attraction at the same time as a short-range steric repulsion is generated due to the protruding hairs (Fig. 5*a*). This mechanism, first proposed by Frens and Overbeek[61], can explain quantitatively the observed monotonic short-range repulsion and absence of adhesion in the case of silica[62]. Such protruding surface groups should not be considered as fixed or rigid, but more as a surface layer of flexible, polymer-like segments, that can be expanded or collapsed by changing the solution conditions, as occurs with adsorbed polymers[63]. For example, in the case of the negatively charged 'co-ion' groups on silica, these would be expected to collapse in the presence of multivalent metal ions such as calcium[64] thereby also reducing the short-range repulsion, as observed[65].

Similar mechanisms, but in terms of the finite sizes of the adsorbed hydrated counterions, rather than the co-ions, have been shown to account quantitatively for the enhanced monotonic repulsions measured between mica surfaces in concentrated electrolyte solutions[66,67]. Again, an outward shift of the surface charges by only a few ångströms is sufficient to remove the van der Waals adhesion at contact, and replace it with a strong short-ranged repulsion that appears to extend some tens of ångströms; and the finite sizes of the hydrated counterions further enhance the repulsive osmotic component of the double-layer force via an excluded volume effect[67]. In all of the above cases, the experimental results could be accounted for quantitatively without invoking any additional repulsive hydration force. We do not consider the first layer of water molecules bound to a surface as producing a hydration force or solvation force. This primary hydration shell has nothing to do with water–water but rather with water–surface interactions, and is therefore not a characteristic or intrinsic property of water. Such solute–solvent interactions arise in all non-ideal binary systems resulting in excluded volume effects that are well understood physically and thermodynamically. Recent direct measurements of the viscosity of water and electrolyte solutions adjacent to silica[68] and mica[69] surfaces have also shown that the non-slip plane is located no further than one water layer from these surfaces—again consistent with the absence of water structuring at these surfaces.

In the case of surfactant and lipid bilayers, above the chain melting transition the molecules have significant thermal flexibility within the bilayer. At the interface molecules protrude out from the bilayer[48,56,70] head groups change conformation[28], and there are also collective undulation modes[71,72] (Fig. 5*b*). In the presence of an opposing surface these motions become restricted, resulting in a monotonically repulsive force[27,28,48,72]. The similarity in the range and magnitude of the short-range repulsive forces measured between certain bilayers and various other macromolecules and surfaces across water has often[9,31] been taken as an indication that they must be due to water structure. But what was missing from this analysis, and most previous analyses, was an appreciation that the short-range entropic forces between mobile surface groups and ions are also not very different (known as the osmotic pressure limit) and that even the simplest theoretical models of entropic thermal fluctuation interactions can account, both quantitatively and qualitatively, for most of the experimental observations on amphiphilic systems[27,28,48,73] and—in the case of lipid bilayers—down to the last one or two water molecules from each bilayer surface[32,55].

Forces arising from water structure

How, then, *does* water structure influence the forces between molecules or surfaces? As illustrated in Fig. 3, the presence of structure in the liquid generally gives rise to an oscillatory force, but only between smooth rigid surfaces. This is the basic structural force in all liquids[19,38,74,75]. In more complex situations involving rough or chemically heterogeneous surfaces, or soft amphiphilic or biological surfaces, we should expect a monotonically varying component due to the 'smearing out' of the oscillations. However, experiments with other surfaces and liquids show that surface roughness and surface fluidity reduce the range and magnitude of

