Watching molecular motion at interfaces
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1. Introduction and Theory

1.1 Thesis Overview
In this thesis, the reorientational dynamics at interfaces for a model biophysical system and for an aqueous mineral surface are investigated. Chapter 1 provides a general introduction to protein side-chain dynamics followed by an overview of the theory behind the measurements performed. Next, Chapter 2 presents a brief overview of the experimental methods used to measure the time-resolved transients presented in this thesis. Chapter 3 describes the application of time- and polarization-resolved SFG to L-leucine adsorbed at the air-aqueous interface to determine how a leucine monomer reorients itself. Molecular dynamics simulations support the experimental findings. In Chapter 4, the work on leucine is continued to study the reorientational dynamics of hydrophobic leucine side chains within model amphiphilic Leucine-lysine (LK) peptides with different secondary structures adsorbed to the air/water interface. Chapter 5 presents work at the buried silica-water interface. First, it is shown through phase resolved SFG that there are non-hydrogen bonded water species present at the interface. The reorientational dynamics of this species are subsequently measured and are found to be similar to those of the free OH at hydrophobic interfaces. The appendix contains additional measurements for LK peptides, leucine, sodium decanoate, and dodecanol taken at the air-water interface. Additionally, a brief overview of local field factors in SFG along with a short section about phase resolved SFG is presented. An outlook is also provided into protein side chain-surface interactions along with suggested future experiments.

1.2 Introduction
Molecular level orientational fluctuations are responsible for many processes ranging from solvation\textsuperscript{1-3} to the globular rearrangement or folding of proteins.\textsuperscript{4} These dynamic reorientational interactions play out on a diverse range of timescales ranging from sub-ps for the reorientation of
water molecules around a solute to ms for the aforementioned case of protein folding.\textsuperscript{5,6} By studying different ranges of motion from sub-ps to ms, a molecular movie of sorts can be assembled, and importantly, the molecular function of these moieties can be deciphered.\textsuperscript{7}

This thesis focuses on the study of sub-ps molecular orientational dynamics at aqueous interfaces. In these experiments, a molecule is first vibrationally labeled and the motion is subsequently followed in real time. We attempt to decipher the rates of fast molecular motions which occur during the lifetime of our molecular, vibrational label. Fluctuations in protein side chains specifically at interfaces have, until recently,\textsuperscript{8} been difficult to experimentally study, and different model systems ranging from leucine monomers to leucine side chains within model peptides are presented. In the last chapter of this thesis, we move away from proteins and we investigate ps reorientational fluctuations of non-hydrogen bonded water molecules in contact with a hydrophilic surface. Based on the spectroscopic evidence, it is revealed that non hydrogen bonded water is present near the interface, and dynamically, these water molecules reorient on similar time scales to dangling OH groups at both the water/air and extended hydrophobic interfaces. This is important because a) the intrinsic reorientation of non-hydrogen bonded water also is thought to play an important role in the “hydrophobic effect” which is highly relevant for protein folding, and b) it allows for future investigations of protein side-chain- surface interactions on chemically modified surfaces.

Biology is not static, and its principle actors cannot be described by merely glancing at the time-averaged equilibrium picture. On average geological timescales on Earth, life in its current form has not existed; in an average multicellular organism’s lifetime, any given cell may not exist. Life is dynamic, and in order to follow these dynamics as they happen, it is pertinent to observe the dynamics as they unfold. To begin to grasp the complexity of the molecular basis of life, monitoring its dynamics as they happen allows for insight into dynamic molecular function. Proteins are biology’s construction crew, and they are responsible for a wide range of
tasks ranging from cell signaling and cross-membrane transport to enzymatic catalysis. These dynamic protein interactions contribute to the dynamic environment present in cells.

Proteins are large globular assemblies of amino acids, and protein dynamics can occur on a plethora of time scales. Large motional rearrangements such as folding take place on ms down to ns timescales. Protein side chains whose dynamics dictate much of protein function also occur on a variety of dynamic time scales ranging from ns for large rearrangements to sub-ps for hydration dynamics of water molecules reorienting in the neighborhood of protein side chains. These dynamic interactions are all interdependent, and access to different dynamic ranges can help build up the overall dynamic picture of protein function.

Typically, side chain dynamics are followed in solution by means of magnetic resonance techniques such as NMR and EPR. Changes in chemical environment can be tracked by following the side chain dynamics, which are usually measured indirectly through linewidth measurements and ultimately quantified through the NMR order parameter $s^2$ (or equivalently $O^2$).\textsuperscript{11-14} This order parameter is directly proportional to the conformational entropy of a series of side chains. The higher the order parameter of a particular sidechain is, the more rigid the side chain. Inversely, lower order parameters are associated with a larger range of motion and a higher degree of disorder. NMR studies have shown that side chain dynamics are not necessarily correlated with the side chain’s location in the protein matrix as
both rigid order parameters have been found for side chains at the surface and low order parameters have been measured in the protein core. Extensive studies on protein side-chain dynamics have revealed that it is likely that the dynamic ps-ns fluctuations are responsible for protein function. A well-known example of this comes to play in allostERIC regulation of enzymes in which protein side chains undergo a variety of conformational changes in order to accommodate a binding event, and these allostERIC interactions also regulate cell signaling. Solution-state NMR has hinted that changes in conformational entropy upon binding are of utmost importance in enzymatic catalysis. A classic example of the likely importance of side-chain dynamics is shown can be found in the work of Lee for the cadomulin complex.

