# Label-Free Chiral Detection of Melittin Binding to a Membrane

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In the study presented here, the intrinsic chirality of melittin, a hemolytic peptide isolated from bee venom, is used to measure its affinity for a planar-supported lipid bilayer (PSLB) of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphotidylcholine (POPC). We believe this is the first time that chirality originating from the secondary structure of a biomolecule has been used for detecting molecular binding at a surface by second harmonic generation (SHG).

Since all proteins in their native conformation contain, to some degree, highly ordered secondary structures (i.e., $\alpha$-helices and $\beta$-sheets), these intrinsic chiral components can be used as spectroscopic probes to measure protein adsorption to a surface. Measuring surface chirality cannot be achieved with conventional linear spectroscopic methods, such as circular dichroism (CD) and optical rotary dispersion (ORD), due to path length and surface concentration limitations. However, nonlinear spectroscopies such as SHG possess the requisite surface selectivity and sensitivity to detect chirality in surface films.[1,2]

SHG is a multiphoton spectroscopy in which an optical field of sufficient intensity at frequency $\omega$, is directed at a surface resulting in the generation of a second optical field at twice the frequency $2\omega$.[3] The induced nonlinear polarization at the surface giving rise to SHG can be expressed as:

$$P^{NL}(2\omega) = \chi^{(2)} E(\omega)_1 E(w)_2 \qquad (1)$$

where $\chi^{(2)}$ is a 27-element third-rank tensor that describes the interfacial response to the applied electric fields $E(\omega)_1$ and $E(w)_2$. The symmetry of the interface also plays a large role in determining the nonlinear response. The interface between two isotropic media possesses $C_{\infty v}$ symmetry, but when chiral molecules are present, an additional symmetry requirement is imposed, reducing the symmetry to $C_\infty$. Transformation of $\chi^{(2)}$ under $C_\infty$ symmetry and introduction of the piezoelectric contraction[3] results in the following symmetry-allowed combinations; $\chi_{zzz}$, $\chi_{zyy} = \chi_{zxx}$, $\chi_{yzy} = \chi_{xzx} = \chi_{yyz} = \chi_{xxz}$, and $\chi_{xyz} = -\chi_{yzx}$, where $\chi_{yyz}$, $\chi_{zyy}$, and $\chi_{zzz}$ are due to achiral properties, and $\chi_{xyz}$ describes the chiral response. The $\chi^{(2)}$ elements, which are macroscopic parameters, are related to the molecular properties of the interface by $\chi = N\langle \alpha^{(2)} \rangle$, where $\alpha^{(2)}$ is the molecular hyperpolarizablity and $N$ is the number of molecules.

The typical analysis of surface chirality with SHG is performed analogously to linear CD spectroscopy, in that the differential SHG signal from left- versus right-circularly polarized incident light is examined. SHG-CD has been used to characterize surface chirality in a number of systems.[4−6] The use of SHG-CD for the investigation of chirality in biological molecules at surfaces has so far been limited to the heme center in cytochrome $c$[2] and the indole chromophore in a dipeptide of tryptophan.[7] One drawback of this approach is that in order for a chiral response to be observed with SHG-CD, a significant phase difference between the chiral and achiral terms must exist.[5] However, by isolating only the magnitude of $\chi_{xyz}$, a direct measure of surface chirality can be obtained.

We have adopted a strategy to selectively isolate the chiral SHG (C-SHG) response by implementing a counter-propagating beam

geometry.[8] The SHG intensity as a function of incident angle ($\theta$) and polarization state ($\gamma$) of the fundamental beams for a counter-propagating geometry can be written as:

$$I_x^{2\omega} \propto \sin^2(\gamma) \cos^2(\gamma) f_y^2 f_z^2 (\chi_{r,xyz}^2 + \chi_{i,xyz}^2) \qquad (2)$$

$$I_y^{2\omega} \propto \sin^2(\gamma) \cos^2(\gamma) f_y^2 f_z^2 (\chi_{r,yzy}^2 + \chi_{i,yzy}^2) \qquad (3)$$

where the $f$'s represent the geometric Fresnel coefficients for the incident light fields. The susceptibility elements $\chi_{r,xyz}$ and $\chi_{i,xyz}$ are the real and imaginary components due to chirality, and $\chi_{r,yzy}$ and $\chi_{i,yzy}$ are the real and imaginary components of the achiral response. Due to conservation of momentum and the coherent nature of the SHG process, the generated SH signal is emitted along the $z$-axis or surface normal. By judicious choice of the input and output polarizations, the chiral (eq 2) and achiral (eq 3) SHG signals can be isolated independently. Another advantage of this optical configuration is that linearly polarized light can be used to observe this phenomenon, which eliminates the need for a high-fidelity circularly polarized light source. In the optical arrangement described here, the measured SHG intensity in the $x$-polarization is directly proportional to the degree of surface chirality.

As a model system, the intercalation of melittin into PSLBs of POPC in the presence of phospholipase A$_2$ (PLA$_2$) has been investigated by C-SHG. Melittin contains 26 amino acid residues with the sequence GIGAVLKVLTTGLPALISWIKRKRQQ.[9] The structure of melittin in solution and in a membrane environment has been determined through NMR spectroscopy,[10] high-resolution X-ray crystallography,[11] and CD spectroscopy measurements.[12] In an aqueous solution, melittin exists as a random coil, but upon intercalation into a lipid membrane environment, it undergoes a structural change to form a well-ordered $\alpha$-helix.[13] It is the chirality of this secondary structure which has been used to detect the association of this peptide to a PSLB of POPC.