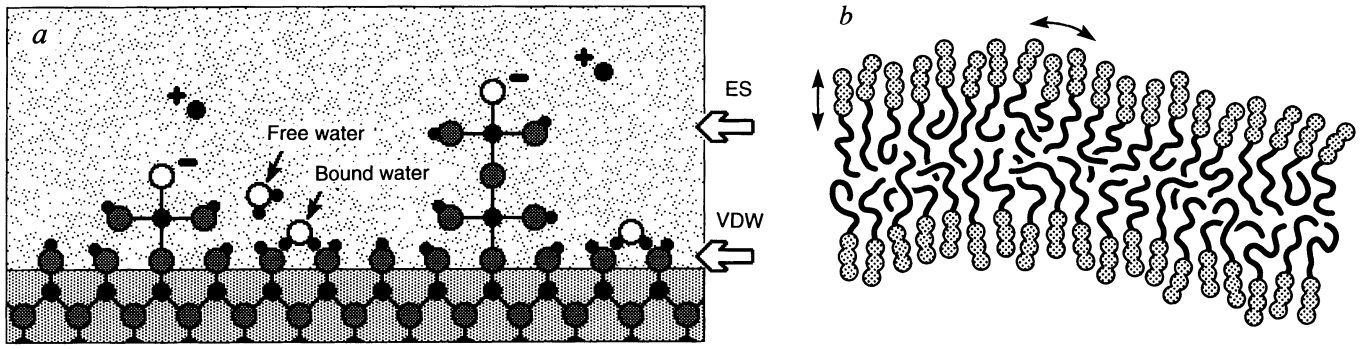

FIG. 5 *a*, Silica-like surfaces in water exposing silanol (Si–OH) and silicilic acid ($Si–O^-$) groups, where the surface charges are located a few ångströms out from the solid–liquid interface (grey spheres, Si; white spheres, O). A difference of as little as 5 Å between the plane of electrostatic (ES) charge and van der Waals (VDW) plane is enough to totally alter the DLVO interaction between two surfaces over the last 50 Å before contact, transforming the expected short-range attraction (dotted curve in Fig. 4*a*) into a monotonic repulsion (solid curve in Fig. 4*a*).[61,62]. *b*, Lipid bilayer or biological membrane in the fluid state in water, where the constituent molecules are in continuous thermal motion, including protrusion and headgroup tilting motions (indicated by vertical and horizontal arrows), and where the membrane as a whole also undergoes gross bending and thickness fluctuations. When two such membranes approach each other, the confinement of these thermal fluctuation modes gives rise to a short-range monotonic repulsion that is analogous to the osmotic pressure of a solution or to the normal pressure of a gas[27].

the oscillations[76,77] and that in binary liquid mixtures the range is less than in either of the pure liquids[78]. We have previously argued[27] that for amphiphilic surfaces there is no firm evidence, experimental or theoretical, in favour of a monotonically repulsive structural force. We now suggest that this may be generally true for all surfaces. First, we illustrate in Fig. 6 how a force arising from surface-induced water structuring effects could be expected to be attractive. This may be the origin of the hydrophobic interaction[79], although the experimental situation is still unclear and we do not wish to discuss the origin of this still elusive interaction here. Attractive hydrophobic forces are sometimes thought to be a negative hydration force. However, just as in the case of DLVO theory or the Lennard-Jones potential, attractive and repulsive forces can have very different origins, so the repulsive and attractive non-DLVO interactions seen in water should not be assumed to be related *a priori*.

The molecular mechanism giving rise to a structural force involving oriented dipolar water molecules is shown in Fig. 6. We consider a surface that interacts strongly with the first layer of water molecules in direct contact with it. This strong interaction may be due to physical or chemical binding, or to a good complementary fit between the water and surface groups. But regardless of the specific mechanism responsible for ordering or orienting the first layer of water molecules, this interaction must be recognized as arising from water–substrate or solvent–solute forces and not from a pure hydration (water–water or solvent–solvent) interaction. These come in only beyond the first or 'primary' hydration layer. Figure 6*a* shows the electrical field lines arising from the first layer of oriented water molecules. We may note that the field is highly non-uniform in the x, y (in-plane) and z (out-of-plane) directions. The field changes direction across one unit cell on the surface, which implies that the field cannot be described in terms of a mean-field at any point z from the surface (by applying Gauss' law, it can be shown that the average field in the z-direction is zero).