For the cadomulin complex, various methyl containing residues have been shown to undergo changes in conformational entropy upon binding to form a Calcium complex. Side chain order parameters have been calculated for several specific methyl-containing side chains, and this side chain order parameter is seen to change upon binding events. Of note, the methyl-containing residues undergo large changes in the measured order parameter for the bound versus the unbound state. Backbone groups are seen to undergo no marked change in NMR order parameter, but side chain conformational entropy is seen to undergo significant changes during binding events. This generalized order parameter reflects the conformational entropy of a series of protein side chains, which is seen to drastically change upon complexation. Changes in protein side-chain orientational dynamics, in this case, reflect changes in the local chemical environment of the protein. While magnetic resonance techniques can provide a wealth of information about side chain dynamics, there are two caveats: direct access to dynamics is not possible, and the access is limited to solution state events. In situ probing of interfaces is difficult, and a wealth of chemically specific information can be extracted by turning to surface-specific vibrationally labeled probes of the dynamics. By studying the dynamics of proteins at surfaces, steps can be taken beyond the structure-
function paradigm towards a functional understanding which includes dynamics.

1.3 Nonlinear Polarization

Before progressing to discuss how the orientational dynamics can be assessed at the interface, it is pertinent to discuss the sum frequency spectroscopic probe which is used to follow the motion. This thesis primarily takes advantage of even order nonlinear optical techniques to help understand molecular reorientational dynamics at model liquid surfaces. Prior to the advent of inherently surface sensitive even-order nonlinear spectroscopies, surface science was generally carried out under \textit{ex situ} ultra-high vacuum (UHV) conditions using techniques ranging from temperature programmed desorption to X-ray and electron diffraction techniques. Techniques based on vibrational spectroscopy allow in situ access to the chemistry, but surface specificity comes from studying inherently well-prepared monolayers, and the probing depth can go to hundreds of nm. Even order nonlinear optical techniques are inherently surface specific to the interface between centrosymmetric media under the electric dipole approximation. The following section is based primarily on the works of Shen,\textsuperscript{17,18} Hirose,\textsuperscript{19} Lambert,\textsuperscript{20} and Boyd.\textsuperscript{21} The field of nonlinear optical spectroscopy is a vast one which has been at play since the invention of pulsed laser sources.\textsuperscript{22-24}

Consider a molecule with permanent dipole moment $\mu_0 = -q \cdot r$, where $q$ is the charge and $r$ indicates direction. A light source with an oscillating electric field $E(r,t)$ is incident upon this molecule, and in response to this driving field, the molecule’s valence electrons will begin to oscillate at the frequency of the driving field, and an electric dipole moment $\mu(r,t)$ will be induced which is proportional to the driving electric field.

$$\mathbf{p} = \mu_0 + \alpha \cdot \mathbf{E} \quad (1.1)$$

The proportionality constant $\alpha$ is the polarizability of the molecule. Over a macroscopic range throughout a macroscopic volume, individual
contribute to the electric dipole sum up to become the macroscopic polarization density $P^{(1)}$. In S.I. units, the polarization density is thus:

$$P^{(1)} = \varepsilon_0 \chi \cdot E \quad (1.2)$$

where $\varepsilon_0$ is the vacuum permittivity and $\chi$ is the electric susceptibility. We will return to equation (1.2) after considering the anharmonic perturbation introduced by adding a strong coherent driving field.

Under the electric dipole approximation, i.e. ignoring magnetic dipoles and higher order terms in the multipole expansion, even-order nonlinear optical processes such as sum frequency generation can probe interfaces between bulk centrosymmetric materials. A nonlinear optical response may be induced in a material at electric field strengths above 1 kV/cm such as those readily available from a pulsed laser source. The molecular response is no longer harmonic because the intense fields make it such that the molecular response can no longer oscillate with the driving field.

The microscopic polarization can then be expanded into a Taylor series expansion about the applied electric field:

$$p = \mu_0 + a_i E + \beta^{(2)} : E E + \beta^{(3)} : E E E \quad (1.3)$$

where $\beta^{(n)}$ represent the $n^{th}$ order hyperpolarizabilities.

