Watching molecular motion at interfaces

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3. Reorientational dynamics of leucine monomers at the air-water interface

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Acknowledgements: Y.Y. Yimer performed the computational work related to MD simulations and extracted the reorientational rates. M. Donovan performed all the experiments, analyzed and interpreted the data ran numerical simulations with parameters from molecular dynamics. T Weidner, J.P, and M Bonn supervised the work.

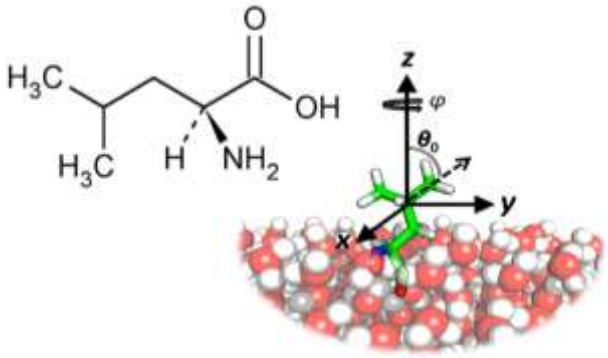
ABSTRACT: Many proteins throughout nature consist of leucine containing residues. Here, we take a first step toward understanding interfacial protein side chain dynamics by measuring the reorientational dynamics of leucine monomers. In this chapter, we demonstrate that ultrafast reorientation dynamics of leucine amino acids at interfaces can be recorded in situ and in real time using polarization- and time-resolved pump-probe sum frequency generation (SFG). Combined with molecular dynamics simulations, time resolved SFG was used to probe the reorientation of the isopropyl methyl groups of L-leucine at the air-water interface. The data show that the methyl units reorient diffusively at an in plane rate of $D_{\varphi} = 0.07$ rad^2/ps and an out of plane rate of $D_{\theta} = 0.05$ rad^2/ps.

3.1 Introduction

The key to protein function lies not only in protein structure but also in protein dynamics[9]. Different dynamical timescales correspond to a wide variety of protein motions.[9] Folding, for example, occurs on nano- to microsecond time scales.[10] Protein side chains undergo torsional motions occurring on pico- to nanosecond timescales.[9,44] It has been shown that side chain dynamics play a role in enzymatic catalysis[44] and protein-protein interactions[11]. Moreover nuclear magnetic resonance (NMR) studies have related changes in side chain entropy to the local environment of a protein complex.[11] Backbone[45,46] and side chain dynamics have been widely studied in bulk utilizing spin techniques like NMR and electron paramagnetic resonance, vibrational spectroscopy[47] and electronic spectroscopy.[48] However, this information is lacking for proteins at interfaces. At the same time, understanding how proteins interact with surfaces is of utmost importance in such varied fields as biomineralization, membrane proteins, biomaterials and biomechanics.[49] Dynamic protein side chain interactions are of utmost importance at interfaces mainly because the protein side chain is directly in contact with the interface.[50,51] In particular, methyl containing residues can display markedly different dynamics which correspond to changes in local chemical environment.[11]

Leucine residues are present in a variety of peptides and proteins throughout nature, and in order to unlock the side chain dynamics in large proteins, it is essential to first develop and test new methods to track the motions of a constituent piece of the puzzle. Toward understanding side chain dynamics, we have chosen to study the dynamics of L-leucine at the air/water interface as a model system (see Scheme 3.1). Even though the dynamics of L-leucine is likely different from leucine side chains within proteins, it is still an ideal model system, since its adsorption behavior is well known from a number of previous static sum frequency generation (SFG) studies.[50,52-58] SFG vibrational spectroscopy is ideally suited to probe the equilibrium and dynamic properties of interfacial species.[18,19,35,50,51,54,55,59-68] Its surface sensitivity is linked to the fact that even-order nonlinear optical processes are forbidden in centrosymmetric bulk media under the electric dipole approximation.[24] As such, second- and fourth-order SFG are sensitive to interfacial order and orientation.

SCHEME 3.1: Leucine adsorbed to the air-water interface.



The orientation of the indistinguishable methyl groups can be characterized by a mean angle θ_o within an angular spread $\Delta\theta$. Reorientation can occur within the plane of the surface in the φ coordinate independently of out of plane reorientation bounded by $\Delta\theta$.

Richmond and Shen have previously determined the orientation and saturation coverage of L-leucine at the oil-water and air-water interface respectively[54,55]. Cremer et al. have determined the structure of related isopropyl at the air/water interface.[18] Furthermore, much work has been done to determine the orientation of aliphatic side chains of leucine/lysine peptides at both hydrophobic and hydrophilic interfaces.[35,50,56,69,70,31] Leucine was concluded to bind to the air/water interface with the isopropyl group oriented towards the vapor phase and the polar head group pointing towards the water (Scheme 1).

While the static properties of leucine have been well studied, dynamic information is clearly lacking. Models for the effect of reorientational motion on SFG spectra have been proposed, and it has been suggested that reorientational motions can be inferred indirectly by comparing the linewidths of the methyl asymmetric stretch using different polarization combinations as has been done by the Benderskii and Fourkas groups.[66,71,72] However, only a time-resolved pump-probe experiment allows to directly quantify these dynamics.

