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Ultrafast Reorientational Dynamics of Leucine at the Air–Water Interface

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Supporting Information

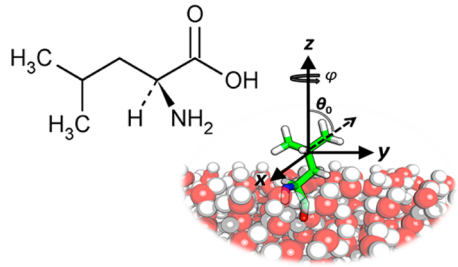
ABSTRACT: Ultrafast dynamics of protein side chains are involved in important biological processes such as ligand binding, protein folding, and hydration. In addition, the dynamics of a side chain can report on local environments within proteins. While protein side chain dynamics have been probed for proteins in solution with nuclear magnetic resonance and infrared methods for decades, information about side chain dynamics at interfaces is lacking. At the same time, the dynamics and motions of side chains can be particularly important for interfacial binding and protein-driven surface manipulation. We here 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_{φ} = 0.07 rad^2/ps and an out of plane rate of D_{θ} = 0.05 rad^2/ps.

The key to protein function lies not only in protein structure but also in protein dynamics.[1] Different dynamical time scales correspond to a wide variety of protein motions.[1] Folding, for example, occurs on nano- to microsecond time scales.[2] Protein side chains undergo torsional motions and rotamer transitions occurring on pico- to nanosecond time scales.[1,3] It has been shown that side chain dynamics play a role in enzymatic catalysis[3] and protein–protein interactions.[4] Moreover nuclear magnetic resonance (NMR) studies have related changes in side chain entropy to the local environment of a protein complex.[4] Backbone[5,6] and side chain dynamics have been widely studied in bulk utilizing spin techniques like NMR and electron paramagnetic resonance, vibrational spectroscopy, and electronic spectroscopy.[7] However, this information is lacking for proteins at interfaces. At the same time, understanding how proteins interact with a surface is of utmost importance in such varied fields as biomineralization, membrane proteins, biomaterials, and biomechanics.[8] Dynamic protein side chain interactions are of importance at interfaces mainly because protein side chains are directly in contact with the interface and are likely key players for specific interactions of proteins with hard and soft tissue, for example, recognition of specific mineral surface, cell membrane receptors, and functional biomaterials.[9,10] In particular, methyl-containing residues can display markedly different dynamics which correspond to changes in local chemical environment.[4]

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 (Scheme 1). Even though the dynamics of L-leucine are 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.[9,11−17] SFG vibrational spectroscopy is ideally suited to probe the equilibrium and dynamic properties of interfacial species.[9,10,13,14,18−30] 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.[31] As such, second- and fourth-order SFG are sensitive to interfacial order and orientation.

Richmond and Shen have previously determined the orientation and saturation coverage of L-leucine at the oil–water and air–water interface, respectively.[13,14] Cremer et al.

Scheme 1. Leucine Adsorbed to the Air–Water Interface[a]



[a]The orientation of the indistinguishable methyl groups can be characterized by a mean angle θ_0 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 $\Delta\theta$.

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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.[9,15,24,32,33,31] Leucine was concluded to bind to the air−water interface with the isopropyl group oriented toward the vapor phase and the polar headgroup pointing toward 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 line widths of the methyl asymmetric stretch using different polarization combinations as has been done by the Benderskii and Fourkas groups.[27,34,35] However, only a time-resolved pump−probe experiment allows to directly quantify these dynamics.