The polarization density $P$ is analogously expanded in a Taylor series about the driving field.

$$P = P^{(1)} + P^{NL}$$

$$P^{NL} = P^{(2)} + P^{(3)} + P^{(4)} + \ldots$$

$$P^{NL} = \varepsilon_0 (\chi^{(2)} : E E + \chi^{(3)} : E E E + \ldots) \quad (1.4)$$

The nonlinear polarization density $P^{NL}$ is the source term for a variety of nonlinear optical phenomena of which even order processes such as sum frequency generation and pump-probe sum frequency generation are
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included. In general, the induced polarization density of a material serves as the source of the electric field $E$ generated in a material in response to a driving field. Classically, this process is governed by the electromagnetic wave equation which can be derived directly from Maxwell’s equations. Including the nonlinear source term in the wave equation, it reads:

$$-\nabla^2 E + \frac{1}{c^2} \frac{\partial^2 E}{\partial t^2} = \frac{-4\pi}{c^2} \frac{\partial^2 P}{\partial t^2}$$  \hspace{1cm} (1.5)

1.4 Sum Frequency Generation

Next, we turn to the specific example of sum frequency generation (SFG). SFG is one of the mixing processes which give rise to the second order polarization density $P^{(2)}$. Consider a sinusoidal driving field $E$ consisting of two circular driving frequencies $\omega_1$ and $\omega_2$ with $E_1$ and $E_2$ respectively:

$$E = E_1 \cos \omega_1 t + E_2 \cos \omega_2 t$$  \hspace{1cm} (1.6)

Taking trigonometric identities and equation (1.4) into account, the second order polarization density is:

$$P^{(2)} = \varepsilon_0 \chi^{(2)} (E_1 \cos \omega_1 t + E_2 \cos \omega_2 t)^2$$

$$= \varepsilon_0 \chi^{(2)} (E_1^2 \cos^2 \omega_1 t + E_2^2 \cos^2 \omega_2 t + E_1 E_2 \cos(\omega_1 + \omega_2)t + E_1 E_2 \cos(\omega_1 - \omega_2)t)$$  \hspace{1cm} (1.7)

Equation 1.7 shows the many different contributions to the nonlinear polarization including optical rectification, second harmonic generation, difference frequency generation, and SFG. In general, in an SFG experiment, all of these contributions will be present. Spatial and spectral filters can help properly detect the sum frequency signal. The surface SFG response satisfies the phase matching condition that only the in plane
component to the momentum is conserved: \( k_{1||} + k_{2||} = k_{SFG||} \). In addition, due to energy conservation:

\[
\omega_1 n_1(\omega_1) \sin \theta_1 + \omega_2 n_2(\omega_2) \sin \theta_2 = \omega_{SFG} n_{SFG}(\omega_{SFG}) \sin \theta_{SFG}
\]

Equation (1.8) states this momentum conservation where \( \omega_i \) are the frequencies of the fields, \( n_i \) the frequency dependent refractive indices and \( \theta_i \) the angles of incidence. In addition, of course, energy has to be conserved in the SFG process, i.e. \( \omega_1 + \omega_2 = \omega_{SFG} \).

For electric dipole interactions, the second-order electronic susceptibility tensor vanishes for centrosymmetric materials because in general, the susceptibility tensor arising from a dipole source term is polar i.e. tensors are variant under inversion. In order to satisfy requirements of the inversion operation in a centrosymmetric material, this means that all odd rank tensors representing centrosymmetric bulk systems should be zero. Consider indices \( i, j, k \) which represent Cartesian coordinates \( x, y \), and \( z \) being such that the \( z \) axis is parallel to the surface normal and the \( xy \) plane lies in the plane of the surface. Equation 1.9 shows this inversion operation considering a tensor which is composed of dipole vectors in the Cartesian coordinate system shown in figure 1.1.
Figure 1.1 Schematic showing a surface SFG experiment. Two incident linearly polarized beams induce a nonlinear polarization, and this nonlinear polarization emits a SF beam. Through polarization control of the incident beams and polarization analysis of the exit beam, SFG is sensitive to molecular orientation.

\[ \chi^{(2)}_{i,j,k} = \chi^{(2)}_{-i,-j,-k} = -\chi^{(2)}_{i,j,k} = 0 \quad (1.9) \]

At the interface between centrosymmetric materials, the inversion symmetry is broken, and equation (1.9) no longer holds. As a result, \( \chi^{(2)} \) is nonzero, and thus, interfacial molecules are SFG active. SFG has proven to be a technique sensitive to fractions of a monolayer. The SFG response is detected in reflection geometry to maximize the dipolar response and minimize the effects of higher order multipoles or magnetic dipoles which are not necessarily zero in bulk materials.\(^{17,26}\)

1.5. Vibrational SFG Spectroscopy

The chemical specificity of the SFG probe depends upon the frequencies of the incident beams. Vibrationally sensitive SFG spectroscopy involves the spatiotemporal mixing of a vibrationally resonant mid-infrared beam...
with a typically nonresonant near-infrared (800 nm) or visible upconversion beam. The mixing generates a coherent, directional signal whose angle of reflection conserves momentum in the surface plane. Figure 1.2 shows a schematic of the SFG process between a vibrationally resonant IR pulse and a nonresonant visible upconversion pulse. The spatiotemporal overlap of the IR and upconversion pulse generates a second order nonlinear polarization. In cases in which one of the beams is resonant with a molecular transition, the emitted SFG signal may be resonantly enhanced. For experiments performed in this thesis, the mid IR beam is typically resonant with a molecular vibration, and the measured SFG response reflects this. When the upconversion beam is 800 nm in wavelength, the sum frequency signal generally covers a red range of wavelengths (620 nm for the dangling OH to 700 nm for Amide resonances for example). This may be readily dispersed by a spectrometer onto a CCD array which detects the intensity spectrum.

