PHYSIOLOGY 26: 381–392, 2011; doi:10.1152/physiol.00021.2011

Water in a Crowd

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In many situations, form biology to geology, water occurs not as the pure bulk liquid but rather in nanoscopic environments, in contact with interfaces, interacting with ionic species, and interacting with large organic molecules. In such situations, water does not behave in the same manner as it does in the pure bulk liquid. Water dynamics are fundamental to many processes such as protein folding and proton transport. Such processes depend on the dynamics of water's hydrogen bonding network. Here, the results of ultrafast infrared experiments are described that shed light on the influences of nanoconfinement, interfaces, ions, and organic molecules on water hydrogen bond dynamics.

In biology, water is found in crowded environments rather than as a pure bulk liquid. Water occurs at the interfaces of cell membranes, at the surfaces of proteins, in transmembrane protein ion channels, and inside molecular chaperones such as GroEL/ES. Water is also found in crowded environments in many nonbiological systems such as the proton transport channels in polyelectrolyte fuel cell membranes, in the pores of ion exchange columns, in zeolites, and at the surfaces of minerals. In such nonbulk water, the dynamics of water are distinctly different from those of water in the pure bulk liquid.

Water is a remarkable molecule. It is very small with a similar mass and size to methane. However, methane is a gas at room temperature. It has melting and boiling points of 91 K and 112 K, respectively. This is in contrast to water with melting and boiling points of 273 K and 373 K, respectively. The difference between methane and water is hydrogen bonding. Water can form up to four hydrogen bonds with other water molecules in an approximately tetrahedral arrangement. The hydrogen bonding among water molecules produces an extended network, but this network is not static. Hydrogen bonds are constantly forming and breaking. In bulk water, the time scale for hydrogen bond randomization through concerted formation and dissociation of hydrogen bonds (11) is approximately two picoseconds (2×10^{-12} s) (2, 5, 15). It is the constant reformation of the hydrogen bond network that makes many processes in water possible, such as protein folding and ion solvation.

When water interacts with an interface, an ion, or a large molecule, the hydrogen bond dynamics change. For such systems, there are a number of important questions. How does interaction with an interface change water dynamics? Does the shape of the interface matter? Does the chemical nature of the interface, for example, ionic vs. non-ionic, matter? How much do ions influence water dynamics? For water confined in environments with nanometer length scales, is there a substantial difference between 10-nm and 1-nm characteristic sizes? To shed light on these issues, it is necessary to have experiments that can directly probe water dynamics in systems with the appropriate sizes and geometries and on suitable time scales.

Because water is a small molecule, its motions are very fast. To directly investigate water dynamics requires experiments that can operate on the appropriately fast time scale, that is, picoseconds. In biology and most other water-containing systems, the processes of interest happen under thermal equilibrium conditions on the ground electronic state. Therefore, it is important to be able to observe water dynamics using methods that do not change the inherent characteristics of the system. Ultrafast infrared (IR) experiments performed on the hydroxyl stretch of water can be used to measure the dynamics of water molecules under thermal equilibrium conditions (30). Although there are a variety of ultrafast IR experiments that are useful in the study of water dynamics, IR vibrational pump-probe experiments are the main methods discussed here. The pump-probe experiments are used to measure the vibrational lifetime of the hydroxyl stretch of water and the orientational motions of the water molecules (15–18). For a water molecule to reorient, it must break and reform hydrogen bonds (11, 12). Therefore, measurements of orientational dynamics probe hydrogen bond network dynamics.

Orientational Dynamics at Interfaces of Large Reverse Micelles

Reverse Micelles and the IR Spectra of Confined Water

Many studies of water confined on nanometer-length scales have focused on reverse micelles.

FIGURE 1 shows schematic illustrations of a reverse micelle. A reverse micelle has a surfactant in a bulk organic solvent with a very small amount of water. Because the bulk organic solvent is hydrophobic, the charged or polar surfactant head groups turn inward, and the water forms a pool in the center. The system of water confined in reverse micelles formed by the surfactant Aerosol-OT [AOT; sodium bis(2-ethylhexyl) sulfosuccinate] is particularly useful because AOT reverse micelles are very well characterized. FIGURE 1A is a illustration of an AOT reverse micelle. The chemical structure of AOT is shown in FIGURE 1C. AOT forms monodispersed spherical reverse micelles in isooctane and other organic solvents over a large range of water content from essentially dry up to ~60 water molecules per AOT (31). The number of water molecules per AOT is conveniently described using the w_0 parameter, $w_0 = [H_2O]/[AOT]$. The smallest reverse micelles have radii of <1 nm (50–100 waters), whereas the largest have radii of up to 14 nm (~400,000 waters). With this large size range, it is possible to change the relative amount of the water interacting directly with the interface (see FIGURE 1B) from a large fraction to a small fraction of the total by increasing the reverse micelle size.

