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# Water Dynamics in the Hydration Layer around Proteins and Micelles

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## Contents



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## 1. Introduction

This review deals with dynamics of water molecules in the hydration layer that surrounds self-assemblies and proteins in aqueous solutions. This topic has not only seen a vigorous upsurge of interest in the past decade[1−5] but also has been a subject of investigation for almost half a century now. The basic motivation behind such studies is that they provide valuable information regarding the structure and dynamics of hydration layers and also about the dynamics of self-assemblies and biomolecules themselves. Perhaps the perception about this problem was aptly voiced by Robinson et al. a few years ago when they observed that this "is the most important problem in science that hardly anyone wants to see solved."[6] While one may certainly argue over the superlative used and the skepticism voiced, the need for a better understanding of this important problem was apparent. Fortunately, considerable progress has been made in recent years by the combination of a host of experimental, theoretical, and computer simulation techniques. We aim to review a part of this progress. Because the existing literature is huge, my review may not be exhaustive but I hope to address at least some of the key issues.

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Biman Bagchi (born 1954) received his undergraduate degree from Presidency College, Calcutta University (W. Bengal, India), in 1976. He obtained his Ph.D. degree from Brown University (Providence, RI) in 1981 with Professor Julian H. Gibbs as his advisor. He was a Research Associate at the James Franck Institute, University of Chicago (1981–1983), where he worked with Professors David W. Oxtoby, Graham Fleming, and Stuart Rice, and the University of Maryland (with Robert Zwanzig) before returning to India in the fall of 1984 to join the faculty of the Indian Institute of Science, Bangalore. He is currently the Amrut Mody Chair Professor of Chemical Sciences and is a Fellow of the Indian Academy of Sciences and also of the Third World Academy, Trieste. His research interests include molecular relaxation and transport in liquids, phase transitions, biophysical chemistry, supercooled liquids, and polymers.

A myriad of anomalous features in the dynamics of neat water have always posed interesting challenges to theoreticians and experimentalists alike. For example, while the viscosity of neat water at room temperature is quite large (for such a small and light molecule) and the rotational relaxation of a water molecule is quite slow (as compared to, for example, acetonitrile), the polar solvation dynamics (SD) in liquid water is exceedingly fast.[4,7−27] Clearly, the extended hydrogen bond (HB) network, small moment of inertia, high dipole moment, and high dielectric constant of water combine to give dynamics ranging over wide time scales.

The characteristic features of water molecules near an interface,[2−4] where the HB network gets locally disrupted, differ significantly from those of bulk and thus pose another set of interesting dynamical problems. This is an important issue because water molecules are found in abundance at the interfaces of proteins and DNA, and they control the structure, function, and reactivity of many natural and biological systems.[28−30] Micelles and reverse micelles, microemulsions and foams, lipid vesicles, and hydrogels are some examples of systems where water at the interface is different from that in the bulk. Without the hydrophobic/hydrophilic interactions, the protein structure is not stable and it cannot function. The shell of water around DNA is essential for replication and transcription. Intercalation of anticancer drugs into DNA also involves active participation of water molecules.[31] Water-mediated interactions play an important role in biomolecular recognition processes.[32] However, the detailed role of water in many of these processes is yet to be established.

To emphasize the difference between water molecules at the surface of biomolecules and self-assemblies and those in the bulk, one often represents the former as a hydration layer, which is assumed to be a distinct entity. In Figure 1, we schematically illustrate the so-called hydration layer. Obviously, such a layer is partly real and partly imaginary but it serves a useful purpose. To characterize this layer, one would like to know the time scale of motion of water molecules within it. The dynamics of water in this hydration layer is partly determined by and reflected in the HB lifetime kinetics, which in turn depends on the structure of the layer induced by the macromolecular surface.

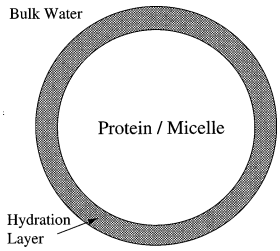


**Figure 1.** Schematic representation of the hydration shell around a protein/micelle. The hydration shell can be typically 4−8 Å thick. The shapes in real systems are seldom perfectly spherical.

In the case of a protein, earlier studies have frequently considered the hydration layer as rigid or "icelike", to give rise to an increased "effective radius" of the protein.[28] The experimental reason for such an assumption was the observation that the rotational correlation time of myoglobin in aqueous solution was found to be 33 ns in contrast to the estimate of 14 ns, which was given by the Debye−Stokes−Einstein (DSE) relation using the crystallographic radius.[24,33] This implies a nearly 40% increase in the hydrodynamic radius of myoglobin over the crystallographic radius, which is indeed surprising. The simplest explanation proposed is that the hydration layer is so rigid that it rotates along with the protein.[1,28] Given that the protein itself rotates slowly, this layer must be really rigid and icelike! Early nuclear magnetic resonance (NMR) studies, despite giving long residence times (of the order of 300 ps or so) for the water molecules in the layer,[34] rightly questioned the concept of a rigid, icelike hydration layer (more recent magnetic relaxation dispersion studies[35] give a much shorter time scale as discussed below).

As a result of many different experimental and theoretical studies,[1−7] we now have a different but (hopefully) more complete picture. It is found that while water molecules near biomolecules or self-assemblies indeed slow noticeably, the hydration layer remains dynamically active and by no means rigid or icelike. Interestingly, one finds the coexistence of both fast (of the order of a few picoseconds) and slow (estimates vary from tens of picoseconds to nanoseconds) dynamics of water molecules in the hydration layer. The origin of this bimodal dynamics can be (partly) rationalized in terms of "bound" (by hydrogen bonding, to macromolecular surface) and "free" water molecules in the layer.[36] The interconversion (that is, the HB breaking) kinetics among these species determines the amplitude and also the time scales of motion in the hydration layer. The biphasic nature of the dynamics has led to the interesting suggestion that the dynamics of interfa-

cial water can be considered similar to that of the bulk but at a substantially lower temperature.[37]

While the hydration layer may be a well-defined entity when studied at a length scale much larger than the diameter ($\sigma_w$) of a water molecule, the same is not true at a molecular length scale. Because the hydration layer is at the most 2−3 (water) molecular diameters thick (for proteins and micelles), probing the dynamics of this layer is difficult. To make matters worse, often different experimental probes of hydration layer dynamics have given rise to different results, causing some amount of confusion in this area. Let us elaborate on this. While quasi-elastic neutron scattering (QENS) studies find a slow decay in the layer with a time constant in the range of hundreds of picoseconds (ps),[37] a more recent magnetic dispersion relaxation method of using water-$^2$H and $^{17}$O nuclei finds the absence of any significant slow decay component.[35] SD studies, however, show the presence of both short and long relaxation times.[24] The varying results may arise from the fact that different experimental techniques probe and measure different physical quantities.

The questions that one would like to ask about hydration layers and issues, which require comprehensive understanding, can be summarized as follows. (i) What are the time scales involved in the relaxation of the hydration layer? How nonexponential is the dynamics? That is, how many time constants are needed to describe a given relaxation process? (ii) How different are the single particle properties, like translational and rotational diffusion coefficients, of individual water molecules in the hydration layer from those in the bulk? (iii) How rigid really is the layer? That is, how slow is the collective water density fluctuation in the hydration layer? Is it at all justifiable to consider this layer as stuck to the macromolecule during its motion, as often assumed in hydrodynamic calculations of the rotational and translational frictions? (iv) How different are the HB lifetimes near the surface? What determines HB lifetime on protein surface? What happens to the intramolecular vibrational modes of water? (v) What is the origin of the ultraslow component observed in the polar SD and reported recently in several studies?[4] (vi) Another important issue is the dynamics of water about a specific region, for example, around a particular group (hydrophilic vs hydrophobic) of amino acids. This is quite demanding information on water dynamics. (vii) Finally, one would like to understand the static and dynamic effects of hydration layer in the biological processes, such as catalysis and molecular recognition.

Answers to some of the above questions are now available, even though many details are still missing. Most of the experimental techniques measure at the most an average over the hydration layer, and this has hindered further understanding. Note that detailed information of water dynamics in the hydration layer is necessary to understand many aspects of biological activity.

Dynamics of water molecules in restricted geometries, like micelles and reverse micelles, are topics of great interest in their own merit, but they also mimic biological systems in certain aspects. For example, a micellar surface is usually charged and hydrophilic. The surface is simpler than a protein surface since the former is homogeneous and perhaps more ordered. The similarity gets further extended if one considers dynamics. One finds that water dynamics slows down on a micellar surface also,[38] and one hopes to gain insight into the time scales involved and also the plausible reasons behind it from such studies.

Therefore, the present review includes the following topics: (i) a brief summary of water dynamics in the bulk, with emphasis on the aspects relevant to the main theme of the review; (ii) experimental studies on dynamics of water (translational, rotational, and vibrational motions) in the protein hydration layer by using techniques such as QENS, NMR, dielectric dispersion, and also SD using probes located near a protein surface; (iii) dynamics in the hydration shell of micelles and in the water pool of reverse micelles and microemulsions. (these systems exhibit slow SD time scales often extending up to tens of nanoseconds); (iv) recent computer simulations studies, which have shown unequivocally that while water molecules in the hydration layer indeed show several anomalous behavior, such as subdiffusive translational diffusion[39,33] and markedly nonexponential orientational relaxation, relaxation remains rather fast; (v) the few purely theoretical studies (involving mainly analytical treatments) that exist on water dynamics at the hydration layer; and (vi) finally, a list of unsolved problems is provided in the penultimate section.

Several reviews have appeared in the literature on different aspects of dynamics of interfacial water.[2−4,8−21,24−27] The focus of the present review is on the microscopic aspects of dynamics of water molecules in aqueous protein solutions and self-organized assemblies. That is, studies devoted to neat water or to other solvents will not be discussed in detail. An attempt will be made to give due importance to the large body of computer simulation studies that have been devoted to understanding water dynamics in complex systems. These studies, when combined with experimental results and theoretical analysis, provide a much better picture of the microscopic aspects of dynamics. An essential ingredient of this new understanding has been the study of the dynamics of hydrogen bonding of interfacial water with the polar headgroups (PHGs) at the surface of self-organized assemblies and proteins.[40,38,41−48] This will also be reviewed.

## 2. Dynamics of Pure Water: Overview

The uniqueness of water originates largely from its hydrogen-bonded network. Each water molecule is capable of forming four HBs. The average HB coordination number at room temperature of an individual water molecule is 3.5, which implies that water is a giant gel or cluster of water molecules.[49] Near to the surface of a micelle or a protein, this HB network gets locally modified. An important technique to understand this change is to study the vibrational dynamics of water molecules near the surface. We now briefly review essential and relevant aspects of dynamics in neat water.

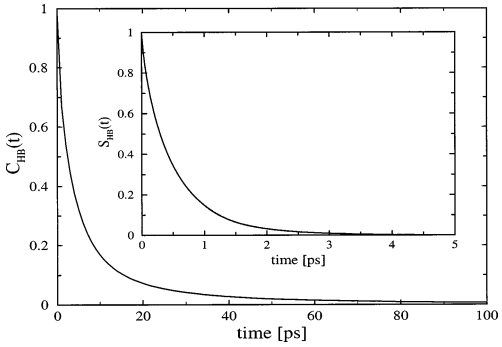


**Figure 2.** HB lifetime correlation function, $C_{HB}(t)$ (defined by eq 1) for a pair of water molecules in the bulk. The inset shows the same for $S_{HB}(t)$ function (defined by eq 2). Reprinted with permission from ref 40. Copyright 2002 American Physical Society.

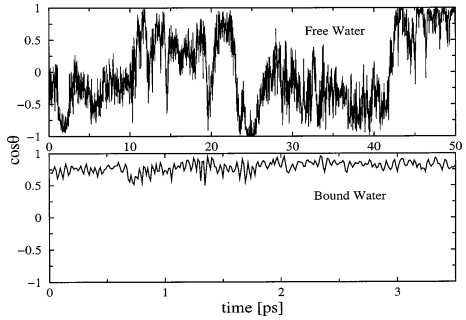


**Figure 3.** Projection of cos$\theta$ of the angle $\theta$ of a tagged water dipole (in the bulk of the simulated water) with the $Z$-axis of a space fixed frame (upper figure). In the lower panel, the same has been shown for a doubly hydrogen-bonded bound water (bound to the surface of a CsPFO micelle) molecule.

