In this chapter we present results of two-dimensional infrared (2D IR) spectroscopy experiments on the OH-stretch vibrational mode of water molecules near hydrophobic solutes. The 2D spectroscopic results are compared with molecular dynamics (MD) simulations to obtain a microscopic picture of hydrophobic solvation. Our experiments demonstrate that the hydrophobic groups induce a dramatic slowing down of the hydrogen-bond dynamics of the solvating water molecules. This change in dynamics perfectly correlates with dramatic decrease of the orientational and translational mobility of the water molecules. We conclude that the molecular jumping mechanism that is responsible for the reorientation in bulk liquid water is switched off in the hydrophobic hydration shell.

The current chapter is based on the following publications:


4.1. Introduction

Ever since Langmuir suggested that hydrophobicity is an important factor for folding proteins into compact globular structures, physical and protein chemists have been actively engaged in experiments to get a grasp of the hydrophobic force. A particular appealing model for hydrophobic hydration was provided by Frank and Evans, who suggested that the large entropy loss on dissolution of a small apolar molecule in water, is due to the formation of an ordered water structure around the solute, coined by them as “iceberg”. This evocative iceberg-model has, since its launch in 1945, inspired many researchers to explore hydration dynamics in small molecules and proteins, both experimentally and by molecular dynamics simulation studies.

It is difficult to obtain dynamical information about the hydrophobic solvation by traditional structural methods. Neutron diffraction, for instance, cannot discriminate between structures fluctuating in time, and (quasi-) static structures with a distribution of conformations, because this technique inherently integrates observables in time. NMR experiments have shown that the rotational diffusion of water molecules in a hydrophobic surrounding on average slows down as compared to neat water. However, NMR lacks the adequate temporal resolution to detect the rotational motions directly, and therefore the time-integrated correlation time constant of the whole solution can be determined. This average time constant can be translated into a rotation time of the hydration shell only assuming a certain size of this hydration shell and that the bulk is not affected.

Although ultrafast optical experiments can, in principle, selectively map out the dynamics of a strongly coupled water layer to a hydrophobic molecule by exploiting vibrational coupling effects, such experiments have not been reported yet. Rezus and Bakker, however, recently demonstrated that in concentrated water solutions of amphiphilic molecules carrying several hydrophobic groups, the observed bimodal decay of the rotational anisotropy (which is a measure for water rotations) of a water vibration can be exploited to distinguish the dynamics of water in the hydration layer from that of water in the bulk. In these experiments the rotational anisotropy partly decays with a time constant of 2.5 ps and then levels off for times longer than 10 ps. While the decay
time was independent of the solute concentration, the offset level scaled linearly with concentration at low solute concentrations, and saturated at high concentrations. This effect was attributed to “freezing” of the rotational water dynamics in the vicinity of the hydrophobic groups. The mechanism behind this immobilization was suggested to be the molecular jump mechanism proposed by Laage and Hynes. In this model, reorientational motions of water molecules (in bulk liquid phase) do not occur in Brownian-like small diffusional steps but in large (~60°), quick (~200 fs), but relatively seldom – once in 2-3 ps - angular jumps. This highly-concerted mechanism involves the approach of a fifth hydrogen-bonded partner of the rotating water molecule and the temporary formation of a bifurcated hydrogen bond. Reorientational freezing was thus explained as resulting from a steric effect: the hydrophobic solute prevents a fifth water molecule from approaching a tetrahedrally-coordinated water molecule.

This interpretation triggered some controversy because classical MD simulations as well as a temperature-dependent NMR study of water in hydrophobic environments predicted no signature of water being significantly immobilized in the solvation shell. In particular, according to the MD simulations, the hydrophobic groups only take away a part of the solid angle in which a water rotational jump could have occurred (“excluded volume” picture), which leads to a rather moderate slowdown of the reorientation (only by 50%). Therefore, it was concluded that hydrophobic groups should only be weak water reorientation retardants.

