Communication

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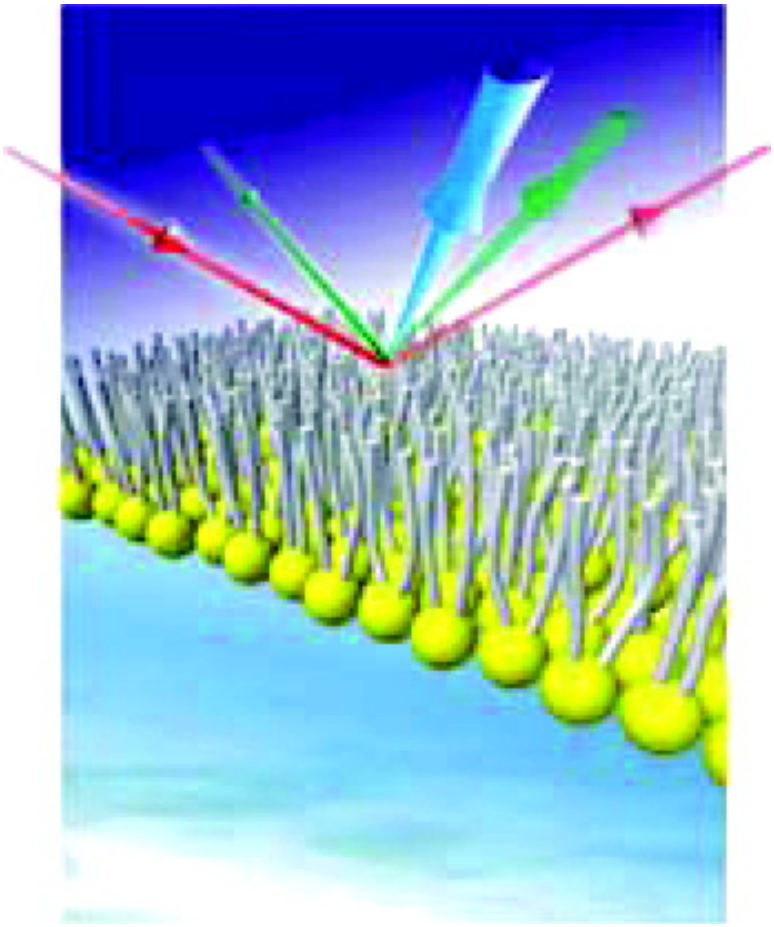

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Membrane-Bound Water is Energetically Decoupled from Nearby Bulk Water: An Ultrafast Surface-Specific Investigation

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The driving force behind a number of entropically unfavorable biophysical processes, such as protein folding, membrane formation, and cellular transport, has been attributed to the orientation and structuring of water molecules in the vicinity of biomolecules.[1,2] Membrane-bound water is of particular interest, as the functionality of lipid membranes is known to be intrinsically linked to hydration, and hydration of lipids has important structural and functional consequences[4] for the membrane. The hydration dynamics[5] and the water−lipid interaction strength[6] are, for instance, closely related to the membrane fluidity and the molecular organization of the lipids.

The surface-specific technique of vibrational sum frequency generation (VSFG) has enabled the investigation of the vibrational spectrum of ∼1 monolayer of water molecules directly interacting with the lipids, owing to its unique selection rules. Similar to the water−air interface,[3] the spectrum of the water−lipid interface is characterized by essentially two broad peaks in the hydrogen-bonded region between 3100 and 3500 cm^{-1} (see Figure 1). Recent VSFG studies have shed important light on the interaction of water molecules with lipid surfaces,[7] addressing questions of water hydrogen bonding structure, orientation, and the effect of the lipid headgroup on the organization of membrane-bound water.