## 2.1. HB Lifetime Dynamics

This is currently an area of great interest because the dynamical response of water is intimately connected with the lifetime of HBs. Recent work of Luzar and Chandler[43,44] has elucidated many aspects of HB lifetime dynamics in neat water, and this has been subsequently extended to explore bond dynamics in complex situations, like electrolytes[46] and protein[50] and micellar surfaces.[40,42] The lifetime of a HB is usually described in terms of the HB lifetime correlation functions,[46,51−53] denoted by $C_{HB}(t)$ and $S_{HB}(t)$, which are defined by the following expressions:

$$C_{HB}(t) = \langle h(0)\, h(t) \rangle / \langle h \rangle \quad (1)$$

$$S_{HB}(t) = \langle h(0)\, H(t) \rangle / \langle h \rangle \quad (2)$$

where $h(t)$ is the HB lifetime function, which is unity if the HB between a pair of water molecules is intact at time $t$ and zero if it is broken. $H(t)$, on the other hand, is unity only if the tagged bond has remained continuously nonbroken from time $t = 0$ to the present time $t$. Thus, $C_{HB}(t)$ allows HBs to be broken and reformed in the time interval $t$ while $S_{HB}(t)$ does not allow such reformation. Both of these functions are found to depend on the criteria of HB forming/breaking, although the numbers obtained from different criteria are not too different. The lifetime of $S_{HB}(t)$ is only about 0.5 ps at $T = 300$ K while that of $C_{HB}(t)$ is about 6.5 ps.[40] The decay of these two functions is shown in Figure 2. The much longer lifetime given by $C_{HB}(t)$ is due to the reformation of the bond after it has broken. Because $C_{HB}(t)$ allows for long sojourns after breaking, $C_{HB}(t)$ may give too long a value for the lifetime.

## 2.2. Rotational and DR

Despite the HB network in bulk water, orientational relaxation in a water molecule appears to be dominated by a single exponential[55] component (although a recent femtosecond midinfrared study reports a biexponential decay[54] with time constants of 0.7 and 13 ps). In fact, unambiguous determination of the single particle rotational correlation time is hard to obtain experimentally. A combination of experiments and computer simulation studies indicates that the time constant of the dipole moment time correlation function (a first rank spherical harmonic) is close to 2 ps at 293 K.[55] A plausible reason for this magnitude of time constant is as follows. A water molecule, on the average, is hydrogen bonded to four other water molecules. At least two of these bonds must break for a significant rotation to take place. As discussed above, it is safe to assume that the HB lifetime in water is about 1−2 ps. In Figure 3, we show the time evolution of cos$\theta$, $\theta$ being the angle of a tagged water dipole with the $Z$-axis in the space fixed frame of simulated water in the bulk.[56] Occasional large jumps are due to HB breaking. In the same figure, we also show the trajectory of a typical surface water molecule, which is doubly hydrogen bonded to a cesium perfluorooctanoate (CsPFO) micelle—This will be discussed in more detail later. Here, note the restricted angular motion of the doubly hydrogen-bonded (to the surface) water molecules.

The dielectric relaxation (DR) spectrum of pure water has been investigated in considerable detail by different experimental techniques, such as dielectric loss and, more recently, by a terahertz technique.[57−63] The DR of water has also been investigated by computer simulations.[64−71] However, the computational efforts have been relatively less successful because of the difficulty of simulating polarizable water molecules.[72,73] We refer to the review by Guillot[64,65] for a recent summary of major computational efforts.

The complex dielectric function $\epsilon(\omega)$ is usually decomposed into the real and imaginary parts

$$\epsilon(\omega) = \epsilon'(\omega) - i\epsilon''(\omega) \quad (3)$$

where $\epsilon'(\omega)$ and $\epsilon''(\omega)$ are the real and imaginary parts, respectively. At room temperature, the real part $\epsilon'(\omega)$ (the permittivity factor) of pure water is nearly 80 at a few MHz and about 1.8 at 10000 GHz.[59,74] The imaginary part $\epsilon''(\omega)$ corresponds to absorption (dielectric loss) and exhibits a peak at a certain characteristic frequency $\omega_m$. The DR time $\tau_D$ is equal to $2\pi/\omega_m$. The dielectric spectrum of pure water in the low frequency region consists of two relaxing components, with time constants of 8.2 and ~1 ps, respectively; the former is responsible for about 90% of the low frequency relaxation.[4,59,74] The

8.2 ps component is believed to be related to the rotational correlation time of 2−3 ps. The increase in the value of $\tau_D$ over the rotational correlation time can be understood quantitatively in terms of micro−macro relation, which provides a relationship between the orientational correlation time (a microscopic, single particle property) and the DR (a collective phenomenon). Simple continuum model arguments give the following relation between the two relaxation times[25,75]

$$\tau_R = \frac{2\epsilon_0 + \epsilon_\infty}{3\epsilon_0}\frac{\tau_D}{g_K} \quad (4)$$

where $\epsilon_0$ and $\epsilon_\infty$ are the static and the infinite frequency dielectric constants of the solvent (here, water), respectively, and $g_K$ is the well-known Kirkwood's $g$-factor with a value equal to 2.8.[76] For water, $\epsilon_0 = 78.5$, $\epsilon_\infty = 4.86$, and $\tau_D = 8.3$ ps at 300 K.[59,74] Therefore, one gets $\tau_R = 0.25$ and $\tau_D = 2$ ps. That is, the macro−micro relation predicts the Debye relaxation time to be significantly longer than the single particle relaxation time.

Although the relationship between the single particle and the collective orientational relaxations has been a subject of repeated discussions in the past, it should be noted that the former remains elusive because no experiment measures the relaxation of a dipole of a single molecule. NMR and IR experiments measure relaxation rates for other vectors within the molecules, and the results of these experiments can differ from DR measurements in more than one way. Nevertheless, the success of a relation like eq 4 is impressive.

Many high frequency modes contribute to the dielectric spectrum of water beyond the Debye relaxation regime. As already discussed, this spectral region is extensively investigated by far-infrared spectroscopic techniques and simulations.[64,77−80] In addition to the 200 $cm^{-1}$ band due to the intermolecular O···O stretching and the 650 $cm^{-1}$ band due to libration, there are a few high frequency IR bands, which are of relatively lesser weight as compared to the former two. These high frequency modes are underdamped and, therefore, are described differently.[77,81]

## 2.3. Vibrational Relaxation

Because vibrational relaxation is a sensitive probe of water environment and dynamics, considerable efforts have been made to understand both vibrational phase and energy relaxations in the bulk water. The focus of the present section is on the rapid progress made in the past decade, both in experimental and in theoretical studies. This area has been reviewed recently by Dlott,[82,83] Hynes,[84−87] Bakker,[88,89,54] Ohmine,[90] and Elsaesser.[91,92] Therefore, I will give only a brief discussion, mainly to complement the other studies discussed in the review.

A water molecule is characterized by three intramolecular vibrational modes—the symmetric and the antisymmetric O−H stretches and the H−O−H bend. In the liquid state, the frequencies of these intramolecular vibrational modes get shifted from their gas phase values.[84,82] Figure 4 shows the three (symmetric, antisymmetric, and bending) normal modes of water and heavy water. Vibrational dynamics in water can be categorized into two parts, vibrational energy transfer and the dephasing (features associated with modulation of the OH stretching frequency). Dlott et al. used ultrafast IR-Raman spectroscopy to study the vibrational energy relaxation (VER) in water and heavy water.[82] They found that the lifetime of the OH stretch in water and HDO is ∼1 ps whereas the same of the OD stretch in $D_2O$ is ∼2 ps. Perhaps it was Rey and Hynes[87,84] who first pointed out that VER of the O−H (and the O−D stretch) could occur via the off-diagonal anharmonic coupling with the overtone of the bending mode. Such off-diagonal anharmonic coupling can be efficient because the fundamental of the O−H stretch is off-resonance with the first overtone of the H−O−D bend by 530 $cm^{-1}$, which is close to the librational mode. On the basis of the seminal work of Rey and Hynes, Lawrence and Skinner[93] have further extended our understanding of this important problem. These authors have shown that the ultrafast vibrational phase relaxation of O−H stretch can be understood from the conventional Kubo−Oxtoby[94] theory of frequency modulation time correlation function. Recently Nibbering and Elsaesser[91] extensively reviewed the experimental and theoretical investigations and discussed the potential of nonlinear vibrational spectroscopy for microscopic understanding of HB dynamics in the liquid state.

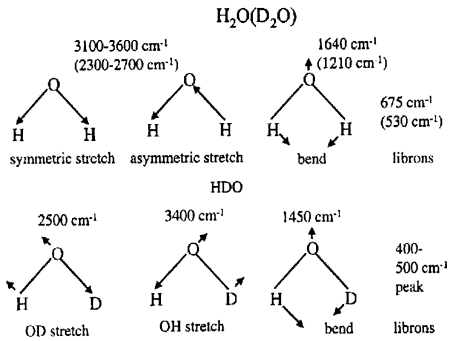


**Figure 4.** Normal modes of water, heavy water ($D_2O$ wavenumbers in parentheses), and HDO. Reprinted with permission from ref 82. Copyright 2000 American Chemical Society.

Both intra- and intermolecular vibrational dynamics of water are expected to get modified significantly near a heterogeneous surface. We shall discuss later that this indeed happens. This modified vibrational spectrum and dynamics can then be used to extract microscopic information about the hydration layer.

## 2.4. Solvation Dynamics

SD measures the time-dependent response of water to a newly created polar species. The quantity measured is the solvation energy, which is a collective quantity. However, one has, on the average, a separation of space associated with a separation of time scale, which makes SD a useful technique. In general,

the ultrafast, the sub-100 fs[95] component (in water and acetonitrile) is dominated by the fully collective response while the slowest, 1 ps component derives a significant contribution from the nearest neighbor water molecules.

The temporal evolution of solvent polarization relaxation may be described by the nonequilibrium function $S(t)$, defined by[8,10−12]

$$S(t) = \frac{\nu(t) - \nu(\infty)}{\nu(0) - \nu(\infty)} \tag{5}$$

where $\nu(t)$ is the frequency denoting the position of the emission spectrum whose time dependence describes the red shift of the spectrum after excitation. Here, $\nu(t)$ is determined either by taking the maximum of the spectrum (if the spectrum is symmetric) or by the average over the spectrum, that is, $\nu(t) = \int d\nu \nu I(\nu, t)/\int d\nu I(\nu, t)$, where $I(\nu,t)$ is the time- and frequency-dependent emission spectrum. $S(t)$, as defined in eq 5, varies from unity at time $t = 0$ to zero as time goes to infinity. Note that $S(t)$ so measured may contain a contribution from solute coordinates also, in particular from solute self-motion, such as rotation and translation, which may accelerate the rate of solvation.[4]

The solvation time correlation function is often equated to the auto time correlation function of energy fluctuation.[8] This is usually termed $C(t)$ to distinguish it from $S(t)$. Thus, $C(t)$ is defined as[4]

$$C(t) = \frac{\langle \delta E(0)\, \delta E(t) \rangle}{\langle \delta E(0)\, \delta E(0) \rangle} \tag{6}$$

where $\delta E(t)$ is the fluctuation in solvation energy from its equilibrium value at time $t$. Within the linear response approximation, $S(t) \approx C(t)$.[8] Therefore, we have made no distinction between the two here.

