# How to keep dry in water

Water is notorious for behaving strangely, yet it is possible that we don't yet know the half of it. The question of what water looks like close to biological surfaces has provoked heated debate for decades. A flurry of recent papers[1-3] lends support to the idea that this 'vicinal' water may be markedly different from the bulk – but there is, as yet, still no consensus in sight.[4]

The cell is a crowded place (Figure 1), and amid the crush of biological macromolecules and membranes the water of the cytoplasm might rarely achieve thicknesses of more than a few molecular layers. Might this confinement between macromolecular surfaces induce behaviour very different from that of the bulk liquid?

The question of how water behaves near hydrophobic species is especially pertinent, since it has been long recognized that hydrophobic surfaces attract one another in water. This hydrophobic interaction is one of the key stabilizing forces of protein folding and of multi-subunit assemblies.

Liquid water is held together by a random, fluctuating three-dimensional network of hydrogen bonds. This unique liquid-state structure is responsible for many of water's anomalies, and it has been long thought to play a role in the hydrophobic interaction. Close to a hydrophobic surface the hydrogen bonding pattern is disrupted, without the compensatory interactions that might operate at hydrophilic surfaces. How does water cope with this loss of stabilization?

The classic model of hydrophobic interactions[5] proposes that around hydrophobes water molecules lock into a more rigidly defined cage that preserves hydrogen bonding in an 'ice-like' solvation shell. The attraction then results from the entropic gain as this solid-like water is liberated from the region between two hydrophobes when they come together. It is an appealing picture, but turns out not to be supported by any evidence of enhanced ordering of water around small hydrophobic molecules.[6]

Modern theories of inhomogeneous fluids show that in fact one needn't invoke water structure to anticipate that strange things will happen at interfaces. Capillary condensation in narrow pores shows how confinement shifts the phase diagram of a fluid. Conversely, when the interactions between a liquid and a surface are relatively unfavourable (as with water and hydrophobes), there may be a depleted fluid density at the interface, leading in the extreme case to the formation of a gas-like layer (drying)

Lum *et al.*[7] predicted that this is just what should happen between hydrophobic objects of various shapes (plates and cylinders, for example, approximating protein surfaces): if the surfaces are large enough, capillary evaporation creates a bubble between them. This

theoretical model included none of the geometrical nuances of a hydrogen-bonded network; it was generic to any confined fluid.

Yet water will not give up its special status so readily. There is, for example, the mystery of the long-ranged hydrophobic attraction. Distinct from the shorter-ranged force that binds proteins, this interaction between hydrophobic surfaces seems to extend over distances of up to 100 nm – many hundreds of molecular diameters. It has compelled some researchers to seek explanations, fantastic at face value, in terms of some kind of extended, collective ordering of the intervening water molecules.

The long-ranged hydrophobic interaction was potentially unified with the issue of drying at a single interface by the suggestion of Attard and coworkers[8] that the force might arise from the growth and bridging of sub-microscopic bubbles between the surfaces. The bridging meniscus would pull the surfaces together. Tyrrell and Attard reported the observation of pancake-shaped 'nanobubbles' using an atomic force microscope.[9]

Might the formation of these bubbles be promoted by partial drying at the interface – that is, by depletion of water density in this region? At least two of the new studies support this notion. Noting that the AFM might itself have nucleated the bubbles in the earlier work, Steitz *et al.*[1] have used a less invasive technique – neutron reflectivity – to look at the interface. Neutrons are strongly scattered by deuterium, and the researchers look at $D_2O$ in contact with deuterated polystyrene, a hydrophobic polymer. The scattering densities of these two substances are nearly equal, so any inhomogeneities at the interface should show up in an otherwise 'uniform' sample.

The researchers see a surface layer 2-5 nm thick with a density about 6-12 percent lower than that of bulk $D_2O$, which they interpret as evidence of partial drying. (It is hard, however, to rule out the possibility of an apparent density deficit arising by surface migration of protonated impurities, which scatter neutrons more weakly.) They also use an AFM to reveal a surface covered with flat bubbles, 50-120 nm wide and up to 18 nm high. They propose that this thin depletion zone acts as a precursor to the nanobubbles (which might still be nucleated by the AFM tip itself).

Jensen *et al.*[2] find a similar depletion zone in X-ray reflectivity measurements of the interface of a heavy-alkane monolayer floating on the surface of water. Again, the density depletion is around 10 percent of the bulk water density, corresponding to about one $H_2O$ molecule for every 25-30 $\text{Å}^2$ of surface. But the depletion layer appears to be thin – less than 1.5 nm, perhaps reduced by the 'capillary waves' that will ruffle the surface of the free monolayer.

Another neutron-reflectivity study has been conducted by Schwendel *et al.*[3], who confess that their studies on contrast-matched mixtures of $D_2O/H_2O$ against alkylated surfaces show a density deficit in the first 2 nm or so that is so large as to be unphysical. They suspect that nanobubbles or other air inclusions might be distorting the results.

Thomas and coworkers at Oxford (personal communication) have also found a water depletion layer at a hydrophobic surface using neutron scattering, extending over a thickness of about 1.8 nm. They are confident that their surface is free of nanobubbles, and fairly so that protonated impurities are not responsible for the result.

Thus these studies start to produce a consistent picture of hydration at hydrophobic interfaces: a thin layer of low-density, somewhat gas-like water that precedes complete capillary evaporation between two such surfaces, and which helps to nucleate nanobubbles that create an apparently long-ranged attraction. But that may not be the end of the story. For example, Yaminsky and Ohnishi[4] argue that experimental imperfections (protrusions from the surfaces) rather than nanobubbles, create the long-ranged hydrophobic force, and claim that their surface-force measurements show nothing inconsistent with the standard theory of colloidal interactions (that is, nothing unique to water) down to separations of 3 nm or so. And would we necessarily expect 'depleted' water to be really more gas-like, or instead more ice-like, with enhanced ordering, as originally supposed?[5] And what are the consequences for the hydration environment of proteins, where, for example, a local change in water density might be expected to affect important quantities like pH and salt concentration?

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