Pump-probe variants of SFG – time[42,73,37,40,74-76] and polarization resolved SFG (TP-SFG)[37-39,77] have been used to study dynamics of specifically interfacial molecules. Reorientational dynamics of dangling OH groups at the water-air interface have been directly measured using TP-SFG,[37] and this motion has been compared with reorientational diffusion influenced spectroscopic models developed by both Nienhuys[38] and the Fayer group[38,78]. Time resolved SFG has also been used to study peptide backbone dynamics.[79] Dynamics experiments on lipid alkyl chains at interfaces, related to the side chain dynamics followed here, have also been reported.[41,80] These studies reported vibrational relaxation rates, but molecular reorientation was not resolved because of fast intramolecular energy transfer from CH_3 to CH_2 modes, i.e. the energy had already dissipated before the molecular reorientation took place. The methyl and methylene groups within the leucine isopropyl group are separated. This architecture suppresses the intramolecular energy dissipation through CH_2 groups and should therefore provide lifetimes long enough for reorientational studies.

Here, we employ TP-SFG to monitor the reorientation dynamics of the isopropyl methyl groups of leucine at the air / D_2O interface in real time. The results are interpreted with the help of molecular dynamics (MD) simulations. For the experiments a broad mid-IR pump pulse was spectrally centered on the methyl asymmetric (AS) stretch to label the molecule with a vibrational excitation in that mode, and the linear polarization of the pump pulse was alternated between s and p polarization. Transient SFG spectra are recorded as a function of both pump polarization and pump-probe delay time. The decay of the transient signal occurs due to both vibrational relaxation and reorientational motion. Pump pulses are aligned parallel (orthogonal) to the transition dipole moment induced by the probe pulse, and subsequently, excited molecules move out of (into) the probe window giving rise to pump-polarization dependent recovery of the time-dependent SFG probe signal.[37] A comparison of the two time-dependent signals obtained with orthogonal pump polarizations provides the timescale of reorientational motion. [37,39]

3.2 Experimental Results

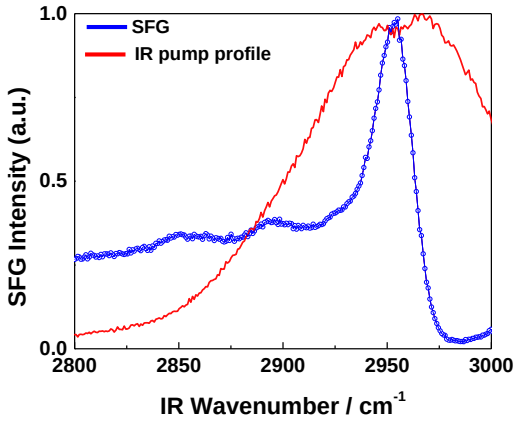


Figure 3.1 *Static sps SFG spectrum (blue) for positively charged L-leucine at saturation coverage[54]. The profile of the excitation pulse is shown in red.*

Figure 3.1 shows a static SFG spectrum of the C–H stretching region of a 0.8 M solution of positively charged L-leucine at pH 0.1 at saturation coverage[54] adsorbed to the air-water interface in the sps (s-polarized SFG, p-polarized VIS, s-polarized IR) polarization combination. The observed spectrum is similar to previous spectra reported for this moiety.[50,52,54,55,57] Differences in the shape of the non resonant background are likely explained by differences of the experimental setup. The main resonance at 2960 cm^{-1} corresponds to the in-plane component of the asymmetric stretching vibration (AS).[59,81] Moreover, small unresolved contributions are observed at the red end of the spectrum. The aliphatic C–H region is subsequently depleted by a strong mid-IR pump pulse centered at 2970 cm^{-1} (red curve in Fig. 1).

Figure 3.2 shows the results from a TP-SFG measurement with pump pulses parallel (s) and perpendicular (p) to the IR probe pulse for the sps probe (see SI for ssp data). The (s)-pumped data (blue) display a larger

bleach and faster recovery than

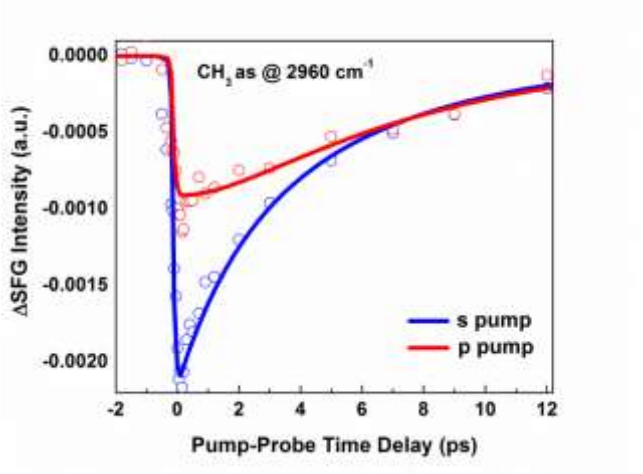


Figure 3.2 *TP-SFG Mid-IR pump, SFG sps probe traces for s (blue) and p (red) pumped methyl groups. Signals are integrated over a spectral range of 2945 cm^{-1} to 2975 cm^{-1}. Plotted is the difference in integrated intensity of (I_{SF}(Pumped) – I_{SF}(Unpumped)) divided by the nonresonant I_{SF} from a z-cut quartz reference. Curves simulated according to the model described in ref [38] using MD simulation results are shown along with experimental data (circles).*

the (p)-pumped (red) data. The initial (zero delay) anisotropy can be explained by noting that the methyl groups aligned parallel to the pump field are most efficiently excited, while methyl groups aligned orthogonally to the excitation pulse will produce a smaller differential SFG signal[37]. The anisotropy shows that the leucine side chains are well aligned with respect to the interface.

