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An SFG Study of Interfacial Amino Acids at the Hydrophilic SiO_2 and Hydrophobic Deuterated Polystyrene Surfaces

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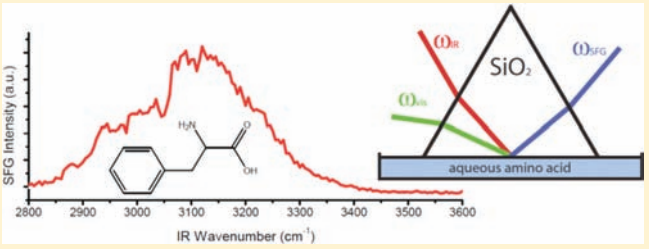
Supporting Information

ABSTRACT: Sum frequency generation (SFG) vibrational spectroscopy was employed to characterize the interfacial structure of eight individual amino acids—L-phenylalanine, L-leucine, glycine, L-lysine, L-arginine, L-cysteine, L-alanine, and L-proline—in aqueous solution adsorbed at model hydrophilic and hydrophobic surfaces. Specifically, SFG vibrational spectra were obtained for the amino acids at the solid−liquid interface between both hydrophobic d_8-polystyrene (d_8-PS) and SiO_2 model surfaces and phosphate buffered saline (PBS) at pH 7.4. At the hydrophobic d_8-PS surface, seven of the amino acids solutions investigated showed clear and identifiable C−H vibrational modes, with the exception being L-alanine. In the SFG spectra obtained at the hydrophilic SiO_2 surface, no C−H vibrational modes were observed from any of the amino acids studied. However, it was confirmed by quartz crystal microbalance that amino acids do adsorb to the SiO_2 interface, and the amino acid solutions were found to have a detectable and widely varying influence on the magnitude of SFG signal from water at the SiO_2/PBS interface. This study provides the first known SFG spectra of several individual amino acids in aqueous solution at the solid−liquid interface and under physiological conditions.



1. INTRODUCTION

Biomaterials, defined as "nonviable material used in a medical device, intended to interact with biological systems",[1] remain an active area of research in the academic, medical, and biotechnology communities because of their crucial role in maintaining and improving our everyday quality of life. A number of biomaterials have been developed over the past decades, which are used to accomplish tasks as complex as keeping coronary arteries from collapsing or as simple as allowing an individual to trade in glasses for contact lenses. A vital part of the rational design of biomaterials is understanding their surface properties and their interactions with biological fluids.[2] When a foreign material is introduced into the body, nonspecific protein adsorption upon the material's surface occurs and can result in an immune response within the host. A common and fundamental approach to developing better biomaterials has been through the functionalizing of surfaces to exhibit specific properties that have been found to reduce or eliminate a host organism's immune response. However, the rational design of materials exhibiting these properties has been proven to be challenging due to the vast array of small molecules, proteins, and cells within an organism.

Our approach to this problem is to start with small, model systems which are experimentally tractable and gradually build toward systems with increasing complexity as we learn more about the fundamental parameters governing the interactions between biomaterials and biomolecules. By studying amino acids—the building blocks of peptides and proteins, and arguably the simplest biomolecules—at the liquid−solid interface, we seek to understand the chemical and physical interactions of these molecules with surfaces and, hence, build an understanding of elementary biomolecule adsorption processes. In the past, there were few experimental techniques available to surface scientists to probe the chemistry and surface physics of biological interfaces under physiological conditions. However, several surface sensitive analysis techniques, particularly sum frequency generation vibrational spectroscopy (SFG),[3−58] second harmonic generation spectroscopy (SHG),[59−62] quartz crystal microbalance (QCM),[63] and surface plasmon resonance (SPR),[64,65] have proven valuable to studying the interactions between surfaces and biomolecules in their native systems free from contributions from a bulk or surrounding environment.

Of the above techniques, SFG vibrational spectroscopy has recently been demonstrated to be an important probe to determine the interfacial structure of adsorbed molecules. Recent advances in SFG techniques have enabled the detection of amino acids at the liquid−solid interface.[6,66−68] In this study, we employ SFG vibrational spectroscopy to characterize the adsorption and interaction of eight amino acids, L-phenylalanine, L-leucine, glycine, L-lysine, L-arginine, L-cysteine, L-alanine, and L-proline, dissolved in an aqueous solvent at the hydrophobic d_8-polystyrene (d_8-PS) and hydrophilic SiO_2 model surfaces.

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Each of these particular amino acids were specifically selected for this investigation based on a number of factors including: (i) the availability of existing and directly comparable SFG experimental data, (ii) structural uniqueness, (iii) ubiquity in human and animal tissue, and (iv) the ability to participate in hydrogen bonding interactions with the aqueous PBS solvent. Specifically, individual amino acids and small model peptides, including arginine, phenylalanine, leucine, lysine, and proline residues, were studied at the aqueous liquid−solid interface by Somorjai et al.[26,68] Glycine was studied because of its chemical simplicity as a "fundamental" amino acid. We believe that the interpretation of more complex amino acids will be simplified if the SFG spectra of the simplest amino acid is measured and interpreted, allowing direct comparisons to more complex amino acids. Additionally, glycine is a primary component of collagen which serves as a critical biological building block and structural scaffold for many types of human and animal tissue including muscle, skin, hair, organs, and bone. Alanine was included in this study because the structural simplicity of its methyl side chain was suspected to potentially be able to provide experimental SFG data that could be used to clarify the spectral differences between asymmetric methylene C−H stretches and terminal methyl stretches which have been previously reported to have very similar stretching frequencies.[26,31,69] Lastly, cysteine was studied because of its chemical simplicity coupled with the hydrogen-bonding capability of its side chain. As a result, the investigation of cysteine was suspected to provide unique insights into the structure and sensitivity of interfacial water species to larger, hydrogen bonding biomolecules, which are potentially co-adsorbed at the aqueous liquid−hydrophilic/hydrophobic solid interface.

We observe C−H vibrational modes from seven of the eight amino acids at the d_8-PS interface. We interpret this in terms of interfacial ordering of the adsorbed amino acids. At the SiO_2 interface, no C−H vibrational modes were observed, which we have interpreted as having a lack of order of the adsorbed amino acids. SFG has been used before to show ordering of peptides and proteins at hydrophobic surfaces (and a lack of ordering at hydrophilic surfaces), and the present study demonstrates a similar phenomenon with amino acids.[31,53,70] Additionally, we demonstrate that certain amino acids can have a strong influence on interfacial water response, at both hydrophobic and hydrophilic interfaces, while others have very little influence. Finally, this experimental investigation documents the first known evidence of SFG activity of several individual amino acids at the solid−liquid interface under physiological conditions.

2. EXPERIMENTAL SECTION

2.1. SFG Experimental Measurements. SFG experiments were conducted using a Continuum (Santa Clara, CA) Leopard D-20 Nd:YAG laser with a pulsed 1064 nm output at 20 Hz and with 20 ps pulse width. This pulsed, near-infrared output was then sent to a LaserVision (Bellevue, WA) Optical Parametric Generator (OPG)/Optical Parametric Amplifier (OPA) system. The OPG/OPA system produced a 532 nm visible beam, ω_{vis}, as well as a tunable infrared beam, ω_{IR}, with an output of 2800−3600 cm^{-1}. Both of these beams were directly used in SFG measurements at pulse energies of ∼200 μJ. As shown in Figure 1, equilateral prism sample substrates made of fused SiO_2 were utilized with experimental beam orientations of 65° and 42° off surface normal for the incoming visible and infrared beams, respectively. These angles were specifically chosen to maximize the collection of SFG signal generated at the liquid−solid interface while minimizing wavelength dependent fluctuations of the Fresnel coefficients over infrared wavelengths.[71] The prism substrates were placed on top of a pH 7.4 phosphate buffered saline (PBS)/biomolecule solution filled Viton O-ring in a clean Petri dish to prevent solvent evaporation and contamination during measurement. SFG signal generated at the solution−prism interface was subsequently detected by a photomultiplier tube before being processed and recorded on a desktop computer. All SFG measurements were conducted in the $S_{SFG}S_{VIS}P_{IR}$ polarization combination and the signal-to-noise ratio for the data included herein is approximately 50. A more detailed theoretical background of the SFG process is provided elsewhere,[72,73] and a description of the OPG/OPA SFG experimental system as well as the background for using the chosen SFG sample substrate arrangement is provided in previous publications.[31,68,69,71,74]

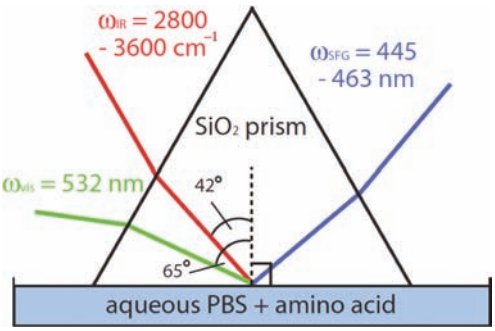

Figure 1. Schematic of the sample interface. The 532 nm and IR light are incident on the prism/solution interface at 65° and 42° relative to the surface normal, respectively.

