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Orientation determination of interfacial bent α-helical structures using Sum Frequency Generation vibrational spectroscopy

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ABSTRACT

Sum Frequency Generation (SFG) has been shown to be a powerful and versatile technique in studies of proteins/peptides at surfaces and interfaces. Recently SFG was successfully applied in studies of interfacial macro-molecules with increasing size and complexity. In this report we continued to employ bond additivity model and group theory to demonstrate the importance of both the inter-helical tilt angle and the lengths of the helical segments assembling the structures being studies. Specifically, a newly improved SFG data analysis of multiple α-helical structures on melittin was used to interpret the SFG experimental observation and also verified the findings with the recent insights brought by other spectroscopic techniques.

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1. Introduction

Sum Frequency Generation (SFG) signal in the amide I band of the α-helical secondary structure was first observed in 2005 by Chen and coworkers [1]. Since then SFG data analysis methods have been continuingly developed and applied to a variety of biological molecules of different levels of complexity including melittin, magainin 2, cecropin P1, fibrinogen, alamethicin, MSI-78, tachyplesin I, cytochrome, human islet amyloid polypeptide, and heterotrimeric G-protein [2–7]. The orientation analysis of simple biological molecules consisting of a single α-helical segment has been successfully carried out on small peptides such as magainin 2, alamethicin and MSI-78 [3–5]. Due to their simple structural properties and interaction schemes, these analyses were carried out using just the SFG signals in *ssp* and *ppp* polarization combinations. For more complex structures/interaction schemes which inherently consist of more orientational parameters, SFG *ssp* and *ppp* signals are not sufficient to provide target orientational information because these two pieces of information can only be used to solve for a single orientation parameter if the absolute peptide coverage is unknown. In such cases, complementary spectroscopic techniques (typically ATR-FTIR), as well as additional computational ab-initio simulation and mathematical approaches/information theories, have been required to provide extra orientational information about the interfacial species [8–11]. Even though these complementary approaches have certainly been useful in the interpretation of various biological secondary structures at the interfaces, they all have intrinsic limitations in the computational algorithms or methods of approximation. For instance, the transition Raman polarizability of an oscillator can only be approximated due to its indefinite number of virtual excited states in the Raman process.

A systematic study employing group theory and the bond additivity model in the orientation determination of single α-helical structures of different lengths using ssp and ppp SFG amide I signals has been previously reported [12]. However, to my knowledge, there have been no published reports of direct and formal determination of the interfacial orientation of a general multi-helical structure. There have been a few recent SFG studies on bent helical structures [13–15]; however, the computational details the successive Euler's transformation implementations in the calculation of the net dipole moment and the polarizability tensor were not explicitly discussed. In addition, these studies assume strong vibrational coupling among all the backbone C=O in the protein molecules; which may not be true if there are substantial symmetry point group interruptions among helical units or when they are distant from each other. Although the orientations of cytochrome b5 in model lipid bilayers have been reported recently, the validity of the analysis relied primarily on the assumed internal cancelation of the SFG hyperpolarizability which arises from helical segments pointing in opposite directions [11]. Unfortunately, a general multi-helical structure may not possess such conformation, notably pardaxin, melittin, and cytochrome P450.

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There have also been a number of excellent SFG studies on peptides/proteins using both the amide I band and the C-H (or C-D) vibrational modes in the 2800–3100 cm^{-1} (or 2000–2200 cm^{-1}) regime. These studies tackle the orientation problem from different aspects such as investigating certain particular amino acid residues [7] or by studying the hyperpolarizability of the backbone C=O bonds. The latter approach can be performed either qualitatively or quantitatively depending on the information being sought [6,16,17].

For quantitative studies, the analyses may become unexpectedly complicated and the level of accuracy depends significantly on the models being implemented in the calculation. Theoretically, the α-helical amide I band was shown to consist of two orthogonal vibrational modes A and E [18]. The double mode added to the complexity of calculating the multi-helical molecular hyperpolarizability. This study successfully investigates the significant impact of the molecular twist angle on the SFG amide I band signal obtained while this twist angle has been omitted in a few over-simplified SFG analyses of bent-helical structures [10,19]. In addition, the inter-helical twist angle was confirmed to be not a crucial factor contributing to the SFG I_{ppp}/I_{ssp} value, which is commonly used in the orientation analysis of helical structures. Finally, we took into account the length of the helical motifs in the data analysis to characterize the molecular orientation of melittin in lipid bilayers and verify the findings with current literature. The molecular orientation of melittin is of great interest since it gives direct hints to the peptide mode of action, which is still being hotly debated. Traditionally, two main peptide-lipid interaction models have been proposed: barrel stave [20] and carpet/toroidal pore models [21,22]. More recent studies observed a more sophisticated interaction picture in which melittin adopts a dual orientation distribution [10,23] or the pore formation occurs as a transient process [24,25]. In this present study, an orientation distribution that aligns with recent studies on the mode of action of melittin was proposed, on the basis of the kink in the molecular structure of melittin being taken into account and the experimental evidence that the peptide adopts a single δ orientation distribution reported recently using dual-fluorescence spectroscopy [26]. It is worth emphasizing that the analysis in this present study may not be able to describe the peptide/lipid interaction in more complex cases in which the single δ orientation distribution condition is not met; in such cases, further parameters can be sought using other optical spectroscopic techniques such as ATR-FTIR or FWM.

