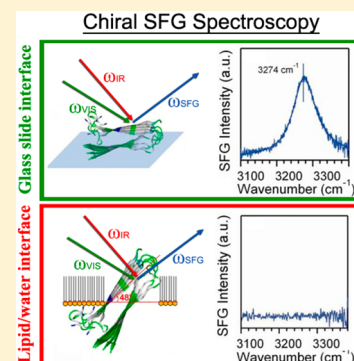


Characterization of Parallel β -Sheets at Interfaces by Chiral Sum Frequency Generation SpectroscopyLi Fu,^{*,‡} Zhuguang Wang, Brian T. Psciuk, Dequan Xiao,[†] Victor S. Batista, and Elsa C. Y. Yan^{*}

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Supporting Information

ABSTRACT: Characterization of protein secondary structures at interfaces is still challenging due to the limitations of surface-selective optical techniques. Here, we address the challenge of characterizing parallel β -sheets by combining chiral sum frequency generation (SFG) spectroscopy and computational modeling. We focus on human islet amyloid polypeptide aggregates and a de novo designed short polypeptide at lipid/water and air/glass interfaces. We find that parallel β -sheets adopt distinct orientations at various interfaces and exhibit characteristic chiroptical responses in the amide I and N–H stretch regions. Theoretical analysis indicates that the characteristic chiroptical responses provide valuable information on the symmetry, orientation, and vibrational couplings of parallel β -sheet at interfaces.



Assembly of parallel β -sheets at interfaces is critical for the design of ordered chiral macromolecular architectures¹ and for understanding structure/function relations in amyloid aggregates.^{2,3} The assembly occurs at aqueous interfaces of various materials, such as lipid membranes or biosensors, which could be remarkably different from that occurs in the bulk solutions. Using conventional characterization techniques such as Fourier transform infrared (FTIR), Raman, and circular dichroism (CD) spectroscopy, it is often difficult to obtain information about protein secondary structures exclusively at the interface while simultaneously eliminate the interference from background of molecules such as lipid and water. Therefore, there is a need to develop surface-selective optical techniques for the characterization of macromolecular structures at interfaces.

In recent years, sum frequency generation (SFG) spectroscopy has gained increasing attention as applied to studies of proteins,^{4–13} DNAs,^{14–17} and other biomolecules.^{18–22} SFG is often sensitive to the interfacial contributions and is background-free from the bulk solution,²³ allowing for studies of molecular structure, orientation, and dynamics at interfaces. In addition, SFG can selectively probe chirality that is gaining tremendous attentions in probing biomolecules by nonlinear optics such as SFG.^{24,25} We have applied chiral SFG by probing the N–H stretch and amide I bands to distinguish protein secondary structures.^{26–28} We have monitored, in situ and in real time, the misfolding of human islet amyloid polypeptide (hIAPP) into parallel β -sheets via an α -helical intermediate at the lipid/water interface,^{26,28,29} and proton exchange in antiparallel β -sheets at interfaces.³⁰ We have also demonstrated that chiral SFG can be used to determine the orientation of the parallel β -strand of hIAPP aggregates at interfaces by analyzing the chiral amide I spectrum.^{28,31,32}

Nonetheless, rigorous testing of theoretical descriptions about chiral SFG response of macromolecules using experimental data under various conditions are still scarce, but much is needed to move the SFG field forward particularly for applications in biological and material sciences. Here, we use parallel β -sheet as a model to demonstrate the synergic approach of combining theory and experiments to analyze the chiral and achiral SFG response of parallel β -sheet, constructing a framework for further developments of theoretical and experimental approaches for analyzing other protein secondary structures, such as an antiparallel β -sheet, α -helix, and 3_{10} -helix, and even beyond protein secondary structures, such as DNA and polysaccharide.