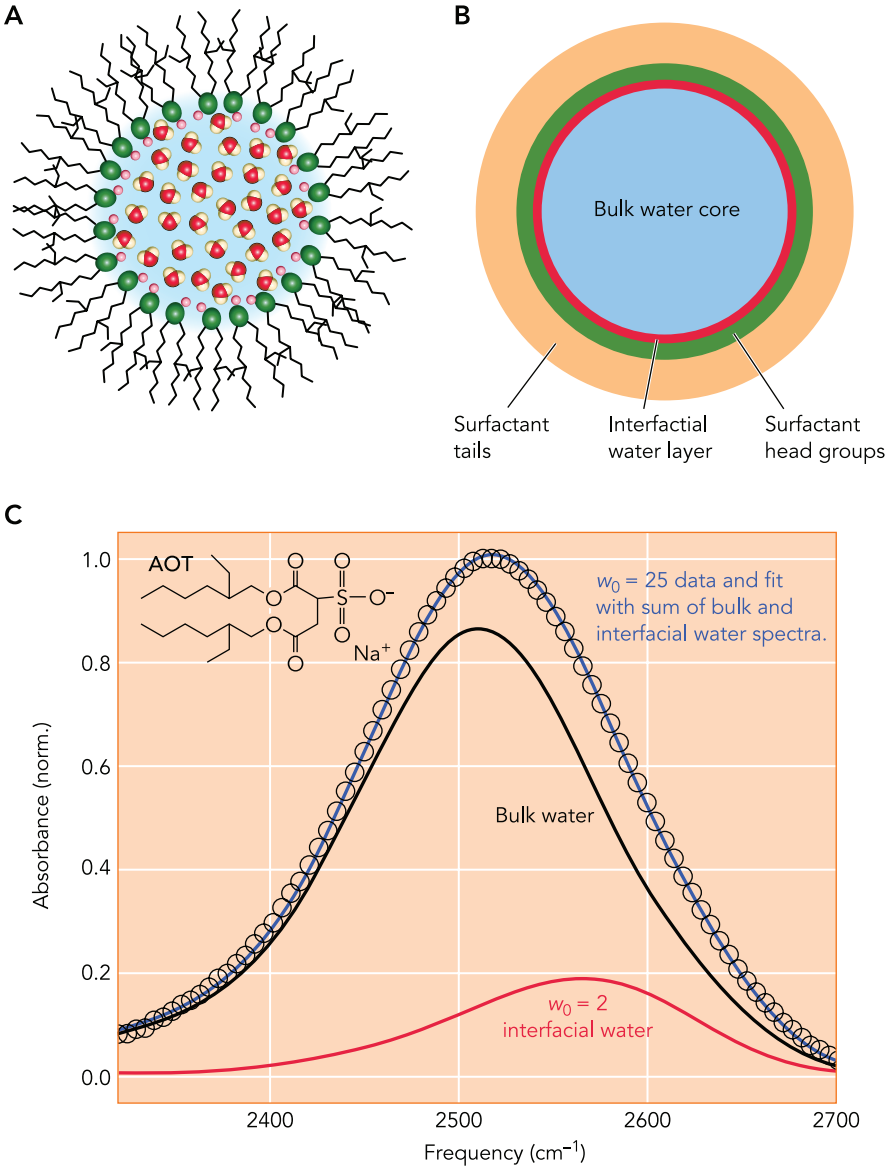


FIGURE 1. **Diagrams of micelles and FT-IR spectra**
A: an illustration of an AOT reverse micelle. The center is a nanoscopic pool of water, which is surrounded by the surfactant's negatively charged sulfonate head groups with associated sodium cationic counter ions. The tails of the surfactants are in a nonpolar organic phase. *B*: a diagram of a large reverse micelle showing the water core and the interfacial water layer. *C*: the FT-IR spectrum of OD stretch in $w_0 = 25$ (circles). Also shown is the fit (blue curve) that is the weighted sum of the bulk water spectrum and the $w_0 = 2$ spectrum, which is used as a model of the spectrum of interfacial water. The $w_0 = 2$ spectrum has a large shift to high frequency. The AOT structure is also shown.

The properties of water confined in relatively large AOT reverse micelles can be described using a core/shell model in which a shell of water molecules interacting directly with the interface have an absorption spectrum, vibrational lifetime, and orientational dynamics (water rotation) that are distinct from the more bulk-like water found in the center of the reverse micelle water pool (4, 7, 16–18, 23). As discussed below, orientational dynamics require the breaking and making of hydrogen bonds. Therefore, measurements of orientational relaxation provide direct information on hydrogen bond dynamics.

Below, the dynamics of the orientational relaxation of water molecules directly in contact with the interfaces of large reverse micelles will be explicated. Large reverse micelles (w_0 = 46, 37, and 25, with radii = 10, 8.5, and 4.5 nm) are useful for this type of study to ensure that a significant bulk-like water pool exists in the center (see FIGURE 1). Laage and Hynes have shown that the orientational dynamics of a water molecule depend on the motions of water molecules in its first and second solvation shell, that is, a particular water molecule's rotation depends on the water molecules immediately surrounding it (first solvation shell) and the water molecules surrounding the first solvation shell (second solvation shell) (11, 12). Based on this model, the water in the core of a large reverse micelle should have bulk characteristics because the water is spatially well separated from the interface and has first and second solvation shells that also do not interact with the interface. This criterion does not apply for much smaller reverse micelles (18).

All of the experiments on water were conducted on the OD (D is deuterium) hydroxyl stretching mode of dilute HOD in H_2O. The OD stretch is used to eliminate vibrational excitation transfer, which can cause artificial decay of the orientational correlation function (rotational observable) (9, 26). In addition, using dilute HOD permits the optical absorbance of the sample to be controlled and eliminates having two hydroxyl stretches, the symmetric and antisymmetric modes, that absorb at different frequencies. MD simulations of HOD in bulk H_2O demonstrate that dilute HOD does not change the properties of water, and the dynamics of HOD report on the dynamics of water (1).

The OD stretching mode of HOD absorbs IR light at ~4 μm (2,500 cm^{-1}). The exact frequency depends on the system and the local environment of the water molecules. FIGURE 1C displays absorption spectra of the OD stretch of HOD in H_2O for w_0 = 25, which has ~11,500 water molecules in the nanopool. The large reverse micelles have spectra that are only somewhat different from that of bulk water. As they get smaller, the spectrum shifts to the blue (higher frequency). These spectra are similar to that of bulk water because each has a large core of bulk-like water (see FIGURE 1B) and a relatively small fraction of water at the interface. In contrast, the spectrum of w_0 = 2 (~40 water molecules) is very different than that of bulk water (see FIGURE 1C), with a substantial blue shift, because essentially all of the water molecules are interacting with the interface. The spectrum of w_0 = 2 is used as a model for the interfacial water spectrum. The spectra of the large reverse micelles are composed of a bulk water spectrum and an interfacial spectrum. The blue shift increases as the reverse micelle becomes smaller because a larger fraction of the water is interacting with the interface. FIGURE 1C shows the spectrum of w_0 = 25 (circles) as well as the spectra of bulk water and w_0 = 2 interfacial water. The solid curve through the circles is the fit to the w_0 = 25 spectrum, only adjusting the relative amplitudes of the bulk water and w_0 = 2 water spectra. The fit is clearly very good. The spectra in FIGURE 1C show that, by conducting the time-dependent IR experiments on the blue side of the large reverse micelle spectra, a significant fraction of the data will come from the interfacial water.

How Water Rotation and Vibrational Lifetimes are Measured

Ultrafast infrared spectroscopy has been used to study water in a variety of nanoconfined systems, in which the dynamics of water are dominated by the effects of the interface (7, 16–20, 23). The first experiments that will be discussed are the rotation (orientational relaxation) of water molecules at the interfaces of large reverse micelles. The orientational relaxation of the HOD molecules can be measured because the absorption of IR light by the OD hydroxyl stretching mode depends on the orientation of the molecule. The transition dipole determines the coupling of the light to the vibration. It is a vector along the OD bond. If the polarization of the light (direction of the light's electric field) is along the OD bond, the probability of exciting the OD stretch is maximum. If the polarization of light is perpendicular to the transition dipole (OD bond direction), the probability of exciting the vibration is zero. It is the directionality of the absorption probability that makes it possible to measure the orientational relaxation (rotation) of the HOD molecules using polarization selective ultrafast IR pump-probe experiments.

