

## Hydrophobic Solvation: A 2D IR Spectroscopic Inquest

ARTEM A. BAKULIN,<sup>†</sup> CHUNGWEN LIANG,<sup>†</sup>  
 THOMAS LA COUR JANSEN,<sup>†</sup> DOUWE A. WIERSMA,<sup>†</sup>  
 HUIB J. BAKKER,<sup>‡</sup> AND MAXIM S. PSHENICHNIKOV<sup>†,\*</sup>

<sup>†</sup>Zernike Institute for Advanced Materials, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands, <sup>‡</sup>FOM-Institute for Atomic and Molecular Physics, Kruislaan 407, 1098 SJ Amsterdam, The Netherlands

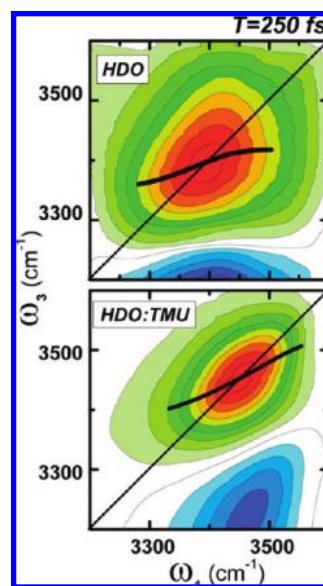
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### CON SPECTUS

For decades, the enigma of the hydrophobic force has captured the imagination of scientists. In particular, Frank and Evans' idea that the hydrophobic effect was mainly due to some kind of "iceberg" formation around a hydrophobic solute stimulated many experiments and molecular dynamics simulation studies. A better understanding of hydrophobic interactions will aid understanding in many contexts including protein structural dynamics and functioning in biological systems.

In this Account, we present results of two-dimensional infrared (2D IR) spectroscopy experiments on the OH-stretch vibrational mode of water molecules near hydrophobic groups in concentrated solutions with tetramethylurea (TMU). The frequency of the OH vibration is a sensitive probe for environmental dynamics and, in particular, for the strength of the hydrogen bond. Two-dimensional IR spectroscopy can trace time correlations of the vibrational frequency at the scale of hundreds of femtoseconds and thus provides valuable insight into the effect of hydrophobic solutes on the dynamics of a hydrogen-bond network. We compare the 2D spectroscopic results with molecular dynamics (MD) simulations to obtain a microscopic picture of hydrophobic solvation.

We observe two different types of hydrogen-bond dynamics in the water/TMU mixtures. We attribute the "fast" (~100 fs) dynamics to highly coordinated water molecular-jump reorientations and assigned the "slow" (>1 ps) dynamics to water translational motions that are strongly suppressed by the TMU molecules. Molecular dynamics simulations demonstrate a clear correlation between the slowed dynamics and the translational mobility of water. This finding indicates that the molecular-jump reorientations are switched off near hydrophobic groups. The fifth water molecule, which is required to form a defect state in the tetrahedral surroundings, cannot approach the hydrogen-bonded pair to initiate the molecular jump. As a result, the rate of the jumping events sharply decreases, which, in turn, strongly slows the rotation of the water molecules. Our findings suggest that water molecules in the hydrophobic solvation shell do not exhibit an increased tetrahedral ordering compared with the bulk but that the hydrogen-bond dynamics in the two cases are different. This result also indicates that consideration of a hydrogen bond's dynamics could be critical for its definition.



### Introduction

Ever since Langmuir suggested that hydrophobicity is an important factor for folding proteins into compact globular structures,<sup>1</sup> physical and protein chemists have been actively engaged in experiments to get a grasp of the hydrophobic

force. A particularly appealing model for hydrophobic hydration was provided by Frank and Evans,<sup>2</sup> 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".<sup>3</sup> This evocative iceberg model has, since its

launch in 1945, inspired many researchers to explore hydration dynamics in small molecules<sup>4</sup> and proteins,<sup>5</sup> both experimentally and by molecular dynamics simulation studies.<sup>6</sup>