All SFG spectra on the hydrophobic deuterated polystyrene surface were fit to the following equation:

$$I_{SFG} \propto |\chi^{(2)}|^2 = \left|\chi^{(2)}_{NR} + \sum_q \frac{A_q}{\omega_{IR} - \omega_q + i\Gamma_q}\right|^2 \quad (1)$$

where $\chi^{(2)}$ is the second-order nonlinear susceptibility, $\chi^{(2)}_{NR}$ is the nonresonant component of $\chi^{(2)}$, and A_q, ω_q, Γ_q are the strength, frequency, phenomenological damping factor of the qth vibrational mode.

2.2. QCM. QCM experiments were used to confirm the presence of amino acids adsorbed at the SiO_2 interface. A detailed description of the experimental methods has previously been described.[68] Briefly, a Q-Sense D300 instrument was used with SiO_2 QCM sensor crystals. The Sauerbrey equation, $\Delta m = -C(\Delta f/n)$, and the third harmonic of the resonant frequency were used to relate the frequency change to the adsorbed mass. In the Sauerbrey equation, Δm is the adsorbed mass, C is the mass sensitivity constant of −17.7 ng cm^{-2} Hz^{-1} for a 5 MHz resonance, Δf is the change in frequency upon addition of the amino acid, and n is the overtone number. The sensitivity of this instrument is approximately 2 ng/cm^2, which is an order of magnitude less than a monolayer based on geometric arguments for the systems studied herein.

2.3. Chemicals. All experiments were conducted at pH 7.4 and solutions were made with phosphate buffered saline (PBS) from Sigma-Aldrich (cat. no. P-5368). All amino acids used were purchased directly from Sigma-Aldrich and were used as received. Biomolecule solutions of L-phenylalanine (cat. no. P5482) and L-leucine (cat. no. L8912) were both used at 5 mg/mL in PBS. Solutions of L-proline (cat. no. P5607), L-lysine (cat. no. L5501), and L-arginine (cat. no. A8094) were all studied at 15 mg/mL in PBS. Finally, L-cysteine (cat. no. C7352), L-alanine (cat. no. A7489), and glycine (cat. no. G8790) were investigated in PBS at final solution concentrations of 25, 40, and 100 mg/mL, respectively. The experimental amino acid concentrations used in this study were selected to maximize SFG signal while still maintaining solubility.

Table 1. Adsorbed Mass at the Aqueous Liquid−Solid Interface Determined by QCM

amino acid	concentration (mg/mL)	interface	adsorbed mass (ng/cm^2)	95% confidence interval (ng/cm^2)
L-phenylalanine	5	PS	57	4
L-phenylalanine	5	SiO_2	63	4
L-arginine	15	SiO_2	196	20
L-alanine	40	PS	469	33
L-alanine	40	SiO_2	467	20
L-cysteine	25	SiO_2	245	22

All SFG measurements were carried out in pure PBS or amino acid/PBS solutions at pH 7.4 and maintained at 20 °C.

2.4. SFG Sample Preparation. SFG sample substrates were prepared from fused SiO_2 equilateral prisms which were cleaned by soaking for 12 h in a solution of concentrated H_2SO_4 (97%) (Sigma-Aldrich) and Nochromix (Godax). Subsequently, the SiO_2 prism substrates were washed with distilled, deionized water prior to being cleaned using a Herrick (Ithaca, NY) oxygen plasma cleaner for 60 s (18 W RF, 200 mtorr O_2). The O_2 plasma treatment was employed to remove any residual organic surface contaminants and to maximize the extent of Si/SiO_2 prism surface oxidation. After plasma treatment, the clean SiO_2 prisms were either used directly for SFG measurements of the SiO_2/PBS interface or were prepared with deuterated polystyrene (d_8-PS) thin films. The thin films were prepared on the SFG prism substrates by spin-coating 3% weight solution of d_8-PS (MW ≈ 300 000) (Polymer Source) dissolved in toluene (Sigma-Aldrich, cat. no. 34866). The spin-coating was followed by annealing at 120 °C for 12 h to ensure surface flatness and to fully remove any residual toluene solvent from the spin coating solution. The thickness of the resulting d_8-PS films was determined to be ∼105 nm by ellipsometry and atomic force microscopy.

3. RESULTS AND DISCUSSION

3.1. General Discussion of SFG Spectra. SFG spectra of aqueous interfaces in the region between 2800 cm^{-1} and 3600 cm^{-1} contain broad O−H vibrational features, with peaks centered at 3200 cm^{-1} and 3400 cm^{-1}, that have been the subject of considerable discussion.[75−79] Most recently, using phase-sensitive SFG, the 3200 cm^{-1} peak has been attributed primarily to the "ice-like" four-coordinated water structure, while the 3400 cm^{-1} peak was concluded to be mainly due to a more "liquid-like" less than four coordinatied water arrangement.[75] However, a simulation study, correlating the SFG strength to the projection of the OH bond onto the surface normal and the bulk electric field distribution, cautions against ascribing intermolecular configurations to these spectral features.[80] While this region of the spectrum is quite different at the PBS/d_8-PS interface, its interpretation is equally complex, which is reflected in the discussion of individual amino acid SFG spectra in the following section.

The interpretation of the signals observed in this region is complicated by the amino acid themselves. At pH 7.4, the amino acids studied here will be zwitterionic, with a negative COO^- group (that may be capable of hydrogen bonding to other amino acids or interfacial water) and a positive NH_3^+ moiety. The NH_3^+ can have two modes, symmetric and asymmetric, which can appear between 3030 cm^{-1} and 3450 cm^{-1}.[81] Cyclic dimers of amino acids have also been recognized to have a unique spectral signature in this region.[82] Lastly, lysine and arginine (both net cations) have an amine or guanidino moiety on their side chain, respectively, which also can give rise to SFG intensity in this region.[13] The signals generated from these vibrations can be broad or narrow, and can destructively or constructively interfere with interfacial water signals. We discuss their possible assignments on a case-by-case basis below.

3.2. SFG at the Hydrophobic Deuterated Polystyrene Surface. *3.2.1. Phosphate Buffered Saline (PBS).* The SFG spectrum of pure pH 7.4 PBS at the hydrophilic−hydrophobic PBS/d_8-PS liquid−solid interface is presented in the background spectra of Figures 2−9. In the background SFG spectra of these data plots, the d_8-PS/PBS interface shows a broad spectral feature centered at ∼3100 cm^{-1}. This broad peak is attributed to a continuum of O−H stretching vibrational modes of interfacial water and is consistent with previous SFG studies of PBS solutions at the hydrophobic d_8-PS surface under similar conditions.[26,31,68,69,74,83] Although in principle the background SFG spectra in Figures 2−9 should be quantitatively reproducible, we have observed small spectral differences between different samples. We believe this is due to surface contaminations gathered during sample preparation.