Vibrational and reorientational dynamics of the methyl units jointly determine the signal recovery rates for s and p-pumped traces. The observed difference in rate constant for the orthogonally pumped pulses is primarily due to side chain reorientation, since vibrational relaxation should be independent of the linear polarization of the pump pulse.[41] Approximating the s and p pumped traces by simple single-exponential kinetics, we recover time constants of $(k_{1s})^{-1}$ = 4.2 ps and $(k_{1p})^{-1}$ = 6.9 ps respectively (see SI for mono-exponential fits). The s pumped trace recovers faster, since the

recovery is governed by both vibrational relaxation (rate k_1) and reorientational motion out of the plane of polarization, i.e. $k_{1s} = k_1 + k_{eff,diff}$, where we have defined an effective reorientation rate $k_{eff,diff}$. Similarly, for p-polarized pump, we can approximate the overall rate by: $k_{1p} = k_1 - k_{eff,diff}$. The two observed lifetimes then give estimated diffusion time of $(k_{eff,diff})^{-1} = 21.5$ ps, and a vibrational lifetime $T_1 = (k_1)^{-1} = 5.2$ ps.

3.3 Molecular Dynamics Simulations

For a more detailed analysis of the interfacial reorientation dynamics, we complement the SFG experiments with MD simulations. To computationally determine the molecular ordering and reorientational diffusion of the methyl groups of leucine at vacuum/water interface, we performed all-atom MD simulations at 298 K. The simulation box contained 40 leucine molecules and 40 chlorine counter ions in an aqueous solution containing 540 water molecules (see SI for further details). Representative snapshots of leucine molecules at vacuum/water interface are shown in Figure 3.3(a). As suggested by previous SFG experiments[54,55], leucine molecules show strong ordering at the interface and expose the methyl groups towards the vacuum.[50,54-56] Figure 3.3(b) shows the tilt (θ) angle distributions of the two individual methyl groups and an average of both methyl groups. As seen in figure 3.3(b), θ shows a normal distribution with peak values near 60°. The average orientation was determined to be $\theta_0 = 64°$ with a distribution width of $\Delta\theta = 27°$. This angle is in reasonable agreement with values determined experimentally

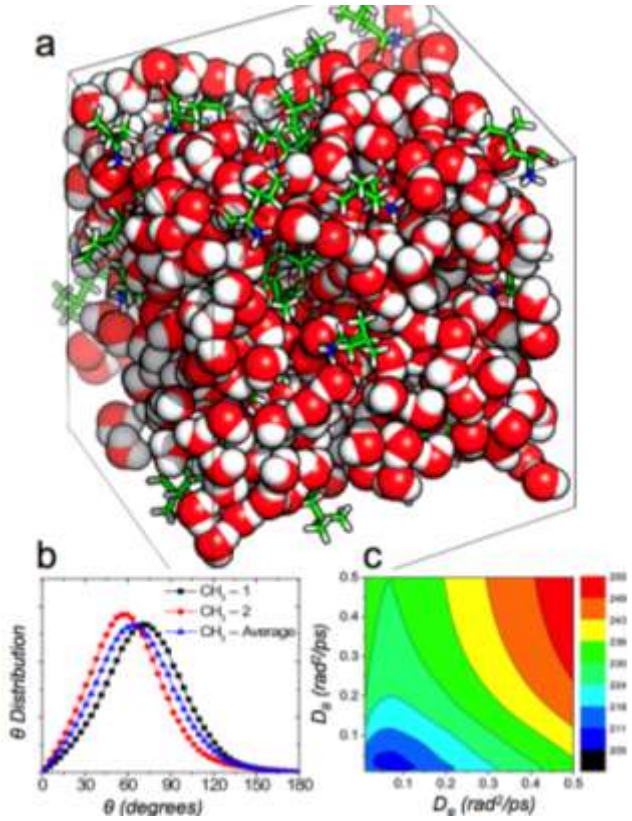


Figure 3.3 *Molecular dynamics simulations of Leucine at air/water interface at 300 K. Snapshot of leucine molecules at vacuum/water interfaces: view (a) of the simulation box. (b) Leucine molecule methyl groups angle θ distributions (c) Variation of* χ^2 *with* D_φ *and* D_θ *for methyl groups at the interface.* χ^2 *has the minimum value at* $D_\theta = 0.05 \pm 0.009$ rad^2/ps *and* $D_\varphi = 0.07 \pm 0.003$ rad^2/ps.

by Shen *et al.*, who reported an angle of 39° for leucine.[54] The angular distribution of the two methyl units is almost identical. In view of the structural degeneracy, we will not differentiate between the groups in our model and use the ensemble averaged values (Fig. 3(b)). Contrary to the tilt angles, the methyl groups' φ angles are completely random at the interface (see SI Figure S6).

The reorientational in-plane (D_φ) and out-of-plane (D_θ) methyl diffusion coefficients at the vacuum-water interface were determined by first

numerically guessing values of the diffusion coefficient to generate a numerical solution to the two dimensional rotational diffusion equation and then calculating and minimizing the square of residuals, χ^2, between the time-dependent methyl group population distributions directly obtained from the simulation as a function of θ and φ (see SI for details). We determined that χ^2 has the minimum value at $D_\theta = 0.05 \pm 0.009$ rad^2/ps and $D_\varphi = 0.07 \pm 0.003$ rad^2/ps (Fig. 3(c)), which shows the leucine methyl groups reorient – on average – at comparable rates in and out of plane.