It is important to realize that rotational anisotropy measurements are sensitive to the rate of switching rather than to individual switching event that takes 200 fs to occur. However, giving one order of difference between the two time constants, there could be many other processes competing with the molecular-jump switching (like librations and/or O-O bending), which can partially contribute to the loss of anisotropy. Therefore, the molecular-jump hypothesis calls for an independent verification by directly observing the switching event itself. A number of recent studies have demonstrated that the frequency of an OH/OD oscillator is a more sensitive probe to environmental fluctuations than the direction of its dipole moment. MD simulations predict that the frequency sweep during the switching event is ~100 cm⁻¹ and that the switching probability increases drastically at blue-shifted frequencies. The frequency sweep magnitude of 100
and switching time of $\sim 200$ fs require an adequate balance between temporal and frequency resolution. This is provided by femtosecond 2D IR correlation spectroscopy \cite{12, 13, 22, 23} as was demonstrated in pioneering 2D IR experiments on molecular switching in water by Tokmakoff and coworkers \cite{20}.

In this Chapter, we report on a 2D IR spectroscopic study of the OH stretching mode of water molecules in the vicinity of the hydrophobic groups of tetramethylurea (TMU) and trimethyl-amine-oxide (TMAO). We observed two different components in spectral dynamics: extremely fast ($<200$ fs) in the blue wing of the OH-absorption spectrum and considerably slower ($>1$ ps) in the central part of the spectrum. The fast dynamics, which are characteristic for the molecular-jump type of OH-bond switching, are active even at the lowest water concentrations. The slow ($>1$ ps) translational dynamics are strongly suppressed by the presence of the solute molecules. The magnitude of this suppression is in perfect correspondence with the rotational water immobilization data, previously measured in the transient anisotropy experiments \cite{14, 15}. We also present MD simulations that show a clear correlation between the slowed-down dynamics and translational water mobility. These simulations provide a molecular basis for the hypothesis of the switched-off molecular-jump reorientations near hydrophobic groups: the fifth water molecule, which is required to form a defect state, cannot approach the hydrogen-bonded pair to initiate the molecular jump. Consequently, the rate of the jumping events sharply decreases which leading to rotational immobilization of water.

4.2. Experimental

The light source for the 2D IR experiments was an optical parametric amplifier providing 70 fs pulses centered about the OH stretching mode of the HDO molecule at 3410 cm$^{-1}$. Data acquisition and processing were performed as described by Fayer and coworkers \cite{24}.

As a sample, we used a mixture of TMU or TMAO (Aldrich, 99% pure), heavy water (Aldrich, 99.9% pure), and isotopically-substituted water (HDO:D$_2$O). In all measurements the HDO concentration in D$_2$O was kept as low as 5% to avoid effects of intra- and intermolecular coupling of OH oscillators. For sake of simplicity the HDO:D$_2$O mixture will be referred to as water. Solutes were added
to water at concentrations 0-10 mol/kg and stirred for 1 minute at room temperature. In the experiments, the solution formed a thin ~40 μm film in a wire-guided jet. To quantify the solute concentration, we, following Ref. 15 introduce a parameter \( w \) that equals the ratio of number of solute and water molecules \( w \); \( w \) was ranging from 0 to 1. All experiments were carried out at ambient temperature.

To quantify heterogeneity in the spectral dynamics, we used an analysis based on the concept similar to hole-burning 25 (see also part 1.4.2.). For each excitation frequency \( \omega_1 \) a position of maximum \( \omega_{3\text{Max}} \) along the \( w_3 \)-axis is found. The diagonal position of the maxima (i.e. \( \omega_{3\text{Max}} = \omega_3 \)) is characteristic for the initial excitation. With an increase of the waiting time \( T \) the burned frequency hole broadens and migrates to the equilibrium position, i.e. \( \omega_{3\text{Max}} = \omega_3^0 \), where \( \omega_3^0 \) is the absorption maximum (fig. 1-9c). Therefore, the time evolution of the hole is described as:

\[
\omega_{3\text{Max}}(T) = \omega_0 - (\omega_0 - \omega_3) \cdot f(T, \omega_1)
\]  

(4-1)

with the boundary conditions \( f(T = 0, \omega_3) = 1 \) and \( f(T \rightarrow \infty, \omega_3) = 0 \). The frequency-dependent function \( f(T, \omega_1) \) defines how fast the hole, being initially burned at the frequency of \( \omega_1 \), is equilibrated. This function can be derived directly from Eq.4-1 by, for instance, calculating the slope value function as 26:

\[
\frac{\partial \omega_{3\text{Max}}(T)}{\partial \omega_1} = f(T, \omega_1)
\]  

(4-2)

Note that in the case of Gaussian (or homogeneous) dynamics, the slope value becomes frequency independent and exactly equal to the normalized frequency-frequency correlation function (ffcf) 27. We stress that in a more general case of non-Gaussian (or heterogeneous) dynamics the slope value function is not a ffcf and therefore should not be taken as such. Nonetheless, the dimensionless quantities of \( f(T, \omega_1) \) are quite suitable for direct comparison of hole dynamics at, for instance, different frequencies (i.e. among different sub-ensembles) and concentrations.