3.2.2. L-Phenylalanine. The SFG spectrum of L-phenylalanine (5 mg/mL) in PBS at the hydrophobic d_8-PS/PBS solid−liquid interface is shown in Figure 2. Each data point in this plot is the average of 500 laser shots. Upon the addition of L-phenylalanine, an enhancement in the SFG signal of water is observed and attributed to the presence of solvated amino acid species at the hydrophobic interface. This is likely due to a change in the net orientation of O−H bonds in the interfacial region. Additionally, four C−H vibrational peaks were fit in this spectrum at infrared frequencies of 2875, 2939, 2991, and 3049 cm^{-1}. The first and second peaks at 2875 and 2939 cm^{-1} are attributed to a CH_2(s) and a combination C−H and Fermi resonance[6] modes of the carbon atom connecting the phenyl group to the amino acid backbone, respectively. The third peak at 2991 cm^{-1} is assigned as either a C−H combination band[84] or a CH_2(as) vibrational mode.[6] Lastly, the mode centered at 3049 cm^{-1} is attributed to an aromatic C−H ν_2 stretch from the phenyl group.[6] This ν_2 aromatic SFG peak from phenylalanine was observed at 3064 cm^{-1} at the hydrophilic−hydrophobic CCl_4/D_2O buffer liquid−liquid interface by Watry and Richmond,[85] but the CH_2(s) vibrational mode at 2875 cm^{-1} was not reported in that study. Phillips et al. also observed an aromatic C−H stretch at 3050 cm^{-1} attributed to phenylalanine in the SFG spectrum of an amphiphilic peptide containing only phenylalanine and arginine residues.[26] The presence of L-phenylalanine at the PS/PBS interface was further validated by QCM, yielding an adsorbed mass of 57 ± 4 ng/cm^2 at a 5 mg/mL concentration as presented in Table 1. Assuming monolayer coverage, this concentration corresponds to a molecular surface area for L-phenylalanine of 48 Å^2 on the hydrophobic PS surface

3.2.3. L-Leucine. The SFG spectrum of L-leucine (5 mg/mL) in PBS at the hydrophilic−hydrophobic PBS/d_8-PS liquid−solid interface is shown in Figure 3. In this figure, every data point is the average of 500 experimental measurements. Three C−H stretching vibrational modes were fit in this spectrum. While previous studies have

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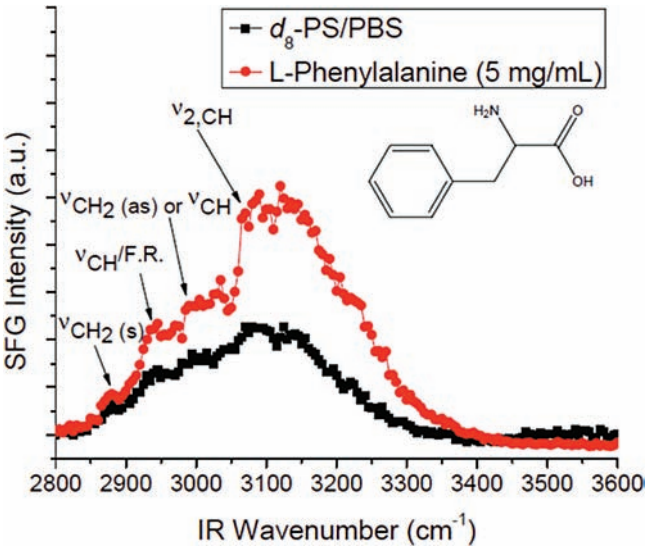

Figure 2. SFG spectrum of d_8-PS/PBS solid−liquid interface before (black squares) and after (red circles) addition of L-phenylalanine (5 mg/mL). The vibrational modes at 2875 and 2939 cm^{-1} are attributed to CH_2 symmetric and a C−H combination band/Fermi resonance, respectively. The peak at 2991 cm^{-1} is identified as a C−H combination band or a CH_2 asymmetric stretch. Finally, the largest peak at 3049 cm^{-1} is indicative of the aromatic C−H ν_2 stretch of the amino acid phenyl side chain.

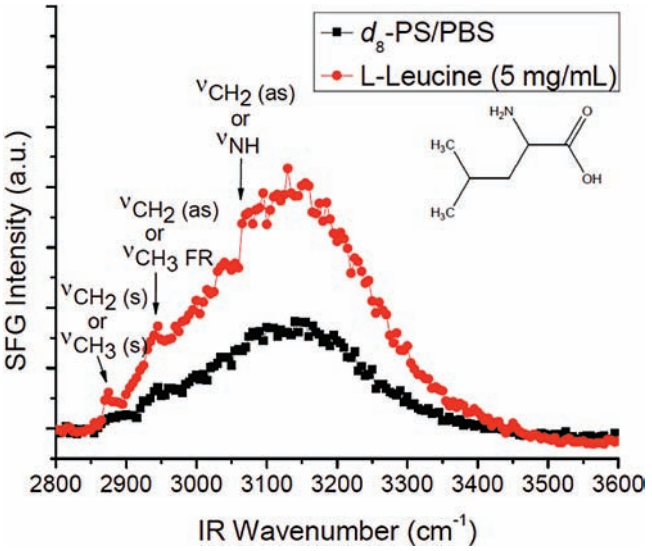

Figure 3. SFG spectrum of the d_8-PS/PBS solid−liquid interface before (black squares) and after (red circles) the addition of L-leucine (5 mg/mL). The vibrational mode at 2874 cm^{-1} is attributed to either a CH_3 symmetric stretch from the terminal methyl groups or a CH_2 symmetric stretching mode. The larger peak at 2942 cm^{-1} is indicative of a CH_3 Fermi resonance or a CH_2 asymmetric stretch. The vibrational mode fit in the spectrum at 3056 cm^{-1} may be due to a highly perturbed CH_2(as), N−H dimer, or N−H stretching vibrational mode.

assigned the peak centered at 2874 cm^{-1} for L-leucine as a CH_3(s) mode,[26,31,69,74,85−87] it may also originate from a CH_2(s) mode or a combination of both CH_2(s) and CH_3(s) vibrations. These previous studies have also identified the vibrational mode observed at 2942 cm^{-1} as a CH_3 Fermi resonance, but it may also originate

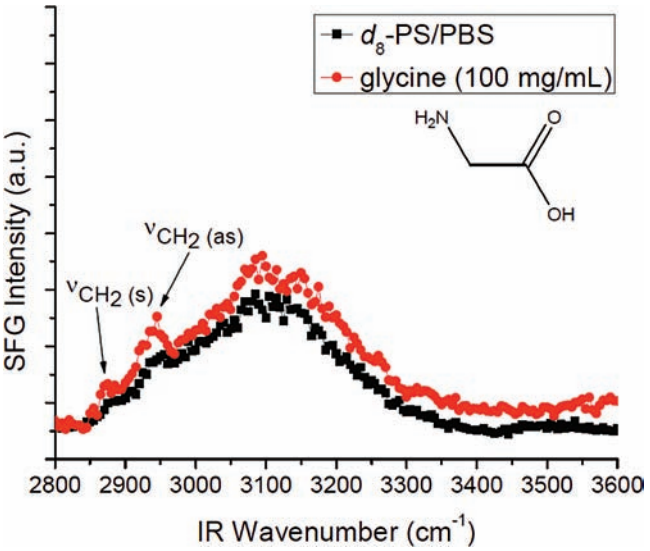

Figure 4. SFG spectrum of the d_8-PS/PBS solid−liquid interface before (black squares) and after (red circles) the addition of glycine (100 mg/mL). The vibrational mode at 2873 cm^{-1} is attributed to a CH_2 symmetric stretching mode, and the larger peak at 2939 cm^{-1} is identified as originating from a CH_2 asymmetric stretch.

from an asymmetric CH_2 stretching mode. The spectrum of L-phenylalanine (which has no CH_3 groups) shown in Figure 2 has a peak at 2939 cm^{-1} which leaves open the possibility of this being a CH_2(as) mode of L-leucine. Additionally, a peak was fit to this spectrum at 3056 cm^{-1}, and while other SFG studies have seen similar C−H modes originating from nonaromatic species, the origin of this mode is unclear.[83] However, there is evidence that this peak may be indicative of highly perturbed CH_2(as) modes, N−H cyclic dimers,[82] or N−H stretching modes from the amino acid backbone. Interestingly, several additional peaks previously observed in SFG studies (of L-leucine amino acid and peptides with L-leucine residues) at 2847, 2895, 2910, and 2960 cm^{-1} were not observed in this study.[26,31,69,74,85,87] This could be a consequence of the number of peaks we choose in our fitting of the spectra. Conversely, these vibrational modes may not be ordered under the experiment conditions chosen here. Similar to the spectrum of phenylalanine, the SFG spectrum of the d_8-PS/PBS interface shows a substantial increase in SFG signal from interfacial water with the presence of L-leucine. This phenomenon is also attributed to a change in the net orientation of interfacial water due to the presence of the solvated amino acid at the hydrophobic solid−liquid interface.