The first theoretical estimate of solvation time was obtained by generalizing the continuum model of Born and Onsager[25] by representing the dynamical properties of the solvent through a frequency-dependent dielectric constant, $\epsilon(\omega)$, which is sometimes approximated by the simple Debye formula[25]

$$\epsilon(\omega) = \epsilon_\infty + \frac{\epsilon_0 - \epsilon_\infty}{1 + i\omega\tau_D} \tag{7}$$

where $\epsilon_0$ and $\epsilon_\infty$ are the zero and infinite frequency values of the dielectric constant, respectively, and $\tau_D$ is the Debye relaxation time. With the above expression for the dielectric function, the continuum model predicts that SD of a newly created ion and that of a dipole proceed exponentially, with time constants given by[11,12]

$$\tau_L^{ion} = \left(\frac{\epsilon_\infty}{\epsilon_0}\right)\tau_D \tag{8}$$

$$\tau_L^{dipole} = \left(\frac{2\epsilon_\infty + \epsilon_c}{2\epsilon_0 + \epsilon_c}\right)\tau_D \tag{9}$$

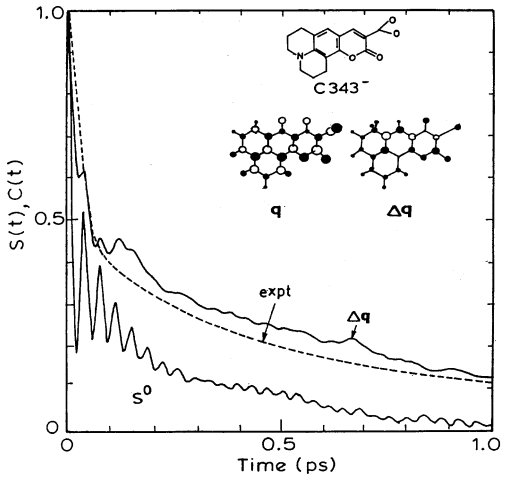


**Figure 5.** Comparison of solvation time correlation function $S(t)$ (defined by eq 5) and $C(t)$ (defined by eq 6) for dye C343 in water. The dashed line shows the experimental result (labeled as expt). The MD simulation result is labeled as $\Delta q$. Also shown is a simulation for a neutral atomic solute with the Lennard−Jones parameters of the water oxygen atom ($S^0$). The experimental data were fitted to eq 10 (using the constraint that the long time spectrum match the steady state fluorescence spectrum) as a Gaussian component (frequency 38.5 $ps^{-1}$, 48% of total amplitude) and a sum of two exponential components: 126 (20%) and 880 (35%) fs. Reprinted with permission from ref 95. Copyright 1994 Nature Publishing Group.

where $\epsilon_c$ is the dielectric constant of the solute probe. For water, $\epsilon_0 = 78.5$, $\epsilon_\infty = 4.86$, $\epsilon_c$ is typically between 2 and 4, and $\tau_D = 8.3$ ps at 300 K.[59,74] Thus, the value of the longitudinal relaxation time $\tau_L^{ion} \simeq 0.5$ ps. That is, even the continuum model predicts an extremely fast solvation in water! Clearly, the reason for the small value of the predicted solvation time is due to the large value of the static dielectric constant.

A time-dependent fluorescence Stokes shift (TDFSS) has been applied to a large number of liquids. In an important paper, Jimenez et al.[95] reported the results of SD of the excited state of the dye, Coumarin 343 (C343). Their result is shown in Figure 5. The initial part of the solvent response of water is extremely fast (few tens of femtoseconds) and constitutes more than 60% of the total solvation. The subsequent relaxation occurs in the picosecond time scale. The decay of the solvation time correlation function, $S(t)$, is fitted to a function of the following form[95]

$$S(t) = A_G\, e^{-t^2/\tau_G^2} + B\, \cos(\alpha t)\, e^{-t/\tau_1} + C\, e^{-t/\tau_2} + D\, e^{-t/\tau_3} \tag{10}$$

where $A_G$, $C$, and $D$ are the relative weights of the initial Gaussian and the subsequent exponential decay processes and $\tau_G$, $\tau_2$, and $\tau_3$ are the corresponding relaxation time constants. The second term in eq 10 takes into account the oscillatory features of the $S(t)$ observed beyond the Gaussian decay in theoretical calculations and simulations.[96,97] The early simulation studies also predicted a very fast initial component with a Gaussian time constant less than 20 fs.[96] Jimenez et al.[95] experimentally detected a

Gaussian component (with a time constant of 28 fs, 48% of the total amplitude) and a slower biexponential decay with time constants of 126 (20%) and 880 (35%) fs, respectively. Several other experimental and simulation studies on SD of large dye molecules as well as electrons in water have demonstrated that the dynamics of solvation in water is indeed ultrafast and occurs in the femtosecond scale.[17,98−111] More recently, higher order nonlinear optical measurements such as three pulse photon echo peak shift measurements have been carried out to study the SD.[112−114]

Fleming and co-workers[115,116] have carried out extensive study of three pulse photon echo from the dye molecule eosin in water. They have found that a substantial amplitude (about 60%) of aqueous solvation occurs within 30 fs. A three-exponential fit (up to 100 ps) to the data of eosin in water yields time constants of 17 fs (73%), 330 fs (15%), and 3 ps (12%). Analysis of the experimental data led Lang et al.[115] to attribute this ultrafast solvation to the high frequency intermolecular vibrational/librational modes of water—the hindered translational band at 180 $cm^{-1}$ due to the HB network and the 600 $cm^{-1}$ band due to libration. It was further pointed out by Lang et al.[115] that the generalized continuum model theory of Song and Chandler[117] could provide a satisfactory description of the solvation experimental data, provided the full frequency dependence of the dielectric function, $\epsilon(\omega)$, is taken into account from experiment. Song and Chandler further found that the solvation response was sensitive to the shape and charge distribution of the probe. The excellent agreement at short times of the generalized continuum models with the experimental results suggests that the ultrafast component of SD is indeed collective in nature where a large number of water molecules participate. Note that this explanation is different from the inertial response of single water molecules. In the latter case, the collective polarization potential (the solvation potential) is not the driving force of the relaxation. These results of Marcus, Song, and Chandler[117] are in agreement with the microscopic analysis of Nandi et al.[81] who observed that if the rotational memory kernel is obtained from the full $\epsilon(\omega)$, a near quantitative agreement with experimental data could be obtained.

## 3. Experimental Studies of Water Dynamics in the Hydration Layer

The first signature that the dynamics of water molecules at the surface of a biomolecule are substantially different from those in the bulk actually came from the measurements of the rotational and translational diffusion coefficients of water in aqueous protein solutions. Analysis based on hydrodynamic formula (like Stokes−Einstein and DSE[28,118]) showed that an explanation of the observed values required a larger than actual radius of the protein to be used in the Stokes expression of the friction (from hydrodynamics).[28,118] Earlier studies of nuclear Overhausser effect (NOE),[34] as already mentioned, gave an upper limit of 500 ps of the residence time for most of the water molecules in the layer.[34]

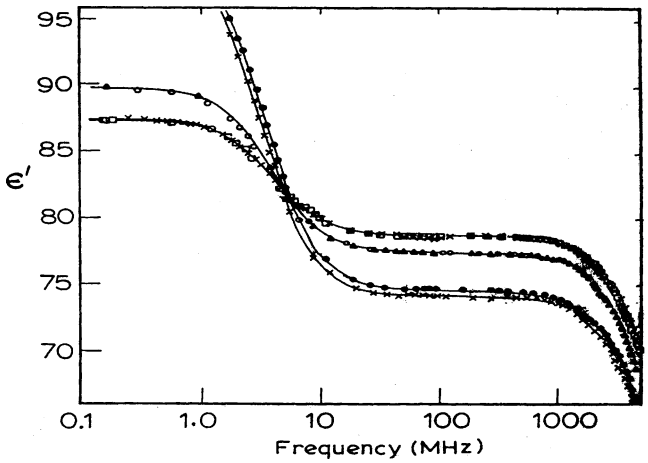


**Figure 6.** Real part of the complex frequency-dependent dielectric function [$\epsilon'(\omega)$] of aqueous myoglobin solution for different concentrations. Concentrations are (from top to bottom) 161, 99, and 77 mg/mL at 293.15 K. The symbols denote experimental results while the solid line is a fit to the theory of Nandi and Bagchi. Reprinted with permission from ref 4. Copyright 2000 American Chemical Society.

However, more recent studies by Halle and coworkers[35] have given a much shorter time. Other sources of information about biological water are X-ray diffraction and neutron scattering[119] experiments. Both give a measure of the number of bound water molecules, but it is only the latter that can give information about the dynamics, both in dry powder and in solution. In the following, we first review experimental techniques and results and then turn to theoretical and computer simulation studies.

### 3.1. Dielectric Dispersion

DR was probably the first method employed to study the relaxation in the aqueous solutions of biomolecules and self-assemblies. Although dielectric dispersion contains (mostly orientational) a response from all of the molecules (water, biomolecules, and ions), the assignment to the orientational relaxation of individual species is possible when they are well-separated in the time scales.

The dielectric spectra of aqueous protein solutions exhibit anomalous dielectric increments[120,121] where the value of the static dielectric constant of the solution is significantly larger than that of pure water. A typical experimental result illustrating the dielectric increment is shown in Figure 6, where the real part of the frequency-dependent dielectric constant of myoglobin is evident. Both the increment at zero frequency and the overall shape of this curve have drawn a lot of attention.[122,123] There are certain universal features in the DR spectra of aqueous protein solutions. One usually finds two distinct loss peaks near 10 MHz and 10 GHz.[124] These two peaks should correspond to the protein and the bulk water relaxations, respectively, as observed in the recent simulation studies of Boresch et al.[125](see Figure 10). The additional high frequency dispersions, observed within the range of 10 MHz to 10 GHz, are often referred to as $\delta$ dispersion ($\delta_{11}$ and $\delta_{22}$ dispersions). While the two peaks near 10 MHz and 10 GHz are high and distinct, the dispersion occurs in the plateau

region of the dielectric spectra and has relatively less weight. Dachwitz et al.[124] suggested that the dispersion was due to the bound water and internal motions of myoglobin. Similar results were obtained for other proteins.[124] Mashimo et al.[126] assigned the low frequency process to the relaxation of bound water and the high frequency process to the relaxation of free water.

Recent DR studies of water in reverse micelles provide information on the mobility of the water molecules in the nanometer-sized pools.[127,128] Terahertz (THz) spectroscopic studies in the frequency range of 0.2−2 THz show that the amplitude of the DR in the water pool is substantially smaller than that in bulk water.[127]

Several comments on the above results are in order. The nearly universal presence of a component in the 30−50 ps range[24] in protein solutions may be attributed to a dynamic equilibrium between the bound and the free water at the interface. Detailed studies (both experimental and simulation) regarding the influence of specific amino acid residues on the dynamics of neighboring water molecules need to be carried out to further our understanding on this important issue.

## 3.2. Rotational and Translational Dynamics

### *3.2.1. NMR Studies*

As is well-known, NMR is a versatile technique, which has been widely used to study not only the structure but also the rotational (single particle) and translational dynamics of water molecules, both in the bulk and near a surface. NMR has provided highly useful local information about the dynamics of water molecules in the hydration layer, which are unique in their specificity.[34,35,129−132] Koenig and Schillinger[133] first observed the magnetic field dependence of water proton spin−lattice relaxation rates with increasing field strength. They considered a chemical exchange model in which the relaxation was assumed to be the weighted average of the relaxation rates of water molecules free in solution and of those that were presumed to be bound to the protein and rotated with the rotational correlation time of the protein. Polanszek and Bryant[134,135] took a completely different approach to characterize the motion of water molecules at the protein surface. These authors used a nitroxide spin label to place a large electron spin magnetic moment on the protein surface. The magnetic field from the unpaired electron is approximately 1000 times larger than that from the protons, so that it was possible to isolate the paramagnetic contribution to the water proton relaxation easily. In this case, the relaxation mechanism is an electron magnetic dipole−water proton−dipole interaction. Because the electron spin relaxation time of the nitroxide radical is long, the correlation time for the intermolecular coupling is that for the relative translational motion of the proton−electron pair. Because protein moves very slowly as compared to water, the effective correlation time for the coupling is the translational displacement correlation time for the water near the nitroxide ion on the protein surface. Measurement of the water proton relaxation rate over a wide range of magnetic field strengths permits extraction of the translational diffusion constant of the water molecules residing near the nitroxide.[134,135] The relaxation is expected to be dominated by the surface effects within approximately 10 Å of the surface.[134,135] The diffusion constant of the surface water was found to be lower than that of the bulk value by a factor of 5. Bryant has reviewed the NMR relaxation of water proton in protein powder system.[136] These authors conclude in the study[137,136] that the motion of water molecules at the protein surface is certainly slower than that in the solute free solvent, but it remains orders of magnitude faster than motions in a rigid ice lattice even in samples hydrated to levels well below what is generally thought to be the full hydration complement of the protein.