In the pump-probe experiment, two very short pulses (60 fs, 60 × 10^{-15} s) of IR light tuned to the OD vibrational frequency are directed into the water sample. Because the IR pulses are very short, they have a very broad spectrum. The spectrum of the pulses is broader than the water spectra

shown in FIGURE 1C, and therefore all of the OD vibrational oscillators can absorb the light. In the experiment, the first pulse, called the pump, excites some of the OD vibrations. A second pulse, the probe, is brought in so that it overlaps spatially in the sample with the volume excited by the pump. The probe is brought in at variable delay times after the pump. Without the pump, when the probe passes through the sample, some of it will be absorbed, so the transmitted pulse has less amplitude than the incident pulse. When the pump pulse precedes the probe pulse in time, some of the ODs have been excited into the first vibrationally excited state. The excitation of OD vibrations leaves fewer molecules in the ground state to absorb the probe, so the transmission of the probe is increased.

First, consider what happens if the water molecules could not rotate. The vibrationally excited state has a finite lifetime. The excited state population created by the pump will decay to the ground (lowest) vibrational state. As the ground state is repopulated, the transmission of the delayed probe pulse will decrease because the absorption of the sample increases. The signal in the pump-probe experiment is the difference in the transmission of the probe with the pump on and with the pump off. As the probe delay is increased, the signal decreases because of population relaxation, $P(t)$. A plot of the signal vs. time delay of the probe will give the vibrational lifetime. The vibrational lifetime of the OD hydroxyl stretching mode is very sensitive to the environment of the OD and can provide useful information, as discussed below. By selecting the appropriate polarizations of the pump and probe beams, it is possible to measure the vibrational lifetime even if the molecules are rotating. For bulk water, the data are fit very well by the single exponential decay, and the fit yields a vibrational lifetime of 1.8 ps.

In addition to population relaxation, the water molecules rotate, which also affects the pump-probe signal. As discussed briefly above, the pump pulse is polarized light, which results in more molecules being excited with their OD bond vectors (transition dipoles) pointing along the pump polarization than perpendicular to it. At very short time, before the molecules have a chance to rotate, the increase of transmission of the probe pulse when its polarization is parallel to the pump pulse polarization is greater than when it is perpendicular. It can be shown that, before orientational relaxation, the pump causes an increase in transmission for parallel probe polarization that is three times greater than for the perpendicular probe polarization. As time goes on, orientation relaxation will randomize the direction of all of the OD bond vectors. Molecules initially parallel to the pump polarization will have equal probability of pointing in any direction at long time, and molecules perpendicular to the pump polarization will also point in all directions at long time. In fact, molecules initially pointing in any direction will randomize their directions at long time. For a probe pulse with polarization parallel to the pump pulse, there will be a decay of the signal as initially excited molecules rotate to other directions and are replaced by molecules that are not excited. For the probe polarization perpendicular to the pump polarization, there will be an increase in signal as excited molecules rotate to the perpendicular direction. Therefore, it is possible to measure the rate of orientational relaxation by making two time-dependent measurements, one with the probe parallel to the pump polarization and one with the probe perpendicular to the probe polarization. In addition to the time dependence from the rotations, there is also the time-dependent population relaxation.

When the polarized pump pulse first excites the sample, the sample has an anisotropic distribution of excited vibrations, that is, more are excited with their OD bond vectors along the pump than perpendicular to it. As time goes on, the anisotropy decays because of the orientational motions. This orientational relaxation causes the anisotropy, $r(t)$, to decay. Measuring the time-dependent anisotropy gives $r(t)$, which is directly related to the orientation relaxation time. For bulk water, $r(t)$ (orientational relaxation) decays as a single exponential with a time constant of 2.6 ps. This is the time constant for water molecules to completely randomize their direction. The mechanism for the reorientation, which involves many water molecules, will be discussed below.

Water Rotation at the Interfaces of Large Reverse Micelles

As illustrated in FIGURE 1B, large reverse micelles will have two types of water molecules, those that are bulk-like water in the core of the reverse micelles and water at the interface. We know the population relaxation time (1.8 ps) and orientational relaxation time (2.6 ps) of bulk water. In reverse micelles, we want to obtain the dynamics of water molecules interacting with the interface. There are now two ensembles of water molecules, bulk water and interfacial water (see FIGURE 1B). Therefore, when we do a pump-probe experiment, the data will contain contributions from both types of water, bulk water and interfacial water. We have developed a theoretical procedure for extracting the interfacial component from the data. In part, we can do this because we know the population relaxation time and orientational relaxation time of bulk water. For bulk water, both of these are single

exponential decays. For the large reverse micelles, the population relaxation is a biexponential decay, with bulk water and interfacial water components. Since we know the bulk water population decay constant, it is straightforward to obtain the OD hydroxyl stretch lifetime for water molecules at the interface (17, 18). The data are taken and fit at a variety of wavelengths on the blue (high frequency) side of the absorption line (see FIGURE 1C). We find that the interfacial water lifetime is independent of the wavelength, but the amplitudes of the two components change with wavelength because the amplitudes depend on how much contribution each type of water makes at a particular wavelength (see FIGURE 1C).

The method for extracting the interfacial water orientational dynamics is more complicated. The anisotropy decays are not single exponential as they are in bulk water. Rather, they have a complex shape that depends on the bulk water and interfacial water lifetimes and orientational relaxation times. FIGURE 2A shows model calculations of orientational anisotropy decays. In the calculations, the known bulk water lifetime and orientational relaxation decay time are used. Also, the lifetime of interfacial water (given below) is used. The only thing that is varied is the interfacial water orientational relaxation time. As can be seen in FIGURE 2A, the shapes of the curves are non-exponential and sensitive to the interfacial orientational relaxation time, τ_{int}. Because the vibrational lifetime is relatively short and the signal decays with the lifetime, we can only obtain data for the short time part of the curves, which is indicated by the dashed line in FIGURE 2A. Nonetheless, because of the sensitivity of the curve to the interfacial orientation relaxation time, the data we can measure is sufficient to determine τ_{int}.

