



*Molecular Orientation at Biological Interfaces: Water and Lipids Studied Through Surface-Specific Vibrational Spectroscopy*

R.E. Pool

# SUMMARY

Interfaces—the boundary surfaces and, as such, the interaction sites between materials—are of high interest in the scientific community. This fact may seem trivial, a straightforward consequence of their ubiquity in every regime of the physical world. This does not imply, however, that interfacial systems are easily studied. In many cases, the arrangement of atoms or molecules at an interface is different from that in the continuous ‘bulk’ material, and as a consequence processes unfold in a different way at interfaces than they would in the bulk. This distinctive interfacial region may be arbitrarily small, down to a single molecular or atomic layer in size. The observables of interfacial phenomena are thus at risk of being lost in their bulk background. For a thin water layer of one millimeter thickness, for example, only one in a million water molecules can be considered to be part of the interface.

In this thesis, several experiments are described to study interfacial systems by means of vibrational sum-frequency generation (SFG) spectroscopy. This technique makes use of an infrared (IR) laser source to excite the vibrational modes of a molecule. A second laser beam, typically of near-infrared frequency and not resonant with any optical transition in the sample, is combined with the IR beam to create an SFG signal with a frequency equal to the sum of the two incident beams. The main advantage that SFG has over traditional IR or Raman techniques in the study of interfaces is its surface specificity. Being a two-photon process, the generation of the sum-frequency signal only occurs in materials that have a broken symmetry. For most materials, symmetry is only broken at the interface, making SFG an ideal tool to focus on just the interfacial properties.

In the first three chapters of this thesis, the theoretical and experimental background of SFG spectroscopy is described, starting with a historical overview of its development. In this overview a range of possible applications was presented, leading to the introduction of the lipid interfaces that are central to the experiments described in the last three chapters of this work. Chapter 1 continues by describing how the spectral information of an SFG measurement can be used to obtain insight into the molecular structure of the interfacial system.

Chapter 2 provides a selection of the physical principles that are fundamental to the occurrence of the sum-frequency signal. This theoretical context is presented to provide the reader with a better understanding of the possibilities and limitations of SFG spectroscopy. From the theoretical description of the SFG response we move on to discuss the model that is used to perform quantitative analysis of the SFG data.

The experimental setup is described in chapter 3, highlighting the princi-

ple of IR generation through optical parametric amplification and providing a description the of sample preparation protocol.

ISOTOPIC DILUTION AT THE AIR-WATER INTERFACE. The first experiment described in this thesis explores the most common aqueous interface, that between pure water and air. The SFG spectrum of this interface consists of two spectral features: a broad peak associated with the hydrogen-bonded water, behaving much like bulk water, and a narrow feature at higher frequency arising from OH bonds sticking into the air. Since these bonds are not hydrogen bonded, we often refer to them as the ‘free OH’.

In the introduction we mentioned that it is common practice to replace water ( $\text{H}_2\text{O}$ ) by heavy water ( $\text{D}_2\text{O}$ ) to move the water’s resonances into a more accessible spectral region. When doing so, it is assumed that the molecular organization and superstructure within the sample is not affected by this exchange. Chapter 4 shows that calculations that include the quantum mechanical properties of  $\text{H}_2\text{O}$  and  $\text{D}_2\text{O}$  indicate that this assumption is not correct: when mixing water and heavy water, significantly more free OH bonds exist than free OD bonds. This prediction is derived from path integral calculations that describe the effect of the uncertainty principle for the position of an atom on the trajectory that it describes. The D atom, being significantly heavier than the H atom, has a more well-defined position. The calculations indicate that this localization effect results in stronger hydrogen-bonding of the D atom than of the H atom. Because of this asymmetry, an HOD molecule at the interface will have a higher probability to be hydrogen-bonded to the D atom and having a free OH bond than vice versa.