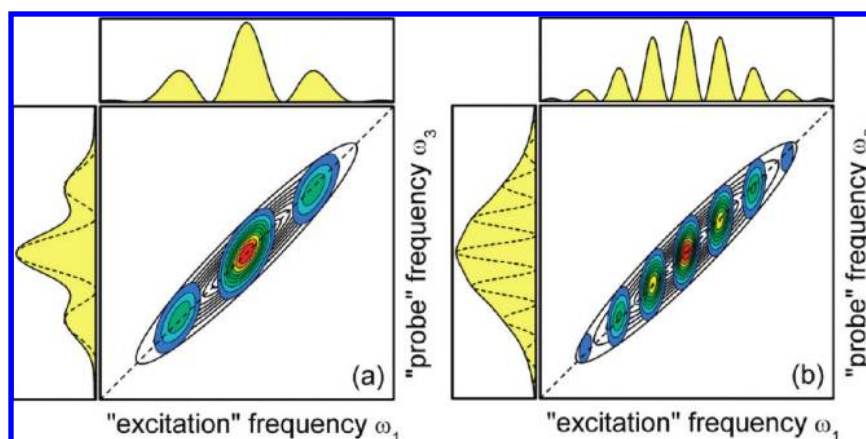
It is difficult, if not impossible, to obtain dynamical information about hydrophobic solvation by traditional structural methods. Neutron diffraction,<sup>7,8</sup> for instance, cannot discriminate between structures fluctuating in time and (quasi-)static structures with a distribution of conformations, 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 compared with neat water.<sup>9–11</sup> However, NMR lacks 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,<sup>12,13</sup> such experiments have not been reported yet. Rezus and Bakker, however, recently demonstrated that in concentrated 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.<sup>14,15</sup> 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.<sup>16</sup> In this model, reorientational motions of water molecules do not occur in Brownian-like small diffusional steps but in large ( $\sim 60^\circ$ ), quick ( $\sim 200$  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.<sup>14,15</sup>

This interpretation triggered some controversy because classical MD simulations,<sup>17</sup> as well as a temperature-dependent NMR study<sup>11</sup> of water in hydrophobic environments, predicted no signature of water being significantly immobilized in the solvation shell. In particular, according to the MD simulations,<sup>17</sup> 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<sup>16</sup> rather than to an individual switching event that takes 200 fs to occur. However, given one order of difference between the two time constants, there could be many other processes competing with the molecular-jump switching (like librations 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 of environmental fluctuations than the direction of its dipole moment.<sup>18,19</sup> MD simulations predict that the frequency sweep during the switching event is  $\sim 100$   $\text{cm}^{-1}$  and that the switching probability increases drastically at blue-shifted frequencies.<sup>20,21</sup> The frequency sweep magnitude of  $100$   $\text{cm}^{-1}$  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<sup>12,13,22,23</sup> as was demonstrated in pioneering 2D IR experiments on molecular switching in water by Tokmakoff and co-workers.<sup>20</sup>

In this Account, 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). We demonstrate that, regardless of the TMU concentration, there are two different kinds of 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 TMU molecules. The magnitude of this suppression is in perfect correspondence with the rotational water immobilization data, previously measured in the transient anisotropy experiments.<sup>14,15</sup> We also present MD simulations that show a clear



**FIGURE 1.** Two-dimensional correlation spectroscopy as a multiple hole-burning process for short (a) and long (b) delays between the excitation pulses. The upper panels show the photoexcitation spectrum, while the left panels depict the result of the spectral evolution of the burnt holes (color map in the central panels) due to spectral diffusion during the waiting time  $T$ . The dashed curves are given for direct comparison of written and read spectral holes. The contour lines show the normalized 2D spectrum resulting from multiple measurements for numerous excitation pulse separations.

correlation between the slowed 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 leads to rotational immobilization of water. Our findings strongly suggest that water molecules in a hydrophobic solvation shell do not possess an increased tetrahedral ordering compared with the bulk; rather their dynamics are different than those in the bulk water.