To determine the interfacial water orientational dynamics, first we determined the interfacial population relaxation time. We did this for a number of sizes of reverse micelles. We find for these large reverse micelles, that is micelles with a bulk water core, that the vibrational lifetimes for w_0 = 46, 37, and 25 are 3.9 ± 0.5, 4.6 ± 0.5, and 4.3 ± 0.5 ps, respectively. Within experimental error, the values

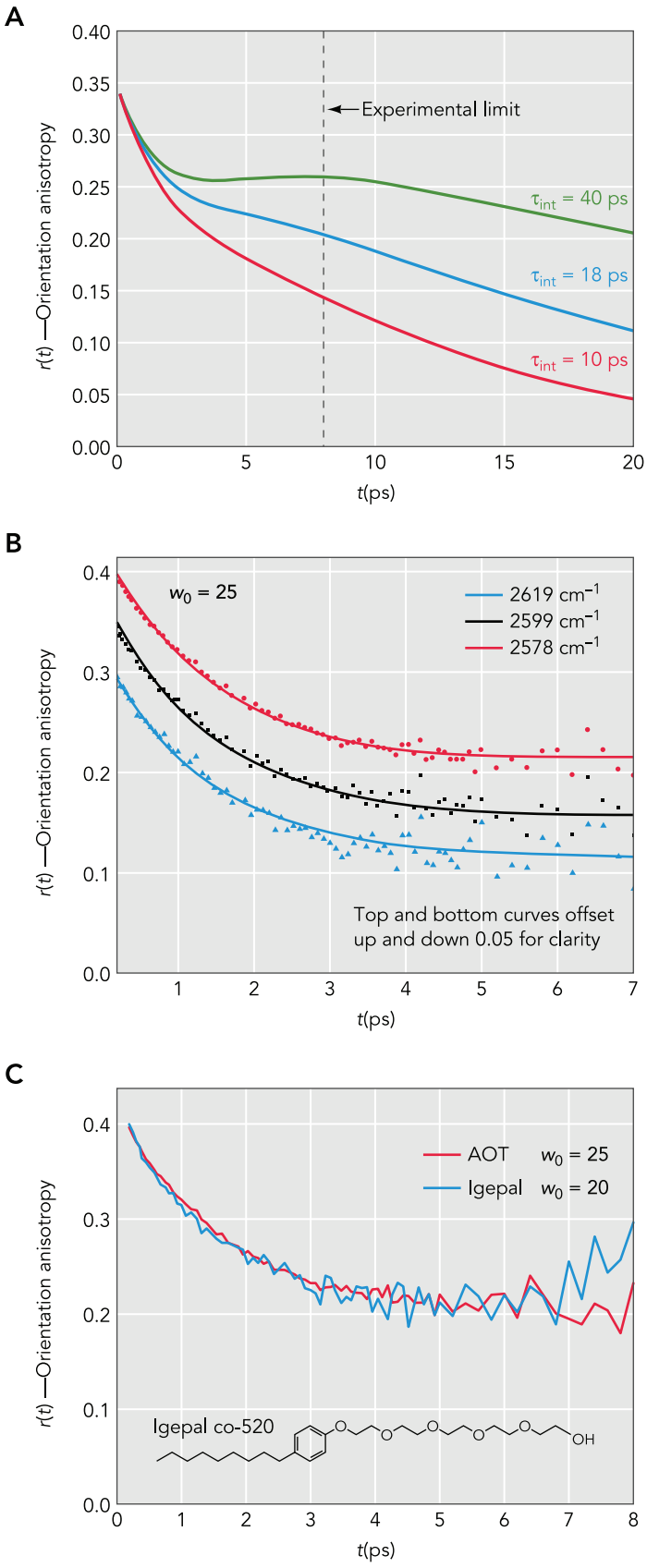


FIGURE 2. **Orientational anisotropy**
A: model calculations for the orientational anisotropy decay for a system with two ensembles of water. One ensemble has the bulk water vibrational lifetime and orientational relaxation time. The other has a slower lifetime and a slower orientational relaxation time that are associated with interfacial water. The shapes of the curves are highly nonexponential and are very sensitive to the interfacial orientational relaxation time. The dashed line shows the typical experimental limit of a measurement, which is restricted by the vibrational lifetime. *B*: orientational anisotropy decays of water in AOT w_0 = 25 reverse micelles at three wavelengths (points), nonexponential fits to the curves (solid curves). The curves have been offset up and down for clarity of presentation. *C*: orientational anisotropy (rotation) data for water in AOT w_0 = 25 and Igepal w_0 = 20 reverse micelle water nanopools. These reverse micelles have the same size nanopools. The chemical structure of Igepal co-520, a neutral surfactant is also shown.

are the same: 4.3 ps. Since vibrational lifetimes are very sensitive to the local environment, the size-independent value is the first indication that, for these large reverse micelles, the water-surface interactions do not change with size. This value is used in the analysis of the interfacial orientation relaxation time.

FIGURE 2B displays anisotropy data for the w_0 = 25 reverse micelle at three wavelength (points) along with fits to the data. The data are analyzed using the method we developed for two component systems (17, 18). This is the same method used to generate the model calculations shown in FIGURE 2A. We know the bulk water lifetime (1.8 ps) and orientational relaxation time (2.6 ps). We also know the interface vibrational lifetime (4.3 ps). In addition, at each wavelength, we know the relative amplitudes of the bulk water and interfacial water contributions from the amplitudes obtained from the biexponential fits to the lifetime data at each wavelength. The result is that there is only one adjustable parameter to fit the data at a range of wavelengths. Because there is only one adjustable parameter and the shape of the curve is very sensitive to τ_{int} (see FIGURE 2A), by simultaneously fitting many wavelengths the resulting errors in the fit are relatively small, even though the data are fit over a restricted time range. As can be seen in FIGURE 2B, the fits are very good.

The orientational relaxation times for w_0 = 46, 37, 25, and 16.5 are 18 ± 3, 18 ± 3, 19 ± 3, and 18 ± 3 ps, respectively. Therefore, within experimental error, the interfacial orientational relaxation times are independent of size, and all are 18 ps. This should be compared with 2.6 ps, the value for bulk water. Interaction with the interface of large spherical AOT reverse micelles slows orientational relaxation substantially but less than an order of magnitude compared with bulk water. The slowing of the orientational relaxation time shows that hydrogen bond rearrangement at the interface is much slower than in bulk water. For the large reverse micelles, the size of the nanoscopic water pool does not matter because the radius of curvature of all of the interfaces is large and all have a significant bulk-like water core. Therefore, the local interactions of water molecules with the interface are very similar.

How Much Does a Charged vs. Uncharged Interface Matter?