\[ \text{Figure 1.2 Left schematic of reflection geometry SFG experiment. SFG propagates in the phase matched direction. Right: Energy level diagram for a vibrationally resonant IR-Vis SFG experiment} \]
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In a standard SFG experiment, the detected intensity is proportional to the modulus squared of the second order polarization. Of particular note is the polarization sensitivity implicit in the $\chi^{(2)}$ tensor. Since the susceptibility tensor is a $3 \times 3 \times 3$ tensor (i.e. rank 3), it contains 27 elements, but symmetry considerations vastly reduce the total number of detected tensor elements.

$$I_{SFG} \propto |P_{i,SFG}^{(2)}|^2 \propto |\chi_{i,j,k}^{(2)}; E_{j,\text{vis}}E_{k,\text{IR}}|^2$$

$$I_{SFG} \propto |P_{i,SFG}^{(2)}|^2 \propto |\chi_{i,j,k}^{(2)}|^2 I_{\text{vis}}I_{\text{IR}}$$

(1.10)

$I_i$ represents the intensity of the beam $i$. For the case of an isotropic liquid interface with $C_{\infty v}$ symmetry, there are seven nonzero components of the susceptibility tensor, four of which are independent: $\chi^{(2)}_{xxz} = \chi^{(2)}_{yyz}$, $\chi^{(2)}_{zxz} = \chi^{(2)}_{zym}$, $\chi^{(2)}_{zxz} = \chi^{(2)}_{zyz}$, $\chi^{(2)}_{zzz}$. Different elements of the susceptibility tensor are probed through selective polarization control of the incident beams and by selectively analyzing the detected SFG beam. Generally, the polarization combinations used in an SFG experiment are labelled from highest frequency to lowest frequency beam, for example $s$ SFG, $s$ VIS, and $s$ IR being referred to as $ssp$. Referring to figure 1.1 again, the polarization control in an SFG experiment is demonstrated. For the Cartesian coordinate system shown in Figure 1.1, beams polarized parallel to the plane of incidence along the $y$-$z$ axis are $p$ polarized and beams polarized perpendicular to the plane of incidence and parallel to the surface plane in the $x$ direction are $s$ polarized. Since for an isotropic surface plane, $x$ and $y$ are interchangeable, the assignment of the surface plane depends upon user preference for right or left handed coordinate systems. Taking into account the right handed coordinate system from figure 1, the $ssp$ polarization combination probes the $\chi^{(2)}_{xzz}$ tensor element. The reader may further refer to the appendix in chapter 6 for a more detailed overview of the relationship between the elements of the $\chi^{(2)}$ susceptibility tensor and standard isotropic polarization combinations typically measured in a surface SFG experiment.
The form of the second order susceptibility tensor is the orientational number average of the second order molecular polarizability tensor.

$$\chi_{i,j,k}^{(2)} = N_s \langle \sum_{k,l,m} R_{\theta} R_{\phi} R_{\psi} \beta_{k,l,m}^{(2)} \rangle$$

In this instance, $N_s$ specifies the number density of molecules at the surface, and $\langle \ldots \rangle$ implies an orientational ensemble. The $k, l, m$ indices are summed over the molecular Cartesian coordinates generally labeled as $a, b, c$. $R_i$ are rotation matrices about the tilt $\theta$, azimuthal $\phi$, and twist angles $\psi$.

The second order hyperpolarizability near a vibrational resonance is proportional to the product of the infrared transition dipole vector $\mu_i$ and the molecular polarizability tensor $\alpha_{jk}$ or, equivalently, the Raman moment. Away from electronic resonance, the $\chi^{(2)}$ effectively becomes the sum of nonresonant and resonant contributions.

$$\chi^{(2)} = \chi^{(2)}_{nr} + \chi^{(2)}_{res}$$

The nonresonant contribution is negligible at dielectric interfaces but can be appreciable for metallic substrates due to the large free electron response and/or surface plasmons. At an infrared resonance, the resonant contribution to the second order susceptibility is:

$$\chi^{(2)}_{res} \propto \sum_n \frac{A_n}{\omega_{IR} - \omega_n + i\Gamma_n}$$

$A_n$ refers to the amplitude of the $n$th vibrational mode $\omega_{IR}$ the frequency of the IR excitation, $\omega_n$ the frequency of the infrared transition, and $\Gamma_n$ is the damping constant or equivalently linewidth of the transition. Whenever the frequency of the excitation IR approaches that of an infrared transition, the signal is resonantly enhanced, and thereby a spectrum of surface molecules may be obtained. Experimentally, the information presented in this thesis is acquired through the mixing of a spectrally broad (temporally short) mid-IR pulse which excites a multitude of vibrational modes within its spectral bandwidth. A spectrally narrow (temporally long) 800 nm pulse is used to upconvert the spectrum, and the spectral resolution is given by either the
bandwidth of the upconverting pulse or the natural linewidth of the molecular transition.