## Experimental Approach

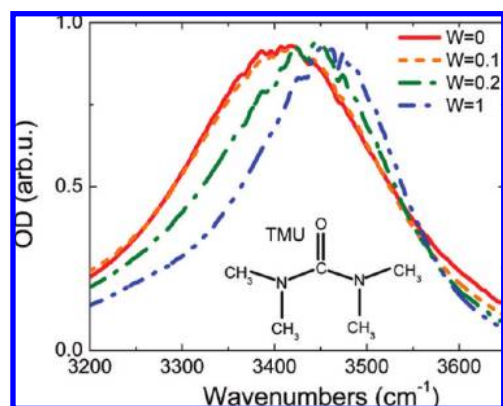
The essence of 2D spectroscopy can be best explained<sup>24</sup> in terms of a multiple pulse hole-burning experiment (Figure 1). In such an experiment two ultrashort pulses, separated by a certain time delay, generate a population grating (in frequency space)<sup>25</sup> in the absorption spectrum of the sample, in other words, burn multiple holes in the absorption line. These holes evolve in time and shift toward the equilibrium position because of spectral diffusion. As a result, the holes widen, while their central frequencies shift to the absorption center (color map in the central panels). Consequently, different holes begin to overlap spectrally, and therefore the resulting hole pattern read by the delayed "probe" pulse is not identical to the imprinted one (left panels). Increasing the time delay between the excitation pulses leads to a finer grating and to increased blurring of the holes. For a given separation between the excitation pulses, this fading also occurs for a longer probe delay (called the waiting time). In both cases, the

correlation between the initial and final frequency modulation is reduced (Figure 1a,b). Multiple hole-burning experiments, performed for all possible excitation pulse delays, result in a single 2D spectrum, which represents the correlation between the initial excitation frequency along the horizontal  $\omega_1$ -axis and the probe frequency along the vertical  $\omega_3$ -axis (contour lines in Figure 1). Such mapping balances the required temporal and frequency resolution in monitoring the spectral diffusion processes with the ultimate temporal resolution determined by the excitation probe pulse duration.

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  $3410\text{ cm}^{-1}$ . Data acquisition and processing were performed as described by Fayer and co-workers.<sup>26</sup> As a sample, we used a mixture of TMU (Aldrich, 99% pure), heavy water (Aldrich, 99.9% pure), and isotopically substituted water HDO, which formed a thin  $\sim 40\ \mu\text{m}$  film in a wire-guided jet. In all experiments, the HDO concentration in  $\text{D}_2\text{O}$  was kept as low as 5% to avoid effects of intra- and intermolecular coupling of OH oscillators. For sake of simplicity, the HDO/ $\text{D}_2\text{O}$  mixture will be referred to as water. To quantify the TMU concentration, we, following ref 15, introduce a parameter  $w$  that equals the ratio of the number of TMU and water molecules;  $w$  ranged from 0 to 1. All experiments were carried out at ambient temperature.

## Experimental Results

Figure 2 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 indistinguishable



**FIGURE 2.** Absorption spectra of the water/TMU mixtures in the OH-stretch region. Different curves correspond to the various water/TMU molar ratios:  $w = 0$  (—),  $w = 0.1$  (---),  $w = 0.2$  (-·-), and  $w = 1$  (···) with FWHMs of 240, 240, 210, and 180  $\text{cm}^{-1}$ , respectively. The inset presents the TMU molecular structure.

from that of neat water. In the range  $0.1 \leq w \leq 1$ , the spectrum shifts to the blue and slightly narrows. This implies that at TMU concentrations  $w \leq 0.2$ , 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<sup>15</sup> and in line with the results of earlier NMR studies.<sup>10</sup>

Figure 3 displays experimental 2D IR spectra 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).<sup>24,27</sup> 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<sup>28</sup>). This process has been discussed in detail for HDO in  $\text{D}_2\text{O}$ <sup>20</sup> (our  $w = 0$  case) and for HDO in  $\text{H}_2\text{O}$ .<sup>23</sup> 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 the water dynamics.

A more careful inspection of the 2D spectra reveals the following phenomenon: dephasing defined as the antidiagonal broadening of the 2D spectra is noticeably faster at the blue-

shifted  $\omega_1$  frequencies compared with the red or central parts. The difference in elongation implies that the dynamics are frequency-dependent, that is, heterogeneous. Tokmakoff and co-workers 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.<sup>20</sup>

To quantify heterogeneity in the spectral dynamics, we used an analysis based on the concept of hole-burning.<sup>28</sup> For each excitation ("hole-burning") frequency  $\omega_1$  a position of maximum  $\omega_3^{\text{max}}$  along the  $\omega_3$ -axis is found (black curves in Figure 3). The diagonal position of the maxima (i.e.,  $\omega_3^{\text{max}} = \omega_1$ ) 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, that is,  $\omega_3^{\text{max}} \equiv \omega_0$ , where  $\omega_0$  is the absorption maximum. Therefore, the time evolution of the hole can be described as