4.3. Experimental Results

Figure 4-1a shows the IR absorption spectra of the samples in the OH-stretch spectral region at different TMU concentrations. For concentrations \( w \leq 0.1 \) (i.e. less than 1 TMU molecule per 10 water molecules) the spectra are virtually
Figure 4-1. Absorption spectra of the water:TMU (a) and water:TMAO (b) mixtures in the OH-stretch region. Different curves correspond to the various water:solute molar ratios: w=0 (solid), w=0.1 (dashed), w=0.2 (dash-dotted), and w=1 (dash-dot-dotted). The insets present the TMU and TMAO molecular structures. The OD-stretch absorption lies out of the observation window.

indistinguishable from that of neat water. In the range 0.2≤w≤1 the spectrum shifts to the blue and slightly narrows. This implies that at TMU concentrations w≤0.2, the water hydrogen-bond network can accommodate the solute molecules without being notably perturbed. The blue shift of the spectrum and its narrowing with further increase of TMU concentration can be assigned to the formation of low-dimensional water structures in the voids between hydrophobic groups of solute.

These results are consistent with the previously reported concentration behavior of the OD absorption spectrum \(^15\) and in line with the results of earlier NMR studies \(^10\). Figure 4-1b shows linear absorption spectra of pure HDO:D\(_2\)O and of TMAO w=0.2 solutions. It is seen that the OH absorption spectra look quite similar with only a minor red shift. Probably, associated with slightly stronger hydrogen bonds in the solution or/and with the effect of ionic hydrophilic groups.

Figure 4-2 displays experimental 2D IR spectra (see part 1.4.2.) of the OH-stretch for a variety of waiting times and concentrations. In the following analysis, we will focus only on the bleaching of the fundamental transition (red contours). The 2D data can be qualitatively interpreted by comparing the antidiagonal cross-sections of the 2D peaks (homogeneous broadening) with the diagonal cross-sections (inhomogeneous broadening) \(^28,29\).
Figure 4-2. 2D absorption spectra of water-TMU solutions at different waiting times $T$ and concentrations $w$. All spectra are normalized to their maximal values. The red-colored contours represent bleaching of the ground state and stimulated emission from the first excited state. The blue-colored part depicts the induced excited-state absorption which is shifted by $\sim 190 \text{ cm}^{-1}$ along the $w_3$-axis due to the anharmonicity of the OH stretch vibration. The isocontours are drawn at 10% steps of the maximal amplitude. Thick black curves connect maxima at each $w_3$ cross-section.
At early waiting times, the antidiagonal cross-sections are quite narrow; for longer times they become considerably broader. This general trend is observed at all TMU concentrations and shows that environmental fluctuations destroy the memory for the frequency of the initial excitation (a process known as dephasing \( ^{25} \)). This process has been discussed in detail for HDO in D\(_2\)O \( ^{20} \) (our \( w=0 \) case) and for HDO in H\(_2\)O.\(^{23} \) In addition to this general trend, there is an important concentration-dependent effect: the higher the TMU concentration, the slower the dephasing. This directly shows that even moderate amounts of TMU considerably slow down the water dynamics.

A more careful inspection of the 2D spectra reveals the following phenomenon: dephasing defined as the anti-diagonal broadening of the 2D spectra is noticeably faster at the blue-shifted \( \omega_1 \) frequencies as compared to the red or central parts. The difference in elongation implies that the dynamics are frequency-dependent, i.e. heterogeneous. Tokmakoff and coworkers demonstrated that such heterogeneity is directly connected to hydrogen-bond switching that occurs in the blue spectral region, and which is accompanied by a large frequency sweep \(^{20} \).

