Molecular orientation at biological interfaces: Water and lipids studied through surface-specific vibrational spectroscopy
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3 EXPERIMENTAL MATERIALS AND METHODS

3.1 INFRARED GENERATION

The vibrational SFG data described in this thesis require an intense, pulsed source of IR radiation. The current standard in high-powered pulsed amplified systems is the titanium sapphire laser, Ti:Sapph for short. The output of a Ti:Sapph laser is centered around 800 nm, and can be converted to other wavelengths by an optical parametric amplifier (OPA). At the center of an OPA system is a nonlinear crystal. A seed pulse is generated, usually by creating white light from the amplifier’s 800 nm output and filtering out unwanted frequencies. The remaining broadband pulse is combined in the crystal with an 800 nm beam in what is called the first amplification stage. Not only spatial overlap is required, but also temporal overlap, which can be challenging when using ultrashort (∼35 fs) laser pulses but can be achieved by means of stable micrometer delay stages. Rotating the crystal influences the phase-matching conditions between seed and amplification. If phase matching is optimized for the seed frequency, a significant part of the 800 nm light will undergo a difference frequency generation (DFG) process, splitting into the a component that has the same energy as the seed, and a component of a frequency matching the difference in energy between the seed and the 800 nm pulse. Of the two resulting frequencies, the higher-frequency one is traditionally called the signal, while the lower-frequency one is called the idler. After the first amplification stage, one of the beams is usually discarded, and the other amplified within the crystal by a new pass of 800 nm light of much higher intensity than during the first stage. Having obtained a high signal and idler intensity, these two beams may now be combined outside the OPA in a second DFG crystal, which allows for the generation of even lower frequencies. We will now describe the process of IR generation quantitatively and in more detail, considering the OPA system shown in figure 3.1.

The IR excitation source used for the experiments described in this thesis is based on a stable and efficient OPA system that has been built and described by Hamm et al. [68]. It must be noted that the quantitative description given here specifically describes our OPA system rather than the general limit of the system’s potential: the use of other geometries and optical materials may strongly alter the characteristics of an OPA system. In our case, the input of the system is 1.2 mJ of a (∼35 fs) pulsed 800 nm amplified laser system. Two different models have been used throughout this thesis: a Coherent Legend and
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Figure 3.1. Experimental geometry of the optical parametric amplifier (OPA). Image adapted from [68].

A Spectra Physics Spitfire Ace, which performed practically identical for the purposes described here. In figure 3.1, the 800 nm beam is seen to enter the OPA system from the lower left. After two mirrors, it encounters a window that reflects a few percent of its power. This small signal, indicated by an encircled Roman numeral I in figure 3.1, passes a λ/2-plate and a cube polarizer. This set of optics has two functions: firstly, they change the polarization of the light from s to the p-polarization needed in the nonlinear crystal. Secondly, with this geometry the light intensity can be sensitively controlled by rotating the λ/2-plate. This control is of importance in the next step, focusing the beam onto a 3 mm thick sapphire plate, where white light is generated. When the intensity is too high, the white light becomes unstable and distorted, but at the right intensity a nicely symmetric circular spectrum is obtained, indicating a neat beam profile. This is this seed pulse, and it continues to the nonlinear medium, a 1 mm-thick barium borate (BBO) crystal.a

Meanwhile, most of the 800 nm light has travelled through the first window, over a delay stage and onto a second beam splitter. A slightly larger fraction of about 10 % is now reflected and focused with a f = 50 cm lens into the BBO crystal. This is the first amplification stage, indicated by a Roman numeral II in figure 3.1. It combines with the seed to form the first signal and idler

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aIn the original OPA developed by Hamm et al. the crystal was 4 mm in thickness. Since then, the development of shorter-pulsed laser systems has increased the light intensity that the crystal can be exposed to. To prevent the crystal from being damaged by this high intensity, it should be chosen thinner to minimize the path length of the radiation within.
light, with frequency in the ranges of 1200 to 1600 nm and 1600 to 2400 nm, respectively. Their combined intensity is typically about 8 $\mu$J. More important than the beam power at this stage is the beam profile, which is determined by the alignment and ultimately greatly affects the intensity and stability of the generated IR radiation. The signal is blocked, and the idler reflected back by means of a refocussing mirror mounted on a delay stage. When passing the BBO crystal on the way back, the idler is overlapped by the second amplification stage, indicated by a Roman numeral III in figure 3.1. This intense beam, still having an energy of about 1 mJ, is not focused onto the BBO because that would surely damage the crystal. Rather, it is narrowed by a telescope consisting of two mirrors, and then collinearly sent through the crystal along with the idler of the first amplification. With a proper alignment, a signal+idler intensity of up to 300 to 350 $\mu$J can be obtained, implying a conversion efficiency of around 30 %. Although the near-IR signal and idler cannot be seen with the naked eye, they are intense enough to generate a strong, visible second harmonic. While this simplifies alignment of the OPA, a practical problem posed by the diverse frequencies, along with the complex experimental geometry, is that it is difficult to efficiently protect your eyes from the potentially dangerous laser pulses.