One important question that has remained unanswered, however, pertains to the *dynamics* of membrane-bound water. Much of the information on the hydrogen-bond dynamics of membrane-bound water remains hidden in the vibrational spectra as the response is broad and featureless in the hydrogen-bonded region, owing to the large local variations in the hydrogen-bond network. The recently developed technique of femtosecond time-resolved sum frequency generation spectroscopy (tr-SFG) allows one to study interfacial water dynamics directly. In a tr-SFG experiment, the O−H stretch vibration of water molecules is excited by an intense infrared pulse, after which the vibrational relaxation of specifically the interfacial water molecules can be monitored in real-time using an SFG probing scheme. Using tr-SFG, it has been shown that water molecules at different water interfaces[8,9] exchange vibrational energy very rapidly with bulk water, independent of the precise interfacial structure.

In this communication, we study membrane-bound water with tr-SFG and demonstrate that the relaxation dynamics of membrane-bound water are dramatically different from water at other interfaces.[8,9] Our results demonstrate that lipid-bound water is energetically decoupled from the bulk.

A Langmuir−Blodgett monolayer of the lipid 1,2-dimyristoyl-*sn*-glycero-3-[phospho-L-serine] (DMPS, sodium salt, Avanti lipids) was prepared on an ultrapure water subphase (Millipore water, 18 MΩ·cm resistivity) at a surface pressure of 25 mN/m (details in Supporting Information). The static, frequency-resolved SFG spectrum of water adjacent to the DMPS monolayer is shown in Figure 1. The tr-SFG setup has been discussed elsewhere[8] (details in Supporting Information). Briefly, a ∼30 μJ femtosecond (120 fs pulse duration) infrared pump pulse tuned to the O−H stretch vibration excites ∼10% of the water molecules to their first vibrationally excited state. Owing to the anharmonicity of the O−H stretch vibration, the excited state SFG signal is shifted out of the frequency window. Hence, for as long as the vibrational excitation resides on the water molecules, the SFG intensity is reduced, and the recovery of the SFG intensity reflects vibrational relaxation. The T_1 vibrational lifetime of the O−H stretch vibration has been shown to be a sensitive probe of the local water surroundings.[10]

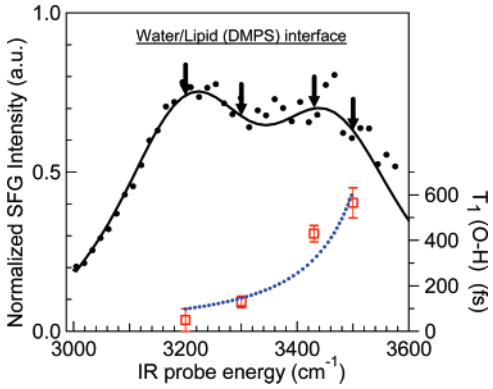

Figure 1. Upper trace: static SFG spectrum of the water/DMPS interface. The water/air spectrum has been reported elsewhere.[3] A fit to the data points is shown by the solid line, and the arrows indicate the pump IR frequencies in the tr-SFG experiments. Lower trace: relaxation times at different frequencies within the SFG band reveal a marked frequency dependence of the relaxation. Dotted line: result of a model calculation explained in the text.

The resulting IR pump−SFG probe transients at different wavelengths within the hydrogen-bonded regime are plotted in Figure 2 for the water−air and water−lipid interfaces. For the former, it was shown that the dynamics are dominated by ultrafast energy exchange of interfacial water with bulk water,[8] so that all transients are characterized by just two distinct time constants obtained from bulk studies:[11] T_1 (190 fs) and an energy thermalization time, $T_{thermalization}$ (500 fs), accounting for the eventual heating of the sample due to the excitation process, which also explains the long time signal offsets. The fact that the transients can be described by the bulk time constants and the observation that these time constants are the same at all wavelengths can be explained by ultrafast transfer of vibrational energy between the surface and the bulk,[9] in analogy to water at the hydrophilic and hydrophobic silica/water interface.[9]