The obtained diffusion coefficients, together with the simulated tilt angles, are used to model the effect of molecular reorientation on the transient SFG signal.[37,38] In order to compare the rate of molecular reorientations derived from MD simulations to the experimental response of the TP-SFG traces, we numerically simulate the solutions to the rotational diffusion equation utilizing the diffusion coefficients and tilt angles derived from MD to calculate the transient SFG response. For the calculation we chose a vibrational lifetime of T_1 = 6.25 ps for the AS methyl stretch because it closely resembles previous lifetimes for methyl symmetric stretching modes[41,80] and best describes the experimental data. Details and limitations of the numerical simulations, such as additional possible relaxation channels and the molecular geometry, are summarized in the SI and ref. [38,41]

In Figure 3.2, the numerically simulated SFG signals are compared to experimental TP-SFG traces. The simulated and experimental traces match very well, implying that the diffusion rates obtained from the simulations accurately capture the leucine dynamics. The out of plane diffusion rate is directly related to the out of plane reorientational rate constant by $k_\theta = D_\theta / \Delta\theta^2$.[38] Based on this model, out of plane reorientation occurs within 4.4 ps. In plane reorientational diffusion occurs on a scale of $(D_\varphi)^{-1}$ = 14.4 ps, but the effect of in plane reorientation on the SFG signal itself can be evaluated by considering the in plane anisotropy which decays at a rate[38] of $4D_\varphi$ = 0.28 rad^2/ps or equivalently with a rate constant of 3.6 ps (SI Figure 3-S3). These timescales are comparable to fast internal motions (5-80 ps) of methyls in hydrophobic leucine residues in contact with the hydrophobic core of a protein obtained from NMR measurements.[82]

It is interesting to compare our results to previous TP-SFG studies of the CH_3 stretch of methyl groups of long chain alcohols self-assembled at surfaces.[39,80] No anisotropy was reported for the methyl stretch of that system, which was attributed to efficient inter- and intramolecular coupling pathways between the different methyl (and possibly methylene) modes.

This coupling is apparently enhanced for the alkyl chains reported in ref [36] compared to the leucine side chains, where the isopropyl methyl units are not neighboring methyl groups. This effect is also apparent from the accelerated vibrational energy relaxation for the alkyl chains – 3.6 vs. 6.25 ps for leucine.

In summary, this communication establishes the real-time observation of molecular reorientation for an amino acid side chain at an interface. We show that time resolved SFG spectroscopy can provide detailed information about side-chain dynamics when combined with MD simulations. An effective diffusion coefficient of 21.5 ps is calculated from the experimental values, and additionally, from MD simulations, we determined in- and out-of-plane molecular reorientation coefficients of $D_{\varphi} = 0.07 \pm 0.003$ rad^2/ps and $D_{\theta} = 0.05 \pm 0.009$ rad^2/ ps respectively.

3.4 Supporting Information

3.4 a. Experimental

For further details about the experiment, refer to Chapter 2. The pump is spatially overlapped with the spatiotemporally overlapped probe pair. Energies of 5, 4, and 20 μJ are utilized for the mid IR probe, visible upconversion, and mid IR pump pulse respectively at respective angles of 43, 53, and 55 degrees from the surface normal. Mid-IR pump, visible SFG is spatially filtered by an iris before the probe SFG signal is separated into pumped and unpumped components by a vibrating galvano-mirror synchronized to the mechanical chopper at 500 Hz. The signal is then sent to a spectrometer (Princeton Instruments) and spectrally dispersed onto a peltier cooled EMCCD (Andor) where the pumped and unpumped signals are simultaneously monitored using LabView (National Instruments) software. Both the time zero and instrument response are obtained by measuring the time dependent $\text{Infrared}_{\text{pump}}\text{Infrared}_{\text{probe}}\text{Visible}$ (IIV) response from a gold sample. Time zero is defined as the maximum of the IIV intensity, and the Gaussian FWHM (154 fs) of this temporal IIV signal determines the instrumental response time. S pumped spectra are acquired for one minute, and subsequently, an automated λ/2 plate rotates the pump polarization to p before the p pumped signal is acquired for 1 minute and

subsequently, the measurement moves to the next delay point. 33 scans were averaged for each pump probe trace. The sample trough is rotated at approximately 8 rpm to avoid sample damage due to laser heating. Solutions at 0.1 pH of 0.8 M L-leucine (Acros Organics) in D_2O were prepared by adding HCl to the leucine solution. This low pH was chosen to maximize the available signal intensity in accordance with the work of Ji and Shen .[54] D_2O is used to avoid interference between hydrogen bonded water and CH stretches. 33 scans were averaged for each pump probe trace. For normalization purposes, the nonresonant SFG signal from z-cut quartz is taken as the IR profile. Plotted signals for ΔSFG equal to (SF Pumped – SF Unpumped) / (SF Quartz) where SF refers to the SFG signal integrated over the spectral window from 2945 to 2975 cm^{-1}. The signals are rescaled to show the modulation of the SFG signal when the pump is on.