NOE intensities get modulated by dipole−dipole interactions between protons of protein and water in the hydration layer. Because this interaction varies as $R^{-6}$, where $R$ is the separation between the two protons, the dynamical information extracted from NOE has earlier been assumed to be local in character. Measurements of magnetization transfer using NOE have thus been used to obtain the residence time of the hydration water.[34] Note that the residence time of water molecules in the hydration layer immediate to the protein is not easily available by other techniques and is a valuable information to quantify the rigidity of the layer. However, NOE studies seem to predict rather long residence times, of the order of 300−500 ps, for water molecules in the hydration layer.[34] Such long residence times can be appropriate only for water molecules strongly bound in a cavity of a protein. As pointed out recently by Halle, the earlier NOE measurements derived significant contributions from distant water molecules as well because the number of contributing water molecules increases as $R^2$ and the characteristic time for orientational modulation of the internuclear vector $\mathbf{R}$ also increases as $R^2$.[138] Thus, earlier estimates from NOE might not be reliable for the residence time of the water molecules. More recent magnetic relaxation dispersion (NMRD) studies[35] involving water oxygen-17 find a much shorter residence time, in the range of 10−50 ps. Modig et al.[35] have reported the hydration dynamics of the cyclic peptide oxytocin and the globular protein BPTI in deeply supercooled solutions (between −10 and −30 °C). Analysis of the results suggests that more than 95% of water molecules in contact with biomolecular surface are no more than 2-fold motionally retarded than those in the bulk.[35] This is certainly a remarkable result, which seems to be in contradiction with results from several previous studies, which concluded slower dynamics.[4] More such studies using other proteins will be quite useful to confirm the generality of this result. It is interesting to note how these recent developments (particularly results from the NMRD technique and computer simulations) have changed our perception about the dynamics of the hydration layer, from a rigid icelike layer to a dynamically mobile, somewhat slower than bulk but still active, region.

The slow dynamics in the water pool of reverse micelle was actually discovered a long time ago by Wong et al.[139] by using $^1$H NMR spectroscopy. These authors found that the rotational correlation time of water lengthened by 2 orders of magnitude in the small water pools of heptane−AOT−water reverse micelles.

Thus, to summarize, modern NMR methods find signatures of slow dynamics at the protein hydration layer, although the extent of slowness is less than what was surmised before. However, the new NMR methods need to be further developed to obtain a time correlation function so that we can capture more details of the dynamics.

#### 3.2.2. QENS Experiments

Inelastic neutron scattering experiments have been widely used both in the liquid and in the solid states to measure structure and dynamics at molecular length scales.[140] In an incoherent inelastic neutron scattering experiment, the measured quantity is the self-dynamic structure factor $S_s(Q, \omega)$, which gives information, as in the liquid state, of the self-diffusion coefficient of the water molecules. $S_s(Q, \omega)$ is the Fourier transform of the intermediate self-scattering function $F_s(Q, t)$, which is defined by

$$F_s(Q, t) = \frac{1}{N} \langle \sum_{i=1}^{N} e^{iQ\cdot[\boldsymbol{r}_i(t)-\boldsymbol{r}_i(0)]} \rangle \qquad (11)$$

where $\boldsymbol{r}_i(t)$ is the position of the $i$-th scatterer at time $t$ and the sum is over all of the scatterers. The symbol $\langle \ldots \rangle$ denotes averaging over initial distribution. If one is probing only the long time center of mass motion, then in the absence of hopping, that is, jump motions, $F_s(Q, t)$ is given by $\exp(-DQ^2t)$, where $D$ is the translational diffusion coefficient of individual water molecules. The presence of jump motions modifies this limiting behavior.[140] At shorter times, QENS derives contributions from both vibrational and rotational motions. Because of recent developments, the time scales accessed by QENS can now range from picoseconds to several nanoseconds. Thus, one can probe not only translational motion but also vibration and rotation. As hydrogen atoms make a much larger contribution to the scattering cross-section than most elements and isotopes, this technique is a powerful tool to study dynamics of proteins. When solvent water is replaced by deuterated water, scattering comes from nonexchangeable protein atoms. Thus, one can study protein dynamics (which occurs at a slower time scale than surrounding water) and also the dynamics of the hydration water.

Several studies using QENS have appeared recently.[141−143] Russo et al.[141] have reported dynamics of hydration water in a completely deuterated pentaalanine peptide at different levels of hydration (7, 30, 50, and 90%) and of dried powder, which contained only one structural water molecule. Even this lonely water molecule was found to move rather fast with an orientational correlation time of 2.2 ps—only two times longer than that of bulk water in the ambient conditions.[141] Thus, there was no really slow water molecule in this system, which was perhaps expected for this system. With a higher level of hydration, rotational dynamics of water approached that of bulk water, again as expected. Paciaroni et al.[143] have presented an interesting QENS study of protein dynamics on the picosecond time scale of lysozyme solvated in glycerol at different water contents, $h$ (g water/g lysozyme). For all $h$, one finds a well-visible low frequency vibrational bump. The quasielastic scattering can be decomposed into two Lorentzian components, corresponding to motions with characteristic time constants of 15 and 0.8 ps.

Tarek et al.[142] have presented results of an interesting study where QENS and molecular dynamics (MD) simulations were combined to study dynamics of native and molten globular states of α-lactalbumin in aqueous solution. The emphasis of this and a related study[144] was on interpretation of QENS studies on aqueous protein solutions. These studies provided detailed descriptions of the mean square displacement of the protein residues and also the role of hydration water on protein motion. These studies have indicated that at the protein interface, water behaves like a "bulk supercooled liquid" in the sense that $F_S(Q, t)$ of water shows a two-step (fast and slow) relaxation with a plateau between.[37] The fast relaxation is over in less than a picosecond while the slow relaxation is approximately a stretched exponential having relaxation time in the hundreds of picoseconds or even in nanoseconds. This slow dynamics has been attributed to center of mass motions of water molecules following the rearrangement of the cage.

Recently, Ladanyi and co-workers have studied the water motion in reverse micelles of aerosol OT using MD simulations.[145] They also calculated the self-intermediate scattering function $F_S(Q, t)$ for water hydrogens and compared the time Fourier transform of the same with the QENS dynamic structure factor $S(Q, \omega)$ and found good agreement between the simulation and the experiment. They have calculated the separate intermediate scattering functions for rotational and translational motion. They have found that the decay of translational scattering function is nonexponential, indicating that this behavior is due to lower water mobility close to the interface and also to confinement-induced restrictions on the range of translational displacements.

Thus, QENS finds signatures of a slow decay in the hydration layer at long times. This decay is considerably slower than what one finds in bulk water at ambient conditions.

### 3.3. Vibrational Spectroscopy

As discussed in the previous section, the O−H (O−D) stretching vibrational spectrum of water molecules exhibits exceptional sensitivity toward hydrogen bonding. This sensitivity has been used as spectroscopic probes for testing solute−solvent interactions, for example, the influence of $Na^+$ and $Cl^-$ ions on solvent structure in electrolyte solutions.[89] Recently, Khoshtariya et al.[146] have applied the difference HO−H and DO−D IR and near-IR vibrational spectroscopy to aqueous or almost dry protein samples

to study dynamics of protein hydration layer.[146] Both native and thermally unfolded bovine serum albumin (BSA) have been studied. BSA "dry films" exhibit two kinds of intense and very broad O−H (O−D) subbands centered at 3260 and 2840 $cm^{-1}$ (for O−D, at 2350 and 2050 $cm^{-1}$, respectively).[146] The first of these two bands has been assigned to the O−H (O−D) stretch of the water molecules where the H(D) of O−H (O−D) is involved in strong hydrogen bonding with other interfacial water molecules. It has been suggested that these water molecules form a connected two-dimensional network on the surface. The second band has been assigned to the O−H (O−D) stretch where the hydrogen of O−H (O−D) is involved in hydrogen bonding with the polar groups of the protein. It was found that the first band is much wider than the second band.

The above nice analysis, supporting the existence of a two-dimensional network of hydrogen-bonded water molecules in the hydration layer, appears to agree with the work of Careri et al.[147−149] who suggested a two-dimensional percolating network in the surface of lysozyme with migrating protons in order to explain the anomalous conductivity and dielectric spectroscopy of dry powders.

IR spectroscopy has been extended to two dimensions (taking the cue from NMR) and applied to the study of structure and dynamics of aqueous protein solutions.[150,151] These new techniques manipulate vibrational coherences of bonds to extract information of protein structure and dynamics. Until recently, two-dimensional IR-COSY and THIRSTY spectroscopies have mostly been applied to the amide region of small peptides and proteins[150] in the 1550−1700 $cm^{-1}$ region. More recently, these techniques have been applied to larger proteins. However, the emphasis till now has been on understanding structure and dynamics of proteins rather than of the hydration water.

To summarize, the vibrational spectroscopy of hydration layer provides a physical picture in terms of a two-dimensional percolating network of HBs among water molecules at the protein surface. This percolating network could make the hydration layer somewhat rigid and slow. This in turn can explain the slow dynamics observed by QENS and SD. Much more work is required to fully quantify this observation.

## 3.4. SD

At a protein surface, the time dependence of the solvation energy of a newly created probe derives contributions from many sources, not only from the surface and the bulk water molecules but also from the amino acid side chains and from ions (as they always tend to be present in experimental systems). This makes the analysis of SD of a protein solution very difficult. In one of the first studies on SD in the surface of protein in aqueous solution, Fleming and co-workers investigated three pulse photon echo responses of the probe dye eosin in aqueous protein solutions of lysozyme.[115,116] This technique provides frequency (that is, energy gap) fluctuation time correlation functions but requires extensive numerical analysis of the data. This study revealed several new results. It was found that although the ultrafast (sub-100 fs) component observed in bulk water continued to play a dominant role even in protein surface, there appeared several slow components, which were found to depend on the time scale of observation. Nevertheless, two slow components, one in the range of 100 ps and another about 500 ps, were observed in this study. The experimental result of solvation time correlation function of eosin in lysozyme is shown in Figure 7. In a series of experiments, Zewail and co-workers[24,152,153] have examined SD of excited tryptophan as a natural probe in several proteins by using TDFSS. The advantage of using tryptophan as a probe is 2-fold. First, it is a natural probe, so the conformation of the protein is not disturbed and the solvation of the native state is explored. Second, one can study proteins where the tryptophan is partly or fully exposed to water, and so, SD studies allow one to directly probe the response of biological water. Zewail and co-workers have found a slow component in the solvation time correlation function, which was in the range of 20−40 ps.[24,152,153] This is more than an order of magnitude slower than the bulk response. The results of Zewail and co-workers are shown in Figure 8 for the protein Subtilisin Carlsberg (SC). The inset in the same figure shows faster solvation when the probe is dansyl bonded and placed at a distance of 6−7 Å from the protein surface. The time resolution of the last set of experiments was much lower than the ones employed in ref 95 and, therefore, missed a significant portion of the faster dynamics. Bhattacharyya and co-workers[154] have reported studies of SD of a covalently bound probe to protein glutaminyl-tRNA synthetase (GlnRS), both in its native and in its molten globule state. The SD was found to have two slow components, one major component at 40 ps and a minor at 580 ps.[154] The decay of response function is shown in Figure 9. Recently, Guha et. al.[155] reported slow SD at the active site of GlnRS by using the fluorescence probe, acrylodan. This result could have important implications in enzymatic catalysis.

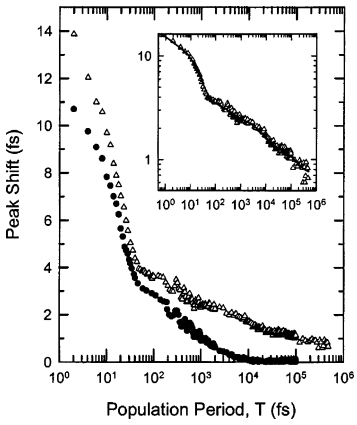


**Figure 7.** SD study of dye eosin in water by third order photon echo spectroscopy. The peak shift data of eosin in water (solid circles) and lysozyme complex in water (open triangles) are shown. The inset shows the lysozyme data (open triangles) with fits (solid line). Reprinted with permission from ref 116. Copyright 1999 American Chemical Society.

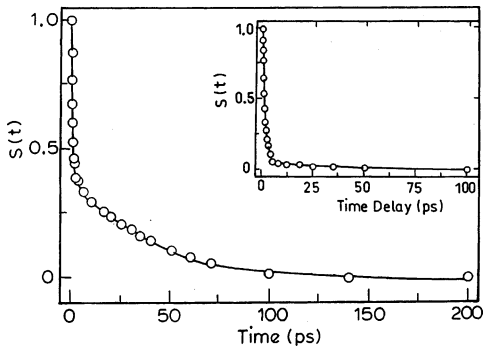


**Figure 8.** Solvation time correlation function for tryptophan probe in the surface of the protein SC. The inset shows the same for Dansyl-bonded SC where the probe is 6−7 Å away from the surface. Reprinted with permission from ref 24. Copyright 2002 American Chemical Society.