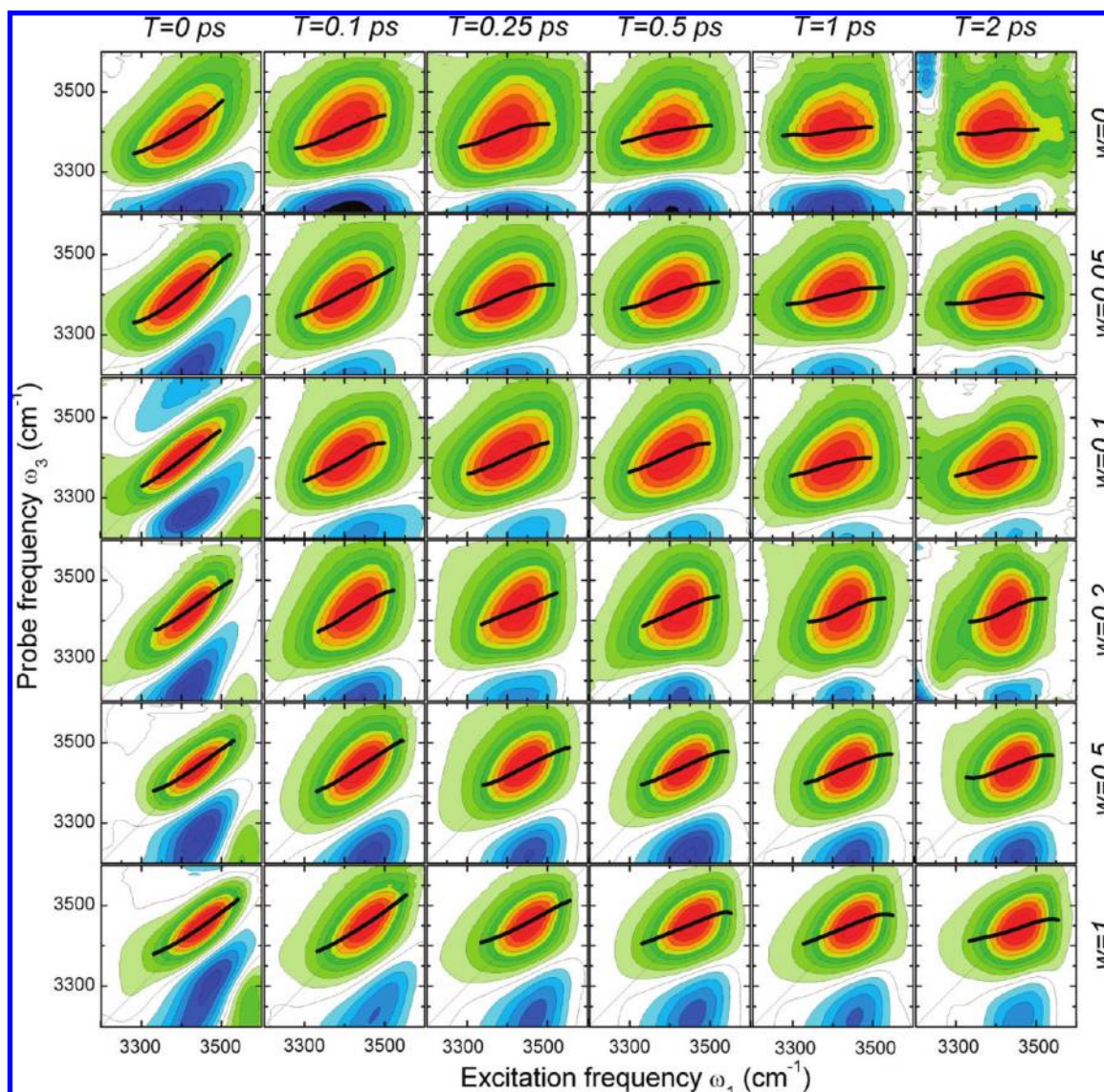
$$\omega_3^{\text{max}}(T) = \omega_0 - (\omega_0 - \omega_1)f(T, \omega_1) \quad (1)$$

with the boundary conditions  $f(T = 0, \omega_1) = 1$  and  $f(T \rightarrow \infty, \omega_1) = 0$ . 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 1 by, for instance, calculating the slope value function as<sup>29</sup>

$$\frac{\partial \omega_3^{\text{max}}(T)}{\partial \omega_1} = f(T, \omega_1) \quad (2)$$

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).<sup>30</sup> 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 subensembles) and concentrations.