1.6 Time-Resolved SFG Spectroscopy

In order to probe vibrational dynamics and processes associated with those dynamics like molecular reorientation, an experiment needs to be performed which allows direct access to those population dynamics. A typical frequency resolved broadband SFG experiment allows access to time-averaged molecular information at the interface. In the absence of inhomogeneous linewidth broadening, energetic details such as vibrational coupling and reorientational dynamics are contained in the spectrum through the frequency components $\omega_n$ and the linewidth $\Gamma_n$ as presented in equation (1.13). The linewidth $\Gamma_n$ of a particular transition is directly related to the decay rate of the macroscopic polarization induced by the mid-IR pulse. In the time domain, this is simply the dephasing time of the free induction decay, $T_2 (= 1/2\pi\Gamma)$.\(^{27}\) Is it possible to just turn to the time domain and measure the free induction decay of the SFG signal? Since the homogeneous dephasing time is contained contributions from the population lifetime $T_1$ of the molecular vibration i.e. the and the pure dephasing time $T_2'$ related to the decay of the coherence induced by the mid-IR pulse i.e.\(^{28}\) For a purely homogeneously broadened system:

$$\frac{1}{T_2} = \frac{1}{2T_1} + \frac{1}{T_2'} \quad (1.14)$$

For inhomogeneous broadening, eq. 1.14 does not hold. Measuring only the decay of the SFG free induction decay does not necessarily disentangle vibrational population dynamics from pure dephasing. It measures the decay of the induced polarization, and the measurement of free induction decay is essentially just a time domain equivalent to frequency resolved SFG spectroscopy. In order to measure processes associated with population dynamics or reorientation of molecules, we take advantage of pump-probe spectroscopy in which we first populate an excited state and then follow that excited state in real time as it relaxes back to the ground state.
Pump-probe SFG spectroscopy was first demonstrated in the early 1990s for measuring the rate of vibrational energy transfer from molecules adsorbed to metallic surfaces under UHV conditions. Before the availability of table-top amplified femtosecond (fs) lasers, the technique was limited to systems with ps dynamics, and additionally, matters were further complicated by the low repetition rate of the available laser systems. With the advent of amplified high repetition femtosecond laser sources, pump-probe experiments at surfaces have been carried out at liquid surfaces starting with time-resolved second harmonic (SHG) measurements of solvation and orientational dynamics by Eisenthal’s group. More recently, McGuire and Shen demonstrated IR-pump, SFG probe spectroscopy at the water surface. Recent reviews by the Hamm and Tahara groups provide further details about how the experiments and modeling are more explicitly carried out. For a rigorous theoretical treatment, the reader may refer to works by Mukamel and Hamm.

A schematic of a pump-probe SFG process is shown below in figure 1.3. An intense mid IR pulse selectively excites a vibrational subset of molecules from the $\nu = 0$ ground state to the $\nu = 1$ first excited state, and the population dynamics may be followed while the selectively excited sub-ensemble of molecules relaxes back to the ground state. The relaxation processes associated with following molecules relax back to the ground state is done by first exciting the molecules with a pump pulse and then later watching some time later what fraction of molecules is still in the excited state. By changing the delay between pump and probe, the fraction of excited versus ground state molecules can be continuously monitored, and a measurement of the population dynamics can be obtained.
Figure 1.3 Schematic of an IR pump, SFG probe experiment. An intense mid-IR pump pulse preferentially excites molecules from the ground state to the first excited state. At some waiting time $\tau$ later, there are fewer molecules available for SFG. Energy diagram courtesy Ruth Livingstone.

The pump pulse will reduce the intensity of the effective $\chi^{(2)}$ tensor because the tensor is proportional to the number density of molecules at the surface as shown in equation (1.11) i.e. $\chi^{(2)} \propto N_s$. Under the influence of a pump pulse which excites molecules from the ground state to the first excited state, the difference in the ground state $\chi^{(2)}$ versus the excited state $\chi^{(2)} \Delta \chi^{(2)}$ will be reflected in the measurement, and this quantity is proportional to the number density difference between ground and excited states. This apparent decrease in the effective $\chi^{(2)}$ will manifest itself as a reduction (bleach) in the probe SFG signal. The population dynamics may be followed by physically scanning the delay between the IR pump and SFG probe pair at some pump-probe delay time $T$ allowing the system to equilibrate back to the ground state. The delay is scanned by changing the path length difference between the mid IR pump pulse and the SFG probe pulse pair. In the absence of intermediate states, the time dependence of the signal reflects the population dynamics of the ground state ($N_0(t)$) and the excited state ($N_1(t)$).