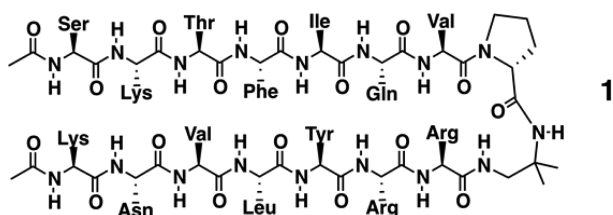
We use hIAPP aggregates as well as a de novo designed short peptide (peptide 1) as model systems. The hIAPP is a 37-amino acid peptide hormone, secreted by human pancreatic β -cells, which forms parallel β -sheet amyloid aggregates upon interactions with negatively charged lipid molecules.³³ The peptide 1 (Scheme 1), developed by Gellman and co-workers, has a D-proline amino acid linker confining two β -strands into a parallel β -sheet structure.³⁴

We combine experiments and theoretical modeling to obtain the chiral SFG spectra of both the N–H stretch and amide I regions and we show that the chiral optical responses of parallel β -sheets are determined by three factors, including the molecular symmetry, vibrational coupling, and the molecular orientation at interfaces. Our analysis agrees with the SFG spectra simulated by using the molecular hyperpolarizability calculated by ab initio quantum chemistry methods. Our results

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Scheme 1. Peptide 1 with a D-Proline Amino Acid Connecting Two Parallel β -Sheet Strands

illustrate a quantitative approach for analyzing SFG spectra to extract information about structures, orientation, and vibrational coupling of parallel β -sheet structures at interfaces, which should be useful for studies of other macromolecular structures at interfaces.

Figure 1 shows the chiral SFG spectra of hIAPP aggregates at the lipid/water interface and at the air/glass surface in the amide I and N–H stretch vibrational regions. The peak intensities and positions of the bands, shown in Table 1, were obtained by fitting the spectra to the Lorentzian function

$$I_{\text{SFG}} \propto \left| \chi_{\text{NR}}^{(2)} + \sum_q \frac{A_q}{\omega_{\text{IR}} - \omega_q + i\Gamma} \right|^2 \quad (1)$$

where I_{SFG} is the sum frequency intensity, $\chi_{\text{NR}}^{(2)}$ is the nonresonant second-order susceptibility,³⁵ ω_{IR} is the incident IR frequency, and A_q , ω_q , and Γ_q are the amplitude, resonant frequency, and damping factor of the q th vibration mode, respectively.

Figure 1 shows that hIAPP aggregates exhibit a significantly different chiral optical response when probed at the lipid/water interface or on a glass slide. The chiral amide I band of hIAPP at the lipid/water interface shows a peak at 1622 cm^{-1} and a shoulder at 1660 cm^{-1} (Figure 1A), assigned to the B

Table 1. Spectral Parameters Obtained by Fitting the Amide I Bands, Shown in Figure 1

		hIAPP lipid/water (Figure 1A)	hIAPP glass slide (Figure 1B)
A mode	$\chi_{\text{NR}}(\text{a.u.})$	0.02 ± 0.003	-0.189 ± 0.017
	$\omega_A(\text{cm}^{-1})$	1660 ± 1.3	1667.4 ± 1.4
	$A_A(\text{a.u.})$	-2.1 ± 0.16	-8.2 ± 1.1
	$\Gamma_A(\text{a.u.})$	16.0 ± 1.1	17.0 ± 1.2
B mode	$\omega_B(\text{cm}^{-1})$	1622 ± 1.3	1621 ± 0.1
	$A_B(\text{a.u.})$	3.9 ± 0.06	27.3 ± 0.2
	$\Gamma_B(\text{a.u.})$	13.6 ± 0.3	14.2 ± 0.1

(asymmetric stretch) and A (symmetric stretch) modes, respectively, of the amide I band (Scheme 2).^{36,37} On the glass slide, the peak is at 1621 cm^{-1} (B mode) and the shoulder at 1667 cm^{-1} (A mode) (Figure 1B). Although turn structures have characteristic amide I vibrational frequency also at $\sim 1660 \text{ cm}^{-1}$, the contribution should be negligible according to the low surface population in the hIAPP structures and previous reports in literature³⁸ (see Supporting Information). Thus, as an approximation, we neglect the contribution from β -turns and only consider contribution from the amide I A mode to the $\sim 1660 \text{ cm}^{-1}$ peak. The intensity ratio of the B mode ($\sim 1620 \text{ cm}^{-1}$) relative to the A mode ($\sim 1660 \text{ cm}^{-1}$) is much smaller than the ratio for the aggregates at the lipid/water interface (Figure 1A). More dramatic differences are observed for the N–H stretch spectra, showing a peak at 3301 cm^{-1} on the glass slide (Figure 1B) but no signal at the lipid/water interface (Figure 1A).