3.4 b. Single-Exponential Fits

Single exponential fits to the experimental data were performed by assuming the CH_3 vibration relaxes back to the ground state. The exponential fit is convoluted with a Gaussian fit to the $Infrared_{pump}Infrared_{probe}Vis$ (IIV) instrumental response function with FWHM of 154 fs to produce the traces shown. Fits were performed using a matlab routine written in house by R. Livingstone. The parallel (S) pumped trace is seen to recover in approximately 4.2 ps whereas the orthogonally P pumped traces recovers in approximately 6.9 ps. Figure 3-S1 shows these fits.

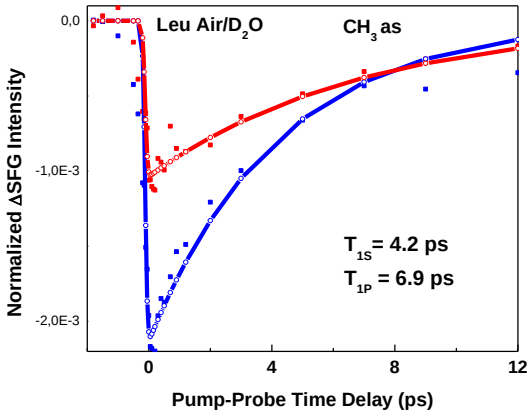


Figure 3-S1 *Single-exponential fits to experimental data.*

3.4 c. Variable vibrational relaxation

Additionally, we used previously published values for interfacial vibrational relaxation of CH_3 asymmetric stretching modes. In figure 3-S2, the experimental data is compared to simulations following Nienhuys' model.[38] The value of vibrational relaxation which most closely follows the experimental relaxation is 6.2ps. Figure 3-S2 shows the effect that different inputs for the vibrational lifetime parameter have on the numerically simulated sps probe signal.

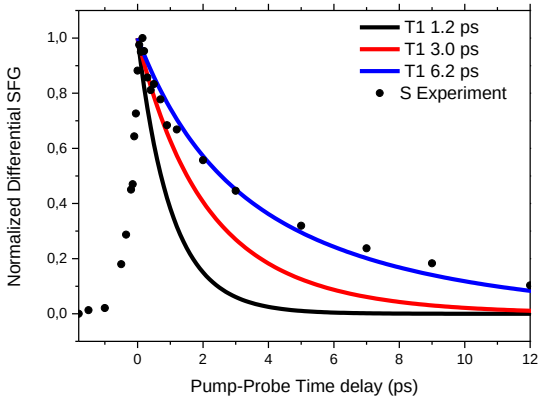


***Figure 3-S2**: Comparison of different values for vibrational relaxation constant using the model from ref.* [38].

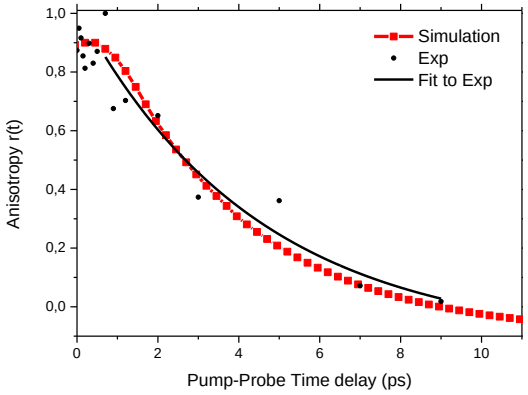


***Figure 3-S3**: Measured and simulated anisotropy decay.*

The effect of in plane reorientation on the measured SFG signal can also be estimated by the decay of the anisotropy decay which was defined by Nienhuys[38] for transient SFG spectra to be:

$$r(t) = {}^{c_-}/_{c_+} = \frac{1}{2} e^{-4D_\varphi t}$$

for the case of sps probe polarization:

$$c_- = \Delta\chi(t)^{(2)}_{xzx:x} - \Delta\chi(t)^{(2)}_{xzx:y} \quad c_+ = \Delta\chi(t)^{(2)}_{xzx:x} + \Delta\chi(t)^{(2)}_{xzx:y}$$

$$c_-/c_+ = \frac{s-p}{s+p}$$

In Figure 3-S3, the value c_-/c_+ is calculated directly from the experimental values and plotted, and s and p refer to s and p pumped transients respectively. The simulated value uses the aforementioned $D_\varphi = 0.07$ rad^2/ ps which is derived from MD simulations. The exponential form of r(t) is convoluted with the IIV instrumental response function to give the plotted simulation curve. In addition a single exponential fit is done from 100 fs to 9 ps on the calculated experimental anisotropy. A resulting single exponential fit leads to a lifetime of 4.4 ps which compares reasonably well to the simulated lifetime of 3.6 ps. An in-plane reorientational lifetime can be approximately just taken to be ($1/D_\varphi$) or 14.4 ps. The single exponential fit to the experimental data thus obtains a rate constant of ($1/4\ D_\varphi$) = 4.4 ps yielding a comparable value of ($1/D_\varphi$) of 17.6 ps or equivalently D_φ = 0.057 rad^2/ps.