$$I_{\text{SFG}} \propto (N_0(t) - N_1(t))^2 = (1 - 2N_1(t))^2$$
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\[ \approx 1 - 4N_1(t) \]  

Pump-probe SFG spectroscopy may be thought of as a five wave mixing process which arises from the fourth order nonlinear polarization term in equations (1.3) and (1.4). If we introduce another mid-IR excitation (pump) pulse with electric field \( E_{IR,pu} \) and corresponding intensity \( I_{pump} \) and consider that the time-dependent signal which is detected arises from the mixing of both the static waiting time independent \( P^{(2)} \) and the dynamic delay time-dependent \( P^{(4)} \) response:

\[
I_{SFG,pu,pr} \propto |P^{(2)} + P^{(4)}|^2 \propto \]

\[
|\chi^{(2)}:E_{vis}E_{IR,pr} + \chi^{(4)}:E_{vis}E_{IR,pr}E_{IR,pu}E_{IR,pu}|^2 \propto \]

\[
|\chi^{(2)}:E_{vis}E_{IR,pr}|^2 + \]

\[
(\chi^{(2)}:E_{vis}E_{IR,pr})(\chi^{(4)}:E_{vis}E_{IR,pr}E_{IR,pu}E_{IR,pu}) + \]

\[
c. c. + |\chi^{(4)}:E_{vis}E_{IR,pr}E_{IR,pu}E_{IR,pu}|^2 \quad (1.16)\]

Since the signal emanating from the pure \( \chi^{(4)} \) response is vanishingly small compared to the other signals, it may be ignored. The measured waiting time-dependent response is given by the cross terms \( \sim \chi^{(2)} \chi^{(4)} \), and when the effects of any coherence generated by the pump pulse may be ignored, the \( \chi^{(4)} \) process may be thought of as a transient \( \Delta \chi^{(2)} \) process where it is now considered that the pump pulse induces transient changes in the \( \chi^{(2)} \) tensor.

Since the \( \chi^{(4)} \) response may be modelled as a \( \Delta \chi^{(2)} \) process, modelling of the orientationally specific response may be done by taking into consideration that azimuthal symmetry is broken in the surface plane thus allowing the creation of new tensor elements which will decay under both the influence of vibrational relaxation and that of molecular reorientation to the isotropic ground state population. In the next section, polarization control of the mid-IR pump pulse is taken advantage of to monitor the influence of reorientational dynamics on the measured transients.
1.7 Time and Polarization Resolved SFG: reorientation

Analogously to fluorescence anisotropy decay using the time-dependent dichroic ratio and bulk sensitive IR polarization anisotropy decay, which probe molecular reorientation in bulk liquid samples, polarization anisotropy may be applied to interfaces through time- and polarization-resolved second harmonic generation$^2,34-36$ and analogously time- and polarization-resolved SFG (TP-SFG) to assess reorientational dynamics at interfaces.$^1,8,37-40$ The inherent asymmetry of the interface complicates matters, but a simple analogy may be made. Linearly polarized light aligned parallel or respectively orthogonal to molecular dipole moments will cause the dipoles which are aligned parallel to the pump to reorient out of the equilibrium orientation distribution and likewise orthogonally aligned molecules orient into the plane of incidence. These motions lead to an apparent speed up or decrease in the measured dynamics. The modeling of the experimental data is not straightforward due to preferential orientation of the dipoles at equilibrium, so in general, a numerical model must be used user input about orientational relaxation to model the data.

The population dynamics which are measured during a pump-probe SFG signal are due to both vibrational relaxation back to the ground state and molecular reorientation. When the pump pulse is oriented parallel to the transition dipole moment of the probe, excited vibrational modes will reorient out of the probing window and the measured signal will correspondingly be dependent on vibrational relaxation and motions of the transition dipoles. Analogously, when the pump pulse is perpendicular to that of the probe’s transition dipole, the excited vibrational motions which lie out of the plane of the probe reorient into the probing window. First we approximate the rates of recovery of the complex decay curves which are pumped parallel and perpendicular to the transition dipole moment as single exponential kinetics. By simplifying the complex orientation and vibration rate dependent trace as single exponential, we can get a semi-quantitative approximation of the orientation rate. A simple exponential approximation$^8$ of the influence of reorientation on the measured population dynamics is given below in equations 1.17 to 1.2
An effective rate of reorientation ($k_{\text{eff, reor}}$) and a vibrational relaxation rate ($k_1$) can be calculated from equations (1.18) and (1.19). The experimentally measured parallel (perpendicular) dynamics are sped up (slowed down) due to reorientation by a factor of $k_{\text{eff, reor}}$ as shown in equations 1.18 – 1.19. If we then infer that the vibrational relaxation rate $k_1$ is calculated from the average of the rate constants of the two traces ($k_\parallel, k_\perp$) as shown in equation (1.17), an effective reorientation rate can be calculated using equation (1.20). While this simple approximation allows for assessing a qualitative trend in reorientation rates at the surface, symmetry breaking at the surface by the pump pulse breaks the azimuthal symmetry at the surface thereby allowing for new tensor elements in $\chi^{(2)}$. In order to interpret the experimental response of a TP-SFG experiment, we turn to molecular dynamics simulations to extract the rates of molecular reorientation, and these results are then used as parameters in a numerical simulation which is used to simulate the transient SFG signal. The brief formalism relating to the influence of molecular reorientation on SFG transients below is presented in more detail in reference.\(^{38}\) The reader can refer to chapters 3 and 4 for further details regarding the molecular dynamics simulations.