The observed differences in the SFG chiral optical response of hIAPP in different spectral regions at various interfaces raise an important question: what determines the selectivity of the SFG response of the parallel β -sheet structure at the interface? We address this question by considering three factors: (1)

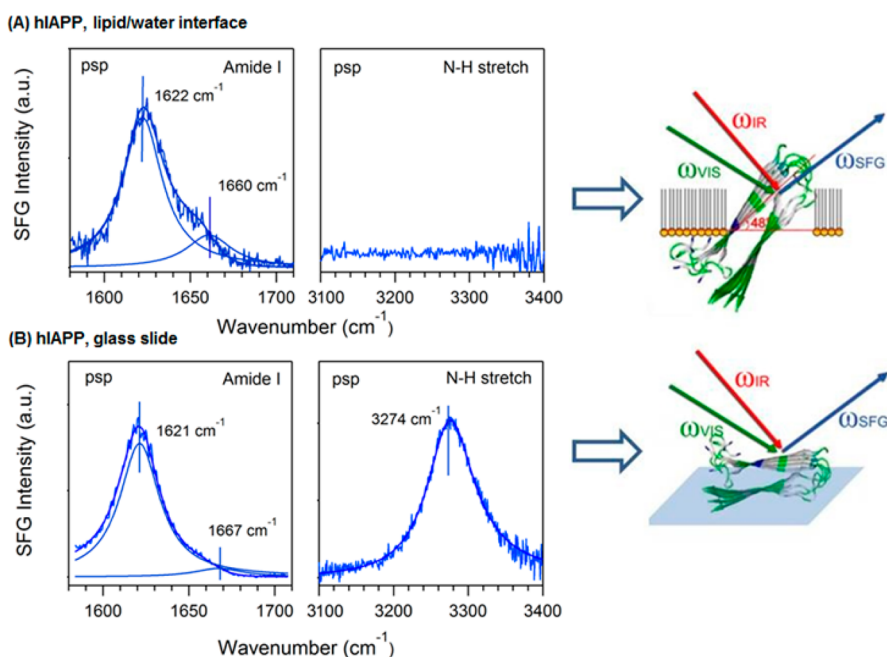
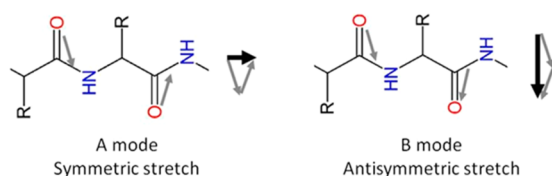


Figure 1. Chiral *psp* SFG spectra of hIAPP aggregates at interfaces, including the amide I (left) and N–H stretch (right) regions, as well as the orientation of hIAPP aggregates at the (A) lipid/water and (B) air/glass interfaces. Color codes: green for hydrophilic residues, white for hydrophobic residues, and blue for a positively charged arginine residue.

Scheme 2. A Mode and B Mode of the Amide I Vibration of Parallel β -Sheets



molecular symmetry, (2) vibrational coupling, and (3) molecular orientation.