2. SFG data analysis – normal vibrational modes of multiple α-helical structures

In fact, this paper is an advancement of a previously developed theoretical background [12,27]; interested readers may find these materials particularly helpful in providing fundamental details for the present study. Therefore, full details on the SFG orientation analysis of single Pauling's α-helical structures of different chain lengths will not all be reiterated here. However, certain concepts which are believed to be crucial for the development of this paper will be selectively discussed.

A sum frequency process can be considered as a hyper-Raman process of which hyperpolarizability is described by a third rank tensor. This hyperpolarizability can be calculated using the transition dipole moment and the transition polarizability tensors. Hence, the normal vibrational modes of the infrared absorption and Raman scattering processes should be thoroughly studied.

The hyperpolarizability tensor of the SFG process can be expressed as a combination of the IR absorption (ω_2) and the Raman scattering (ω_1):

$$\beta_{ijk} = \sum_{v} \langle g|\tilde{\alpha}_{ij}(\omega_{SF})|v\rangle\langle v|\tilde{\mu}_k(\omega_2)|g\rangle \frac{\rho_g^{(0)} - \rho_v^{(0)}}{\omega_2 - \omega_v + i\gamma_v} \quad (1)$$

where $\omega_{SF} = \omega_1 + \omega_2, \omega_v$ is the frequency at which vibrational resonant transition occurs from $|g\rangle$ to $|v\rangle$. The quantity $\frac{\rho_g^{(0)} - \rho_v^{(0)}}{\omega_2 - \omega_v + i\gamma_v}$ appears in the expression as a line-shape function. The ρ values are the fractional populations at the vibrational states; while γ dictates the line width of the spectral peak corresponding to the indicated vibrational transition.

The SFG macroscopic susceptibility tensor element $\chi_{ijk}(i,j,k = x, y, z)$ is related to the SFG molecular hyperpolarizability tensor element $\beta_{lmn}(l,m,n = a,b,c)$ by an Euler angle projection [18,27–29]:

$$\chi_{ijk,q} = N_s \sum_{l,m,n} \langle(\hat{i}\cdot\hat{l})(\hat{j}\cdot\hat{m})(\hat{k}\cdot\hat{n})\rangle \beta_{lmn,q} \quad (2)$$

Eq. (1) reveals the dependence of the hyper-Raman tensor on the IR absorption and Raman scattering. Each hyperpolarizability tensor corresponds to a vibrational mode that is both active among IR and Raman normal modes. Therefore, the number of normal modes of the SFG process is reduced from three to two: A (symmetric, along the z axis) and E (asymmetric, in the xy plane) modes. To illustrate this, the A and E modes are presented graphically in Fig. 1.

It can be seen from Fig. 1 that the overall molecular symmetric A mode is directly dictated by the relative tilt angle between the two adjacent helical segments; whilst it is unlikely that the molecular E mode will be influenced by the corresponding twist angle. The process of calculating the molecular hyperpolarizability tensors could be simplified if these two vibrational modes are well separated spectrally; unfortunately, the vibrational energy of the A and E modes are inherently only a few wavenumbers apart in the SFG spectra. For this reason, the SFG signals of the A and E modes cannot be resolved due to the limited resolution of a typical SFG system. Thus both modes are likely to be contributing to the amide I signal as shown below [10]:

$$\chi_{zzz} = \chi_{E,zzz} + \chi_{A,zzz} \quad (3)$$

$$\chi_{yyz} = \chi_{xxz} = \chi_{E,yyz} + \chi_{A,yyz} \quad (4)$$

$$\chi_{yzy} = \chi_{xzx} = \chi_{zxx} = \chi_{zyy} = \chi_{E,yzy} + \chi_{A,yzy} \quad (5)$$

The calculation of the hyperpolarizability tensors using the bond additivity model and group theory has previously been systematically reported [12]. In this model, the hyperpolarizability tensors are first calculated in the molecular fixed frame, which takes into account the relative positions of the amino acid units. Hence, this analysis is highly dependent on the structural properties of the molecule, as will be discussed later. The microscopic hyperpolarizability tensors are then calculated and transformed into measurable macroscopic quantities in the laboratory fixed frame using the appropriate Euler's transformations [28]. This

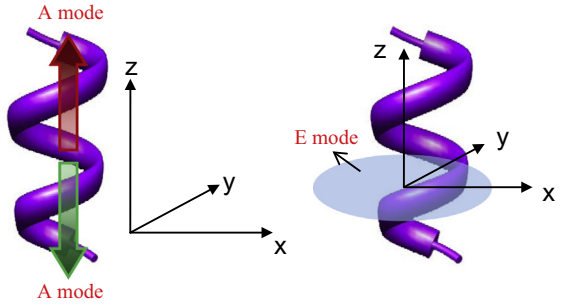

Fig. 1. A (left) and E (right) vibrational mode illustrations of an α-helix.

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