If a second surface approaches the first with its surface water molecules in perfect juxtaposition, a repulsion results (Fig. 6*b*). Thus, any theoretical solution to this problem that assumes mirror symmetry will predict a repulsion, but it will not be computing the interaction of lowest energy. When one of the two surfaces is translated a distance of half a unit cell in the x and y directions, the electric field lines from the two surfaces now add constructively resulting in an attractive interaction[80] (Fig. 6*c*). At first sight, it seems counter-intuitive that two surfaces having their water dipoles pointing towards each other will attract. The answer to this apparent paradox is to be found in the long-ranged character of dipolar interactions. The nearest oppositely oriented water molecules do indeed repel, but the effect is more than compensated for by the water dipoles that are farther away. The complexity of this situation, where a small shift in the relative positions of the two surfaces changes a repulsion into an attraction, illustrates the subtlety that can arise when electrostatic dipolar and molecular structural effects are involved together in determining the final net interaction. Theories that simplify the system by assuming a mean-field or ignoring the possibility of asymmetric or staggered molecular ordering (that may also be continually changing as two surfaces approach each other) may miss the lowest-energy configurations at any given surface separation, and thus predict an erroneous repulsive interaction. Computer simulations, however, are less likely to fall into this trap and, for rigid solid surfaces, have so far have predicted only oscillatory or attractive forces arising from surface-induced water-structuring effects[46,47,81,82]. Recent simulations of fluid bilayer-water surfaces[48] have also found that the decaying polarization and structure of water molecules from bilayer surfaces are determined by the surface electric fields and 'dynamic roughness' of the bilayer surfaces, with no indication of any water-intrinsic decay length.

Repulsive forces due to entropic interactions

Thus we propose that the steep short-range repulsions that are often observed between colloidal particles and biological surfaces in water are not due to a layer of structured water but to the entropic repulsion arising from the confinement of thermally mobile surface groups—the properties of water being essentially the same as in the bulk beyond the first layer of surface-adsorbed water (which may or may not be strongly bound to the surfaces). Thus, water should not be considered to have a different mobility, or dielectric constant or freezing point, near or between two surfaces (apart from effects arising from the normal freezing-point depression of solutions). Nor should one consider that there are thick hydration layers preventing proteins, ligands, receptors and other surface hydrophilic groups from coming into contact with each other. Indeed, it is this very contact that gives rise to the repulsion, which is thus not unlike the repulsive pressure generated by gas molecules colliding with a wall[27] (it is not always appreciated that large osmotic pressures can be produced by very low solute concentrations). In the case of colloidal, micellar, vesicle and cell–cell interactions, including membrane fusion, there is no thick hydration barrier that must be overcome before the surfaces or molecular groups on the opposing surfaces can interact directly with each other; the only real force barrier is the first layer of water molecules directly in contact with the surfaces.