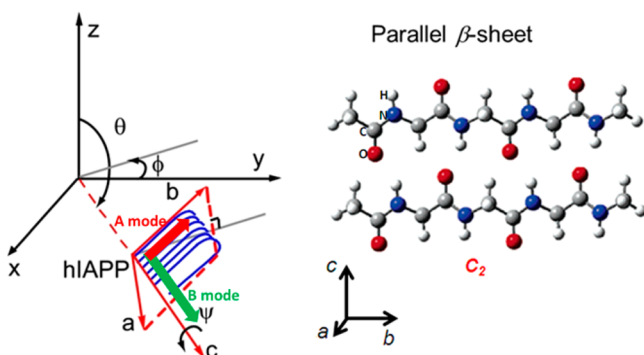
Molecular Symmetry. Molecular symmetry governs the chiroptical responses of interfacial molecules through the hyperpolarizability elements.³⁹ Although a single amide group has C_s symmetry, the chiral SFG response originates from the macroscopic arrangement of multiple amide I groups in protein backbones and, thus, should conform to the symmetry of the secondary structure under study. The parallel β -sheet in our study adopts a C_2 symmetry, giving the following nonzero hyperpolarizability tensor elements: $\beta_{abc} = \beta_{bac}$, $\beta_{cba} = \beta_{bca}$, $\beta_{acb} = \beta_{cab}$, $\beta_{bcb} = \beta_{ccb}$, $\beta_{aba} = \beta_{baa}$, $\beta_{bbb} = \beta_{abb}$, $\beta_{aab} = \beta_{baa}$, $\beta_{ccb} = \beta_{bcb}$ with the molecular coordinates (a, b, c) defined in Scheme 3. Using these nonzero hyperpolarizability elements, one can express the effective susceptibility of SFG in the psp polarization setting (see Supporting Information for derivation)

$$\chi_{psp,q}^{(2)} = -\frac{L_{zyx}}{2} N_s \left\{ \begin{aligned} &\langle \cos^2 \theta \rangle (\beta_{cab} - \beta_{cba}) + \langle \sin^2 \theta \sin^2 \psi \rangle (\beta_{bca} - \beta_{bac}) \\ &+ \langle \sin^2 \theta \cos^2 \psi \rangle (-\beta_{acb} + \beta_{abc}) \\ &+ \langle \sin \theta \cos \theta \cos \psi \rangle (-\beta_{aab} + \beta_{aba} - \beta_{cbc} + \beta_{ccb}) \end{aligned} \right\} \quad (2)$$

where θ is the tilt angle between the c -axis of the β -sheet and the interface, and ψ is the twist angle between the b -axis of the β -sheet and the interface (Scheme 3), N_s is the surface population, and L_{zyx} is the Fresnel factor. Equation 2 serves as a basis for quantitative interpretation of the chiral SFG spectra of the parallel β -sheet.

Vibrational Coupling. The hyperpolarizability elements of the complete parallel β -sheet can be obtained by summation of the contributions of individual amide groups because the N–H stretch modes are typically localized without significant

Scheme 3. Orientation of the β -Sheet of hIAPP Aggregates with $\theta = 90^\circ$ When the Normal to the Interface Is Defined by the z -Axis



coupling.⁴⁰ Thus, eq 2 can be directly applied to describe the chiral N–H signal based on their spatial arrangement of amide groups along the polypeptide backbone.³⁹ In contrast, the amide I modes of parallel β -sheets are coupled and split into two bands corresponding to the symmetric A and antisymmetric B vibrational modes (Scheme 2).³⁷

According to group theory, the elements β_{aab} , β_{ccb} , $\beta_{acb} = \beta_{cab}$ and β_{bbb} are associated with the A mode, whereas the elements $\beta_{abc} = \beta_{bac}$, $\beta_{bcb} = \beta_{bcc}$, $\beta_{cba} = \beta_{bca}$, $\beta_{aba} = \beta_{baa}$ are associated with the B mode. Thus, one can rewrite eq 2 to describe the A and B modes, as follows:

$$\chi_{psp,A}^{(2)} = -\frac{L_{zyx}}{2} N_s \{ \langle \cos^2 \theta \rangle \beta_{cab} - \langle \sin^2 \theta \cos^2 \psi \rangle \beta_{acb} + \langle \sin \theta \cos \theta \cos \psi \rangle (\beta_{ccb} - \beta_{aab}) \} \quad (3)$$

$$\chi_{psp,B}^{(2)} = -\frac{L_{zyx}}{2} N_s \left\{ \begin{aligned} &-\langle \cos^2 \theta \rangle \beta_{cba} + \langle \sin^2 \theta \sin^2 \psi \rangle (\beta_{bca} - \beta_{bac}) \\ &+ \langle \sin^2 \theta \cos^2 \psi \rangle \beta_{abc} + \langle \sin \theta \cos \theta \cos \psi \rangle (\beta_{aba} - \beta_{bcb}) \end{aligned} \right\} \quad (4)$$

Hence, although the N–H stretch of parallel β -sheets can be described by eq 2, the A and B modes of the amide I vibrations are described by eqs 3 and 4, respectively.