AOT reverse micelles have sulfonate ionic head groups that carry a −1 charge and sodium counter ions that have a +1 charge (see FIGURE 1C). The positively charged Na^+ ions are closely associated with the negatively charged sulfonates at the water-surfactant interface of the reverse micelles. To determine the role of interfacial charges, reverse micelles formed from the neutral surfactant Igepal co-520 were studied. The Igepal structure is shown in FIGURE 2C. Igepal forms monodispersed, spherical reverse micelles (14). Igepal w_0 = 20 reverse micelles have the same size water nanopool, i.e., a diameter of 9 nm, as w_0 = 25 AOT reverse micelles. FIGURE 2C shows a comparison of the orientational relaxation data for the two samples (7). Although they are very similar, they are not identical. The Igepal data has an upturn by 8 ps. The model calculations in FIGURE 2A show that it is possible to have an upturn in the data at intermediate times. Fitting the Igepal data in a manner analogous to that used to fit the AOT data gives the interfacial orientation relaxation time (7). The results are Igepal, 13 ± 4 ps; AOT, 18 ± 3 ps; and, for comparison, bulk water, 2.6 ± 0.1 ps.

The orientational relaxation time for water at the nonionic Igepal interface is slightly faster than that of AOT. However, the error bars overlap somewhat. Therefore, it is not certain that they are actually different. Even if they do display some difference, the important point is that going from an ionic interface to a neutral interface at most makes a relatively small difference. Therefore, the presence of the interface has a more substantial influence on the orientational relaxation dynamics of water than on the chemical nature of the interface for these two very different interfaces. As discussed further below, orientational relaxation involves concerted hydrogen bond rearrangement. So, the interface influences hydrogen bond dynamics significantly, but the chemical nature of the interface has less impact on the dynamics.

We performed another type of experiment to address how much ionic species influence water's hydrogen bond dynamics. Ultrafast, two-dimensional infrared (2D IR) vibrational echo chemical exchange spectroscopy was employed to look directly at the influence of ions on water hydrogen bond dynamics (21, 30). Ultrafast 2D IR vibrational echoes are akin to 2D NMR, but the experiment operates on time scales many orders of magnitude faster than NMR. As in a 2D NMR chemical exchange experiment, the 2D IR chemical exchange experiment directly measures the time for interconversion between chemical species that have populations in thermal equilibrium. The 2D IR chemical exchange experiment, operating on the picosecond time scale, directly measures the switching time of water molecules between those hydrogen bonded to ions and those hydrogen bonded to other water molecules. From experiments conducted on bulk water, this time is ~2 ps (1, 2). In the water-ion chemical exchange experiment, the water contained the salt sodium tetrafluoroborate ($NaBF_4$). Water hydroxyls are either hydrogen bonded to BF_4^- anions or to the oxygen

atoms of other water molecules. The measured time for a water to go from being hydrogen bonded to BF_4^- to being bonded to another water molecule is 7 ps (21). Thus, in this direct measurement, ions slow the hydrogen bond rearrangement but only by a factor of three or four. This is consistent with the rather small change observed in going from the AOT charged interface to the Igepal neutral interface.

Both the AOT and Igepal reverse micelles have spherical water pools. Another system that was studied in detail but will only be discussed here briefly is AOT lamellar structures (16). AOT also forms lamellar structures when mixed with water, and the lamellar repeat distances have been characterized by X-ray diffraction (3, 8, 22). The water in the AOT lamellae forms two-dimensional slabs. The slabs are effectively infinite in the two dimensions parallel to the AOT interfaces and bounded by the AOT. Detailed studies of water orientational relaxation at the interfaces for various separations of the AOT layers were made using the same methods as applied to AOT reverse micelles. The water slabs were thick enough so that there was bulk water away from the interfaces. The experiments gave interfacial water orientational relaxation times that were identical to those found for the large AOT reverse micelles within experimental error (16). These results show that in going from a large radius of curvature interface in the AOT reverse micelles to a planar interface did not change the water dynamics.

Why an Interface Slows Water Reorientation

FIGURE 3 shows a cartoon of how water undergoes orientational relaxation in bulk water and the role that an interface plays in slowing the reorientation. The figure is a very qualitative illustration in two dimensions of what are really three-dimensional structures and processes. In bulk water, orientational relaxation involves concerted hydrogen bond rearrangement that results in jump reorientation (11, 12). In the left portion of the figure, a central water molecule is shown making four hydrogen bonds to other water molecules in its first solvation shell. A fifth water moves in from the second solvation shell. As shown in the middle portion of the figure, this additional water molecule allows switching of a number of hydrogen bonds without leaving dangling bonds for any length of time. As indicated by the arrows, in switching hydrogen bonds, the central water (as well as the other waters) will change orientation. This is the jump in orientation that has an average angle of ~60° (11, 12). The right-hand portion of the figure suggests the role that an interface or large molecule plays. The interface blocks many of the pathways for water to move into the first solvation shell, which greatly reduces the number of pathways that can give rise to jump reorientation. The interface eliminates an entire half space of water molecules. In addition, the rough surface topography of the interface also inhibits water molecules from moving into the first solvation shell along a range of paths that are approximately parallel to the interface. Thus the presence of the interface or other large blocking molecule reduces the rate of jump reorientation independent of the blocking species' chemical nature.

Water Interacting with a Large Organic Molecule

Water interacting with much larger molecules will also affect water dynamics. FIGURE 4 displays a ball-and-stick diagram of tetraethylene glycol

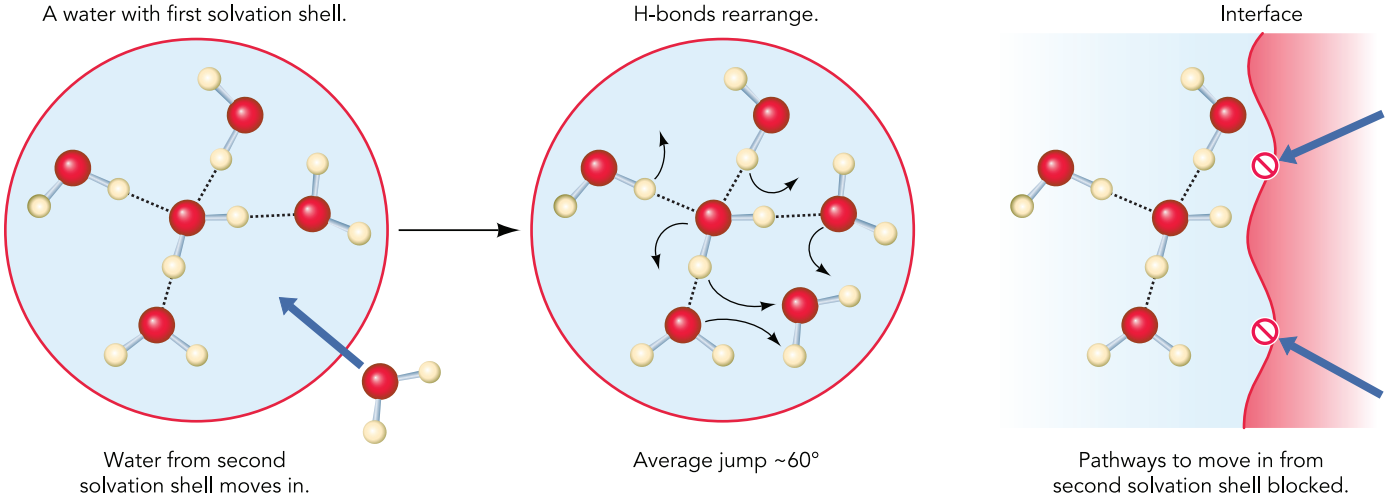
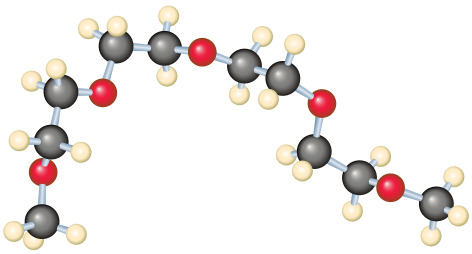