3.4 d Additional Pump-Probe Traces

Previously, efforts by Yamamoto et. Al. and Bredenbeck et. Al. have studied used anisotropic linear IR pulses to monitor reorientational dynamics of long chain alcohols with a single CH_3 group such as 1-heptanol and 1-dodecanol at the air-water interface.[39,41] It was shown through TP-SFG[39] that anisotropic pumping of the CH_3 ss mode through an ssp probe did not lead to any noticeable anisotropy, and with the additional help of 2D-SFG[41,42], it was shown that ultrafast intramolecular energy transfer through the symmetric CH_2 modes was responsible for the lack of anisotropy in the observed time traces. Below in figure 3-S4, we show the results of separate pump-probe anisotropy measurements of the long chain alcohol 1-dodecanol adsorbed to the air-water interface. In panels a and b, the ssp spectrum is shown with the ss mode at 2875 cm^{-1}. The observed

decay of an S and P pumped ss mode is shown, and it matches the literature result.[41] A delayed ingrowth is shown in the S pumped signal, and this is attributed to efficient intramolecular energy transfer. Likewise in c, the sps spectrum of the as CH_3 stretching vibration of 1-dodecanol is shown along with the decay and recovery of the signal in panel d. No significant anisotropy is seen for this AS mode because, as before, intramolecular energy transfer is seen to be accelerated for dodecanol. In addition, one may try the ppp polarization combination, but since this mode probes lots of possible tensor elements of the nonlinear susceptibility tensor $\chi^{(2)}$ and is highly dependent on Fresnel factors, we have chosen to neglect this polarization combination in favor of ssp and sps. In addition to the pump-probe traces shown in the manuscript, we have also studied the pump-probe recovery of the ssp probe SFG signal. In panels a and b of figure 3-S5, the respective static spectrum and pump-probe recovery of the ss CH_3 mode are shown. Again, similarly to the case for the ss and as CH_3 modes of dodecanol, no significant anisotropy is seen in the bleach for the s and p pumped traces. Through our observation, this mode is not a good reporter of the reorientational dynamics of the L-leucine system. As seen in the manuscript, significant anisotropy is seen between the s and p pumped signals of the as CH_3 stretch in the case of L-leucine, and this might also occur because the molecular geometry is such that ultrafast intramolecular vibrational energy transfer is suppressed in this mode allowing for in situ observation of the reorientational dynamics.

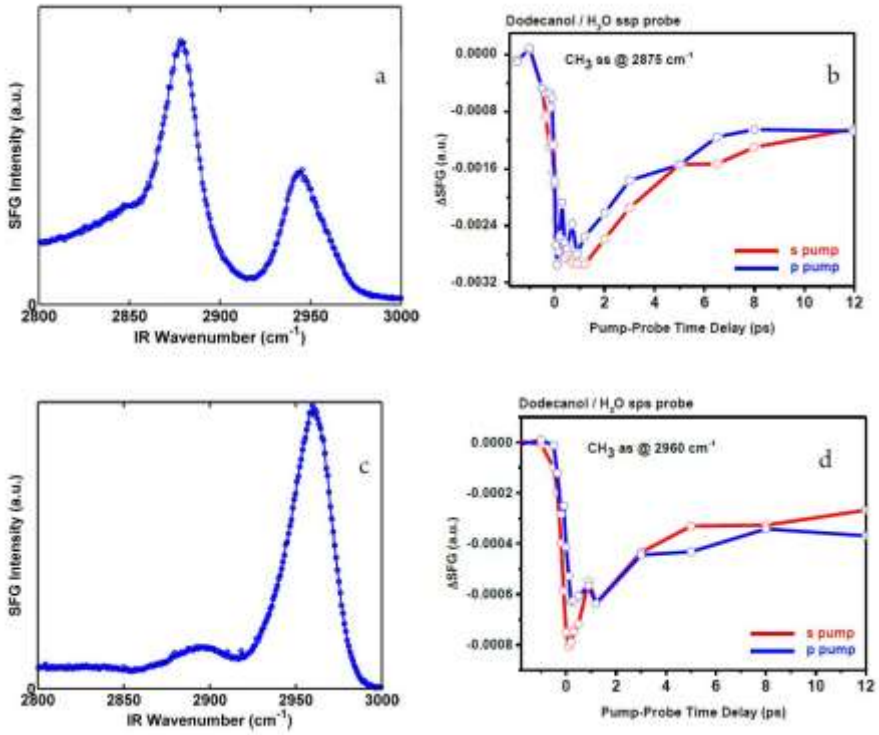


Figure 3-S4*: Static spectra and pump-probe dynamics traces for 1-dodecanol adsorbed to the air-water interface (a) and (b) show the static ssp spectrum and bleach and respective recovery of the pump probe signal over a spectral window of 2865 to 2895 cm^{-1} while panels (c) and (d) show the sps static spectrum and respective recovery of the pump probe signal over a spectral window of 2945 to 2975 cm^{-1} Lines shown in the pump probe traces are guides for the eye.*

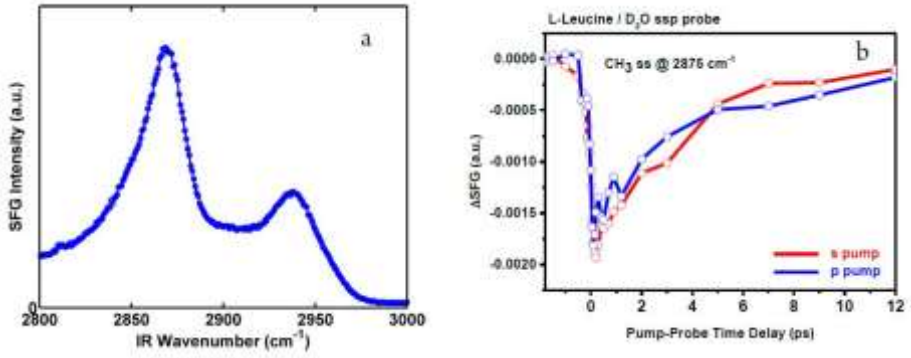


Figure 3-S5: *ssp static spectrum (a) and respective pump-probe recovery (b) for the CH_3 ss mode integrated over a spectral window of 2855 to 2890 cm^{-1} Lines shown in the pump probe traces are guides for the eye.*