3.2.4. Glycine. The SFG spectrum of a solution of the simplest amino acid, glycine (100 mg/mL in PBS), at the hydrophobic d_8-PS surface is presented in Figure 4. Each data point in this plot is the average of 500 laser shots. This SFG spectrum shows two C−H vibrational modes at 2873 and 2939 cm^{-1} which are readily identified as CH_2(s) and CH_2(as) vibrations, respectively, due to the molecular simplicity of glycine. These peak assignments support the attribution of the 2939 cm^{-1} stretch of L-leucine in this study to a CH_2(as) mode. However, the high amino acid concentration necessary to observe SFG activity of glycine amino acid may have influenced the frequency of this CH_2(as) mode and still leaves open the possibility of the Fermi resonance peak assignment of leucine in other studies to stand as reasonable.[26,31,69,74,85,87] The SFG signal from the O−H stretches of interfacial water is largely unchanged in Figure 4 upon the introduction of the amino acid. This is remarkably different than in the case of L-phenylalanine and L-leucine. While the reason for this is

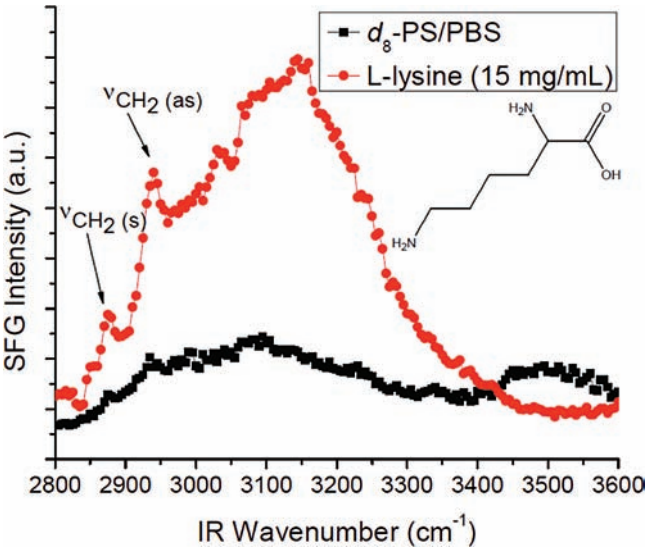

Figure 5. SFG spectrum of the d_8-PS/PBS solid−liquid interface before (black squares) and after (red circles) the addition of L-lysine (15 mg/mL). The vibrational mode at 2874 cm^{-1} is attributed to a CH_2 symmetric stretch and a larger peak at 2942 cm^{-1} is indicative of a CH_2 asymmetric stretch from the lysine side chain. The dramatic increase in signal upon the addition of L-lysine is attributed to a change in the net orientation of interfacial water as well as possible contributions from N−H stretching modes of the acid's terminal amine group or backbone.

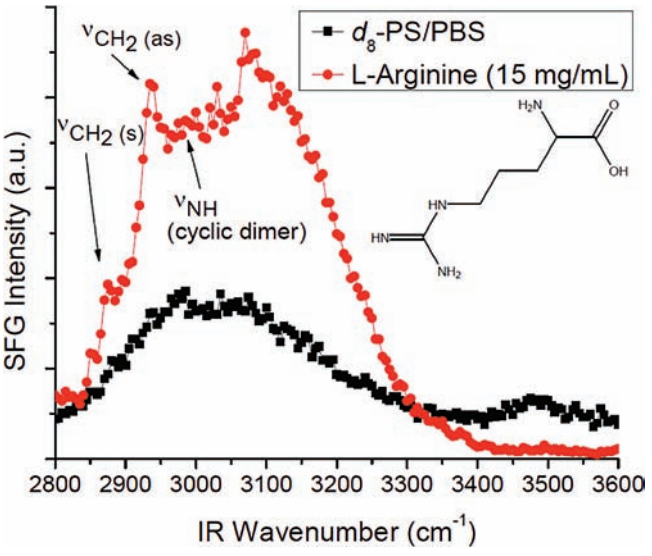

Figure 6. SFG spectrum of the d_8-PS/PBS solid−liquid interface before (black squares) and after (red circles) the addition of L-arginine (15 mg/mL). The vibrational mode at 2880 cm^{-1} is attributed to a CH_2 symmetric stretch and a larger peak at 2935 cm^{-1} is indicative of a CH_2 asymmetric stretch. Another peak centered at 2977 cm^{-1} was fit to the spectrum and is attributed to the formation of a cyclic dimer species forming between two neighboring arginine molecules on the hydrophobic surface. The dramatic increase in signal near 3100 cm^{-1} upon the addition of L-arginine is attributed to change in the net orientation of interfacial water as well as N−H stretching modes of the amino acid's terminal guanidino group or backbone.

unclear, it may be due to the high concentration of glycine used. A similar phenomenon was previously observed in SFG spectrum of

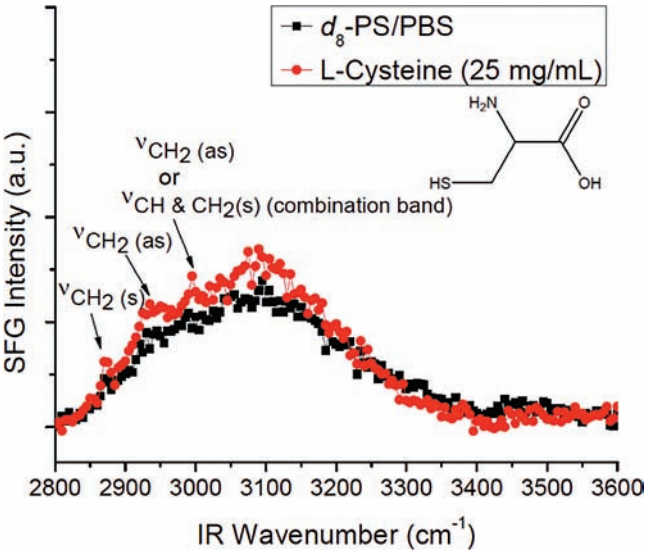

Figure 7. SFG spectrum of the d_8-PS/PBS solid−liquid interface before (black squares) and after (red circles) the addition of L-cysteine (25 mg/mL). The vibrational mode at 2872 cm^{-1} is attributed to a CH_2 symmetric stretching mode, and the peak at 2935 cm^{-1} is identified as originating from a CH_2 asymmetric stretch. A third C−H vibrational mode is observed at 2993 cm^{-1} and is assigned as either a perturbed CH_2(as) mode, or a combination band of the CH_2(s) and CH stretches of solvated L-cysteine molecules.

concentrated L-proline (500 mg/mL in PBS) at the same d_8-PS interface.[68] Alternatively, the relatively minor change in water signal may be explained as a minimal disruption of the solid−liquid interface after addition of the amino acid. However, this case is unlikely due to the presence of C−H vibrational modes in this spectrum.