FIGURE 3. **Schematic illustration of the mechanism for water orientational motion (jump reorientation) and how an interface influences orientational dynamics**
See text for detailed description.

dimethyl ether (TEGDE). Detailed calculations and experiments show that its structure in water is not all trans but more compact (25). The structure shown is approximately the average structure found from examining fifty structures obtained from molecular dynamics simulations (25). The polymer poly(ethylene oxide) (PEO) is a technologically important polymer with a wide range of applications including protein crystallization, ion-exchange membranes, and medical devices. PEO differs from TEGDE in that it has hydroxyl end groups rather than methyls. TEGDE permits the interactions of water with the ether moieties, which can act as hydrogen bond acceptors, and the nominally hydrophobic portions of the molecule to be studied without the presence of the strong interactions of water with terminal hydroxyls (6).

Orientation relaxation of water interacting with individual TEGDE molecules was studied over a wide range of ratios of the water to TEGDE concentrations. The lower portion of FIGURE 4 shows orientation relaxation of a solution of 50 water molecules per TEGDE as well as the orientational relaxation of bulk water for comparison. The water/TEGDE samples were analyzed in the same manner as discussed above in connection with FIGURE 2 (6). The dashed blue curve through the TEDGE data is a fit. Data over a wide range of concentrations at various wavelengths for each sample were fit to determine the orientational relaxation time of water interacting with TEDGE (6). The results yield an orientational relaxation time of 19 ± 4 ps (6). Like the results for water interacting with AOT and Igepal reverse micelle interfaces, and with AOT lamellar interfaces, the orientational relaxation is significantly slower than bulk water (2.6 ps). The measured orientational relaxation times in the four systems discussed are very similar. This again indicates that the presence of an interface or large molecule is more important than their chemical natures or geometries.



Tetraethylene glycol dimethyl ether (TEGDE)

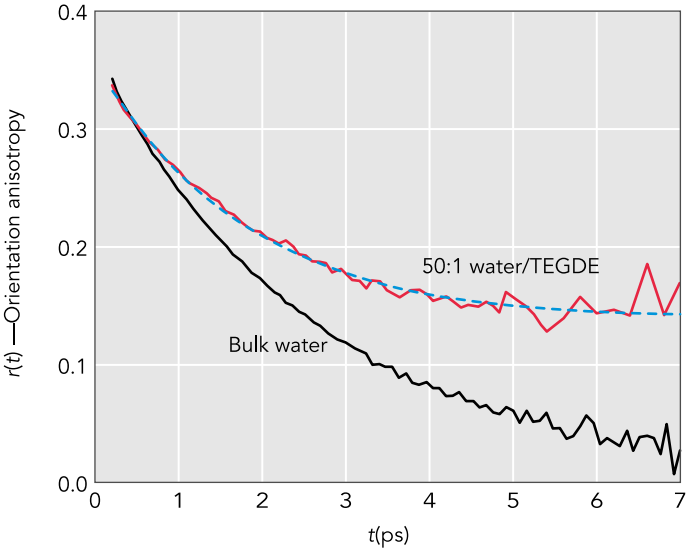


FIGURE 4. **Tetraethylene glycol dimethyl ether and orientational anisotropy decay**
Top: A ball and stick model of tetraethylene glycol dimethyl ether. The ether oxygens (red) are hydrogen bond acceptors. Bottom: the orientational anisotropy decay of HOD in water and TEGDE (50 waters per TEDGE molecule, red curve) and the anisotropy decay of bulk water (black curve). The blue dashed curve is the fit to the non-exponential water/TEDGE data.

Small Water Nanopools

The systems discussed so far, AOT and Igepal reverse micelles, tetraethylene glycol dimethyl ether, AOT lamellae, and water/salt solutions, all have sufficient water in the systems so that there is bulk-like water in addition to water interacting with an interface or ion. Thus water dynamics are of two types, bulk water and interfacial water. In a reverse micelle, such as AOT, and in other systems, the situation changes as the size of the water nanopool becomes small. In AOT reverse micelles, it was found that there are three regimes of water nanopools, large, intermediate, and small (18). The large reverse micelles have cores (a substantial amount of water well separated from the interface) with bulk water characteristics.

As the size of the water nanopool becomes smaller, a larger and larger fraction of the water molecules is interacting with the interface. The influence of the interface propagates out over some distance. In the intermediate regime, the nanopool is small enough that the influence of the interface has not completely died away at the center of the nanopool. This type of system has a core of water molecules, but the core does not have bulk-like properties. In connection with FIGURE 2B, it was found that w_0 = 46, 37, 25, and 16.5 all had interfacial water orientational relaxation times of 18 ps and core relaxation times with the bulk water value of 2.6 ps.

Between the large reverse micelles, $w_0 \geq 16.5$, and the small reverse micelles, $w_0 \leq 5$, lies a transitional region centered about $w_0 = 10$ (diameter of 4 nm). The $w_0 = 10$ reverse micelle is reasonably large; based on geometric considerations, approximately two-thirds of its water molecules are not directly interacting with the interface. However, water reorientation depends on the concerted motions of many water molecules. Although the sulfonate groups in the $w_0 = 10$ are fully

hydrated, the question is whether there are enough water molecules to form a bulk-like core.