**Figure 4-3.** The results of the slope analysis at different TMU concentrations and waiting times (see the legend). In the left and right panels the slope values are averaged over the frequency range \( w_1-w_0 = -25 \pm 25 \text{ cm}^{-1} \) and \( w_1-w_0 = 75 \pm 100 \text{ cm}^{-1} \), respectively, where \( w_0 \) is the central frequency of absorption (figure 4-1). The curves are guides to the eye.
The slope values at the central and blue part of the spectra are presented in figure 4-3. At the waiting time $T = 0$, the dynamics are only slightly concentration dependent, and on average 25% of the initial slope values have been lost (figure 4-3). This loss occurs because the early dynamics are so fast that the system evolves appreciably already within the delay between the excitation pulses. These dynamics were previously assigned to water librations with a typical time scale of 50 fs for bulk water.$^{18,23}$ There is no significant difference between the slope values at different concentrations, both at the central and blue side. Therefore, for the shortest waiting times, the dynamics behind the observed line shapes can be regarded as homogeneous and concentration independent.

At longer waiting times, the behavior at the center and blue side of the absorption line becomes different. At waiting times of 100 and 250 fs a notable dephasing in the blue part of the 2D spectra (figure 4-3) is observed, which is also concentration dependent. For neat water (i.e. $w=0$) this behavior was previously interpreted as a manifestation of the hydrogen bond switching mechanism.$^{20}$ The switching of weakly-bonded (and thus higher-frequency) oscillators to the strongly-bonded configuration is accompanied by a frequency sweep from the blue to the line center, which results in a remarkable decrease of the slope value.

The spectral dynamics at the center part of the spectrum (figure 4-3, left) are very different from those at the blue side. In neat water ($w=0$), molecules excited at the central frequency become almost equilibrated within ~1 ps. The difference between the dynamics in the central and blue spectrum wings arises because the hydrogen bonds are stronger at lower frequencies, which leads to suppression of the molecular-jump reorientation mechanism.$^{30}$ Only after some time the frequency of the OH oscillator drifts to the blue region due to spectral diffusion, at which point the molecular jump mechanism becomes effective.$^{30}$ However, with an increase of the TMU concentration, the equilibration dynamics slow down significantly. For instance, there is very little change in the slope values after 250 fs for $w \geq 0.2$. As a result, the central part dynamics, being similar at short delays ($T<250$ fs) for all concentrations, become quite dissimilar at longer times.

To study the effect of the solute on the molecular-jump reorientation mechanism, we analyzed the dynamics on the blue wing in more detail. By 100 fs, for neat water ($w=0$) ~60% and ~40% of water molecules at the blue side and center, respectively, have undergone a relaxation event (figure 4-3, right). If we assume
the difference to originate from the most probable switching event at the blue side, we arrive at the 20% probability in full accord with MD simulations. This also fully corroborates the previous experimental observations on the extreme swiftness of the switching events.

The switching probability decreases when the TMU concentration increases: for \( w=1 \), there are very little additional dynamics at \( T=100 \) fs as compared to 0 fs which implies that there has been a few switching events yet. The switching events become significant at a waiting time of ~250 fs (green curve), and are largely complete by 500 fs where the slope values become concentration independent. The 1 ps delay does not add much to the dynamics differences: slope values at all concentrations synchronously decrease.

Figure 4-4. 2D absorption spectra of water-TMAO solutions at different waiting times \( T \) and concentrations \( w \). The representation is performed in the same way as in the figure 4-2.
The fact that hydrogen bond switching is observed at all concentrations may suggest that switching also occurs in hydrophobic hydration shells. However, the blue wing of 2D spectra reflects the dynamics of the switching events for those waters that have already been prepared in configurations prone to switching. These molecules might reside in water nanopools formed in the voids formed by hydrophobic ends of the TMU molecules.\textsuperscript{10}

To verify the abovementioned hypothesis of solute clustering effect on the frequency dynamics at blue edge of the spectra, we also performed 2D IR experiments on the water with TMAO – a solute with much less prominent clustering effect (fig. 4-4). For TMAO, the dynamics in the center of the line are similar to those in the water:TMU mixtures. At the same time, dynamics on the blue wing steadily decelerate with the increasing solute concentration indicating

Figure 4-5. Cross-sections through the 2D spectra (Fig.4-4) for bulk water (a), TMAO (b) and TMU (c) solutions (both with $w=0.2$) at detuning of $\omega_0-\omega_d=100$ cm$^{-1}$ with respect to central frequency $\omega_0$. Only the positive part (i.e. due to ground-state bleach and excited-state stimulated emission) of the cross-sections is shown.
that in homogeneous concentrated solutions (where all the water molecules belong
to the solvation shells) molecular jumps are blocked.