The slope values at the central and blue part of the spectra are presented in Figure 4. At 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). 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.<sup>18,23</sup> There is no significant difference between the slope values at different concentrations, both at the central and blue side. Therefore, for the shortest waiting

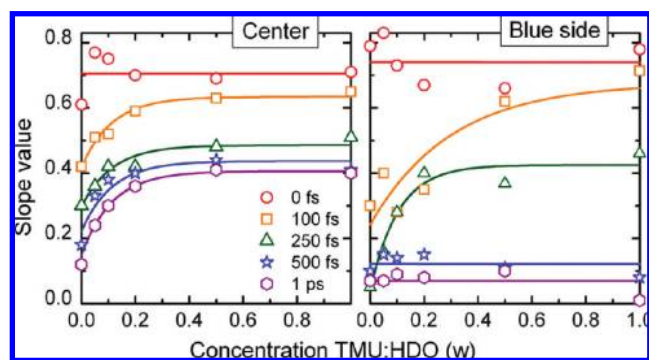


**FIGURE 3.** 2D absorption spectra of water–TMU solutions at different waiting times  $T$  and concentrations  $w$ . All spectra are normalized to their maximal values. The red-colored contours represent bleaching of the ground state and stimulated emission from the first excited state. The blue-colored part depicts the induced excited-state absorption, which is shifted by  $\sim 190\text{ cm}^{-1}$  along the  $\omega_3$ -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  $\omega_1$  cross section.

times, the dynamics behind the observed line shapes can be regarded as homogeneous and concentration independent.

At longer waiting times, the behaviors at the center and blue side of the absorption line become different. At waiting times of 100 and 250 fs, a notable dephasing in the blue part of the 2D spectra (Figure 4) 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.<sup>20</sup> 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 4a) are very different from those at the blue side. In neat water ( $w = 0$ ), molecules excited at the central frequency become almost equilibrated within  $\sim 1$  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.<sup>31</sup> 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.<sup>31</sup> However, with an increase of the TMU concentration, the equilibration dynamics slow significantly. For instance, there is very little change in the slope val-



**FIGURE 4.** 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  $\omega_1 - \omega_0 = -25 \pm 25 \text{ cm}^{-1}$  and  $\omega_1 - \omega_0 = 75 \pm 100 \text{ cm}^{-1}$ , respectively, where  $\omega_0$  is the central frequency of absorption (Figure 2). The curves are guides to the eye.

ues 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 ( $w = 0$ ),  $\sim 60\%$  and  $\sim 40\%$  of water molecules at the blue side and center, respectively, have undergone a relaxation event (Figure 4). 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.<sup>16</sup> This also fully corroborates the previous experimental observations<sup>20</sup> on the extreme swiftness of the switching events.

The switching probability decreases when the TMU concentration increases: for  $w = 1$ , there is very little additional dynamics at  $T = 100$  fs compared with 0 fs, which implies that there have been few switching events yet. The switching events become significant at a waiting time of  $\sim 250$  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 are similar.

The fact that hydrogen bond switching is observed at all concentrations suggests 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.

A key question is then how can water dynamics, which are just slightly retarded on the blue wing of absorption line, be substantially slowed near the absorption line center. From pre-

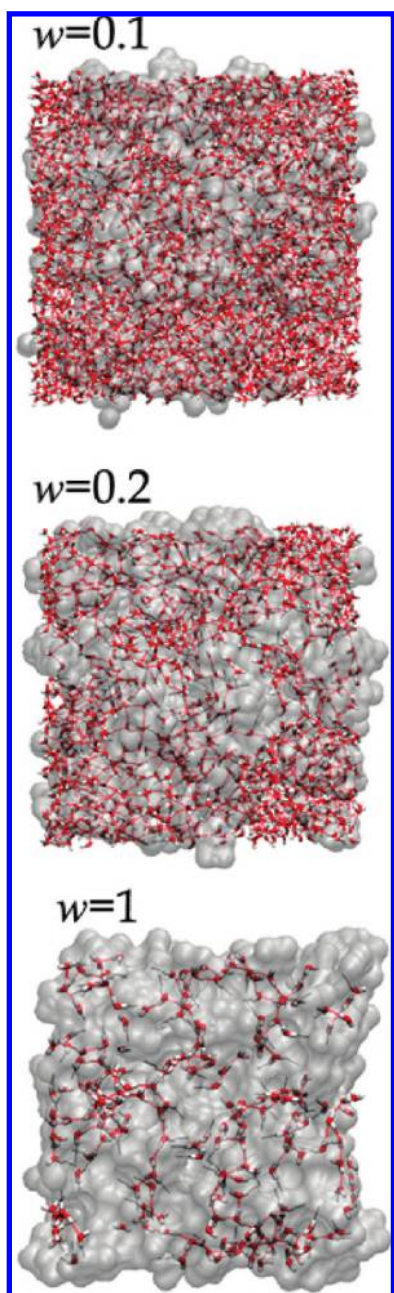
vious studies on solvation dynamics in general and water dynamics in particular, it is known which underlying molecular motions govern each particular time interval. For instance, water librations account for a fast ( $< 100$  fs) initial decay,<sup>18,23</sup> while oxygen–oxygen breathing and bending modes dominate in the 100–200 fs region.<sup>18</sup> After this time regime, translational diffusion of water molecules in and out the first solvation shell becomes important.<sup>32,33</sup> The correlation between the times of slowing of the central part dynamics and the translational dynamics strongly suggests that the translational degrees of freedom become suppressed at higher TMU concentration. To substantiate this conclusion and to make a link between spectroscopic observables and microscopic structures, we performed MD simulations on water–TMU mixtures.