FIGURE 5A shows anisotropy data (points) for $w_0 = 10$. The data has the same qualitative shape as the data for the larger reverse micelles, that is, a faster decay followed by a substantial slowing. The *inset* shows the data to longer time with the full vertical range so that the characteristic shape is more evident. The red curve is the calculated anisotropy decay assuming that the parameters found universally for the larger reverse micelles hold for $w_0 = 10$. Although the disagreement between the data and the calculation is not large, clearly the calculation is not an excellent description of the data. Using the two component model embodied in the model calculations shown in FIGURE 2A, the $w_0 = 10$ data were analyzed but without assuming that the core has the bulk water orientational decay of 2.6 ps (18). The black curve through the data is the result of the analysis. It was found that the core orientational relaxation is 4.0 ps and the interfacial orientational relaxation is 26 ps, which should be compared with 2.6 ps and 18 ps found for the large reverse micelles. The water dynamics are slower in both regions, but there are still two distinct types of water molecules in the $w_0 = 10$ reverse micelle. The influence of the interface slows the core water dynamics, which then act back on the interfacial dynamics, slowing them as well.

In the $w_0 = 2$ reverse micelle (see spectrum in FIGURE 1C), there are only two water molecules per AOT molecule. Hauser et al. (10), using differential scanning calorimetry, NMR, and ESR, suggest that between 4 and 6 water molecules are required to hydrate the sulfonate head group of AOT. Although there may not be a bulk water-like core, HOD molecules in small reverse micelles have their ODs experiencing a variety of environments, as demonstrated by a wavelength dependence to the vibrational lifetime (18).

Although small variations in the local environment of a water molecule may affect the vibrational lifetime, the long-time orientational dynamics are related to the complete structural rearrangement of the hydrogen bonding network due to hydrogen bond switching events. An anisotropy decay for HOD in $w_0 = 2$ reverse micelles is shown in FIGURE 5B with the anisotropy decay of bulk water for comparison. After a very fast initial decay, the anisotropy decay in the reverse micelle has a very slow long time decay. The long time decays at a variety of wavelengths are identical within experimental error (18). This behavior is consistent with the extremely low water content, only ~50 water molecules per reverse micelle for $w_0 = 2$. Essentially, hydrogen bond rearrangement is dramatically slowed because of the energetic penalty required to disrupt the structure of sulfonate groups, water molecules, and sodium ions at the interface.

The long time orientational dynamics are independent of frequency because hydrogen bond switching is a concerted process that depends on new hydrogen bond acceptors moving into the first solvation shell of the reorienting water, not the

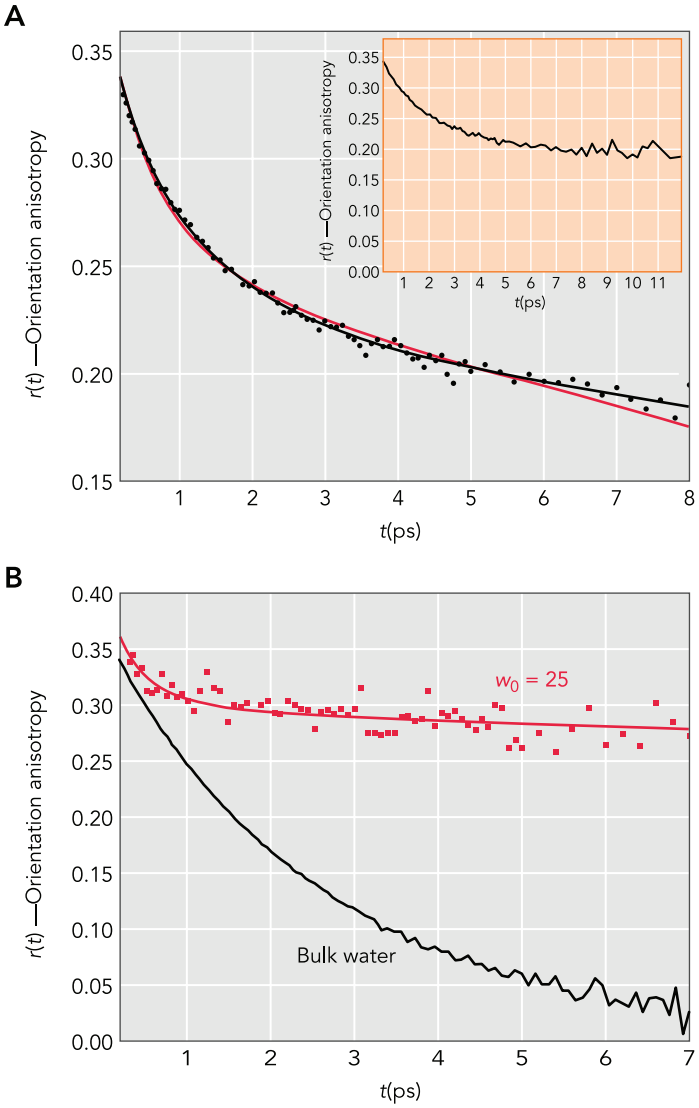


FIGURE 5. **Orientational anisotropy relaxation data**
A: orientational anisotropy relaxation data (rotation, points) of HOD in the water nanopool for the intermediate-sized AOT reverse micelle, $w_0 = 10$. The red curve is a non-adjustable parameter calculation using the results that fit the data from the large reverse micelles. The black curve is the fit without using bulk water parameters for the dynamics of the core water molecules. For this intermediate-size water nanopool, the orientational relaxation of both the core and interfacial water has slowed. *Inset*: the data with expanded scales to reveal the characteristic shape of the two ensemble orientational dynamics (see FIGURE 2A). *B*: orientational anisotropy relaxation data (rotation, points) of HOD in the water nanopool for the very small AOT reverse micelle, $w_0 = 2$ and the decay for bulk water for comparison. The red curve is a biexponential fit to the data. There is a very fast component and a very slow component (>100 ps). The data is not in accord with the two ensemble, core and interfacial water, model. The water in very small nanopools, in which essentially all water molecules are interacting with the interface or interacting with other waters that are directly interacting with the interface, behaves as a single, very slow ensemble.

strength of a particular hydrogen bond (11, 12). In a $w_0 = 2$ reverse micelle, the hydrogen bonding network is completely coupled because so few water molecules are present. Therefore, the long time orientational dynamics are well described by a single ensemble, not a core subensemble and an interface subensemble. The long time anisotropy decays for all frequencies in the $w_0 = 2$ reverse micelle can be fit with the same time constant of 110 ± 40 ps. The large error bars indicate the uncertainty in determining such a long time constant from a limited time range of data. It is interesting to note that the reorientation of water in the $w_0 = 2$ reverse micelle is still an order of magnitude faster than the tumbling time of the reverse micelle itself (24), indicating that water is still able to reorient, albeit slowly.