More clearly it is shown in the Figure 4-5 which depicts the cross-sections through the 2D spectra at the frequency of $\omega_3-\omega_0=100$ cm$^{-1}$ for bulk water and TMU/TMAO solutions (both with $w=0.2$) at different waiting times. For bulk water (fig.4-5a), the spectra are broadened and shifted from the on-diagonal position within 0.1 ps $^{31}$ while for a waiting time of $T=0.25$ ps the spectra already peak at zero detuning. Also, the decrease of the on-diagonal amplitude (i.e. at the frequency of $\omega_3=\omega_1$) is clearly visible when the waiting times increases to 1 ps. These features are characteristic for the hydrogen bond switching mechanism $^{20,31}$.

The hydroxyl oscillators that have higher frequencies find themselves in the unstable configuration where the switching to a new water partner is very probable $^{21}$. This switching is accompanied by a frequency sweep from the close-to-diagonal position to the line center, which results in rapid change of the position of the peak absorption. In sharp contrast to neat water, the TMAO solution demonstrates a completely different behavior (fig.4-5b). After initial (within 0.1 ps) broadening, there are very little dynamics in terms of both spectral shift and broadening. This signifies a substantial slowing down of the blue-frequency dynamics that are directly associated with the molecular jump mechanism. Interestingly, the TMU dynamics (fig. 4-5c) are quite similar to the dynamics of bulk water described above. This means that in this solution the molecular jumping mechanism is still present for a significant part of the water molecules. This observation is consistent with the existence of water nanoclusters in the strongly heterogeneous solution of TMU in water.

To analyze more quantitatively the spectral dynamics on the blue wing for water TMAO solutions we followed a procedure similar to the one developed by Fayer and coworkers for the chemical exchange analysis $^{32}$. In Figure 4-6 we present the temporal evolution of the fraction $\eta$ of high-energy oscillators with respect to high- and average-energy oscillators for different TMAO concentrations after excitation in the blue wing ($\omega_3-\omega_0=100$ cm$^{-1}$). For that, we integrated the positive part of the response in the regions where weakly hydrogen-bonded water ($50<\omega_3-\omega_0<150$ cm$^{-1}$) and mostly strongly hydrogen-bonded water ($-50<\omega_3-\omega_0<50$ cm$^{-1}$) absorb.
Figure 4-6. Fraction $\eta$ of high-energy oscillators after excitation into the blue wing of OH stretch line of isotopically diluted water mixed with TMAO. See the text for the details. Lines are guides to the eye.

The parameter $\eta$ was calculated as a ratio between the first integral and sum of both integrals. Thus, $\eta$ represents the relative number of the OH oscillators that still maintain their initial excitation frequency in the blue region after a certain time and, therefore, have not undergone any switching event. For bulk HDO:D$_2$O, the parameter $\eta$ is small already at short time delays and keeps on decaying quickly with a time constant of $\sim 0.2$ ps. This decay can be attributed to the frequency sweep due to the molecular switching from weakly to strongly hydrogen-bonded configurations. Already after 0.5 ps $\eta$ reaches the offset of $\sim 0.43$ which is close to the value of $\sim 0.4$ calculated for the thermodynamic equilibrium at room temperature. With increasing TMAO concentration, the parameter $\eta$ shows an increase of its initial value and a decay to a higher final level. In addition, the decay appears to become slower. As a result, after 1 ps delay, the OH oscillators are still far out of spectral equilibrium. This indicates that the molecular jumps from weakly to strongly hydrogen-bonded configurations are strongly hindered.

It is interesting to note that the slow residual component in the $\eta$ decay is quite similar to the long component observed in the slope analysis in the center of the line (figure 4-3). That signifies that the mechanisms underlying frequency dynamics on the blue wing of the spectra and in the center of the line are identical, namely, slow diffusive-like rearrangements of hydrogen-bond network. At the
same time, fast large-amplitude switching events are absent as most of the water molecules reside in the solute hydration shell.