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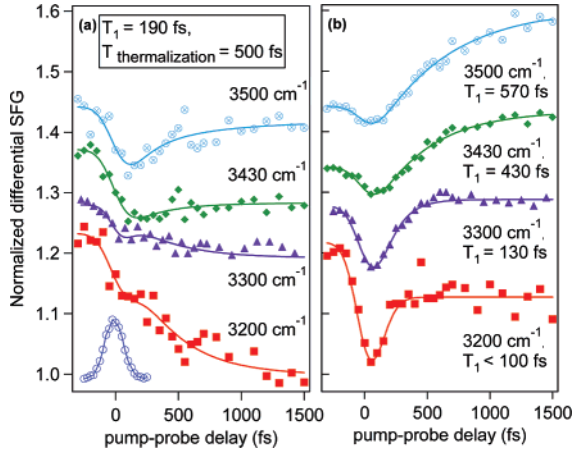

Figure 2. Pump−probe SFG transients for (a) the neat water−air interface and (b) the water−lipid interface. The infrared−infrared−visible SFG cross-correlation trace (lower left) determines the time-zero and illustrates the time resolution of the experiment. All traces are offset from 1.0 for clarity.

Remarkably, Figure 2b reveals that the behavior of water at the water−lipid interface is very different from that at the water−air interface. Unlike the water−air interface, the data can be described by a single-exponential decay, with distinct time constants at different probe frequencies within the hydrogen-bonded regime. A three-level kinetic model (Supporting Information) was invoked to describe the water−lipid transients and extract the vibrational lifetime T_1. The model includes the rapid heating of the sample upon vibrational relaxation, which fully accounts for the long time SFG intensity offsets and its dynamic ingrowth. Vibrational relaxation is extremely fast at lower probe frequencies (T_1 < 100 fs at 3200 cm^{-1}), whereas at higher frequencies (e.g., 3500 cm^{-1}), the dynamics are much slower. This indicates that, in contrast to the water−air interface, the ultrafast vibrational energy communication between surface and bulk does not play a significant role in the relaxation of vibrationally excited membrane-bound water molecules. The resulting slow spectral diffusion leads to distinct relaxation dynamics at different probe wavelengths, as shown in Figure 1. Such heterogeneity of water was reported previously in bulk lipid systems.[12]

The absence of resonant energy transfer implies that vibrational relaxation takes place as for isolated, H-bonded water molecules. Indeed, the vibrational lifetimes obtained from the transients can be described well using the theoretical model that describes relaxation of the O−H stretch into the O−H···O hydrogen bond mode as developed by Hynes et al.[13] The model predicts that the vibrational lifetime, T_1^{OH}, is related to the H-bond strength, for which an accurate measure is the red shift with respect to the free O−H band ($\nu_{OH}^{free} \sim 3650$ cm^{-1}), so that $T_1^{OH} \propto (\nu_{OH} - \nu_{OH}^{free})^{-1.8}$. Stronger hydrogen-bonded O−H groups (with lower stretch frequencies) exhibit faster vibrational relaxation. The fact that this theory describes the data very well (dotted line in Figure 1) and the experimental observation that ultrafast energy transfer is absent demonstrate that membrane-bound water is energetically decoupled from the bulk.

The reason for this energetic decoupling must lie in the fact that water molecules adjacent to the lipid membrane interact strongly with the polar headgroups of the lipids and are localized within the headgroup region. Indeed, molecular dynamics simulations have demonstrated that water penetrates the headgroup region up to the apolar alkyl chain.[14] As a result, water molecules are physically removed from the bulk, and the energy transfer resulting from dipolar interactions between the surface and bulk is not as efficient as for the neat water/air interface. Our results conclusively demonstrate the existence of membrane-bound water: the water molecules detected here with SFG do not simply terminate the bulk but are energetically decoupled from the bulk and, as such, constitute an intrinsic part of the membrane.

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Supporting Information Available: Description of the setup, sample preparation, and data analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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