Orientation. The effect of orientation is analyzed by using eqs 2–4. The B mode (1620 cm^{-1}) does not appear in the achiral spp SFG spectra (Supporting Information Figures S3),³¹ suggesting that its corresponding transition dipole (Scheme 2) along the c -axis of the β -strands is isotropic (i.e., $\theta = 90^\circ$) at the interface (Scheme 3).

Hence, we can further simplify eqs 3 and 4, as follows:

$$\chi_{psp,A}^{(2)} = \frac{L_{zyx}}{2} N_s \langle \cos^2 \psi \rangle \beta_{acb,A} \quad (5)$$

$$\chi_{psp,B}^{(2)} = -\frac{L_{zyx}}{2} N_s \{ \langle \sin^2 \psi \rangle \beta_{bca,B} + (\langle \cos^2 \psi \rangle - \langle \sin^2 \psi \rangle) \beta_{bac,B} \} \quad (6)$$

Thus, the intensity ratio of B and A modes is a function of the twist angle ψ ³¹

$$I_{B/A} = \left| \frac{\chi_{psp,B}^{(2)}}{\chi_{psp,A}^{(2)}} \right|^2 = \left| \langle \tan^2 \psi \rangle \frac{\beta_{bca,B}}{\beta_{acb,A}} + (1 - \langle \tan^2 \psi \rangle) \frac{\beta_{bac,B}}{\beta_{acb,A}} \right|^2 \quad (7)$$

Equation 7, when applied for the chiral amide I signals, can be used to obtain the twist angle ψ of hIAPP aggregates at lipid/water and glass/air interfaces.³¹ We obtain the orientation of the hIAPP aggregates (Table 1) giving the observed intensity ratio $I_{B/A} = |((A_B/\Gamma_B)/(A_A/\Gamma_A))|^2$, which is 4.8 for the lipid/water interface (Figure 1A) and 15.9 for the glass slide surface (Figure 1B). In addition, our ab initio quantum chemistry calculations yield the tensor elements of $\beta_{bca,B}$, $\beta_{bac,B}$ and $\beta_{acb,A}$ revealing the relationship between intensities and orientation of the parallel β -sheet (ψ) (Supporting Information Figure S1). The tensor elements of $\beta_{bca,B}$, $\beta_{bac,B}$ and $\beta_{acb,A}$ are calculated as in

our previous studies,^{31,32} using the “divide-and-conquer” methodology, giving $\beta_{bca,B}/\beta_{acb,A} = 1.2$ and $\beta_{bac,B}/\beta_{acb,A} = -3.8$. At the lipid/water interface, $I_{B/A} = 4.8$ when the twist angle $\psi \sim 48^\circ$ consistent with our previous analysis.³¹ This orientation will bring the hydrophobic β -sheet (white, Figure 1) to the lipid phase and the hydrophilic β -sheet (green, Figure 1) to the water phase, and at the same time, it can position the positively charged arginine residue (blue, Figure 1) right at the level of the negatively charged DPPG lipid headgroup to facilitate electrostatic interactions. Unlike the B mode, whose dipole moment is parallel to the interface, the dipole moment of the A mode is nonparallel (Scheme 3) and generates achiral SFG signals at 1660 cm^{-1} in the *ssp* amide I spectra (Supporting Information Figure S3). For the glass slide surface, the achiral *ssp* amide I achiral SFG signals of both the B and A modes are not observed (Supporting Information Figure S3), suggesting that the average orientation of the dipole moments of both B and A modes are horizontal (i.e., isotropic on the surface). This indicates that the tilt angle $\theta \sim 90^\circ$ and the twist angle $\psi \sim 0^\circ$. This particular orientation yields a larger intensity ratio of B to A modes in the chiral *psp* amide I spectrum as predicted by eq 7, shown in Supporting Information Figure S1,³¹ in agreement with the larger experimentally observed value of $I_{B/A} = 15.9$.