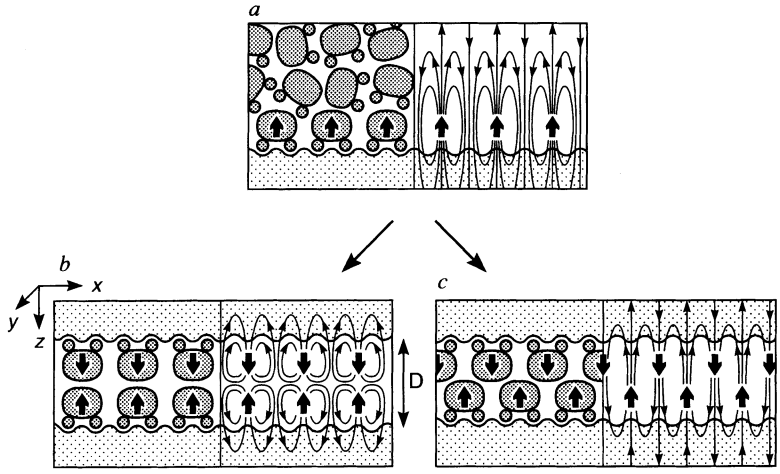

FIG. 6 *a*, Hydrophilic surface with a strongly bound first layer of oriented water molecules, shown by the darkly shaded figures that resemble H_2O molecules. The thick arrows indicate the direction of the oriented water dipoles, and the thin arrows indicate the electrostatic field lines (shown only in the right half of each panel for clarity). It is important to appreciate that these primary binding forces, which can arise from specific ionic, hydrogen-bonding or complementary-fit interactions, act between the surface and water, and that they occur for all substrate–solvent or solute–solvent systems. These forces should be distinguished from those acting between the water molecules themselves (the solvent–solvent and electrostatic forces) which occur beyond the first layer. *b*, Two surfaces as in *a* interacting in registry in water. The resulting electrostatic interaction is an exponentially decaying repulsive force with a decay length λ equal to some fraction of the spacing d between adjacent dipoles on the surfaces ($\lambda \approx d/2\pi$ for a square surface lattice, $\lambda \approx \sqrt{3}d/4\pi$ for a hexagonal lattice)[19,80]. This 'symmetric' situation, in which the mid-plane is a mirror plane of symmetry, is often implicitly assumed in computer simulations. *c*, Most favourable interaction-energy configuration, attained when the two surfaces are allowed to fully relax at any given separation. This asymmetric or 'staggered' configuration gives rise to an attractive interaction of the same magnitude and range, but of opposite sign, as in *b*. This lowest-energy configuration can easily be missed in simplified models of short-range electrostatic forces between surfaces. This schematic diagram shows that the electrostatic dipole–dipole interactions alone cannot give rise to a repulsion. Inclusion of the entropy of rotating and translating dipoles reduces the strength of the attraction[19] but, unlike the entropy of ions in a double layer, it does not change the sign of the interaction.

Likewise, the action of a dehydrating agent or water-structure breaker may be more on the nearby surface than on the water. More generally, one should perhaps look more towards how surfaces rather than solvents may be modified to bring about some desired effect, such as the coagulation of a colloidal dispersion or a better lubricant. And finally, as a solvent and suspending medium, water should be seen as an ordinary liquid, whose molecules have a small size and strong intermolecular interactions with surfaces and solute molecules.

Further experiments and theoretical modelling are required to test the validity and generality of the above hypothesis. Experimentally, this will require more discriminating molecular probes of surface structure, such as specially modified atomic force microscope probes that can function reliably under water, to measure the static and dynamic roughness of surfaces and check for the existence of small protruding surface groups. On the conceptual and theoretical levels, we need an improved description of the molecular-scale properties of surfaces. Theoretical treatments of interactions between surfaces at short range require that the surfaces be treated, not just as hard or soft walls, but with the same molecular detail as are the intervening liquid molecules, including a proper balancing of the interplay between the long-range and short-range intermolecular forces. Given the complexity of these systems, any quantitative analysis will have to rely on computer simulations, which have now matured so that it is becoming feasible to evaluate explicitly the forces between surfaces in addition to the molecular structure. But care must be taken to avoid artificial restrictions, such as assuming mirror symmetry of the surfaces, which can produce results that do not reflect the interaction of lowest energy (compare Fig. 6*b* and *c*). Experimental and theoretical methods have now reached a level of sophistication that the 60-year-old issue of hydration forces—so central to a molecular-level understanding of biological and inorganic systems in water—may soon be resolved. □

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Structure and mechanism of DNA topoisomerase II

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The crystal structure of a large fragment of yeast type II DNA topoisomerase reveals a heart-shaped dimeric protein with a large central hole. It provides a molecular model of the enzyme as an ATP-modulated clamp with two sets of jaws at opposite ends, connected by multiple joints. An enzyme with bound DNA can admit a second DNA duplex through one set of jaws, transport it through the cleaved first duplex, and expel it through the other set of jaws.