3.2.5. L-Lysine. The SFG spectrum of L-lysine (15 mg/mL) in PBS at the d_8-PS/PBS solid−liquid interface is shown in Figure 5. In these spectra, every data point is the average of 600 measurements. Upon introduction of L-lysine, the SFG spectrum fit indicates peaks at 2845, 2848, 2874, and 2942 cm^{-1}. The 2845 and 2848 cm^{-1} peaks have small amplitudes yet are necessary to obtain an adequate fit of the spectrum. They are not identifiable at this point. The stronger vibrational modes centered at 2874 and 2942 cm^{-1} have been previously reported by our group as corresponding to a CH_2(s) and CH_2(as) vibrational modes, respectively.[68] Another vibrational mode may be fit to this spectrum centered at 3040 cm^{-1}, but this peak was not included herein because its presence substantially reduced the quality of the fits for the remaining four C−H vibrational modes. Additionally, the amino acid spectrum shows a substantial SFG signal increase in the water region when compared to the SFG spectrum of the pure PBS/d_8-PS hydrophobic interface. Like L-leucine and L-phenylalanine, the water SFG signal is significantly increased in the presence of L-lysine, and this increase is attributed to a change in the net orientation of the interfacial water. Interestingly, there is an apparent blue-shift in the broad O−H bond peak, which is consistent with the presence of an N−H stretch contributing to the shoulder of the O−H peak.[88,89]

3.2.6. L-Arginine. The SFG spectrum of L-arginine (15 mg/mL) in PBS at the hydrophilic−hydrophobic PBS/d_8-PS liquid−solid interface is shown in Figure 6. Individual data points in these spectra are the average of 300 SFG experimental measurements. The fit of the amino acid spectrum shows CH_2 vibrational modes centered at 2880 and 2935 cm^{-1} which are attributed to symmetric and

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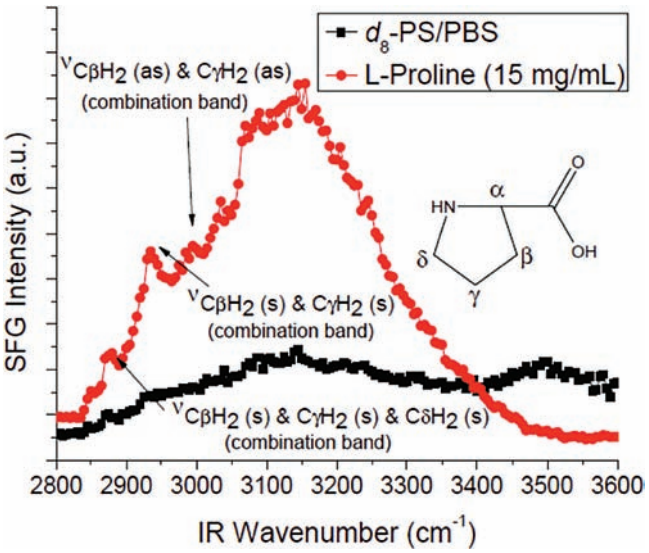

Figure 8. SFG spectrum of the d_8-PS/PBS solid−liquid interface before (black squares) and after (red circles) the addition of L-proline (15 mg/mL). The vibrational mode at 2875 cm^{-1} is attributed to a combination of $C_\beta H_2$ (s), $C\gamma H_2$ (s), and $C_\delta H_2$ (s) modes. The peak at 2934 cm^{-1} is identified as combination mode from $C_\beta H_2$ (s) and $C_\gamma H_2$ (s) vibrations. Finally, the combination mode at 2984 cm^{-1} originates from $C_\beta H_2$ (as) and $C_\gamma H_2$ (as) vibrations. The broad increase of SFG signal in the region 3000−3400 cm^{-1} upon the addition of L-proline is attributed to change in the net orientation of interfacial water as well as N−H stretches of the heterocyclic amino acid.

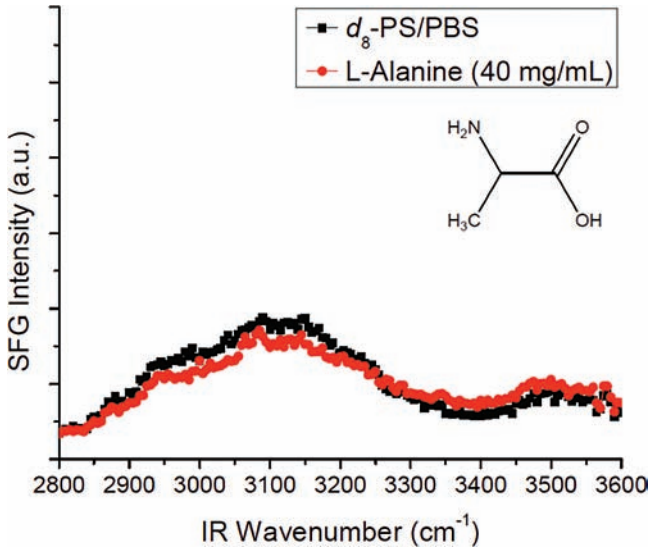

Figure 9. SFG spectrum of the d_8-PS/PBS solid−liquid interface before (black squares) and after (red circles) the addition of L-alanine (40 mg/mL). No C−H vibrational modes are observed, and the water signal is unperturbed after the adsorption of amino acid.

asymmetric CH_2 stretches of the L-arginine side chain, respectively. An additional peak was fit at 2977 cm^{-1} which is attributed to N−H cyclic dimers forming between neighboring pairs of this nitrogen terminated species.[82] A mode may be identified around 3070 cm^{-1}, although we did not fit this peak. The spectrum also shows a substantial increase in the broad O−H peak. Like L-lysine, there is an apparent blue-shift of this peak, which could be attributed to the N−H stretching modes of the charged terminal guanidino group or backbone, in addition to interfacial water.

3.2.7. L-Cysteine. The SFG spectrum of L-cysteine (25 mg/mL) in PBS at the hydrophilic−hydrophobic PBS/d_8-PS liquid−solid interface is shown in Figure 7. Each data point in this figure is the average of 300 laser shots. The L-cysteine amino acid shows CH_2 symmetric and asymmetric stretches at 2872 and 2935 cm^{-1}, respectively. Interestingly, the SFG spectrum of L-cysteine also shows a C−H peak at 2993 cm^{-1} for which the peak assignment is nontrivial. However, recent theoretical studies have predicted a vibrational mode of L-cysteine at this approximate infrared frequency originating from a perturbed CH_2(as) vibrational mode,[90] or from a combination of the CH_2(s) mode and the CH mode from the carbon atom connecting the thiol group to the amino acid backbone.[90,91] Similar to glycine, the SFG spectrum of L-cysteine shows only a minimal change in the O−H signal from interfacial water at the hydrophobic d_8-PS surface.

3.2.8. L-Proline. The SFG spectrum of L-proline (15 mg/mL) is presented in Figure 8 where each data point is the average of 600 measurements. This spectrum shows C−H vibrational modes at the d_8-PS hydrophobic interface at 2875 cm^{-1} indicative of a combination of $C_\beta H_2$ (s), $C_\gamma H_2$ (s), and $C_\delta H_2$ (s) modes, 2934 cm^{-1} also indicative of a combination mode from $C_\beta H_2$ (s) and $C_\gamma H_2$ (s) vibrations, and finally a mode at 2984 cm^{-1} from a combination of $C_\beta H_2$ (as) and $C_\gamma H_2$ (as) modes.[68,92] The asymmetric CH_2 modes are observed at a higher frequency than for all other amino acids in this study due to the strained nature of the five-member proline ring from which they originate. Additionally, the spectrum shows a dramatic increase in the region ∼3000−3400 cm^{-1}. The origin of this signal enhancement is attributed to one vibrational mode from a net orientation change in interfacial water O−H (∼3100 cm^{-1}), and N−H stretching modes from L-proline.[88,89,93] While these are identified as individual modes responsible for the increase in SFG signal, these modes are not distinctive in the broad peak because both the N−H group from L-proline and O−H from the aqueous solvent (carboxylic acid portion of the amino acid is deprotonated at pH 7.4)[94] readily participate in hydrogen bonding which leads to line broadening for each of the three modes to the extent that they are substantially overlapping.

In a previous SFG study of similar L-proline solutions at the hydrophilic d_8-PS interface, we only observed a smaller peak in this region around 3100 cm^{-1}, which was only about half of the intensity of the 2984 cm^{-1} mode.[68] This discrepancy between the L-proline spectra is partially attributed to differences in the solution concentrations used in these studies. It was previously necessary for us to perform our SFG experiments on L-proline at 500 mg/mL concentrations to resolve any spectral features in the 2800−3600 cm^{-1} region, but subsequent experimental improvements allowed the amino acid to be easily measured in this study at a concentration of 15 mg/mL. The substantial reduction of solution concentration used may have reduced the incidence of adsorbate accumulation on the substrate surface, thus, providing a more appropriate liquid−solid interface to probe with SFG by minimizing the probability of signal contributions from a potential multilayer build-up of amino acid molecules.