A key question which should be addressed is – what is the molecular mechanism responsible for retarding dynamics near the absorption line center. From previous studies on solvation dynamics in general and water dynamics in particular, it is known which underlying molecular motions govern each particular time interval. For instance, water librations account for a fast (<100 fs) initial decay \(^{18, 23}\) while oxygen-oxygen breathing and bending modes dominate in the 100-200 fs region \(^{18}\). After this time, translational diffusion of water molecules in and out the first solvation shell becomes important. \(^{33, 34}\) The correlation between the times of slowing down of the central part dynamics and the translational dynamics strongly suggests that the translational degrees of freedom become suppressed at higher solute concentration. To substantiate this conclusion and to make a link between spectroscopic observables and microscopic structures, we performed MD simulations on water-TMU mixtures.

4.4. Molecular Dynamics simulations

MD simulations were performed using the GROMACS package \(^{35}\). The SPC/E \(^{36}\) force field was used for water, and the TMU force field was taken from Belletato et al. \(^{37}\). All bond lengths were kept fixed with the LINCS algorithm, allowing the use of 2 fs integration time steps. A twin-range cutoff of 1.0/1.55 nm was used for the nonbonded/electrostatic interactions, respectively. For the long-range electrostatics the Reaction-Field method was employed\(^{35}\) with dielectric permittivity \(\varepsilon_r = 80\). The temperature and pressure were fixed at 298 K and 1 atm, respectively. After a 2 ns equilibrium run, the total simulation time in production phase spanned 2 ns for each concentration with molecular snapshots saved every 20 fs.

The resulting molecular structures of the water-TMU mixtures are illustrated in figure 4-7 for three representative concentrations. At low water concentration, a network of water molecules penetrates through the voids between the TMU molecules. The water molecules participate in hydrogen bonding\(^{38}\) both with other waters and with the carbonyl group of TMU molecules (figure 4-8a). The decreased number of hydrogen bonds is consistent with the blue shift of the absorption
spectrum at high TMU concentrations (fig. 4-1a). At higher water concentrations, 
the average number of hydrogen bonds to other waters per water molecule 
increases, and larger clusters of water are formed, but these are all interconnected. 
The most likely mechanism for such behavior is the disappearance of alternative 
hydrogen bonded neighbors for the water molecules. This leads to reduced 
flexibility for the water network to rearrangement, and “freezes” the solution in 
the existing hydrogen bond network.

To characterize the molecular translation mobility, the mean square 
displacements $\langle r^2 \rangle$ were calculated for water and TMU molecules separately. 
In all cases these were linear beyond the first picosecond, and the diffusion 
constants were extracted using the Einstein relation $D = 6t / \langle r^2 \rangle$. The water 
diffusion constant decreases quickly when small amounts of TMU are added, from 
the self-diffusion value of 0.23 Å²/ps, to 0.033 Å²/ps at $w = 1$ (figure 4-8b). At the 
same time, the TMU diffusion constant of ~0.03 Å²/ps is largely independent on 
the concentration for $w \leq 1$, and is considerably lower than the self-diffusion 
constant of ~0.13 Å²/ps of neat TMU. As a result, the water-TMU mixtures reveal 
the interpenetrated network of the hydrogen-bonded molecules that are mutually 
immobilized upon dilution with water. When equal amounts of water and TMU

---

**Figure 4-7.** The molecular structure of binary mixtures at three representative concentrations. Water is illustrated with dark (oxygen) and white (hydrogen) atoms. TMU is depicted as a gray transparent medium to expose the hydrogen bond network. Hydrogen are shown as dashed lines.
are present, the diffusion constant of water molecules is a factor of six smaller than in bulk water.

The slope values at the central part of the 2D spectra depicted as a function of concentration (figure 4-3) level off as the average number of hydrogen bonds per a water molecule reduces from ~3.5 to ~2 (figure 4-8a). Remarkably, a perfectly linear correlation is observed between the reduced translational mobility and slope values at the central part of the 2D spectra (figure 4-9). This corroborates the conclusion from the 2D data analysis that the suppressed translational diffusion (as opposed to the number of hydrogen bonds) is responsible for the slowed-down frequency dynamics.

**Figure 4-8.** The averaged number of hydrogen bonds (HB) per a water molecule (a) and the translational diffusion constants (b) as functions of concentration of the TMU-water binary mixture. Open and close symbols stand for water and TMU, respectively.