TYPE II DNA topoisomerases are essential cellular enzymes that function in the segregation of newly replicated chromosome pairs, in chromosome condensation, and in altering DNA superhelicity[1–4]. All type II enzymes, prokaryotic and eukaryotic, belong to a single family (Fig. 1*a*)[5–8]. The eukaryotic enzymes are dimers; the prokaryotic enzymes, bacterial DNA gyrase and DNA topoisomerase IV, are A_2B_2 tetramers. The amino- and carboxy-terminal parts of the eukaryotic enzyme polypeptide are homologous to the gyrase B and A subunits, respectively.

Type II topoisomerases work by cleaving and opening one DNA duplex, passing a second duplex through the opening, and then resealing the break[9–11]. The DNA cleavage is a transesterification between a pair of tyrosyl residues, one in each half of the dimeric enzyme, and a pair of DNA phosphodiester bonds four base pairs apart[3]. In this process, the phenolic oxygens of the tyrosines become covalently linked to the phosphoryl groups at the 5′ ends of the transiently broken DNA, leaving a pair of hydroxyl groups on the recessed 3′ ends. The intervening base pairs then separate, and the pair of tyrosine-linked 5′ DNA ends move away from each other, opening a 'gate' in the DNA double helix for the transport of another double-stranded DNA segment. After the second segment has passed through, the enzyme-mediated gate in the first DNA segment closes. A transesterification between the pair of 3′ hydroxyl groups and the phosphotyrosyl linkages restores the continuity of the DNA strands and breaks the covalent bonds between the enzyme and the DNA. This mechanism requires that the enzyme undergo dramatic conformational changes, involving displacements as large as 35–40 Å (Fig. 1*b*).

Type II topoisomerases depend on ATP binding and hydrolysis for completing the full reaction[1–3]. Non-hydrolysable analogues of the enzymes, such as the β,γ-imido analogue, AMP-PNP, allow one transport event but prevent enzyme turnover[12]. Proteolytic cleavage of the GyrB subunit yields an N-terminal subfragment (the 'ATPase domain'), which binds ATP and AMP-PNP[13], and a C-terminal subfragment termed B′ (refs 14, 15) (Fig. 1*a*). Binding of AMP-PNP causes the ATPase domain to dimerize[16,17]. Proteolytic digestion of *Saccharomyces cerevisiae* topoisomerase II with SV8 protease has shown that binding of AMP-PNP leads to significant conformational transitions[18], probably involving (at least in part) dimerization of the ATPase domains. There are three preferred cleavage sites [(A), (B) and (C) in Fig. 1*a*], but site (A), positioned between the ATPase and B′ regions, is sensitive in the absence of AMP-PNP, and site (B), positioned between fragments B′ and A′, is sensitive in its presence[18]. Eukaryotic DNA topoisomerase II bound to a DNA ring can also form a salt-stable complex in the presence of AMP-PNP[19–22]. The formation of this complex has been attributed to the action of the enzyme as an ATP-modulated clamp, which closes when ATP binds and opens after ATP hydrolysis and release; these ATP-modulated movements are tightly coupled to the transport of one DNA double helix through another[20–22].

Type I DNA topoisomerases perform a different reaction, the transient cleavage of a single DNA strand to allow passage of a second strand through the break[1,2]. The type I enzymes are monomers, and the reaction involves transesterification to a tyrosine, as on each subunit of the dimeric type II enzymes. A 67K (relative molecular mass 67,000) fragment of DNA topoisomerase from *Escherichia coli* has four domains in a ring-like structure[23]. A domain interface can separate to give access to the active-site tyrosine. A hinge-and-gate mechanism has been proposed, with the cleaved DNA strand opening up across this interface[23].

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