The fast anisotropy decay component seen in FIGURE 5B is not due to a fraction of the water molecules that are completely reorienting on a time scale faster than bulk water. Rather, it is caused by local orientational motions of water molecules within a stable hydrogen bonding configuration. This type of restricted, incomplete orientational relaxation is referred to as wobbling-in-a-cone (13). It is associated with the wobbling of the orientation of the water molecules over a limited range of angles within a very slowly evolving hydrogen bonding network (18).

The concerted nature of water hydrogen bond rearrangement is responsible for the transition from two-component dynamics (large- and medium-sized water nanopools) to collective dynamics (very small water nanopools). Laage and Hynes showed that water reorientation occurs through a transition state that involves a bifurcated hydrogen bond that the rotating water molecule forms between the old and new hydrogen bond acceptors (see FIGURE 3) (11, 12). The new acceptor is originally in the second hydration shell of the rotating water molecule, but through concerted motions it moves into the first hydration shell, creating an over-coordinated environment for the rotating water molecule and reducing the energetic barrier for reorientation. This model shows that water reorientation is not a localized process; the reorientation of a single water molecule requires concerted rearrangement of both its first and second hydration shell involving ~16 water molecules (28). If one or more of these 16 water molecules is itself in an environment that is not the same as bulk water, causing it to have dynamics distinct from bulk, it will affect the dynamics of its neighbors. When there are many unperturbed water molecules, such as in large reverse micelles, the effect of the perturbation dies off relatively quickly. However, in small reverse micelles, so many of the water molecules are perturbed that even water molecules not directly interacting with the interface have very slow dynamics.

GroEL is a molecular chaperone that belongs to the chaperonin family (27, 29). To function, it requires the cochaperonin protein complex GroES, which serves as a lid for GroEL. Without a protein in the GroEL cavity, the diameter of the water pool is ~8 nm. Thus the nanopool is sufficiently large that it would be expected to behave like the water pool in a large reverse micelle. Water at the interface will have dynamics that are very different from those of bulk water, but there will be a water core with bulk-like or close to bulk-like water dynamics. When a protein enters the GroEL cavity, it will fill most of the interior cavity volume. For example, for a 60-kDa protein in the cavity, the separation between the GroEL interface and the intracavity protein surface will be very small, <1.5 nm. This is less than the diameter of the $w_0 = 2$ reverse micelle. The water molecules in the space between the two proteins will behave like water in a small reverse micelle. The water dynamics, that is, water's ability to rearrange hydrogen bonds and therefore change the local structure, will be very slow compared with bulk water or even water at the GroEL interface in the absence of an intracavity protein.

Concluding Remarks

Water is ubiquitous in biology and in many other areas of nature. However, in general, water does not exist as the pure bulk liquid. In cells, water interacts with cell membranes, the surfaces of proteins, the interiors of proteins, and many other biological molecular species. Water plays a fundamental role in many processes because of its ability to undergo structural reorganization that makes the processes possible. For example, as a protein folds, water must rearrange to accommodate the changing exposure of hydrophilic and hydrophobic regions to the water.

In this paper, work on water dynamics as it interacts with different types of molecular systems was reviewed. The experimental methods involved ultrafast infrared spectroscopy. The dynamics of water are very fast, picosecond to tens of picosecond times scales. The IR experiments make it possible to examine key features of water dynamics and interactions on the time scales on which the important events occur.

Water's ability to accommodate and participate in many biological and chemical processes arises from its constantly evolving hydrogen bond network. In bulk water, the time for hydrogen bond rearrangement is ~2 ps. This time becomes substantially longer when water interacts with an interface, a large molecule, or an ion. It was found

that the nanoscopic dimensions of the water system are important. In a system in which there is an interface and water extends a considerable distance out from the interface, the interfacial water dynamics are independent of the distance as long as it is sufficiently large. This is the situation in the large AOT reverse micelles and the AOT lamellar structures. In these systems, interfacial orientational relaxation, which is dependent on hydrogen bond rearrangement, takes 18 ps, independent of the size of the nanoscopic water pool. The orientational relaxation time in pure bulk water is 2.6 ps. The chemical nature of the interface is less important than the presence of the interface as shown by the similarity of the orientational relaxation times of interfacial water in large reverse micelles of AOT (charged head groups) and Igepal (neutral head groups). The water in contact with a large molecule, e.g., a polyether, in a large amount of water shows similar behavior to that of water at an interface.

Change does matter but not as much as has been thought previously. The ultrafast 2D IR chemical exchange experiments directly measured the time for hydrogen bond switching between water bound to an anion and water bound to another water molecule. This time was measured to be 7 ps, which again should be compared with the ~2 ps time for water hydrogen bond switching between water molecules in pure bulk water. Thus water interacting with charged amino acids at the surface of a protein will experience two influences, the charge and the interface. The results presented here show both matter, but the presence of the interface is the dominant factor in slowing water dynamics.

When the nanoscopic dimension associated with the water volume becomes small, things change. In the AOT reverse micelle studies, it was determined that the dividing line between large water dimensions and small dimensions is ~4 nm between surfaces. The diameter of an AOT reverse micelle with $w_0 = 10$ is 4 nm. For this water nanopool, the water in the core (center of the pool) is somewhat slower than the bulk water behavior found in larger reverse micelles. The water at the interface is also slowed. This suggests that the interface has some effect out to a distance of ~2 nm. When the size becomes very small, such as in AOT reverse micelles with the diameter 1.7 nm ($w_0 = 2$, ~40 water molecules), the interface strongly influences all of the water molecules. Since hydrogen bond dynamics are a concerted process requiring the rearrangement of the hydrogen bonds of many water molecules, the dynamics become very slow. One water molecule cannot rotate by rearranging hydrogen bonds without others doing so as well. Since none of the water molecules is relatively free to move, the system locks up. Hydrogen bond rearrangement, as manifested in the orientational relaxation time, becomes >100 ps.

The net result is that water interacting with interfaces, large molecules, or ions has dynamics that are substantially slowed. However, the nanoscopic dimension of the water pool is of fundamental importance. Water interacting with an interface behaves very differently if it is in a system in which the characteristic nanodimension is relatively large (>10 nm) vs. one in which it is small (<4 nm). Water dynamics depend on the nature of large molecular structures the water is interacting with, but the dynamics depend to an even greater extent on the size of the nanoscopic water system. ■

This work was supported by the National Institute of General Medical Sciences (2-R01-GM-061137-09), and the Department of Energy (DE-FG03-84ER13251).

No conflicts of interest, financial or otherwise, are declared by the author(s).

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