3.2.9. L-Alanine. The SFG spectrum of a solution of L-alanine (40 mg/mL) in PBS at the hydrophobic d_8-PS liquid−solid interface is presented in Figure 9. Each data point in these spectra is the average of 600 laser shots. Interestingly, the spectrum of L-alanine does not show any SFG active modes at this hydrophobic surface. Furthermore, the background and amino acid spectra are indistinguishable as both show a broad continuum of SFG signal centered around 3100 cm^{-1} attributed to O−H stretching vibrational modes of interfacial water at the hydrophobic solid−liquid

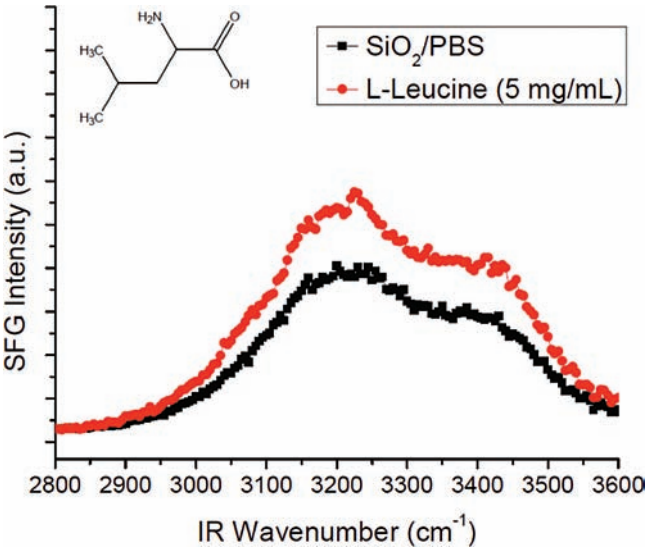

Figure 10. SFG spectrum of PBS/SiO_2 liquid−solid interface before (black squares) and after (red circles) addition of L-leucine (5 mg/mL). No hydrocarbon vibrational modes are observed, but an enhancement in the broad spectral features at ∼3200 and ∼3400 cm^{-1} occurs upon the addition of leucine. This result suggests that the presence of the hydrophobic amino acid can cause a change in the net orientation of interfacial water at the SiO_2/PBS interface.

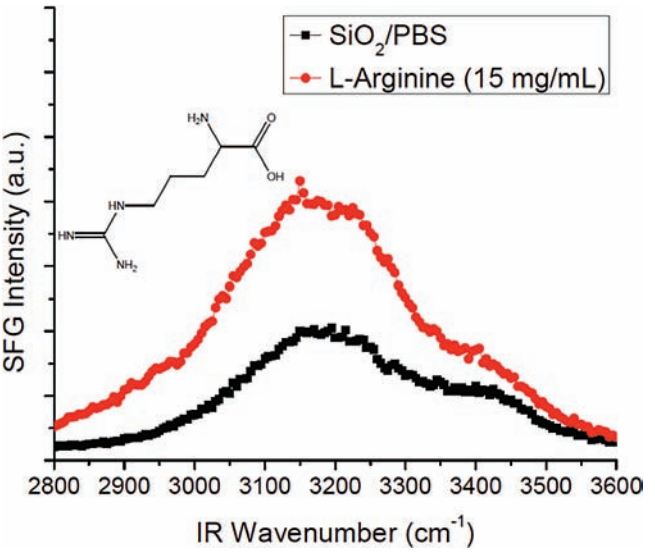

Figure 11. SFG spectrum of PBS/SiO_2 liquid−solid interface before (black squares) and after (red circles) addition of L-arginine (15 mg/mL). No hydrocarbon vibrational modes are observed upon the addition of arginine, but an enhancement and substantial peak broadening occurs in the spectral features at ∼3200 and ∼3400 cm^{-1}. This result suggests that the presence of the L-arginine can cause a change in the net orientation of interfacial water at the SiO_2/PBS interface.

interface. Using arguments based purely on the hydrophobic nature expected to be exhibited by an amino acid with a methyl side chain, one might expect this small molecular species to readily adsorb on to the deuterated polystyrene surface. Therefore, the absence of SFG active C−H modes in this spectrum may be attributed to a lack of interfacial L-alanine ordering and not an all together absence of interfacial amino acid species. This explanation is supported by QCM experiments at the aqueous PS liquid−solid interface which indicated a L-alanine surface concentration of 469 ± 33 ng/cm^2 as presented in Table 1.

3.3. SFG Data at the Hydrophilic SiO_2 Surface. *3.3.1. Phosphate Buffered Saline (PBS).* The SFG spectrum of pure pH 7.4 PBS at the hydrophilic SiO_2/PBS solid−liquid interface is presented in the background spectra of Figures 10−17. The background spectra of these figures show two strong, broad spectral features centered at ∼3200 and ∼3400 cm^{-1} attributed to a continuum of O−H stretching modes of interfacial water. Both of these spectral features are consistent with previous SFG studies of PBS solutions at the hydrophilic SiO_2 surface under similar conditions. Interestingly, these features are similar to those observed at the water−vapor interface.[26,31,68,69,71,73,75,83,95] The interpretation of these two peaks as representing two distinct intermolecular configurations is not embraced here, but the traditional naming convention of "ice-like" (3200 cm^{-1}) and "liquid-like" (3400 cm^{-1}) is used herein for simplicity. None of amino acids investigated at the hydrophilic SiO_2 surface were observed to have clear C−H vibrational modes at this interface. However, the addition of these biomolecules to the hydrophilic solid−PBS interface was found to have a varying and measurable range of influence on the SFG signal from interfacial water as a function of amino acid identity. This agrees with previous work that shows the adsorption of charged species to aqueous interfaces alters the O−H vibrational features.[96] The absence of observable C−H vibrational modes at the SiO_2/PBS interface for these species is attributed to the zwitterionic nature of amino acids in

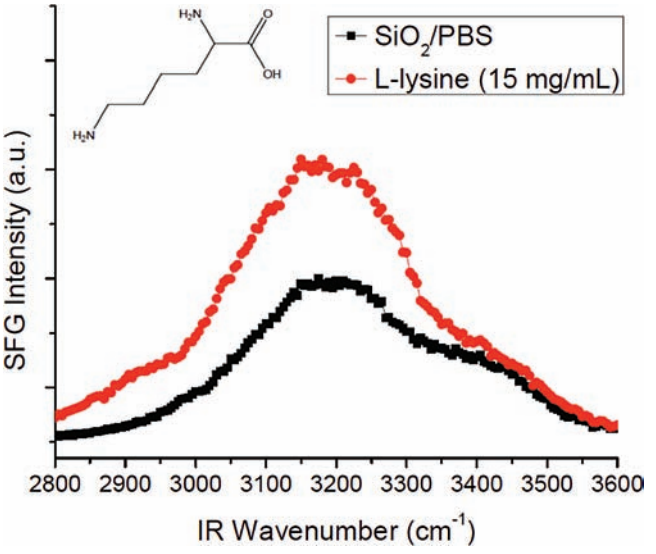

Figure 12. SFG spectrum of PBS/SiO_2 liquid−solid interface before (black squares) and after (red circles) addition of L-lysine (15 mg/mL). No hydrocarbon vibrational modes are observed upon the addition of arginine. However, similar to the SFG spectrum of L-arginine at the SiO_2/PBS interface, this spectrum shows a significant enhancement and peak broadening occurring in the spectral features at ∼3200 and ∼3400 cm^{-1}. This result suggests that the presence of the L-lysine amino acid molecules causes a change in the net orientation of interfacial water at the SiO_2/PBS interface.

aqueous solution at pH 7.4. These amino acids have their amine portions protonated and their carboxylic acid portions deprotonated leaving charged $-NH_3^+$ and $-COO^-$ groups present at the two ends of each molecule, respectively.[94] Yeganeh et al. used SFG to observe SiO_2 in an aqueous environment transitioning (with