Pump−probe variants of SFG, time[36−42] and polarization resolved SFG (TP-SFG),[39,43−46] 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,[39] and this motion has been compared with reorientational diffusion influenced spectroscopic models developed by both Nienhuys[43] and the Fayer group.[43,47] Time resolved SFG has also been used to study peptide backbone dynamics.[48] Dynamics experiments on lipid alkyl chains at interfaces, related to the side chain dynamics followed here, have also been reported.[49,50] These latter 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 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, both as monomer and as a side chain within a peptide, at the air−D_2O interface in real time. The monomer 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.[39] A comparison of the two time-dependent signals obtained with orthogonal pump polarizations provides the time scale of reorientational motion.[39,44]

Figure 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[13] 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.[9,11,13,14,16] Differences in the shape of the nonresonant background are likely explained

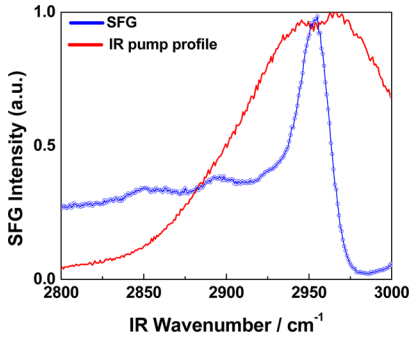

Figure 1. Static sps SFG spectrum (blue) for positively charged L-leucine at saturation coverage.[13] The profile of the excitation pulse is shown in red.

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).[19,51] 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} (Figure 1, red curve).

The upper panel of Figure 2 shows the results from a TP-SFG measurement with pump pulses parallel (s) and perpendicular

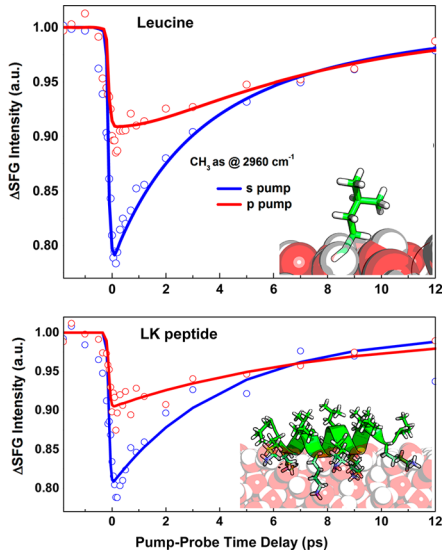

Figure 2. TP-SFG mid-IR pump. SFG sps probe traces for s (blue) and p (red) pumped methyl groups. Signals are integrated over a spectral range of 2945−2975 cm^{-1}. Plotted is the difference in integrated intensity of (I_{SF}(pumped) − I_{SF}(unpumped)) divided by the nonresonant I_{SF} from a *z*-cut quartz reference. The signals are rescaled to show the modulation of the SFG signal when the pump is on. (Top) TP-SFG data for leucine. Curves represent calculations following the model described in ref 43 using MD simulation to model the experimental data (circles). (Bottom) TP-SFG traces for an α-helical LK peptide for comparison. The curves are single exponential fits yielding an effective reorientation time of 15 ps.

(p) to the IR probe pulse for the sps probe (see SI for ssp data) for the leucine monomer. The (s)-pumped data (blue) display a larger bleach and faster recovery than 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

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signal.[39] 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.[49] 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 monoexponential 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., $k_{1s} = k_1 + k_{\mathrm{eff,diff}}$, where we have defined an effective reorientation rate $k_{\mathrm{eff,diff}}$. Similarly, for p-polarized pump, we can approximate the overall rate by $k_{1p} = k_1 - k_{\mathrm{eff,diff}}$. The two observed lifetimes then give estimated diffusion time of $(k_{\mathrm{eff,diff}})^{-1}$ = 21.5 ps, and a vibrational lifetime $T_1 = (k_1)^{-1}$ = 5.2 ps.

To test whether the dynamics observed for leucine amino acids are comparable with side chain dynamics for leucine side chains within proteins, we have recorded TP-SFG traces (Figure 2) for a 0.1 mg/mL solution of a model leucine-lysine (LK) peptide (Ac-LKKLLKLLKKLLKL-OH). LK peptides have been shown to adopt an α-helical secondary structure when adsorbed at the air–water interface with the leucine side chains extending into the vapor phase and the lysine sites oriented toward the water.[52] Monoexponential fits yield $(k_{1s})^{-1}$ = 4.3 ps and $(k_{1p})^{-1}$ = 7.8 ps, respectively, resulting in an estimated diffusion time of 15 ps for the leucine side chains, similar to the diffusion time of 21.5 ps observed for the leucine amino acids. This analysis demonstrates that the dynamics of leucine amino acids are comparable with those of leucine side chains and therefore a useful model system.