## Molecular Dynamics Simulations

MD simulations were performed using the GROMACS package.<sup>34</sup> The SPC/E<sup>35</sup> force field was used for water, and the TMU force field was taken from Belletato et al.<sup>36</sup> 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 and 1.55 nm was used for the nonbonded and electrostatic interactions, respectively. For the long-range electrostatics, the reaction-field method was employed<sup>34</sup> with dielectric permittivity  $\epsilon_r = 80$ . 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 5 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<sup>37</sup> both with the carbonyl group of TMU molecules and with other waters (Figure 6a). The decreased number of hydrogen bonds is consistent with the blue shift of the absorption spectrum at high TMU concentrations (Figure 3). 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.<sup>38</sup> This leads to reduced flexibility of the water network for 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



**FIGURE 5.** The molecular structure of binary mixtures at three representative concentrations. Water is illustrated with red (oxygen) and white (hydrogen) atoms. TMU is depicted as a gray transparent medium to expose the hydrogen-bond network. Hydrogen bonds between water molecules are shown as red dashed lines, while hydrogen bonds between waters and the TMU carbonyl groups are shown as gray dashed lines.

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  $0.23 \text{ \AA}^2/\text{ps}$  to  $0.033 \text{ \AA}^2/\text{ps}$  at  $w = 1$  (Figure 6b). At the same time, the TMU diffusion constant of  $\sim 0.03 \text{ \AA}^2/\text{ps}$  is largely independent of

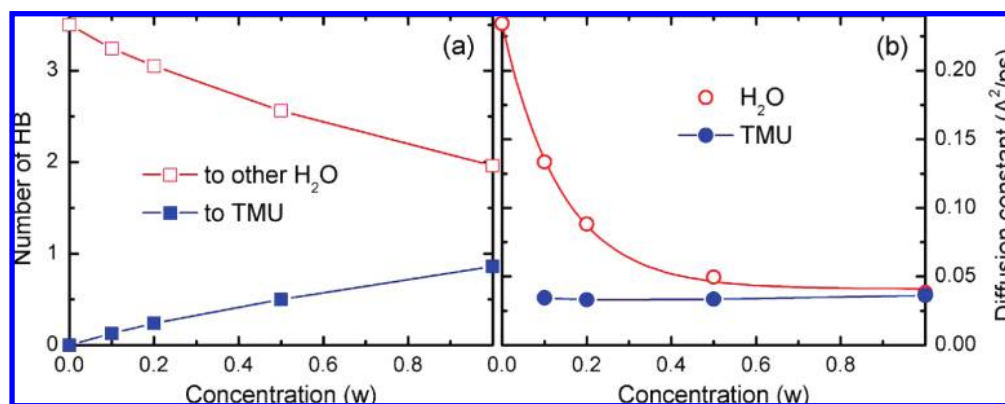
the concentration for  $w \leq 1$  and is considerably lower than the self-diffusion constant of  $\sim 0.13 \text{ \AA}^2/\text{ps}$  of neat TMU. As a result, the water–TMU mixtures reveal an interpenetrated network of hydrogen-bonded molecules that are mutually immobilized upon dilution with water. When equal amounts of water and TMU are present, the diffusion constant of water molecules is a factor of 6 smaller than that in bulk water.