To generate the mid-IR frequencies that are required for vibrational spectroscopy, signal and idler need to be combined in a second nonlinear crystal, silver gallium sulphide (AGS, see figure 3.2). In a DFG stage signal and idler are split by a beam splitter to optimize their time overlap before sending both through the AGS crystal. The difference frequency generated by signal and idler can be controlled by changing the orientation of the BBO and AGS crystals, along with adjustments to the temporal and spatial overlap of the beams within the OPA. In our setup, this results in a mid-IR DFG signal that can be tuned between about 1600 cm$^{-1}$, the amide vibrational region, and 3700 cm$^{-1}$, the free OH vibration. After the AGS crystal, pulse energies are in the order of 5 $\mu$J. IR generation is inevitably a very inefficient process. On the other hand, much higher IR intensities are not always welcome, since lipid samples may be influenced by heating effects even at low IR flux [69].

### 3.2 The Spectroscopy Setup

The placement of the OPA within the entire SFG setup is shown in figure 3.2. In the lower left corner, we see the 800 nm amplifier that both powers the OPA and provides the visible upconversion pulse at the sample. Around 0.6 of the amplifier output is spectrally narrowed to 25 cm$^{-1}$ using a Fabry-Perot etalon. Only about 40 $\mu$J remains after the etalon, but the frequency narrowing is necessary to provide the SFG spectrum with a decent frequency resolution. The frequency resolution is determined by a convolution of the IR and visible pulse, and to obtain high pulse energies and broadband excitation, the IR needs

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bSignal and idler do not temporally overlap before due to a phase difference in their generation within the BBO crystal [64,68].
Figure 3.2. Experimental geometry of SFG detection. 800 nm (VIS) and mid-infrared (IR) laser pulses are combined and focused onto the sample, generating an SFG response that is detected on a CCD camera. The inset shows a side view of the beams combining on the sample.

After the etalon, the 800 nm beam is passed back and forth between a few mirrors to compensate the long distance that the (later) IR travels within the OPA. Only by exactly equating the traveled distance, with the help of a delay stage within the 800 nm beam path, can a pulse generated in the amplifier be recombined at the sample to generate an SFG signal. Close to the sample area, the IR beam passes through a half wave plate and polarizer before being focussed onto the sample together with the 800 nm (VIS) beam. IR and VIS are focused onto the sample by means of lenses with a focal length of 50 mm and 200 mm, with angles of incidence of 45° and 40° with respect to the surface normal. The SFG signal is reflected of the sample surface and the remaining 800 nm light is filtered out by a series of high-pass filters, and guided by a series of lenses and mirrors into a spectrograph (Acton, Princeton Instruments) where it dispersed, via a grating, and focused onto an electron multiplied charge coupled device (emCCD) camera (Newton, Andor). Unless stated otherwise, all spectra reported in this thesis are collected under the ssp-polarization condition (SFG...
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and visible s, IR p).

3.3 Sample preparation and tensiometry

The sample holder most commonly used throughout this thesis is a teflon-coated aluminum trough, with a surface area of 49 cm$^2$ and a volume of 20 mL. For measurements on the air/water interface (chapter 4, it suffices to fill the trough with distilled and filtered millipore water. Regularly, heavy water ($\text{D}_2\text{O}$) is used instead of H$_2$O, because its resonances lie at other frequencies. This can be useful when trying to separate the spectral response of two components, like CH and OH vibrations. Additionally, the IR excitation power obtained with our OPA system is higher at the OD resonant frequencies that at those for OH, which enables measurements with a higher signal-to-noise ratio. For measurements of lipid monolayers, a lipid solution in chloroform (sometimes with methanol) is deposited drop by drop onto the surface with a microliter syringe. In most monolayer studies, the lipid concentration at the surface was established by surface pressure measurements, also known as tensiometry. Tensiometry is an easy to use and useful tool in providing information on the lipid concentration and thermodynamical properties of lipid monolayers. The basic technique is resting a small needle tip onto the surface, attached to an electronic device that records the needle weight, indicating the surface tension. What is commonly called surface pressure can also be interpreted as a change in free energy, as can be made intuitive by noting the matching units for two-dimensional pressure and energy per surface unit area: $[\text{N/m}] = [\text{J/m}^2]$. A change in surface free energy may be associated with the addition of substance to the surface, as is the case when adding phospholipids to the air-water interface, lowering the free energy. A lowering of the surface free energy corresponds to an increase in surface pressure $\pi$, which is defined as relative to that of clean water ($\pi_{\text{water}} = 72.8 \text{ mN/m at room temperature}$) as $\pi = \pi_{\text{water}} - \pi_{\text{surface}}$. The surface pressure is typically a function of surfactant density, but may also change due to the reorganization of the molecular structure at the surface at constant surface density. In general, lipids are deposited onto the surface up to a target value. The relation between the surface pressure and the lipid density is given by an isotherm. For many lipids, these isotherms can be found in literature; alternatively, they are relatively easy to measure by compressing a monolayer of a known quantity of lipid molecules while recording the surface pressure. The shape of the isotherm reveals information about the collective behavior of the monolayer; discontinuities in the curvature indicate phase changes in the lipid monolayer [70].