To obtain more detailed information about the interfacial reorientation dynamics of the leucine amino acids, 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 counterions 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 3a. As suggested by previous SFG experiments,[13,14] leucine molecules show strong ordering at the interface and expose the methyl groups toward the vacuum.[9,13−15] Figure 3b shows the tilt (θ) angle distributions of the two individual methyl groups and an average of both methyl groups. As seen in Figure 3b, θ shows a normal distribution with peak values near 60°. The average orientation was determined to be θ_0 = 64° with a distribution width of $\Delta\theta$ = 27°. This angle is in reasonable agreement with values determined experimentally by Shen et al., who reported an angle of 39° for leucine.[13] 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 (Figure 3b). Contrary to the tilt angles, the methyl groups' φ angles are completely random at the interface (see 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

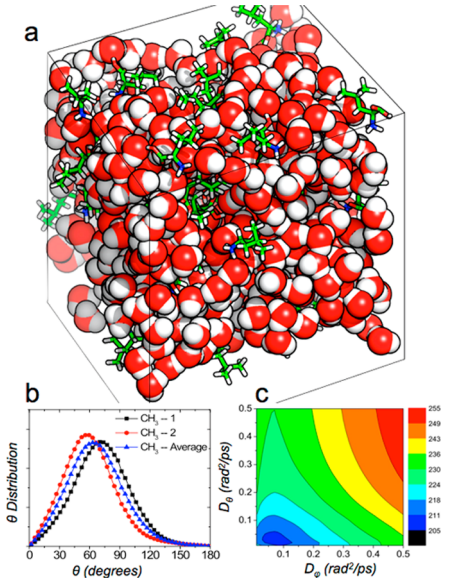

Figure 3. MD simulations of leucine at the air–water interface at 300 K. Snapshot of leucine molecules at vacuum–water interfaces: view (a) of the simulation box. (b) Leucine molecule methyl groups angle θ distributions. (c) Variation of χ^2 with D_φ and D_θ for methyl groups at the interface. χ^2 has the minimum value at $D_\theta = 0.05 \pm 0.01$ rad^2/ps and $D_\varphi = 0.07 \pm 0.01$ rad^2/ps.

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 $D_\theta = 0.05 \pm 0.01$ rad^2/ps and $D_\varphi = 0.07 \pm 0.01$ rad^2/ps (Figure 3c), which shows the leucine methyl groups reorient, on average, at comparable rates in and out of plane.

In Figure 2 (upper panel), 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 $k_\theta = D_\theta/\Delta\theta^2$.[43] Based on this model, out of plane reorientation occurs within 4.4 ps. In plane reorientational diffusion occurs on a timescale of $(\mathrm{D}_\varphi)^{-1}$ = 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[43] of $4\mathrm{D}_\varphi$ = 0.28 rad^2/ps or equivalently with a rate constant of 3.6 ps (Figure S3). These time scales 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.[53]

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.[44,50] 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, we establish the first real-time observation of molecular reorientation for an amino acid side chain at an

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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. Additionally, from MD simulations, we determined in- and out-of-plane molecular reorientation coefficients of $D_\varphi = 0.07 \pm 0.01$ rad^2/ps and $D_\theta = 0.05 \pm 0.01$ rad^2/ ps, respectively. Proteins are not static at interfaces, they are highly dynamic. Similar to protein docking and enzyme−substrate interactions in solution, side chain dynamics will play a critical role for function at surfaces. The methods developed here will pave the way for experiments aimed at, for example, understanding the role of side chain dynamics for specific protein recognition of minerals and cells, targeted enzymatic interactions, and the affinity to artificial biomaterials.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b01878.

Experimental details and data (PDF)

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Notes

The authors declare no competing financial interest.

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