3.4 e. Molecular Dynamics Simulation Details

All-atom molecular dynamics simulations in the canonical (NVT) ensemble were performed to determine interfacial reorientation of leucine at vacuum/water interfaces. The simulation box contained 40 leucine molecules, 40 chloride ions and 540 water molecules, and the simulation box dimension is 30 ÅX30 ÅX60 Å. Using Packmol[83] the initial configuration of the molecules was generated by randomly placing the leucine molecules very close to the vacuum/water interfaces. The MD simulations were performed using GROMACS 4.6.5.[84] For leucine, the AMBER99SB-ILDN[85] force field was used. The leucine molecule is in a cationic state (+1e) (see Figure 3-S6) and the electrostatic point charges of the leucine molecule were parameterized by fitting to HF/6-31G* quantum calculations in Gaussian 09[86] with the RESP[87] method in antechamber.[88] The TIP3P[89] water model modeled rigid water.. Lennard-Jones potentials were shifted to zero at 12 Å. For electrostatic interactions, a cut-off distance of 14 Å was used and beyond the cutoff distance, the long-range interactions were calculated using the Particle mesh Ewald summation (PME) algorithm.[90] Bonds between hydrogen atoms to other atoms were constrained with the LINCS algorithm in order to allow for an integration time step of 2 fs. The temperature of the simulation was kept at 298 K using a stochastic velocity-rescaling thermostat.[91] The system was first minimized over 10 000 steps with the steepest descent method followed by 40 ns of

MD simulation in the NVT ensemble. The last 5 ns of the NVT simulations were used for analysis.

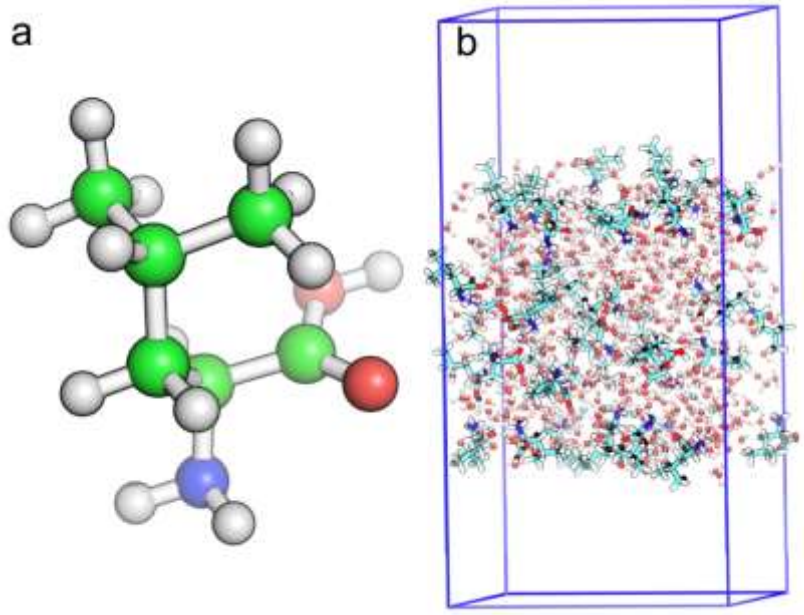


Figure 3-S6*: a) molecular structure of leucine molecule and b) representative snapshot of leucine molecules at the vacuum/water interfaces.*

3.4 f Analysis of MD results

The mass density profile of water was calculated by partitioning the simulation box into 1 Å-thick bins along the *z*-axis and calculating the total mass in each bin per partition volume. A region at which the mass density of water is less than the bulk density (middle region) is defined as an interfacial region. To determine whether a leucine molecule belongs to the interfacial region or not the position of the C_α atom (of leucine) is used as a reference.

As shown in Figure 3-S7, the orientational vector of the methyl group is defined as a unit vector originating from the carbon atom (on the methyl

group) and terminating at the geometric center of the three hydrogen atoms (on the methyl group). Angle θ (polar angle) is defined by the angle between the unit vector and the Z-axis (a vector normal to the leucine-water slab surface). The angle φ (azimuthal angle) is defined as the angle between the orientational vector projection on the xy-plane and the x-axis. Figure 3-S8 shows the azimuthal φ angle distributions of the methyl groups at the air-water interface.