The slope values at the central part of the 2D spectra, depicted as a function of concentration (Figure 4), level off as the average number of hydrogen bonds per water molecule reduces from  $\sim 3.5$  to  $\sim 3$  (Figure 6a). Remarkably, a perfectly linear correlation is observed between the reduced translational mobility and slope values at the central part of the 2D spectra (Figure 7), which 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 frequency dynamics.

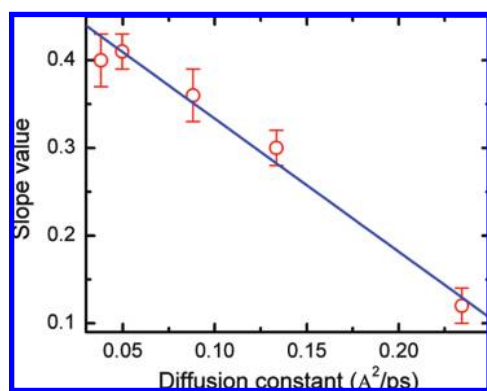
## Discussion

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 2 times longer time constant than in neat liquid water (Figure 4), while another part of the water molecules appear 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”<sup>17</sup>) 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 the TMU solvation shell (Figure 5), for which switching can occur.

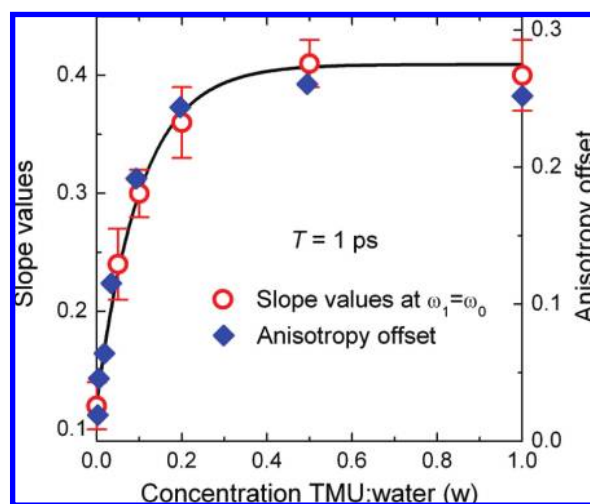
It is interesting to compare the present results to the recent measurements of the rotational anisotropy dynamics of aqueous solutions of TMU. In those studies, the reorientation dynamics of water was observed to show two distinct time constants. At low concentrations, one of these time constants was similar to the reorientation time constant of 2.5 ps of bulk water; the other time constant was  $>10$  ps. The quickly reorienting water molecules likely correspond to the water mole-



**FIGURE 6.** The averaged number of hydrogen bonds (HB) per water molecule (a) and the translational diffusion constants (b) as functions of concentration of the TMU–water binary mixture. Open and filled symbols stand for water and TMU, respectively.



**FIGURE 7.** 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.



**FIGURE 8.** Slope values (circles) at the absorption line centers (averaged over the frequency range of  $\omega_1 - \omega_0 = -50 \pm 50$  cm<sup>-1</sup>) at the waiting time  $T = 1$  ps and the anisotropy offset (diamonds) from ref 15 as functions of concentration. The solid curve is a guide to the eye.

cules that show switching, while the slowly reorienting molecules 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 refs 14 and 15 as a function of the concentration TMU. The result is given in Figure 8: the slope values and the end level of the anisotropy show perfect correspondence. This finding strongly suggests that water molecules that are excluded from switching are indeed the same as those showing a very slow reorientation, even at low concentrations of TMU. This fraction increases linearly with the concentration of TMU, which shows that these water molecules must be part of the hydration shells of the TMU molecules. MD simulations confirm that at the lowest TMU concentration these water molecules form clathrate-like structures around the methyl groups. At higher concentrations, the fraction of slowed water was observed to saturate, especially for TMU solutions (Figure 3 of ref 14 and Figure 8b of ref 15). This means that at high concentrations the number of slowed OH groups per solute molecule decreases. In refs 14 and 15, this effect was explained from

the overlap of the hydration shells of the solute molecules. However, here we find that this saturation is due to the formation of water nanoclusters. At very high concentrations of TMU, the reorientation of the fast reorienting water molecules was observed to somewhat slow in comparison to that of neat liquid water:<sup>15</sup> from 2.5 to  $\sim 3.5$  ps at  $w = 1$ . This agrees very well with the fact that we also observe the switching time getting longer for those water molecules (Figure 4, right panel).