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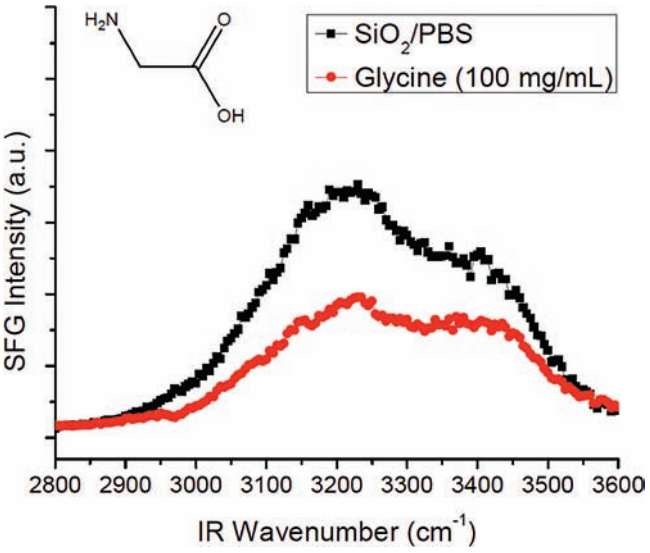

Figure 13. SFG spectrum of the SiO_2/PBS solid−liquid interface before (black squares) and after (red circles) the addition of glycine (100 mg/mL). SFG signals at ∼3200 and ∼3400 cm^{-1} are reduced upon addition of the amino acid.

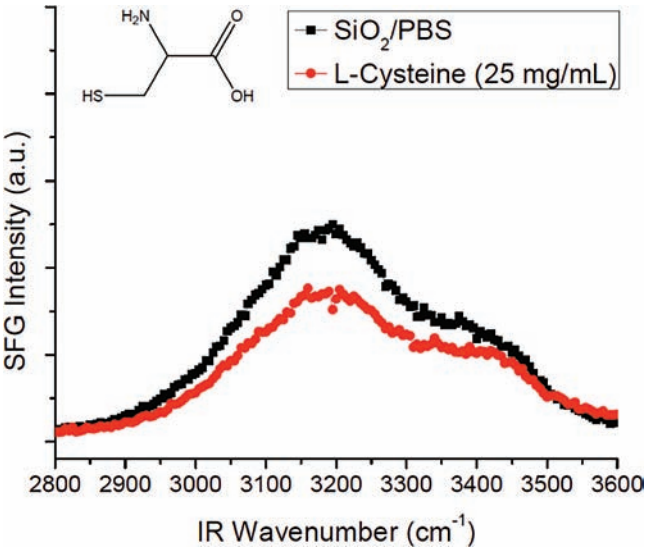

Figure 14. SFG spectrum of the SiO_2/PBS solid−liquid interface before (black squares) and after (red circles) the addition of L-cysteine (25 mg/mL). SFG signals at ∼3200 and ∼3400 cm^{-1} are reduced upon addition of the amino acid.

decreasing pH) from having a negative surface charge to becoming electrically neutral at its isoelectric point, p*I* ∼2.[97] As a zwitterionic amino acid comes close to the negatively charged and hydrophilic SiO_2 surface, columbic forces between the two are expected to drive adsorption via the charged ends of the amino acid instead of through the side chain as expected in the case with the hydrophobic d_8-PS surface. This phenomenon is highlighted in the case of solvated glycine at pH 7.4 where each molecule has a total of two charged moieties and only ten covalently bound atoms.

3.3.2. L-Leucine, L-Arginine, and L-Lysine. At the hydrophilic SiO_2/PBS solid−liquid interface, Figures 10−12 show the SFG spectra of solutions of L-leucine (5 mg/mL), L-arginine (15 mg/mL), and L-lysine (15 mg/mL) in PBS, respectively. Upon the addition of amino acid solutions, each of these spectra show an enhancement in the SFG signal from both the "ice-like" and "liquid-like" peaks relative to the spectrum of pure PBS at the SiO_2 surface. These three amino acids have side chains of substantially differing chemical structure and character making this finding unanticipated. The origin of the water signal enhancement is indicative of a change in the net orientation of interfacial water species. These orientation changes are not surprising, considering the adsorption of zwitterionic amino acids to the charged SiO_2 interface. Each data point in the spectra of L-leucine and L-arginine are the average of 500 laser shots, while the spectrum of L-lysine is the average of 600 measurements.

The adsorbed mass of L-arginine at the SiO_2/PBS interface was determined to be 196 ± 20 ng/cm^2 by QCM measurements shown in Table 1. Therefore, the lack of identifiable vibrational C−H modes in the SFG spectrum of L-arginine is not attributed to a lack of amino acid at the interface. Instead, this lack of vibrational modes in the SFG spectra indicates that the interfacial amino acid species do not have a strong net orientation.

In addition to an increase in signal, the SFG data of L-arginine (Figure 11) and L-lysine (Figure 12) also show a broadening of the peaks at 3200 and 3400 cm^{-1}. This spectral change may be indicative of a weak N−H vibrational mode from these amino acids with the distinctive and shared characteristic of having amine terminated side chains overlapping with the existing O−H modes. The amino terminated chain of L-lysine and the guanidino terminated side chain of L-arginine are both capable of directly participating in hydrogen bonding in aqueous solution and both are expected to be protonated in a solution of PBS at pH 7.4.[94] The presence of these additional charged groups on the amino acids may also lead to an enhancement in the frequency and strength of attractive interactions with the hydrophilic and charged SiO_2 substrate as they approach the substrate in an adsorption event. This finding underscores the sensitivity of SFG vibrational spectroscopy to a species' interfacial orientation and the intermolecular forces that can affect it.

3.3.3. Glycine, L-Cysteine, and L-Alanine. The SFG spectra of glycine (100 mg/mL), L-cysteine (25 mg/mL), and L-alanine (40 mg/mL) at the SiO_2 surface all show a reduction in interfacial water SFG signal relative to their pure PBS background scans presented in Figures 13−15, respectively. This was an unanticipated similarity between the spectra based on their remarkably different molecular structures and varying hydrophilic nature of the three amino acids. The absence of C−H modes from glycine agrees with previous SFG and FTIR experiments on another hydrophilic surface, TiO_2, at the liquid−solid interface.[67,98] The adsorbed mass of L-cysteine and L-alanine at the SiO_2 interface as determined by QCM are shown in Table 1, indicating that these amino acids are adsorbed to the SiO_2 interface. The decrease in SFG signal due to interfacial water species could be due to a change in the net orientation of interfacial water species. However, it may also be attributed to the higher solution concentration of these amino acids than all others in this study. An elevated level of biomolecule solution concentration could result in the displacement of interfacial water molecules. Either of these potential circumstances could directly result in a decrease in the SFG signal intensity observed from water. This hypothesis is supported by the observed reduction in water signal upon addition of a high concentration L-proline at the SiO_2/PBS interface in a previous study.[68] Each data point in the spectra of glycine and L-alanine are the average of 500 laser shots, while the spectrum of L-cysteine is an average of 600 measurements.

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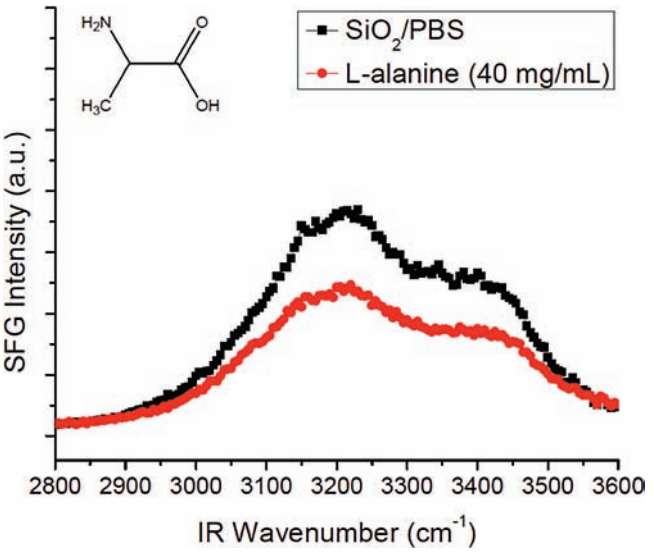

Figure 15. SFG spectrum of the SiO_2/PBS solid−liquid interface before (black squares) and after (red circles) the addition of L-alanine (40 mg/mL). SFG signal at ∼3200 and ∼3400 cm^{-1} are reduced upon addition of the amino acid.