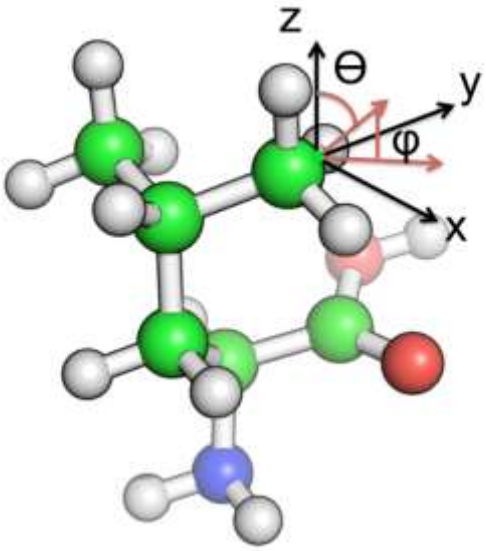


Figure 3-S7*: Definition of orientational angle θ and azimuthal angle φ of methyl group of a leucine molecule.*

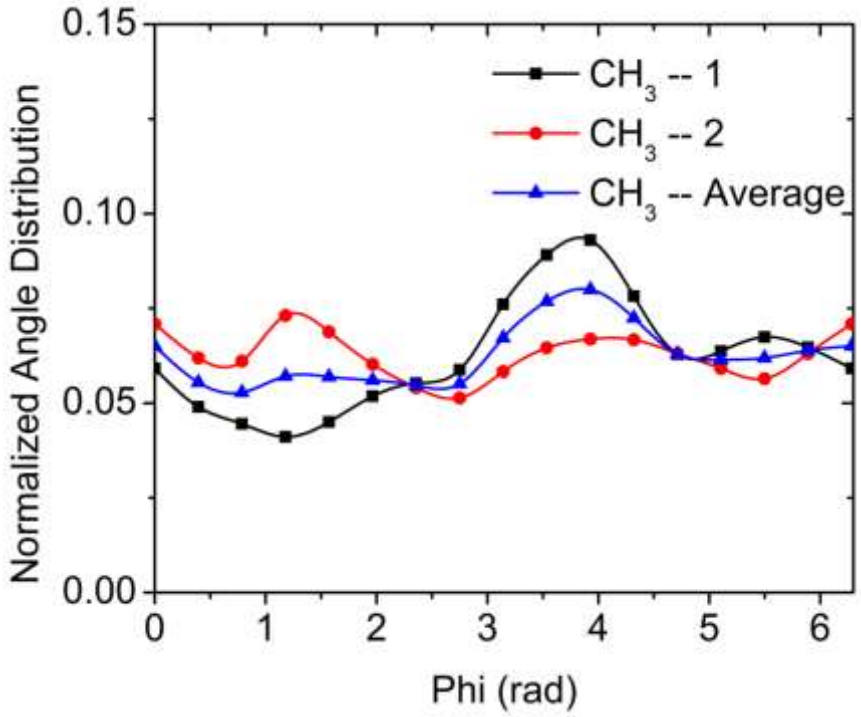


Figure 3-S8*: Azimuthal φ angle distributions at the vacuum water interface: square symbol (first methyl group), circle symbol (second methyl group) and triangle symbol (all methyl groups).*

For the methyl group reorientation, diffusion coefficients D_θ (out of the plane of the surface) and D_φ (in plane of the surface) were calculated following similar procedure previously used by Hsieh et al.[37] In the molecular dynamics simulation we tracked the population of methyl groups that at time 0 are within the range of $0 < \varphi < 180$ and $34 < \theta < 96$ and we observed this population relax towards equilibrium. Using the methyl angle distribution (θ, φ) obtained from the MD simulation as an initial boundary value, a two dimensional diffusion equation (given in Equation 1 of the SI of ref. [37]) was solved for different guessed values of D_θ and D_φ. Then, we calculated the square of residuals, χ^2,to determine the goodness of a fit between the angle distributions (θ, φ) obtained from the numerical solution using the diffusion equation and MD simulation results.

3.4 g. Numerical Simulation Details

In order to simulate the transient SFG response a numerical simulation routine was used based on the model of Nienhuys and Bonn for describing molecular reorientation at liquid interfaces as seen in appendix B of ref. [38]. Briefly, the numerical solution to the rotational diffusion equation can be approximated for small spread angles to be:

$$\frac{\partial \rho}{\partial t} = \frac{D_\varphi}{sin^2\theta_0}\frac{\partial^2 \rho}{\partial \varphi^2} + \frac{D_\theta}{k_B T}\frac{\partial \rho}{\partial \theta}\frac{\partial V}{\partial \theta} + \rho\frac{D_\theta}{k_B T sin\,\theta}\frac{\partial}{\partial \theta}\sin\theta\frac{\partial V}{\partial \theta}$$

The potential V is harmonic and defined as in reference 2 to be:

$$V(\theta) = \frac{k_B\,T}{2(\Delta\theta)^2}(\theta - \theta_0)^2$$

The solution to this equation is related to the SFG response by:

$$\Delta\chi^{(2)}(t) = -\iint \rho_\sigma\,(\varphi, \theta, t)\beta(\theta, \varphi)d\varphi d\theta$$

where $\Delta\chi^{(2)}(t)$ is the transient SFG response and $\beta(\theta, \varphi)$ represents the molecular hyperpolarizability.

The SFG signals are simulated by utilizing the solutions to ρ presented in ref. [38], and this is numerically solved by inputting the diffusion coefficients obtained from MD simulations. In addition, the code simulates a phase matched transient SFG signal utilizing the experimental angles and a vibrational lifetime is estimated according to previously recorded values for methyl stretches at interfaces.[39,42,80] Given the vibrational lifetime and parameters from MD simulation such as in plane and out of plane diffusion coefficients, molecular tilt angle and molecular tilt angle distribution, the transient SFG response is calculated. Afterward, the simulated SFG signal is convoluted with the experimentally determined instrumental response time (IIV cross correlation, width = 154 fs) to produce the traces shown in the main text.