Consider an ensemble of SFG vibrational chromophores which is oriented at a tilt angle $\theta$ with respect to the surface normal ($z -$ axis). The chromophores are isotropically distributed across the x-y plane; that is, the orientational distribution about the azimuthal angle $\varphi$ is invariant under rotations at equilibrium. This can be represented by a unit vector about a central atom, for example, the central carbon of a methyl group or the
oxygen atom in a water molecule. This is schematically demonstrated in Figure 1.4.

![Figure 1.4](image)

**Figure 1.4.** Pump pulse of variable linear polarization incident upon a surface with molecules oriented isotropically along the azimuthal angle $\phi$ and with net dipole orientation. $\theta$ refers to the angle from the surface normal and $r$ the unit vector.

The linearly polarized pump pulse will break the azimuthal symmetry thereby inducing different angular distributions in the surface plane as a function of pump polarization. Let us ignore changes in the tilt angle distribution and only consider changes in the distribution $\rho(\phi)$ along the azimuthal angle $\phi$. The orientational fluctuations may be modeled by the diffusion equation.

$$
\frac{\partial \rho(\phi, t)}{\partial t} = D \frac{\partial^2 \rho(\phi, t)}{\partial \phi^2}
$$

(1.21)

where $D$ is the rotational diffusion constant and in general has units of inverse time. Since in this case, we are referring to diffusion about an angular coordinate, the units are rad$^2$/s.

The generic solution to this diffusion equation is given by the Fourier series:
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\[
\rho(\varphi, t) = a_0 + \\
\sum_{m \geq 1} [a_m \cos(m\varphi) + b_m \sin(m\varphi)] \times \cdots \\
\cdots \times e^{-m^2 Dt} \quad (1.22)
\]

where \(a_0\), \(\cos(m\phi)\) and \(\sin(m\phi)\) are eigenfunctions of the diffusion equation. Since the probability of molecules being excited by the pump pulse is proportional to \(|\mu E(t)|^2\), linearly polarized pump pulses will induce an anisotropic population of molecules at the surface. Two limiting cases of s and p polarization pulse may be considered. When the pump is s polarized along the laboratory x axis defined in figure 1.4, transition dipoles which lie along the x-y plane are primarily excited, and when the pump is p polarized, it is defined such the excitation lies along the laboratory yz plane at an angle of \(\alpha\) with respect to the surface normal which is defined by the angle of incidence of the pump beam. If we additionally consider that the transition dipole moment \(\mu\) is oriented at an angle of \(\theta_0\) with respect the surface normal, \(\mu = \mu_x e_x + \mu_y e_y + \mu_z e_z = \mu_0 \cos\phi \sin\theta_0 e_x + \mu_0 \sin\phi \sin\theta_0 e_y + \mu_0 \cos\theta_0 e_z\) where \(e_i\) are unit vectors along the specified axis. Considering that additionally \(E_s = E_s e_x\) and \(E_p = E_p \sin\alpha e_y + E_p \cos\alpha e_z\), the excitation probabilities for s and p polarized pump pulses then become:

\[
|\mu \cdot E_s|^2 = (E_s \cos\phi \sin\theta_0)^2 = \\
\frac{1}{2} I_s \sin^2 \theta_0 (1 + \cos 2\varphi) \quad (1.23)
\]

and

\[
|\mu \cdot E_p|^2 = \\
I_p (\sin\phi \sin\theta_0 \sin\alpha + \cos\theta_0 \cos\alpha)^2 \quad (1.24)
\]
Considering equation (1.22) and defining the excited state population distributions at time zero to be \( \rho_s(\phi,0) = \rho_{s0} \) and \( \rho_p(\phi,0) = \rho_{p0} \) (where the initial \( \rho_i(\phi,0) \) values are proportional to the intensity of the pump pulses at the interface) we can write for the population distribution of excited molecules under \( s \) and \( p \) polarization:

\[
\rho_s = A_s [1 + \cos(2\phi) e^{-4Dt}]
\]

\[
\rho_p = A_p + A_p \sin\phi e^{-Dt} + A_p \cos(2\phi) e^{-4Dt}
\]

(1.25)