The angles θ and ψ define the orientation of parallel β -sheets at lipid/water and glass/air interfaces. For the N–H stretch, the expression of the effective susceptibility given by eq 2 can be simplified by setting $\theta = 90^\circ$, as follows:

$$\chi_{psp,N-H\text{ Stretch}}^{(2)} = -\frac{L_{zyx}}{2} N_S \{ \langle \sin^2 \psi \rangle \beta_{bca} - \langle \cos^2 \psi \rangle \beta_{acb} + \langle \cos^2 \psi - \sin^2 \psi \rangle \beta_{bac} \} \quad (8)$$

The analysis of eq 8 shows that the chiral N–H signal can be greatly suppressed, yielding an undetectable signal for hIAPP aggregates at the lipid/water interface. First, a twist angle of $\psi = 48^\circ$ is close to 45° , making the term $\langle \cos^2 \psi - \sin^2 \psi \rangle$ approximately zero. Second, β_{bca} and β_{acb} in eq 8 have small contributions to the effective hyperpolarizability $\chi_{psp,N-H\text{ stretch}}^{(2)}$ due to the fact that the individual dipole moment of the N–H stretch projects mostly onto the *c* axis, with a weak projection onto the *a* and *b* axes (Scheme 2). Previous analysis by Simpson and co-workers supports this argument as they found that, for N–H stretch, β_{bac} ($= -1.91 \times 10^{-2}$) is about 10 times larger than β_{bca} ($= 6.82 \times 10^{-4}$) and β_{acb} ($= 1.95 \times 10^{-3}$).³⁹ By substituting these values of hyperpolarizability into eq 8, we simulated the chiral N–H intensity as a function of orientation for a more quantitative understanding of the relationship between the chiral N–H stretching signal and the orientation of the parallel β -sheet at the interface (Figure 2).

Figure 2 shows that when the twist (ψ) angle of the parallel β -sheet approaches $\sim 45^\circ$, the SFG intensity of N–H stretch vanishes, supporting that the disappearance of the N–H signal of the hIAPP aggregates at the lipid/water interface is due to its orientation with $\psi = 48^\circ$, as revealed from the analysis of the chiral *psp* amide I spectrum. On the other hand, it is expected that the chiral N–H stretching signal can be detected from the hIAPP aggregate, if it adopts an orientation deviated from 45° , as shown in Figure 1B for hIAPP aggregates on a glass surface.

As discussed earlier, the achiral SFG spectra show no noticeable amide I signal from the hIAPP aggregates at the glass slide surface (Supporting Information Figure S3), suggesting that dipole moments of both the A and B modes are parallel to the glass surface and that the hIAPP aggregates are lying flat

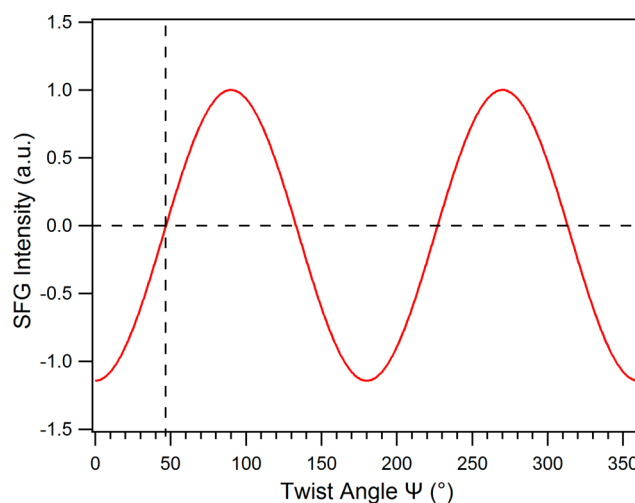


Figure 2. Simulated SFG intensity of the N–H stretch as a function of ψ , with $\theta = 90^\circ$, using the calculated DFT hyperpolarizability elements.³⁹