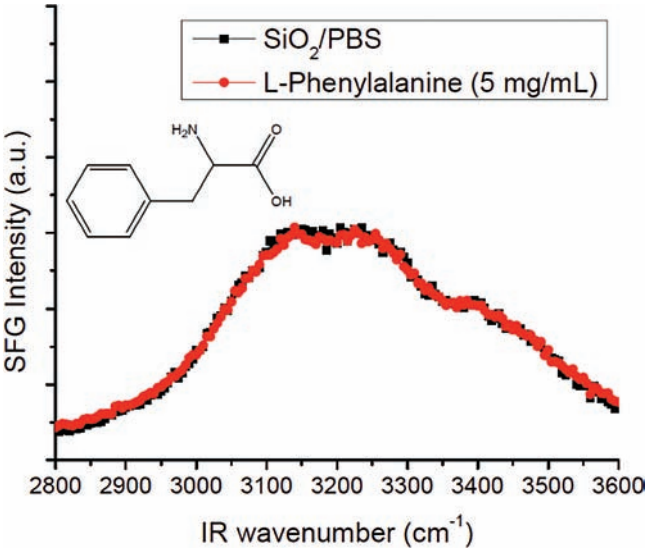

Figure 16. SFG spectrum of PBS/SiO_2 liquid−solid interface before (black squares) and after (red circles) addition of L-phenylalanine (5 mg/mL). No changes in water signal at ∼3200 or ∼3400 cm^{-1} are observed upon the addition of amino acid.

3.3.4. L-Phenylalanine and L-Proline. The SFG spectra of L-phenylalanine (5 mg/mL) and L-proline (15 mg/mL) in Figures 16 and 17, respectively, differ from the other amino acids studied at the hydrophilic SiO_2 surface in that these spectra are not different from that of pure PBS. Neither the "ice-like" water signal at 3200 cm^{-1} nor the "liquid-like" water peak at 3400 cm^{-1} show any change upon the addition of amino acid in either of these spectra. QCM measurements conducted in this study indicated an interfacial concentration of L-phenylalanine at 63 ± 4 ng/cm^2 on the hydrophilic SiO_2 surface as shown in Table 1. This data indicates that the absence of distinguishable SFG vibrational modes following the introduction

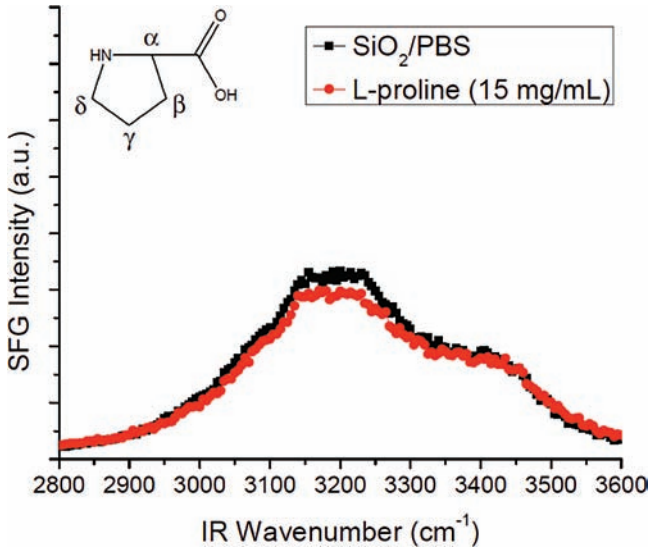

Figure 17. SFG spectrum of the SiO_2/PBS solid−liquid interface before (black squares) and after (red circles) the addition of L-proline (15 mg/mL). No changes in water signal at ∼3200 or ∼3400 cm^{-1} are observed upon the addition of amino acid.

of L-phenylalanine may be primarily due to its surface organization and not to an absence at the hydrophilic solid−liquid interface. In a previous SFG study of phenylalanine at the glassy carbon electrode/D_2O liquid−solid interface, the observation of an aromatic C−H stretch was expected but also was not observed.[54]

It has previously been demonstrated that L-proline does adsorb to the SiO_2 interface at higher solution concentation.[68] The results reported here is in contrast to the previous SFG study of L-proline at much higher concentration (500 mg/mL) where both a C−H vibrational mode and a substantially reduced water signal were observed at the hydrophilic SiO_2/PBS interface in the presence of the L-proline amino acid.[68] In that study, a small peak at 2985 cm^{-1} was identified as originating from an asymmetric CH_2 stretch from the C_β and C_γ carbons on the strained, five-member proline ring.[68,92] Some of the reduction in water signal in that study was attributed to the relatively high concentration of amino acid used which may have led to the displacement of interfacial water molecules from the SiO_2 surface. This hypothesis is supported by the absence in observed change of the water signal upon addition of L-proline at a much lower solution concentration of 15 mg/mL used in this study. Each data point in Figures 16 and 17 is the average of 500 laser measurements.

4. CONCLUSIONS

This investigation demonstrated the feasibility of investigating amino acids, the building blocks of both peptides and proteins, using sum frequency generation vibrational spectroscopy. Specifically, the SFG spectra of aqueous solutions of eight amino acids were obtained at the hydrophilic SiO_2 and hydrophobic d_8-polystyrene surfaces. This finding is significant because it includes the first known SFG signal obtained from several of these individual amino acids in aqueous solutions at the solid−liquid interface and at physiological pH. Seven of the eight amino acids showed identifiable C−H vibrational modes at the hydrophobic d_8-polystyrene surface, while L-alanine was the only amino acid studied which did not show distinct SFG activity at the d_8-PS/PBS interface. However, QCM measurements confirmed the interfacial presence of this chemical

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species indicating this SFG inactivity may be attributed to the interfacial configuration of the L-alanine species on the d_8-PS surface. At the hydrophilic SiO_2 surface, adsorption of amino acids was confirmed by QCM measurements, but no C−H vibrational modes were observed from any of the amino acids studied. The absence of C−H vibrational modes at the SiO_2 interface is most simply interpreted as an indication that amino acid ordering at this interface is weaker than at the d_8-PS interface. However, the presence of amino acids in buffer solutions was found to have a varying influence on the SFG signal from interfacial water at the SiO_2 surface. The spectra obtained at the SiO_2/PBS interface showed enhancement in the SFG signal from interfacial water in the presence of L-leucine, L-arginine, and L-lysine amino acid molecules when compared to background levels. The spectral features from interfacial water were found to decrease in magnitude from levels observed at the pure PBS/SiO_2 interface for solutions of glycine, L-cysteine, and L-alanine. This finding is attributed to these three amino acids being studied at relatively high solution concentrations of 25 mg/mL and above, which may lead to an increase in the adsorbed number of amino acids and their frequency of interactions with interfacial water molecules. Such a circumstance could result in a disruption or displacement of interfacial water molecules at the hydrophilic SiO_2 surface. Lastly, the SFG spectra of both L-proline and L-phenylalanine solutions at the SiO_2/PBS interface showed no evidence of perturbing the organization or concentration of adsorbed water.

Although this work clearly demonstrates the feasibility of using SFG to probe amino acids at surfaces, challenges remain in the effort to elucidate the molecular level interfacial structure of these amino acids. There is some uncertainty regarding the fitting of the spectra and the assignment of vibrational modes. Additionally, SFG spectra cannot be interpreted as simple absorption spectra; the number of adsorbed species, their orientation, and the strength of transition all contribute to the magnitude of the observed spectral features. These issues can be addressed in part by selective deuteration or other, more advanced methods, such as phase sensitive sum frequency generation. We aim to determine the temperature and concentration dependence of various amino acids, and to compare these results to molecular dynamics simulations. It is our hope that this type of study will aid in future work on more relevant and important biomolecules, such as peptides and proteins, and provide some physical insight to the adsorption processes of molecules from solution onto solid surfaces.

■ ASSOCIATED CONTENT

Supporting Information. SFG plots (Figures 2−8) are shown with their corresponding fits and fitting parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

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