In recent MD simulations,<sup>17</sup> it was found that *all* water molecules in solutions of amphiphilic compounds undergo switching albeit somewhat more slowly than in the bulk because 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 of the switching and the reorientation by a factor of 2 only. This result clearly does not agree with the present findings that show a much stronger effect of hydrophobic molecules on the dynam-



ics of the hydration shell. We thus conclude that the effect of hydrophobic groups is 2-fold. These molecular groups 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 (Figure 6a) but the translational mobility decreases. This explanation is supported by the suppressed translational mobility of water (Figure 6b) and its excellent correlation to the hydrogen-bond dynamics (Figure 7).

NMR studies also indicate that water molecules in the solvation shells of hydrophobic molecule 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.<sup>11,39</sup> 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.<sup>7,8</sup> 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 is relatively small: only two water molecules per methyl group<sup>14</sup> 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.

## Concluding Remarks

In this Account, 2D IR spectroscopy and MD simulations have been used to study hydrophobic solvation. The utilization of 2D spectroscopy allowed us to combine a  $\sim 70$  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 mixtures. The first type, “fast” ( $\sim 100$  fs) dynamics, are connected to the molecular-jump water reorientation, while the second type, “slow” dynamics, are associated with translational mobility of water. According to our results, molecular-jump reorientations are still possi-

ble at least for some water molecules in the solution. However, the rate of the reorientation events decreases dramatically 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”.<sup>2</sup> 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<sup>40</sup> and experimentalists<sup>8,11</sup> 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.<sup>11</sup> On the other hand, NMR<sup>9,10</sup> and time-resolved IR studies<sup>14,15</sup> 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 Account seem to resolve this contradiction. The water molecules in the hydrophobic shell are not ice-like frozen because they have at least librational degrees of freedom and some of them can even rotate. However, their translational mobility is severely compromised 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,<sup>41</sup> which proposed that the hydrogen-bond network around hydrophobic groups is not more rigid compared with the bulk but that the hydrogen bond dynamics in the two cases are different. This proposition echoes a recent point regarding the question of whether a hydrogen bond can be defined without considering its dynamics.<sup>42</sup>

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## BIOGRAPHICAL INFORMATION

**Artem A. Bakulin** (M.S. Physics, 2005, Moscow State University) is a Ph.D. student at the University of Groningen. His research includes 2D correlation spectroscopy and ultrafast spectroscopy of conjugated polymers.

**Chungwen Liang** (M.S. Physics, 2008, Fudan University) is a Ph.D. student at the University of Groningen and works on membrane protein channels with molecular dynamics simulation methods and modeling their multidimensional spectra.

**Thomas la Cour Jansen** (M.S. Chemistry, 1998, University of Copenhagen; Ph.D. Chemistry, 2002, University of Groningen) is a researcher at the University of Groningen and develops methods for modeling multidimensional spectra.

**Douwe A. Wiersma** (M.S. Chemistry, 1967, and Ph.D. Chemistry, 1969, University of Groningen; Postdoctoral Fellow at University of Pennsylvania, 1969–1971) is professor of physical chemistry at the University of Groningen and interested in the application of femtosecond four-wave-mixing spectroscopies to liquid state dynamics.

**Huib J. Bakker** (M.S. Chemistry, 1987, Free University of Amsterdam; Ph.D. Physics, 1991, University of Amsterdam and Institute for Atomic and Molecular Physics, AMOLF; postdoctoral fellow at the Technical University of Aachen, 1991–1994) is a group leader at AMOLF and a professor of physical chemistry at the University of Amsterdam. His research work includes the spectroscopic study of the structure and ultrafast dynamics of water interacting with ions and (bio)molecular systems and the mechanism of proton transfer in aqueous media.

**Maxim S. Pshenichnikov** (M.S. Physics, 1983, and Ph.D. Physics, 1987, Moscow State University; postdoctoral fellow at University of Groningen, 1992–1995) is a researcher at the University of Groningen. He is interested in applications of femtosecond spectroscopy to dynamics of liquids and polymers and (non)linear single-molecule microscopy.

## FOOTNOTES

\*To whom correspondence should be addressed. E-mail: M.S.Pchenichnikov@RuG.nl.

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