**Figure 4-9.** Linear correlation between the slope values in the central part of the 2D spectra ($T = 1$ ps) and diffusion constants as derived from MD simulations.
4.5. Discussion

The 2D experimental results demonstrate that water molecules in concentrated TMU solutions show strongly heterogeneous dynamics. Even at very high TMU concentrations a part of the water molecules still show switching behavior, albeit with an approximately two times longer time constant than in neat liquid water (figure 4-3b), while another part of the water molecules appears to be completely excluded from hydrogen-bond configurations where switching can occur. The picture that emerges is that we can crudely distinguish two classes of water molecules in concentrated TMU solutions. Water molecules that still show switching are water molecules that are surrounded by other water molecules and thus can form bifurcated hydrogen-bond structures required for switching. For the molecules that do not switch, the approach of a second hydrogen-bonded partner is severely hindered by the presence of TMU (“excluded volume”\(^{17}\)) and by low translational mobility of nearby water molecules. This inhomogeneous picture of the liquid agrees with the results from MD simulations: even at extremely high TMU concentrations there are nanoclusters with water molecules out of TMU solvation shell (figure 4-7), for which switching can occur. In the case of TMAO the picture is slightly different. Number of molecules which can potentially perform switching monotonically decreases as the solute is added and become almost zero at \(w=0.2\).

It is interesting to compare the present results to the recent measurements of the rotational anisotropy dynamics of aqueous solutions of TMU and TMAO. In those studies the reorientation dynamics of water was observed to show two distinct time constants. At low concentrations, one of these time constants was similar to the reorientation time constant of 2.5 ps of bulk water, the other time constant was \(>10\) ps. The fast reorienting water molecules likely correspond to the water molecules that show switching, while the slowly reorienting molecule correspond to the water molecules that we observe to be excluded from switching. To explore this comparison, we plot the central part slope values at \(T=1\) ps together with the end level (offset) of the anisotropy observed in Ref. \(^{14, 15}\) as a function of the solute concentration. The result is given in figure 4-10. Both for TMAO and TMU there exists an excellent correlation between the slope in the 2D spectrum and the final level of the anisotropy. For TMU exactly the same strong saturation effect is
observed for the spectral diffusion and the molecular reorientation, which unambiguously proves that the water molecules showing slow spectral diffusion and the water molecules showing a slow reorientation are the same.

Now the question arises how the slow spectral diffusion and slow reorientation are connected. The answer to this question is provided by the results of Figure 4-4. At a high concentration of TMAO (w=0.2) excitation in the blue wing of the OH stretch absorption band no longer leads to a strong broadening of the $\omega_3$ response with waiting time. This result shows that for water molecules in the solvation shell of TMAO, the molecular jumping mechanism is suppressed for most of the water molecules. Their hydrogen bonds do not evolve to the bifurcated hydrogen-bond state that is essential for the molecular jumping mechanism for the reorientation. Hence, for these water molecules the primary reorientation mechanism of bulk

![Figure 4-10](image)

**Figure 4-10.** Slope values ($d\omega_{3\text{max}}/d\omega_1$) at a waiting time $T$ of 1 ps as a function of $w$ denoting the concentration of TMAO (Fig. a) and TMU (Fig. b) in HDO:D$_2$O. The slope values (open, left axis) are compared with the slow component of the reorientation measured with polarization-resolved pump-probe spectroscopy (solid, right axis) at different concentrations. The dashes are guides to the eye and the dotted lines represent an extrapolation of the low-concentration data.
liquid water is switched off, and the reorientation will be slow. In contrast to TMAO, for a highly concentrated TMU solution (\(w>0.2\)), excitation in the blue wing still leads to a broad response in \(\omega_3\) (Fig. 4-2), meaning that for this solution the jumping mechanism is still present for part of the water molecules. This observation can be explained from the presence of water nanoclusters in highly concentrated TMU solutions. The water molecules in the nanoclusters show similar dynamics as in bulk liquid water, including the molecular jumping mechanism. For the solvated water molecules, the hydrogen-bond dynamics are slow and the molecular jumping mechanism is no longer active.