with the tilt angle $\theta = 90^\circ$ and the twist angle $\psi = 0^\circ$. This indicates that the two β -sheets (upper and lower, Figure 1) in hIAPP adopt an orientation parallel to the surface, presumably with the more hydrophilic sheet (amino acid 28–37) in contact with the glass slide and the more hydrophobic sheet (amino acid 20–27) in contact with the air phase. With such orientation, eq 8 predicts that $\chi_{psp,N-H\text{ stretch}}^{(2)}$ would not be suppressed, which is consistent with Figure 2 and with the chiral N–H signal shown in Figure 1B.

To analyze whether the chiral N–H signal is common to other parallel β -sheets, beyond the hIAPP aggregates, we analyzed the SFG spectra of peptide 1 (Scheme 1) in a parallel β -sheet structure.⁴¹ Figure 3 confirms that the chiral N–H stretch signal is observed for peptide 1 with parallel β -sheet with characteristic amide I bands (Figure 3). Two peaks at 1636 and 1661 cm^{-1} are fitted into eq 1 (Supporting Information Table S1) and assigned to the amide I B and A modes, respectively. In addition, we conclude that the long *c*-axis of peptide 1 aligns with the interface, with $\theta = 90^\circ$, since the achiral amide I SFG spectra is muted (Supporting Information Figure S2). These results agree with our conclusion that the muted chiral N–H stretch spectrum of hIAPP aggregates at the lipid/water interface is due to the specific orientation of hIAPP with ψ close to 45° .

Our analysis thus demonstrates that the chiroptical SFG response from parallel β -sheets is highly dependent on the molecular orientations at the interface. For the amide I spectra,

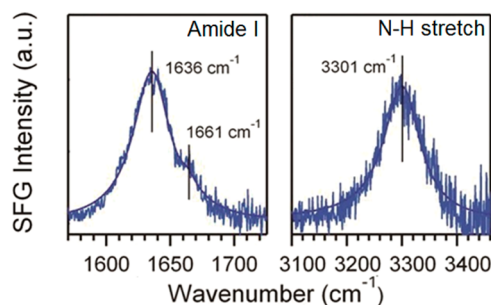


Figure 3. *psp* SFG spectra of peptide 1 at the glass slide surface in the amide I and N–H stretch region.

the intensity ratio of the A and B modes can be used to probe the orientation. The intensity of chiral N–H signal also varies with the orientation and may disappear under specific orientations as observed for hIAPP aggregates.

Conclusions. We have shown how molecular symmetry, vibrational coupling, and molecular orientation determine the chiroptical SFG response from protein secondary structures at interfaces. Our theoretical analysis addresses both amide I and N–H stretching modes for parallel β -sheets at water/lipid and air/glass interfaces and provides a rigorous interpretation of experimental observations. The resulting study of parallel β -sheets clearly demonstrates that the combined theoretical and experimental approach is a valuable methodology for quantitative and systematic investigation of the chiroptical responses of not only protein secondary structures but also synthetic and native chiral macromolecular structures, such as DNA, RNA, polysaccharides, and supramolecular structures at interfaces. Our work demonstrates the capacity of chiral and achiral SFG for revealing fundamental information about protein structures and orientation at different interfaces, building a foundation for future applications of the method to solve a wide range of problems related to protein structures and functions at interfaces.

■ ASSOCIATED CONTENT

■ Supporting Information

Materials and methods. Experimental SFG spectrometer. Intensity ratio of amide I B to amide I A mode in the *psp* spectrum as a function of twist angle (ψ). Spectral parameters obtained by fitting the amide I bands in Figure 3. The *ssp* SFG spectrum of peptide 1 at the glass slide surface. Derivation of the effective susceptibility as a function of hyperpolarizability elements (including the *ssp* SFG spectrum of hIAPP aggregates at the lipid/water and the air/glass interfaces). Contribution of turn structures to the $\sim 1660\text{ cm}^{-1}$ peak in hIAPP spectra. Characterization of peptide 1. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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