This theoretical prediction is confirmed experimentally by recording the SFG spectra of a range of isotopic mixtures of  $\text{H}_2\text{O}$  and  $\text{D}_2\text{O}$  in both the OH and OD spectral region and carefully fitting the data. The ratio between free OH and free OD formation is in quantitative agreement with the calculated values, indicating a difference of up to 10%.

COMPARISON OF DIRECT AND PHASE-SENSITIVE SFG SPECTROSCOPY. After this look into the fundamental behavior of the deuterated water interface, we proceed with a discussion of a technical aspect of SFG spectroscopy. In recent years, several groups have implemented an interference-based (‘heterodyning’) technique to extract the absolute sign of the resonances featured in the SFG spectrum. Whereas in a typical, direct SFG measurement the squared electric field is detected, interference with a nonresonant reference medium introduces linear terms in the SFG signal intensity that can have both positive and negative spectral features. This sign, or *phase* of the complex signal indicates if the molecular bond that it originates from is pointing up or down, and thus provides additional insight into the molecular structure of the studied interfacial system. Chapter 5 provides a thorough theoretical description of the methodology of this phase-sensitive SFG (PS-SFG), deriving the mathematical principles that it is based upon and acting as a step-by-step guide for data analysis. As a case study the water below monolayers constituted of the negatively charged

phospholipid DMPS and the positively charged lipid DPTAP is probed by both traditional (direct) SFG and PS-SFG. The sign of the detected PS-SFG water signal is positive for DMPS and negative for DPTAP, corresponding to the expected alignment of the water dipole along the interfacial electrostatic field. We show that to compare the PS-SFG spectrum to the direct SFG measurement one need not only square the signal, but also correct it for the IR radiation lost when reflecting off the sample surface. By considering the complex refractive index of the sample, in this case approximating it by that of just heavy water, we were able to neatly overlap our direct and PS-SFG data. This quantitative comparability supports a better understanding of PS-SFG, a field of research that is both powerful and growing in application, but still contains uncertainties and would benefit from more standardization across scientific groups.

Chapter 5 is concluded by a comparison of the signal-to-noise (SNR) ratio that is achievable with direct SFG and PS-SFG techniques. We present a simple analysis to show that PS-SFG has a higher sensitivity, giving a better SNR for very weak signals, but performs equally to or slightly worse than direct SFG for high signal intensities.

THE INTERACTION OF CHARGED LATEX NANOPARTICLES WITH A MODEL MEMBRANE. In chapter 6, the interaction of nanoparticles (NPs) with a model membrane is studied by tracking the changes induced in the SFG spectrum. As a model membrane, monolayers consisting of phospholipids with only positive, negative or zwitterionic charges in their head groups were chosen. The NPs were 30 nm polystyrene latex spheres functionalized with either positive or negative charge groups.

The interaction between NPs and the lipid monolayer is clearly identifiable with SFG spectroscopy through changes in the spectral features of the OD and CH stretch vibrations of (heavy) water and lipids, respectively. The spectral changes induced by adding charged NPs to monolayers of opposite charge are significant, while equal charged NPs and monolayers show only minor effects. This result indicates, in agreement with intuition, that the interaction is dominated by electrostatics. Scanning electron microscopy (SEM) images of the deposited system confirmed this finding: a high density of NPs ( $> 100$  per  $\mu\text{m}^2$ ) was found for the oppositely charged NP/monolayer combination, and almost none ( $< 1$  per  $\mu\text{m}^2$ ) for equal charges. From the SFG data, details of the molecular superstructure at the interaction site could be deduced. Negative NPs are shown to bind tightly to the positively charged (DPTAP) monolayer so effectively that charge inversion occurs, as can be observed by a phase change of the water signal. Binding is accompanied by an increase in alkyl chain order. The system with charges reversed, positive NPs binding to negatively charged (DPPG) monolayers, looks very similar in the SEM images. The SFG spectra, however, reveal several interesting differences: the binding of the positive NPs to a negative monolayer induces neither charge inversion nor an increase in alkyl chain order. An explanation for both these differences is presented based on how the lipids' chemical structure may influence the interfacial molecular organization.