In recent MD simulations \(^{17}\) it was found that all water molecules in solutions of amphiphilic compounds undergo switching albeit somewhat slower than in the bulk due to the fact that part of the volume is excluded for the approach of a new hydrogen-bonded partner. At low concentrations of TMU this effect is expected to lead to a maximum slowing down of the switching and the reorientation by a factor of two only. This result clearly does not agree with the present findings that show a much stronger effect of hydrophobic molecules on the dynamics of the hydration shell. We thus conclude that the effect of hydrophobic groups is two-fold. These molecular groups do not only exclude part of the volume available for switching, but fundamentally change the hydrogen-bond dynamics of the solvating water molecules. This can be associated with the formation of a more “rigid” water-solute structure where the average number of hydrogen bonds is lower (fig. 4-8a) but the translational mobility decreases. This explanation is supported by the suppressed translational mobility of water (fig. 4-8b) and its excellent correlation to the hydrogen-bond dynamics (fig. 4-10).

NMR studies also indicate that water molecules in the solvation shells of hydrophobic molecules show different hydrogen-bond dynamics from neat liquid water. For instance, in temperature dependent NMR studies it was found that the activation energy for reorientation in the hydration shell was approximately two times higher than the activation energy of the reorientation in bulk liquid water \(^{11, 40}\). This shows that at low temperatures (a part of) the hydration shells form relatively rigid hydrogen-bonded structures which become much less rigid when the temperature is increased.

The strong effects of hydrophobic molecular groups on the hydrogen-bond and orientational dynamics do not find their counterpart in the structure of the liquid.
Neutron scattering studies show that hydrophobic solutes have surprising little effect on the oxygen-oxygen distances\textsuperscript{7, 8}. Apparently, water molecules can change their hydrogen-bond dynamics and lose their reorientation ability without strongly disrupting the local structure (i.e. the oxygen-oxygen distance). It should be noted, however, that the fraction of water molecules that are slowed down, is relatively small: only two water molecules per methyl group\textsuperscript{14} are affected. The effects of hydrophobic solutes on water dynamics are thus very short ranged, and it is to be expected that a change of the oxygen-oxygen distance would require a much longer range effect, involving many hydration shells.

4.6. Conclusion

In this Chapter, 2D IR spectroscopy and MD simulations have been used to study hydrophobic solvation. The utilization of 2D spectroscopy allowed us to combine a \(~70\) fs temporal resolution with high spectral selectivity. As a result, we succeeded in the separation of two different types of hydrogen-bond dynamics in water:TMU and water:TMAO mixtures. The first type, “fast” (\(~100\) fs dynamics, are connected to the molecular-jump water reorientation while the second type, “slow” dynamics, are associated with reduced translational mobility of water. According to our results, molecular-jump reorientations are still possible only for molecules in the specific five-coordinated configuration. However, the probability to reach such configuration (and, therefore, rate of the reorientation events) decreases dramatically with addition of the solute due to a substantial decrease in the water translational mobility near the hydrophobic groups.

When Frank and Evans coined the term “iceberg”, they referred to a microscopic region around a hydrophobic molecule, in which “water molecules are tied together in some sort of quasi-solid structure”\textsuperscript{2}. Later, the word “iceberg” often began to be taken literally: the hydrating water molecules were assumed to form an ice-like structure, that is, more tetrahedrally ordered than bulk water. This picture was not supported by theoreticians\textsuperscript{41} and experimentalists\textsuperscript{8, 11} alike who came to the conclusion that the hydrogen bonds in the hydration shell of nonpolar solutes are either more broken or do not differ much from those in bulk water\textsuperscript{11}. On the other hand, NMR\textsuperscript{9, 10} and time-resolved IR studies\textsuperscript{14, 15} indicated that the rotational mobility of water molecules in a hydrophobic solvation shell decreases,
bearing some reminiscence to iceberg formation. The 2D IR experiments and MD simulations presented in this Chapter seem to resolve this contradiction. The water molecules in the hydrophobic shell are not ice-like frozen as they have at least librational degrees of freedom, and some of them can even rotate. However, their translational mobility is severely compromised (due to lack of HB partner) and therefore defect configurations required for molecular switching, are lacking, in agreement with results of rotational anisotropy experiments. In this respect, our 2D spectroscopic inquest also provides a microscopic foundation for a previously published empirical model, which proposed that the hydrogen-bond network around hydrophobic groups is not more rigid as compared to the bulk, but that the hydrogen bond dynamics in the two cases are different. This proposition echoes a recent point regarding the question whether a hydrogen bond can be defined without considering its dynamics.

4.7. References

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38. The standard geometrical definition of the hydrogen bond was used: the O⋯O distance is shorter than 3.5 Å and the O⋯OH angle is smaller than 30°.