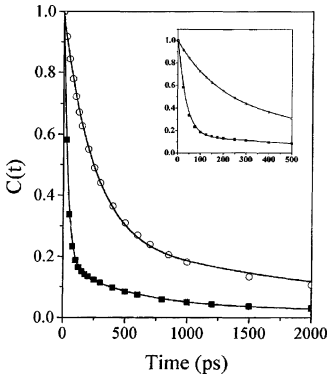


**Figure 9.** Time dependence of solvation time correlation function, $C(t)$ [which is the same as $S(t)$], of the probe 4-(*N*-bromoacetylamino)phthalimide in an AOT microemulsion ($w_0 = 20$, open circle) and covalently bound to GlnRS in 60 $\mu$M GnlRS in phosphate buffer (pH 7.5, filled square). Open circles and filled squares are the experimental data points, and solid lines are the biexponential fits. The inset shows the same for initial decay. Reprinted with permission from ref 154. Copyright 2002 American Chemical Society.

Pierce and Boxer[156] and Baskhin et al.[157] had earlier reported that the SD in the protein environments was nonexponential with a long component with a long time constant of the order of 10 ns. It is interesting to note that this time scale is very close to the nanosecond component of DR earlier observed for the aqueous protein solutions. This set of experiments also suffered from limited time resolution. Thus, all of the dynamics occurring with time constants shorter than 10 ps were not detected.

Thus, SD has given ample evidence of slow processes in the protein hydration layer and in the layers of self-assemblies. The 20−40 ps component seems to be due to the bound ⇄ free dynamic equilibrium.[120,24] SD finds other slow processes, which could be due to the protein side chain motion or due to the motion of the probe itself, as discussed later.

The success in the study of SD in bulk water motivated many studies on aqueous complex systems.[158−171] In addition to the complexity present due to heterogeneity, the study of SD in micelles, reverse micelles, and microemulsions gets further complicated due to probe diffusion. A probe molecule can undergo an excursion over a region of radius of a few nanometers within its excited state lifetime of several nanoseconds. Thus, a fluorescent probe actually reports the property of a microenvironment of radius of a few nanometers (nm)[168−170] and not of a specific site. An additional complexity in the case of micelles is that there are three possible locations of the probe, namely, the bulk water, the "dry" micellar core, and the stern layer.

In an early study in this area, Vajda et al.[171] found that SD of the coumarin probe within cyclodextrin cavity slowed substantially in the long time. This slow was attributed to the quenching of the translational motion of the water molecules inside the cavity. A conclusive understanding may require detailed computer simulation, but it is clear that dynamics in a restricted environment can slow dramatically as compared to that in the bulk. This area has been a subject of intense research.

SD in micelles has been studied using Coumarin 480 (C480) and 4-aminophthalimide (4-AP) as probes.[172,173] Emission properties of the probes in the micelles are very different from those in water and in hydrocarbon, indicating that the probes reside neither in the bulk water nor in the core of the micelles and, hence, are located in the Stern layer of the micelles. Sarkar et al.[172] and Datta et al.[173] studied SD of C480 and 4-AP, respectively, in neutral (TX-100), cationic (CTAB), and anionic (SDS) micelles. It was observed that for SDS, CTAB, and TX-100 the average solvation times were, respectively, 180, 474, and 1456 ps for C480 and 82, 273, and 716 ps for 4-AP. Thus, the long time part of SD in the Stern layer of micelles was found to be 2 orders of magnitude slower than that in the bulk water. For both of the probes, it was observed that the solvation times followed the order TX > CTAB > SDS. Qualitatively, the difference in the solvation times in the three micelles may be ascribed to the difference in their structures[174,175]—the thickness of the hydrated shell for TX-100 (25 Å) is higher than that for SDS and CTAB (6−9 Å). The small angle neutron scattering[175−177] studies indicate that CTAB micelles are drier than SDS micelles. It is interesting to note that the time scale of solvation is similar to the intermediate range of DR times reported by Telgmann and Kaatze[178] who observed several relaxation times in the long (ms), intermediate (10 ns), and fast (0.1−0.3 ns) time scales by using ultrasonic absorption in the 100 kHz to 2 GHz frequency range. The longest relaxation time was attributed to the exchange of a surfactant monomer between the micelle core and the bulk while the fastest to the rotation of the alkyl chains of the surfactants in the core of the micelle. The intermediate relaxation time was not assigned to any particular motion. Note that for TX-100, water molecules can penetrate into deep inside the micelle because they form single or double HBs with the oxygen of the ethoxy group. Such water molecules can give rise to significant slow solvation.

Reverse micelles and microemulsions have a water pool in the core and hydrocarbon chains outside. Reverse micelles are characterized by the radius of the water pool $r_w$ and the molar ratio $w_0$, which is the water to surfactant mole ratio. Levinger et al.[179] studied the SD of a charged dye, C343, in lecithin and AOT microemulsions using femtosecond up-

conversion.[179−182] For lecthin microemulsions, the solvent relaxation displays a very long component, which does not become complete within 477 ps. This observation is similar to the nanosecond dynamics reported by Bright et al.[183] and Sarkar et al.[184] For Na-AOT, the SD reported by Levinger et al. for the charged probe C343 is faster than that reported by Bright co-workers[183] and Sarkar et al. Shirota and Horie[185] also demonstrated that in the AOT microemulsions the SD of acetonitrile and methanol were nonexponential and 1000 times slower as compared to those in the pure solvents. They attributed the nonexponential decay to the inherent inhomogeneous nature of the solvent pools.

The emission spectrum of the probe changes markedly when it is transferred from bulk hydrocarbon to the water pool. The absorption maxima of C480 in *n*-heptane and water are at 360 and 395 nm, respectively, while the emission maxima are at 410 and 490 nm, respectively.[186] The addition of AOT and subsequently water to an *n*-heptane solution of C-480 results in a very prominent shoulder at 480 nm,[184] which can, therefore, be easily assigned to the C480 molecules in the water pool of the microemulsion. Sarkar et al.[184] studied the SD of C480 in AOT/*n*-heptane/water microemulsions. They observed a distinct rise in the nanosecond time scale at the red end of the emission spectra. They observed that in a small water pool ($w_0 = 4$, $r_w = 8$ Å) the solvation time was 8 ns while for a very large water pool ($w_0 = 32$, $r_w = 64$ Å) the response was bimodal with a fast component of 1.7 ns and a slower component of 12 ns. Obviously, these studies missed all of the ultrafast solvation, which occurs in the picosecond (or faster) time scale. Bright and co-workers[183] studied the SD of acrylodan-labeled human serum albumin in AOT microemulsion by phase fluorimetry.[183] They reported that the solvation time was about 8 ns in a small water pool ($w_0 = 2$) and 2 ns in a large water pool ($w_0 = 8$). To explore the effects of ions, Mandal et al.[187] studied the SD of 4-AP in a microemulsion containing neutral surfactant Triton X-100 where no ions were present in the water pool. The neutral Triton X-100 microemulsion also exhibited nanosecond SD.

SD studies have also been carried out in lipid vesicles, which resemble biological cells. They contain an aqueous volume enclosed within a membrane and dispersed in bulk water. Red edge excitation spectroscopy[188−190] has been used to study the state of solvation of a fluorescent probe in the ground state in the unilamellar and multilamellar vesicles. Datta et al.[173] observed that the SD of C480 in DMPC vesicles was nonexponential with two components of time constants 0.6 (40%) and 11 (60%) ns. It should be noted that these studies missed almost the entire portion of the ultrafast, subpicosecond solvation component. So, these percentages describe only the slow decay.

SD in these complex systems is further complicated by the restriction imposed by surfactant−chromophore interactions. In the case of micelles and monolayers, one can study only surfactants that can be located near the surface by special attraction to the PHGs and, therefore, prevented from being fully solvated by water. However, in the case of reverse micelles, any chromophore that is water soluble can be confined and studied.[191]

It is, therefore, important to note that the slow dynamics in orientational relaxation has been observed by other methods as well. Of special interest is the early study by Wong et al.[139] who found, by using $^1$H NMR spectroscopy, that in the small water pools of the heptane−AOT−water reverse micelle system, water rotational diffusion slowed by as much as 2 orders of magnitude.

## 4. Computer Simulation Studies

Because of the molecular length scale heterogeneity, it is difficult to develop any simple analytical description of the structure and dynamics of interfacial water. MD simulations have proved to be a valuable tool in this area. Because the dynamical behavior manifested is rich and diverse, a large number of computer simulation studies have been carried out. In the following, I first summarize some of the results obtained by MD simulations of protein/micelle hydration layers. Bizzarri and Cannistraro[192] have recently presented a nice review of many aspects of hydration dynamics recently. My discussion will, therefore, concentrate primarily on HB lifetime, vibrational dynamics, and also rotational and DRs. However, I shall mention related studies in order to obtain as complete a picture as possible.

### 4.1. Protein Hydration Layer

#### 4.1.1. Rotational and Translational Motions and Residence Time Distribution

The simulation study of the hydration of protein ribonuclease A by Gu and Schoenborn[193] showed that at room temperature and at high hydration, significant translational and rotational motions continue to occur in the layer. Both translational and rotational diffusion coefficients of water molecules in the layer are expected to be correlated with the residence time because the former is a direct measure of the rigidity of the layer. The residence time of water molecules in the hydration layer of myoglobin was found to have a distribution between somewhat less than 30 ps and more than 80 ps; the latter was also the longest run time of the simulation.[193] The water molecules with much longer residence times were those that were either buried inside protein cavities or in the clefts or had multiple interactions with the protein and had higher (than average) binding energies. Water molecules with long residence time exhibit slow orientational relaxations. The binding energy distribution had values ranging from 0.5 to 9 kcal/mol. Gu and Shoenborn[193] found a strong peak in the radial distribution function for hydrogen bonding between protein surface and water molecules. The trajectory of individual water molecules clearly showed two entirely different behaviors—one for the bound state and the other for the moving (free) state. Rapid exchange between the two states was observed suggesting the existence of a dynamic

equilibrium between the two states. In a study of the dynamics of protein hydration layer, Rocchi et al.[194] calculated a layer survival correlation time, which was allowed to decay when a water molecule left or entered the layer. This correlation function was found to decay slowly for the nearest layer. Rocchi et. al.[194] also calculated the average (over the water molecules in the layer) orientational time correlation function and found it to be markedly nonexponential. The orientational time correlation function could be fitted to a stretched exponential with the value of the exponent significantly less than unity. In addition, the average translational motion was found to be subdiffusive. Marchi et al.[195] also found that the rotational relaxation of water molecules in the vicinity of simulated lysozyme was 3−7 times slower than that in the bulk, depending on how the hydration shell was defined. The slow molecules were found to be those water molecules, which had longer residence time near the lysozyme.

Marchi et. al.[195] also reported observation of subdiffusive translational motion of water molecules in the hydration shell. Because the water molecules studied were initially constrained to be in the hydration shell surrounding the lysozyme, one of course expects that the water molecules would exhibit slower initial displacements than those in the bulk. However, the degree of slowing down is hard to predict a priori. Unfortunately, this work did not report the energy distribution of the water molecules in the shell. A simple interpretation of the subdiffusive behavior has been presented in terms of a theoretical model that employs a double-well potential near the surface[39]—the subdiffusive motion originates from recrossing of the tagged molecules into the bound state. Thus, the slowing down in diffusion is not just due to the constraint of being initially in the hydration layer but also due to interconversion between the two states.

#### 4.1.2. HB Lifetime Dynamics

The study of HB dynamics has proven to be a very useful tool to understand the origin of many fascinating features of water dynamics arising from the extended HB network. The formation and breaking of protein−water HB play an important role in determining the functionality of the protein. In MD simulations, one can use either energetic or geometric criteria to define a HB. Recently, Bandyopadhyay et. al.[50] studied the protein−water HB lifetime dynamics using classical atomistic MD simulations. Their study has shown that the structural relaxation of the protein−water HB is much slower than that of the water−water HB. They have correlated this protein−water HB dynamics with the biological activity of the protein. Stillinger et al.[196] and also Berne and coworkers showed that water HB dynamics could slow appreciably, as much as 20%, near a hydrophobic surface.[197]

#### 4.1.3. SD

Recently, Bandyopadhyay et. al.[198] have reported simulation studies of the SD of the polar amino acid residues in each of the three helical segments of the protein HP-36 using classical atomistic MD simulations. They found that the presence of a slow component in the SD, which is an order of magnitude slower than that of bulk. In this work, the authors have established the correlation between the exposure of polar probe residues and the SD of different secondary structures of a protein molecule. Pettitt and co-workers[199,200] presented an elegant physical picture of solvation and hydration of proteins and nucleic acids based on extensive computer simulations and theoretical calculations.