Where:

\[
A_s = \frac{1}{2} \rho_{s0} \sin^2 \theta_0
\]

\[
A_{p0} = \rho_{p0} \left( \frac{1}{2} \sin^2 \theta_0 \sin^2 \alpha + \cos^2 \theta_0 \cos^2 \alpha \right)
\]

\[
A_{p1} = \frac{1}{2} \rho_{p0} \sin 2\theta_0 \sin 2\alpha
\]

\[
A_{p2} = \frac{1}{2} \rho_{p0} \sin^2 \theta_0 \sin^2 \alpha
\]

(1.26)

The effect of vibrational relaxation may be taken into account by multiplying the population distributions \( \rho \) by a factor \( e^{-t/T_1} \) where \( T_1 \) is the vibrational lifetime of the excited chromophore. This lifetime should be independent of orientational relaxation. Knowing the functional form of the distributions \( \rho \), the time dependent SFG response may then be calculated considering the tensorial nature of the SFG response as presented in equations (1.10) and (1.11). The pump induced changes in the second order susceptibility will then be:

\[
\Delta \chi^{(2)}(t) = -\int_0^{2\pi} \rho_\sigma(\phi, \theta_0, t) \beta(\theta_0, \phi) d\phi
\]

(1.27)

The index \( \sigma \) represents the polarization of the beam and \( \beta \) the hyperpolarizability. Considering the eigenvalues \( f_m(\phi) = 1, \sin\phi, \) and \( \cos 2\phi \), a new tensor \( B_m \) can be defined such that:
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\[ B_m = \int_0^{2\pi} f_m(\varphi) \beta(\theta_0, \varphi) d\varphi \]  

(1.28)

Using (1.25) the dynamic susceptibilities may be derived to be:

\[ \Delta \chi_s^{(2)} = -\rho_{s0} (A_p B_0 + A_p B_1 e^{-Dt} + A_p B_2 \cos(2\varphi) e^{-4Dt}) \]

\[ \Delta \chi_p^{(2)} = -\rho_{p0} (A_s B_0 + B_2 e^{-4Dt}) \]  

(1.29)

where the \( A \) coefficients are defined in equations (1.26).

By selectively probing certain elements of the susceptibility tensor through polarization control of the SFG experiment, different parts of the differential susceptibility tensor may be probed. From table 1 of reference,\textsuperscript{38} the rate at which different transient tensor elements decay may be estimated. With the polarization combination sps, which is used in Chapters 3 and 4 to study leucine, the effective intensity probed by this polarization combination is \( I_{sps} \propto |\chi_{zzx}^{(2)}|^2 \) and as an estimate, the decay of the transient sps signal will have a rate of 4D. The transient SFG signals may be calculated by considering that the measured response \( |\Delta \chi_{tot}^{(2)}|^2 = [\chi^{(2)}(t)_{pump}]^2 - [\chi^{(2)}_{unpumped}]^2 \). The relationship of total measured signal to the transient signal will then be \( |\Delta \chi_{tot}^{(2)}|^2 \propto \chi^{(2)}_{unpumped} \Delta \chi^{(2)}(t) \).

Reference\textsuperscript{41} also introduces an anisotropy experiment to extract the measured rates of reorientation by calculating an anisotropy ratio between the differentially pumped probe traces, and this is further discussed in chapters 3 and 4. Unfortunately, the previous approximations neglected the effects of reorientation in the \( \theta \) coordinate. The diffusion equation then becomes a problem of solving the 2-dimensional diffusion equation in spherical coordinates with a harmonic potential governed by the angular spread \( \Delta \theta \) of tilt angles:

\[ \frac{\partial \rho}{\partial t} = \frac{D_\varphi}{\sin^2 \theta} \frac{\partial^2 \rho}{\partial \varphi^2} + \frac{D_\theta}{k_B T} \frac{\partial \rho}{\partial \theta} \frac{\partial V}{\partial \theta} + \frac{\rho D_\theta}{k_B T \sin \theta} \frac{\partial}{\partial \theta} \sin \theta \frac{\partial V}{\partial \theta} \]  

(1.30)
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\[ V(\theta) = \frac{k_B T}{2(\Delta\theta)^2} (\theta - \theta_0)^2 \]  

(1.31)

The solution to this equation may then be obtained numerically through a procedure established in the Nienhuys paper.\textsuperscript{38} Now, reorientational diffusion can occur along the azimuthal and tilt angles. The diffusion rates and angular spreads are determined by molecular dynamics simulations, and this is shown in chapter 3 and 4.

Finally, Figure 4 shows contour graphs which show the evolution of the distribution function \( \rho(\theta, \phi, t) \). On the left side, the azimuthal symmetry at the surface is broken, and this shows different populations for s and p pumping. Notice that not only does the azimuthal distribution change, but the \( \theta \) distribution also is perturbed away from equilibrium. On the right-hand side, in the absence of the pump, no preference is shown in the azimuthal direction while the tilt angle displays its equilibrium distribution.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Simulated population distribution \( \rho(\theta, \phi, t) \) at time \( t=0 \) and time \( t = \infty \)}
\end{figure}