In the experiments performed here, melittin isolated from bee venom (93% purity, Sigma), which contains approximately 1% PLA$_2$ was used. The PSLBs were created using the Langmuir−Blodgett−Schaffer method[14] on a hemicylindrical quartz prism. The laser system used was a Continuum Panther optical parametric oscillator which was pumped with a Nd:YAG laser.

Figure 1 shows a set of SHG polarization anisotropy curves obtained for a POPC membrane and for a POPC membrane with melittin adsorbed. The chiral response has been isolated by measuring the SHG signal with $x$-output polarization while continuously varying the input polarization from 0 to 360°. The incident laser was tuned to 440 nm, with detection of the resulting SHG at 220 nm, which is resonant with the polypeptide backbone in the $\alpha$-helix conformation.[15] The two counter-propagating beams were directed at the surface through the quartz prism at an angle of 66° from the surface normal. The C-SHG polarization anisotropy curve for the POPC membrane shows no response, corresponding to a lack of chirality in the membrane. Upon injection of a 2.0 $\mu$M
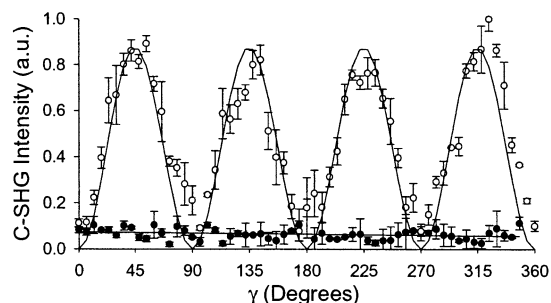
**Figure 1.** C-SHG polarization anisotropy curves for a POPC PSLB (solid circles) and a POPC PSLB after injection of a 2.0 $\mu$M melittin solution (open circles). The solid line for each curve represents the fit to the data using eq 2.
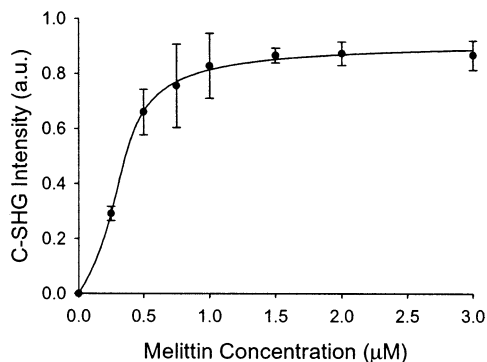


**Figure 2.** C-SHG adsorption isotherm for melittin with PLA$_2$ binding to a POPC PSLB (solid circles) The solid line represents the fit to the data using a Frumkin adsorption isotherm.

solution of melittin with 1% PLA$_2$, a significant increase in the intensity of light at 220 nm is measured for the chiral response. The polarization anisotropy curve for *x*-polarized SHG shows four peaks at values of $\gamma = 45$, 135, 225, and 315°, as predicted by eq 2. The only way to obtain a signal polarized along the *x*-axis is if chirality is present at the surface. The appearance of a measurable *x*-polarized C-SHG signal upon melittin injection strongly suggests that the melittin has intercalated into the POPC membrane and formed an $\alpha$-helix with a net orientation along the surface normal in agreement with CD, NMR, FTIR, and capacitance measurements.[8,16−22] No chiral SHG signal was observed for PLA$_2$ in the absence of melittin.

To quantify the affinity of melittin adsorption to a POPC PSLB, SHG adsorption isotherms were obtained, shown in Figure 2. An increase in the C-SHG intensity is observed as the bulk concentration of melittin is increased, with saturation observed at concentrations greater than 1.0 $\mu$M. A satisfactory fit to the C-SHG intensity data was obtained using a Frumkin adsorption isotherm with a positive interaction constant. An affinity constant ($K_a$) of $(8.3 \pm 1.0) \times 10^5$ M$^{-1}$ was calculated for melittin binding to the POPC membrane. The $K_a$ value measured here is larger than that reported by surface plasmon resonance (SPR) for melittin binding to a 1,2-dimyristal-*sn*-glycero-3-phosphotidylcholine (DMPC) membrane, which was determined to be $3.3 \times 10^4$ M$^{-1}$.[23] The discrepancy in the binding data may result from slightly different membrane structures (DMPC $T_m = 23$ °C and POPC $T_m = -2$

°C). Previous studies have suggested that the more fluid the lipid membrane, the greater propensity for melittin binding.[21] In addition, conductivity measurements have shown that melittin in the presence of PLA$_2$ absorbs and produces defect sites in a POPC membrane at concentration levels of 1.6 $\mu$M.[21] These results are consistent with the C-SHG data presented here.

We have demonstrated for the first time that the intrinsic chirality of proteins can be used to monitor their association to a surface using C-SHG. This has been achieved via the implementation of a novel counter-propagating optical geometry which separates the achiral and chiral SHG response. The results of these studies have far-reaching implications in the use of SHG for the detection of protein adsorption in biological systems and the analysis of protein interfacial phenomena. Studies are ongoing to evaluate C-SHG as a general detection method for proteins and DNA adsorbed to a surface.

**Supporting Information Available:** Experimental description, optical configuration and the Frumkin model used to fit the C-SHG data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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