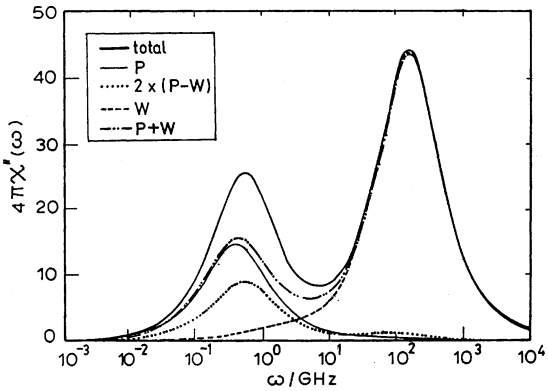


**Figure 10.** Simulated frequency-dependent dielectric loss $4\pi\chi''(\omega)$ [same as $\epsilon''(\omega)$] of the components of the 0.0093 M aqueous ubiquitin solution: protein−protein (P) and water−water (W) self-terms, as well as two times the protein−water cross-term (P − W) $4\pi\chi''\omega$ of the solution as a whole (total) and the sum of the protein and water self-term, i.e., the overall spectrum minus the protein−water cross-term is given (P + W). Reprinted with permission from ref 125. Copyright 2000 American Chemical Society.

#### 4.1.4. DR

Boresch, Höechtl, and Steinhauser[125] calculated the frequency-dependent dielectric properties of ubiquitin solution by a long MD simulation. They observed a significant dielectric increment for the static dielectric constant at low frequencies but a decrement at high frequencies (which is of course expected). When the overall dielectric response was decomposed into protein−protein, water−water, and water−protein cross-terms, the most important contribution was found to arise from the self-term of water. The simulation beautifully captured the bimodal shape of the dielectric response function as often observed in experiments. This is shown in Figure 10, where the relative contributions of the pure and cross-terms, as found by Boresch et al.,[125] are also indicated. We have earlier discussed that the DR of aqueous solutions of proteins shows an anomalous dispersion at frequencies intermediate between those corresponding to rotational motion of bulk water and that of the macromolecule.[125,201−203]

#### 4.1.5. Vibrational Dynamics

A direct probe of the intermolecular interactions between the water molecules and the surface atoms of the substrate is provided by the shift and line width of the vibrational modes of water at the surface. Sometimes, this shift can be very large and sometimes even new intermolecular modes can appear, thereby throwing new insight into the nature

and strengths of interactions. Low frequency Raman and neutron scattering experiments on aqueous protein solutions have shown the presence of excess density of states, called the boson peak, at around 3 meV (24 $cm^{-1}$),[204] which has been corroborated by MD simulations.[205] MD simulations of Cannistraro et al.[205] found signatures of a low frequency collective mode at 1.3 meV, which has been attributed to a hydration water. This boson peak was found at low temperatures (180 K) and was manifested as a broad inelastic peak. This boson peak is found to be rather general and has also been observed in experiments on hydrated proteins. Joti et al.[206] very recently studied the origin of protein boson peak from the energy landscape. They found that the presence of structured water molecules around a protein molecule increased the number of local minima in the protein energy landscape. The peak was found to appear when protein dynamics got trapped in one of the minima. Recently, an elegant analysis of the boson peak in disordered solids was presented by Das.[234] In this mode coupling theory analysis, the boson peak was shown to arise from the coupling between slow density fluctuations and the transverse sound modes. A similar analysis of the protein vibrational modes coupled to slow solvent relaxation is needed to understand the origin of the observed boson peak and the protein−glass transition.[235,236]

## 4.2. Micelles

As already mentioned, computer simulation studies have been an area of intense research activity in the recent past. Detailed simulations have addressed many different aspects of water dynamics and revealed a wealth of information.[38,40−42,201,207−209] Several investigations,[38,41,210] notably that by Watanabe et al.,[210] have been carried out to classify the interfacial water molecules into different categories. The obvious classification is on the basis of the number of HBs that water molecules form with the protein/micelle. On the basis of simulations,[38,41] interfacial water molecules can be categorized into bound (hydrogen bonded) or free. The bound water molecules can sometimes be further classified as single or double bonded (denoted by IBW1 or IBW2), depending on the number of HBs that they form with the PHG of the micelle. Interfacial water molecules, which do not form HB with the PHG micelle/protein, are denoted by interfacial free water (IFW). MD simulations have shown that for the CSPFO−$H_2O$ system, the ratio of IFW:IBW1:IBW2 is 1:8:1.[41]

### 4.2.1. Rotational and Translational Dynamics

Klein and co-workers[210−212] and Pal et al.[207] reported slowing down in water orientational relaxation in the surface of a micelle. Balasubramanian et al.[208,209] carried out fully atomistic MD simulations of a micelle consisting of CsPFO surfactant molecules in water. The CsPFO micelle was stable over a wide temperature range for the duration of the simulation (5 ns). Dramatic slowing down of water dynamics was observed in all of the cases.[207,208] In Figure 11, the orientational correlation function of the interfacial water is shown and compared with the same for bulk

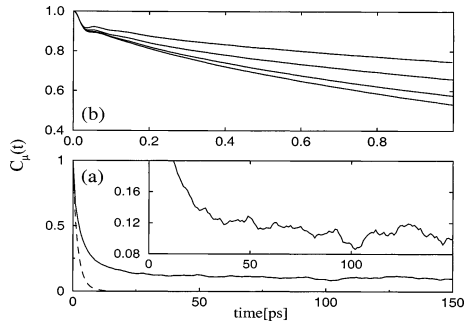


**Figure 11.** Dipolar time correlation function ($C_\mu$) for water molecules at 350 K. Only those molecules that are located within (near) or beyond (far) the specified distance for the period of time mentioned have been considered for this calculation. (a) Continuous line, molecules within 10 Å; dashed line, molecules beyond 25 Å. These were obtained by averaging over five independent trajectories each of length 330 ps. Inset: Near-constancy of the time correlation function for water molecules within 10 Å from the micellar surface in expanded scale. (b) Short time decay of the same function for water molecules in various regions for trajectories of length 2.4 ps. Top to bottom: near 4.5 Å, near 6 Å, near 10 Å, and far 28 Å. Reprinted with permission from ref 208b. Copyright 2002 American Chemical Society.

water. Note the dramatic slow decay in the long time. In the top-half of the same figure, the time dependence of the dipolar correlation function at short times is shown at several distances. The decay becomes faster as the water molecules are located at larger distances from the interface. Similar results have been observed by Bruce et al.[38] in their atomistic MD simulations of water dynamics in aqueous SDS micelle. In the latter work, water orientation was found to exhibit a slow component in the long time, with time constants in the range of 100 ps or above. In their simulation of aqueous CsPFO, Pal et al.[207] found that translational diffusion of water molecules at the micellar surface also slows down by about 20%. Thus, the translational motion was found to be less affected than the rotational motion. This is due to the fact that the average mean square displacement was dominated by the fast moving "free" water molecules in the layer, while the long time slow decay of orientational relaxation was dominated by the "bound" water molecules.

One can use the transition rates between the bound and the free water molecules and the fraction of each to construct a free energy surface for the water species in the hydration layer.[41] Such an analysis shows that the free energy surface is determined by both the entropy and the enthalpy of these molecules. If the entropy of the water molecules can be computed separately, then such an analysis can provide further insight into the nature of water−micelle and water−protein interactions.

### 4.2.2. HB Lifetime Dynamics

Balasubramanian et al.[40] have studied water dynamics at the surface of an anionic micelle of CsPFO using atomistic MD simulations. Their study has shown that the HB between the PHG of the micelle and water molecule has a much longer lifetime—13 times larger than that between two tagged water molecules. The HB lifetime correlation functions

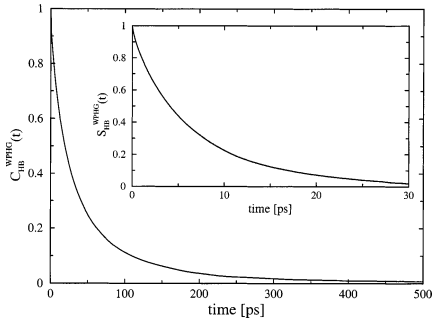


**Figure 12.** HB lifetime correlation function, $C_{HB}^{WPHG}(t)$, between the PHG and the water molecule. The inset shows the same for $S_{HB}^{WPHG}(t)$ function. Reprinted with permission from ref 40. Copyright 2002 American Physical Society.

$C_{HB}^{WPHG}(t)$ and $S_{HB}^{WPHG}(t)$ are shown in Figure 12. This figure is to be compared with Figure 2 where the same functions are shown for bulk water. This result indicates the presence of quasi-bound water molecules on the surface. The lifetime of the latter also slows down by about 25% near the surface. The HB between PHG and water molecules is much stronger than that between any two water molecules.[40]

Berkowitz and co-workers[38] have recently employed MD simulations to study the structure and dynamics of a sodium dodecyl sulfate micelle in water. Their detailed simulations have added to the knowledge of this system obtained from earlier simulations of Klein and co-workers and that of MacKerell.[213] Here also, the water molecules at the interface form HBs with the headgroups of the surfactant, apart from forming HBs with other water molecules. About 60% of the interfacial water molecules are singly hydrogen bonded with the micelle, that is, belong to IBW1 while 33% form two such HBs. A small fraction of the molecules do not form any HB with the micelle.

### 4.2.3. SD

In their simulations of SD in water inside a spherical cavity, Senapati and Chandra[214] observed a marked slowing down in the SD in confined water. Recently, Pal et. al. have studied the SD of aqueous micellar solution of decyltrimethylammonium bromide (DeTAB).[215] They found that the SD of bromide ions exhibits a slow component, which was about 2 orders of magnitude slower than that in bulk. Balasubramanian et. al.[208] studied the SD of Cs ion near the CsPFO micellar surface. The SD of Cs ion too showed similar slow dynamics. The slowest component was found to be 2−3 orders of magnitude slower than that in bulk.[208] In Figure 13, the solvation time correlation function of $Cs^+$ ion at the CsPFO micellar surface is shown. For comparison, the same correlation function computed in bulk water is shown in the inset. Note the large difference in the time scale of the decay. However, here, the slow decay was largely due to the contributions from the PHGs of the micelle and not from slow water dynamics.[208]

### 4.2.4. DR

In an interesting simulation, Senapati and Chandra[214] studied dielectric properties of water inside a

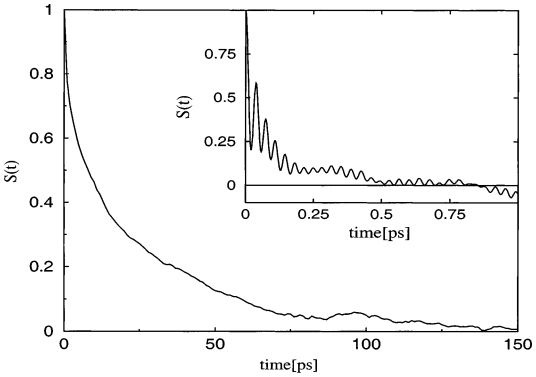


**Figure 13.** Solvation time correlation function, $S(t)$, of cesium ion near the surface (within 10 Å from the surface) of the CsPFO micelle at 350 K. The inset shows the same for bulk water. Reprinted with permission from ref 208a. Copyright 2001 American Chemical Society.

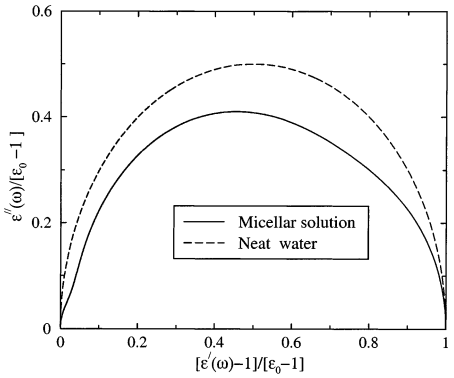


**Figure 14.** Cole−Cole plot of the frequency-dependent dielectric function, $\epsilon(\omega)$, where the imaginary part is plotted against the real part, for water molecules, both in aqueous micellar solution of the CsPFO micelle (solid line) and in neat water (dashed line). Reprinted with permission from ref 201. Copyright 2004 American Institute of Physics.

cavity and showed that the dielectric constant of confined water could be significantly smaller than that of bulk, extended water. They further found that orientational relaxation of water molecules slowed appreciably inside the cavity. This slowness was found to arise mainly from the hydrogen bonding of water molecules to the PHGs of the micelle. Recently, Pal et. al.[201] studied the DR of surface water molecules of the CsPFO micelle. Their study detected a 40 ps component of in the DR spectra.[201] A decay with similar time constant appears to be a universal component observed in a large number of systems. The Cole−Cole plot of their study is shown in Figure 14, which shows a considerable degree of nonexponentiality in the DR spectrum.[201]

### 4.2.5. Vibrational Dynamics

An aqueous macromolecular interface also affects the intermediate frequency modes, namely, the O···O stretching and the librational modes of water that are peaked around 200 and 650 $cm^{-1}$, respectively, in bulk water. Analysis of these modes has been carried out recently at the surface of a micelle.[42] The results have indicated strong effects of the micellar surface. In Figure 15, we present the power spectrum for the different kinds of interfacial water molecules. A blue shift in the O···O···O bending mode of about

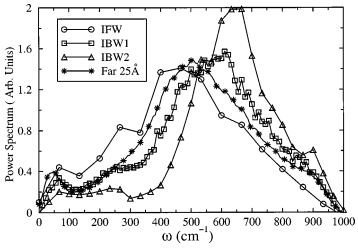


**Figure 15.** Power spectrum for various interfacial water molecule types as compared with that for water molecules that are at least 25 Å away from the micellar surface. The latter behave like water in bulk. Reprinted with permission from ref 42. Copyright 2003 American Physical Society.

40 cm$^{-1}$ has been observed. The librational mode of the water molecule undergoes a blue shift by 100 cm$^{-1}$.[42] These data agree well with recent incoherent, inelastic neutron scattering experiments on aqueous DNA.[216] Lee and Rossky[217] have found a similar behavior for water molecules present near a hydrophobic surface. This indicates that the IFW species might be somewhat buried within the micelle in proximity to the CF2 groups adjacent to the PHG. Experimental studies of vibrational line shape and vibrational relaxation of water molecules at a micellar surface will be greatly welcome.

In addition to the extensive work on protein and micellar hydration layers, several studies[218−225] have investigated water dynamics inside the water pool of reverse micelles. However, a detailed review of this area is beyond the scope of this review, and I just mention, for the sake of completion, a few relevant and interesting studies. Faeder and Ladanyi investigated SD in the water pool of aqueous reverse micelles.[218,219] Their simulations focused on the short time dynamics of solvation, in the range of 1−2 ps. They found that 70% of the solvation remained ultrafast even for moderately sized water pool, although the translational motion of water molecules was found to be significantly reduced. These authors also studied[220] the effects of counterion type ($Na^+$ and $K^+$) on the properties of the interior region of aqueous reverse micelles. Berkowitz and co-workers carried out MD simulations to investigate the structural properties of polyether and perfluoropolyether surfactant-based reverse micelles in supercritical carbon dioxide.[221−223] They calculated the different micellar properties and found that they were consistent with the available experimental data. Cummings and coworkers[224] studied the MD simulations of reverse micelle in supercritical carbon dioxide. They studied how varying the surfactant molecular architecture and chemistry modified the structure and properties of reverse micelles in supercritical carbon dioxide. Klein and co-workers[225] carried out MD simulations to study the microscopic properties of a reverse micelle of the poly(oxyethylene) surfactant $C_{12}E_2$ in nonpolar environments (decane and vacuum). Their simulations revealed that the core water molecules and the oxyethylene headgroups of the surfactants behaved similarly in decane and vacuum simulations. Linse[226] carried out MD simulations to study the aqueous core of a reversed ionic micelle. They found that the water structure was strongly distorted by the strong local field and that the hydrophobic interface played a secondary role.

## 5. Phenomenological Models and Simple Theories

In sharp contrast to the large number of experimental and computer simulation studies reported in the literature, there has been very few purely analytical or model-dependent studies on the dynamics of hydration layer around self-assemblies. Some of the early theoretical studies were carried out by Halle[228] who introduced a simple reaction−diffusion model to understand NMR results. In this model, the bound state was assumed to be close to the protein surface and the free state away from it. Thus, the transition from bound to free was dependent on the location of the reaction surface and the distance from the surface was essentially the reaction coordinate. Thus, one can represent the dynamics of the transition by a reaction−diffusion reaction. This model is ideal to treat the NOE results, which are sensitive to the distance between the protein proton and the water proton.

A statistical mechanical approach to study biomolecular hydration has been initiated by Hummer et al.[229] In this approach, the water near a surface was represented by a local density. This density was then expressed in terms of two- and three-particle correlation functions. A theoretical calculation of this space-dependent process has been carried out by Mukherjee and Bagchi.[39] These authors have numerically solved the reaction−diffusion model to obtain the probability distribution and the time-dependent mean-square displacement. The latter was found to be nonlinear. Interestingly, the model has predicted a transition from a subdiffusive to a superdiffusive behavior, before finally becoming diffusive in the long time.

A somewhat different model was proposed by Nandi and Bagchi.[36] This model was initially proposed to explain anomalous DR of aqueous protein solutions. This model is based on the existence of a dynamic equilibrium between the bound and the free water molecules in the surface of biomolecules or self-assembly. At the center of this model lies the assumption that the water molecules at the surface of proteins can be considered as distinct species because of their strong hydrogen bonding to the biomolecular surface. This equilibrium can be symbolically written as[36,230]

$$\text{bound water} \rightleftarrows \text{free water}$$

Bound water is not a unique species because (i) it is transient and (ii) there is a distribution of the energies of binding of water molecules to the protein surface. Using the dynamic exchange model, an expression for this slow relaxation has been derived. The starting point is a coupled reaction−diffusion equation,[36] which can be solved to obtain the two rate constants, $k_{\pm}$, for dipolar orientational correlation function. These rates are given by[36]

$$k_{\pm} = 0.5[-B \pm \sqrt{B^2 - 4D_R k_{fb}}] \qquad (12)$$

with $B = 2D_R + k_{bf} + k_{fb}$, where $D_R$ is the rotational diffusion coefficient of the free water molecules, $k_{bf}$ is the rate of free to bound transition, and $k_{fb}$ is the rate constant of the reverse process. Typically, the rate constant of free to bound reaction, $k_{bf}$, is larger than that for the reverse process, $k_{fb}$. In the limit when the rate of conversion from bound to free becomes very small, the above expression further simplifies and the two rate constants are given by $2D_R$ and $k_{fb}$. Thus, while one time constant remains fast, of the order of 3−5 ps, the other can slow appreciably, even to the extent of hundreds of picoseconds. The rate constant $k_{fb}$ is of course determined by the binding energy. For the majority of sites, the time constant may range between 10 and 100 ps or so because the binding energies are often small. However, for a few (rare) molecules, the second time constant can be quite long. When the rates of interconversion between "bound" and "free" water molecules are small as compared to $2D_R$, the dynamic exchange model predicts that the two relaxation times can be approximated by two limiting time constants given by[36]

$$\tau_{fast} \approx \tau_s^{bulk} \quad (13)$$

$$\tau_{slow} \approx {k_{bf}}^{-1} \quad (14)$$

In the same limit of large activation energies separating the bound state from the free one, the residence time of the bound water molecules is given essentially by $k_{fb}^{-1}$. This also gives the residence time ($\tau_{res}$) of strongly bound water, that is, $\tau_{slow} \simeq \tau_{res}$. This is an interesting result that shows that the long time component of polar SD is equal to the residence time of the water molecules.

The high activation energy ($E_{act} \gtrsim 5k_BT$) that is required for the validity of eqs 13 and 14 is usually not common, and there may be only a few percentages (about 10%) of water molecules on protein surfaces strictly obeying the condition.[193,227] However, on a charged micellar surface, it is found that a larger percentage (about 20−30%) of water molecules may satisfy the criteria.

The above dynamic exchange model applies mainly to rotational relaxation of interfacial water molecules. As discussed above, computer simulations have repeatedly shown the existence of subdiffusive translational motion of the interfacial water molecules. Recently, a numerical calculation of the translational diffusion of water molecules near a biological interface was carried out by modeling the interaction between the surface and the water molecules by a double well potential where one side of the double well is bounded by a steep increasing potential to mimic the impenetrability.[39] The numerical calculations predict that the initial subdiffusive motion of the bound water molecules could be followed by a superdiffusive motion, before going over to the expected diffusive motion in the long time. This prediction is yet to be verified either by experiments or by computer simulations.

In addition to the work of Halle and that of Nandi and Bagchi, Grigolini and Maestro presented a model of dynamics of constrained water.[230] This model considered dynamics in reverse micelles where slow relaxation arose from crossing (by diffusion) of the hydrophobic layer by the water molecules. This paper could provide a nice and simple explanation of the experimental results that water rotational correlation time increases by nearly 2 orders of magnitude in the heptane−AOT−water reverse micellar system.[139,172]

Clearly, these models are all phenomenological and do not address the microscopic dynamics in the hydration layer. For example, these models cannot address the reduced rate of rotation of the protein, that is, cannot provide an answer for the enhanced friction.

An important ingredient of any microscopic theory should be coupling to the motion of the surface. In addition, the rigidity of the surface is determined by the distribution of the binding energy. However, the rigidity itself should be determined by the bound $\rightleftarrows$ free transition rates. Thus, one requires a self-consistent mode coupling theory to treat many of these effects.[231]

Application of mode coupling theory to describe dynamics of hydration layer is hard because the system is heterogeneous on a molecular length scale (thus, the usual advantage of using the wavenumber-dependent description is not available anymore). A preliminary effort toward such a theory has been made recently.[7,232] The obvious slow dynamical variable in such a theory is the density of water in the hydration layer. This density should have the two components—bound and free. An attempt has been made to derive a microscopic expression for the enhanced dielectric friction on the protein's rotation due to the presence of slow fluctuations in the hydration layer. An important aspect of the work is the recognition that the slow translational diffusion of water molecules in the hydration layer can give rise to large friction.[8,9]

### 6. Water Dynamics near a Hydrophobic Surface

Much of the discussion in the previous sections has concentrated on hydrophilic surfaces, particularly for micelles and reverse micelles where the hydrophilic groups are on the surface to stabilize the aggregation. In proteins, however, a large number of hydrophobic groups are found on the surface, although the majority of the hydrophobic groups form the core. The hydrophobic groups and pockets play important roles in the ligand binding activity and also in the association of proteins. Dynamics of water near a hydrophobic surface can be a lot different from that near a hydrophilic surface as there is no possibility of HB formation with any group on the hydrophobic surface. However, a hydrophobic surface can modify the water dynamics significantly because it can induce a structure in the surrounding layer. In a nice study of HB kinetics in the solvation shell of 16-residue polypeptide, Xu and Berne[197] showed that HBs among water molecules became stronger in the solvation shell of nonpolar groups. The polypeptide studied was the chain of the last 16 residues in the C terminus of the immunoglobin binding protein G (PDB ID 2gbl). The residues Trp43, Tyr45, Phe52, and Val54 formed an

extended flat hydrophobic surface exposed to the solvent. It was found that HB between two water molecules both of which were in the solvation shell of the hydrophobic groups was stronger than that in the bulk by 0.25 kcal $mol^{-1}$, which was the reason for the slow bond breaking of these HBs. In addition to the energy effects, there should be an entropic contribution to the slow down because a hydrophobic surface would lower the entropy of the surrounding water by imposing an order.[237]

DR measurements of cationic surfactants, *n*-methylammonium bromide ($C_n$TAB, $n = 8, 12, 16$), found a slow relaxation around 25 ps, which was attributed to the water molecules within the micelle and in the vicinity of the hydrophobic tail of monomeric octyltrimethylammonium ions.[62] Similar slow relaxation was observed by Kaatze et al.[178] for an aqueous solution of dodecyltrimethylammonium chloride and by Buchner et al.[62] in an aqueous solution of tetrabutylammonium bromide. These authors assigned this dispersion (between 16 and 30 ps) to water molecules surrounding the hydrophobic particles.

Luzar has investigated water HB dynamics close to hydrophobic groups and also noted the slow down[238] in the HB making/breaking kinetics. Luzar has interpreted the results in terms of a time-dependent strain function $n(t)$.

Thus, water dynamics is predicted to be slow both near hydrophobic and near hydrophilic surfaces but more slow near hydrophilic ones due to the possibility of HB with the surfaces. Because of the inherent heterogeneity of a protein surface where polarity varies from residue to residue, it is very difficult experimentally to isolate and identify the slowness due to hydrophobic groups alone. However, there have been studies of self-diffusion of probe dyes in porous Vycor glass in various solutions.[239] One finds that the diffusion of the probe slows down by almost 2 orders of magnitude.[239]

### 7. Origin of the Experimentally Observed Ultraslow (1–10 Ns) Component

As discussed in the earlier sections, several experimental studies have detected an ultraslow (ranging from a few hundred picoseconds to few tens of nanoseconds) component in the SD of an external probe, both in proteins[24] and in self-assembly.[4,172] The origin of this slow component initially posed a big puzzle but is now relatively better understood, although by no means solved.

Theoretical and computer simulation studies of HB lifetime dynamics[40,44,46,238] show that the usually fast HB breaking/reformation process in bulk water can slow at the surface of proteins and micelles. This may be due to a combination of the rigidity of the water network at the surface, coupled with the strong HBs between the PHGs at the surface and the water molecules. Here, two somewhat opposing factors arise. While the strength makes the bond breaking process slow, the rigidity of the interfacial water often forces reformation of the bond in short time. Computer simulation studies[40] show that for the CsPFO micelle, the two HB lifetime correlation functions, $S_{HB}^{WPHG}(t)$ and $C_{HB}^{WPHG}(t)$, differ largely—the latter shows a slow component in the range of 100 ps with an amplitude of 20%—the time value could be even longer if a longer time resolution is used.

However, the experimentally observed ultraslow solvation component does not appear to be coupled to the dynamics of such slow water because their contribution to total solvation energy relaxation is found to be rather small. This view is further substantiated by the fact that such a slow component is totally absent in protein hydration dynamics when a natural probe located near the surface is used.[24] In such cases, the slowest time observed is just about 20−40 ps.[24] The ultraslow component has also not been observed in the computer simulation studies of SD in micelles and reverse micelles. In the latter cases, the slowest component is again less than 100 ps. The orientational relaxation exhibits slower relaxation, as remarked earlier.

One can think of at least three mechanisms that could give rise to decay in the nanosecond time scale. First, the solute probe may itself diffuse after photoexcitation. The free energy for probe redistribution comes from the creation of the large dipole moment in the probe. Second, the bound $\rightleftarrows$ free water equilibrium may still be relevant but now the bound water may be trapped inside the hydrocarbon core. This is possible in the TX-100 neutral micelle, which has a thick hydration shell. Such water will be slow to orient in response to the external field as they would need to overcome packing and would need to break HBs. Third, for SD, a significant contribution to the total solvation energy can come from the macromolecule/self-assembly itself. SD at the surface of CsPFO is dominated by such a contribution.[208] For proteins, side chain motions can occur on a time scale comparable to the ones observed in the solvation experiments.[24] Clearly, further study is required to explore these possibilities. One thing, however, seems clear that the experimentally observed ultraslow component[4,172] may not be due to slow dynamics in the hydration layer.

### 8. Future Problems

In this review, I have attempted to survey the current status of theoretical, experimental, and computer simulation studies of water dynamics at surfaces of natural systems, both chemical and biological. These studies clearly demonstrate that the dynamics of water molecules present in the vicinity of these complex systems are significantly different from those of bulk water. The most remarkable effect is observed in the slowing down in the long time decay of the orientational correlation function of these water molecules. This slow down has been observed in SD of the probe as well. However, one fails to notice a significant signature in the average translational diffusion, which slows down by only 25% or so. The explanation for this disparity is simple—translational diffusion is dominated by the mean square displacement of the fast moving ones. At any time, about 20% of the water molecules in the surface layer may be bound. In fact, this implies that

free water molecules are not that much influenced by the surface. Another signature is the significant blue shift in the frequency of the librational mode and the O−O−O bending mode of the surface molecules. This implies enhanced structure in the surface layer. A recent advancement in the study of water dynamics in complex systems has given rise to several interesting problems for future investigation. A list with a brief discussion on each of the suggested problems is given below.

### 8.1. Vibrational Relaxation of the Water Molecules at the Interface

I discussed earlier that the vibrational frequencies of both intra- and intermolecular bonds of water molecules undergo a noticeable blue shift in the presence of the charged groups of a protein and a micelle. Therefore, these modes can be selectively probed by using femtosecond spectroscopy, as has recently been done for neat water.[54,89] Such studies can provide useful information on both HB lifetime dynamics and protein−water (or micelle−water) interactions.

Vibrational dephasing measures the loss of coherence in a collection of oscillators coupled to a solvent bath. In this phenomenon, both the time scale and the magnitude of the force fluctuations are important. Valuable information about the SD can be extracted from a vibrational dephasing analysis of Raman line shapes if a sensitive Raman probe is used. In recent years, vibrational dephasing has also been investigated directly in the time domain using ultrafast laser spectroscopies such as Raman echo or Raman free induction decay. We are not aware of any such study on vibrational dynamics at a protein surface.

If one can hazard a guess, the vibrational phase relaxation of the O−H stretch is expected to pick up a component, which could be even smaller (that is, smaller than the measured 90 fs component in neat water) due to the enhanced interaction of the interfacial water molecules with the micellar or polar charged/polar groups. However, the slowness of dynamics at the hydration layer can also be observed as a slow long time decay component. Both phase and energy relaxations of the interfacial water molecules can reveal a wealth of information.

### 8.2. Rotational and Translational Motion of Proteins and Micelles

So far, we have concentrated mostly on the dynamics of interfacial water molecules. Needless to say, the presence of slow and structured water molecules at the surface of these systems can exert profound influence on the motion of these molecules themselves. Thus, the rotational relaxation time of myoglobin lengthens by about three times as compared to the value predicted from DSE expression of rotational friction by using the bare crystallographic size and shape of the protein.[24,34] Similarly, the translational motion of a protein also slows down, although not as drastically as its rotational motion. In addition, the slow structured water molecules at the surface of these systems can profoundly affect the internal dynamics, such as vibration or the motion of the amino acid side chains. As mentioned before, one often makes the ad hoc assumption that a protein is surrounded by a thin rigid hydration layer, which increases its size, so that hydrodynamic estimate can be used to explain the experimental results. It has been shown recently that the friction on a rotating protein can be decomposed into a sum of a hydrodynamic friction and a dielectric friction.[33] When an estimate of each of the two is made using standard methods, it has been found that the total friction indeed tracks the experimental value rather well, except for the case of myoglobin, for reasons that are not known presently. Thus, although a beginning has been made toward understanding this problem,[33] a lot remains to be done.

### 8.3. Protein Folding and Protein Association: Role of Biological Water

The dynamics of water around an extended, unfolded protein certainly play a very important role in determining the rate of protein folding. For example, hydrophobic collapse involves movement of water molecules away from the region between two hydrophobic amino acid residues that form pair contact. Similarly, $\beta$ bends also involve water mediation.[233] In both of the examples, the water molecules in close proximity to the protein amino acids are expected to play critical role. This role will involve a subtle balance between enthalpic and entropic forces.

Water molecules in the protein hydration layer have a finite residence time. This residence time has a distribution, depending on the nature of the neighboring protein surface, and this distribution can play a critical role in protein association. The final act of association of two proteins may require partial desolvation around the necessary amino acid residue sites.[240] This is only possible if the residence time of water around these sites is sufficiently short. The residence time is determined by the dynamics in the hydration layer. This correlation between hydration layer and protein association is also an important problem that deserves further study.

### 8.4. Protein–Glass Transition at 200 K: Role of Water Dynamics

Neutron scattering and computer simulation studies[47,236] have shown that all proteins undergo a glass transition around 200 K. Experiments and simulations[141−143] show that below this temperature, the dynamical behavior of proteins changes from anharmonic to harmonic. It has been anticipated that below this temperature, proteins form a glassy state. Note that for most proteins, the enzymatic activity ceases below 220 K.[241,242] It has been argued that water dynamics may hold the key to the understanding of this unusual behavior of proteins. Note that water itself is believed to have a glass transition around 135 K.[243,244] It has also been suggested that water also has an additional transition at a temperature below of 228 K.[243,245,246] Below this temperature, water behaves like a strong liquid while above this

temperature it behaves like a fragile liquid.[245,246] The proximity of this liquid−liquid transition to the protein−glass transition temperature is suggestive. Clearly, at temperatures below 220 K or so, the dynamics of water and protein are highly coupled. A recent computer simulation study has shown that structural relaxation of protein requires relaxation of the water HB network and translational displacement of interfacial water molecules.[50,247] It is, therefore, clear that dynamics of water at the interface can play a very important role. This is an interesting problem that deserves further investigation.

### 8.5. Water-Mediated Molecular Recognition

The recognition of binding sites (the "sticky" regions) of proteins by ligands, inhibitors, substrates, and other proteins is expected to control, to some extent, the biological activity of proteins.[32,248] As a ligand approaches a protein, an important step toward binding may occur in a short time (in a few tens of picoseconds) when the interfacial water molecules will determine the rate, even the outcome of binding. A microscopic theory of molecular recognition would need to discuss the free energy barrier (or rather, the free energy landscape) at the surface that will be experienced by the incoming ligand. Experiments by Zewail and co-workers[249] have already given an indication of the need of such a molecular level description. In their study of molecular recognition by a protein mimic, the cobalt picket fence porphyrin, Zewail et al.[249] found the need to assume an energy landscape, which involved two barriers. The first step was the "absorption" of $O_2$ in the hydration layer of the protein, which was followed by the subsequent binding. This may be a common mechanism in many other cases. A more microscopic treatment of such phenomena will require inclusion of hydration dynamics at the interface.

### 8.6. Water Dynamics at the Surface of a DNA

This is certainly a problem of great topical interest,[199,250] although a great deal of further studies are required to understand water dynamics in the major and minor grooves of DNA. In particular, systematic studies in terms of relevant microscopic time correlation functions (such as the HB lifetime correlation) are needed.

The above list of problems is by no means exhaustive, but it is hoped that it gives a glimpse of many interesting (and challenging, although often very difficult) problems that remain to be understood in the general area of hydration dynamics in complex aqueous systems. This field will surely remain an active area of research in the future, and one can look forward to many exciting new results.

## 9. Conclusion

In the Introduction, we presented a list of issues and questions to be discussed in this review. We conclude by summarizing the answers (many tentative) hopefully provided in this review.

(i) Relaxation in the hydration layer is in general nonexponential. Thus, the fitting of the orientational time correlation function of water needs 2−3 time constants, in contrast to the situation in the bulk.[208] The time constants are considerably separated so that an average value of the time constant may not be meaningful. In the surface of charged micelles, a slow relaxation component of the order of 100 ps appears;[208] such a slow time scale is not significantly present in the protein hydration layer.[50] In the case of micelles, the slow orientation in the long time is due to the water molecules strongly bound to the PHGs.[40−42] Free energy calculations show that about 80−90% of the water molecules at the micellar surface can be classified as bound.[40−42] This percentage is much less at a protein surface.

(ii) The single particle properties, like translational diffusion and orientational relaxation, are quite different in the layer from that in the bulk. The translational motion exhibits a subdiffusive behavior while orientational relaxation, as discussed above, is nonexponential. Both are found to slow down relative to the bulk.

(iii) Number density fluctuation in the layer slows down significantly, signaling increased rigidity of the layer.[215] However, the layer is still dynamically mobile and not icelike.

(iv) The HB lifetime between the polar/charged groups of the protein or surfactant and the surface water molecule is found to be substantially longer than that between two water molecules in the bulk. The HB lifetime correlation function becomes nonexponential. The average HB lifetime is typically 5−10 times longer in the hydration layer than in the bulk.[40]

(v) The ultraslow time scales observed in some experiments[158] might not arise from surface water molecules at all but rather from motion of the probe itself or from protein side chain motion or even from buried water. This is evident from the time scales of water motion and HB lifetime dynamics.

(vi) Protein site specific dynamical studies have just begun.[155,198] Initial studies suggest that water dynamics is sensitive to its immediate environment. While this is expected, a quantitative measure of site specificity has just begun to emerge. A recent computer simulation study by Bandyopadhyay et al.[198] finds evidence of a correlation between water dynamics and biological activity.

(vii) As discussed in the list of future problems presented in the previous section, the hydration layer is expected to participate in many biological and natural processes, ranging from protein folding to molecular recognition and catalysis. This area is certain to see major development in future.

Finally, it appears that all of the different experimental approaches are now converging to provide a unified view of water dynamics on protein and micellar surfaces, although much work is left to be done. This is expected to be an exciting area of future research.

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