Communication

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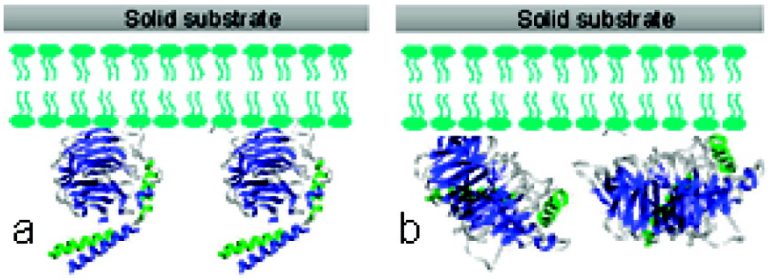

$G\beta_1\gamma_2$ with (a) and without (b) the geranylgeranyl group

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In Situ Investigation of Heterotrimeric G Protein $\beta\gamma$ Subunit Binding and Orientation on Membrane Bilayers

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This paper investigates the binding and orientation of an important signal transduction membrane protein, G$\beta\gamma$, in a membrane bilayer. This is the first time that sum frequency generation (SFG) vibrational spectroscopy has been used to deduce the orientation of a peripheral membrane protein in the membrane environment. Heterotrimeric guanine nucleotide-binding proteins (G proteins) are involved in numerous signal transduction pathways[1] and relay the extracellular signals sensed by G protein-coupled receptors (GPCRs) to down-stream effectors. Heterotrimeric G proteins are composed of three subunits (Gα, Gβ, and Gγ), with Gβ and Gγ forming a tightly associated dimer. In the resting state, G$\alpha\beta\gamma$ exists in the trimeric form. Upon GPCR activation, G$\alpha\beta\gamma$ releases the guanosine diphosphate (GDP) originally bound to the Gα subunit and the subsequent guanosine triphosphate (GTP) binding to Gα induces conformational changes that result in release of the G$\beta\gamma$ subunits.[2] Gα·GTP and G$\beta\gamma$ then can associate with their own or mutual effectors to trigger downstream signaling events (e.g., ion channel opening or initiation of cell transcription machinery). Crystal structures of G-proteins have been solved to better understand how G-proteins perform these complex tasks (e.g., refs 3,4). However crystal structures cannot provide exact information regarding how this membrane machinery assembles and functions on membranes in situ, while our current SFG studies can elucidate membrane orientation information. Such in situ information is difficult to probe using other experimental techniques.

A theoretical calculation based on finite difference Poisson−Boltzmann methods was previously used to shed light on the membrane orientation of transducin G$_t\beta\gamma$. It was predicted that the electrostatic interaction between G$_t\beta\gamma$ and membranes not only enhances its membrane partitioning, but also induces a specific G$_t\beta\gamma$ orientation.[5] Cryo-electron microscopy (cryo-EM) has been applied to study the membrane orientation of the transducin heterotrimer.[6] G$_t\alpha\beta\gamma$ was found to bind to the surface of a tubular lipid bilayer using a very small contact area. The membrane orientation was determined by shape-matching the cryo-EM results to the existing crystal structure. However the tubular lipid bilayer preserved in vitreous ice and the quasi-crystalline nature of this system provide only a semi-in-situ environment.

In this Communication, we investigate how the G$\beta_1\gamma_2$ subunit (Figure 1) binds to and orients on a substrate supported lipid bilayer using SFG. The roles of geranylgeranyl group in G$\beta_1\gamma_2$-subunit and membrane lipid charges on G$\beta_1\gamma_2$ binding to the membrane were both evaluated. This study provides the first step in achieving a better molecular model of heterotrimeric G proteins and their complexes in situ.

G$\beta_1\gamma_2$ was overexpressed using previously established methods.[7] Two forms of G$\beta_1\gamma_2$ were tested, one with the wild-type geranylgeranyl group and, as a negative control, a GγC68S unprenylated (soluble) mutant. Supported lipid bilayers were prepared using the Langmuir−Blodgett/Langmuir−Schaefer method. SFG experimental details are presented in the Supporting Information.

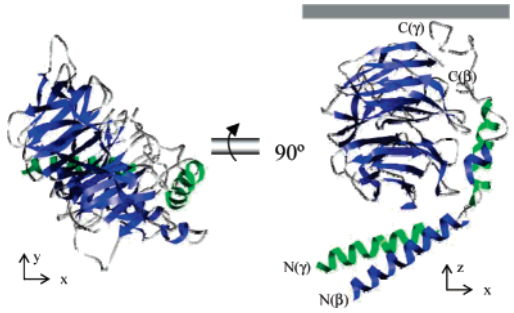

Figure 1. G$\beta_1\gamma_2$ structure and the molecular coordinate system used for molecular hyperpolarizability calculation. Gβ_1 is blue, Gγ_2 is green, loops between α-helices and β-strands in both subunits are gray. The C-terminal residue of Gγ_2 is geranylgeranylated (not resolved in this crystal structure).

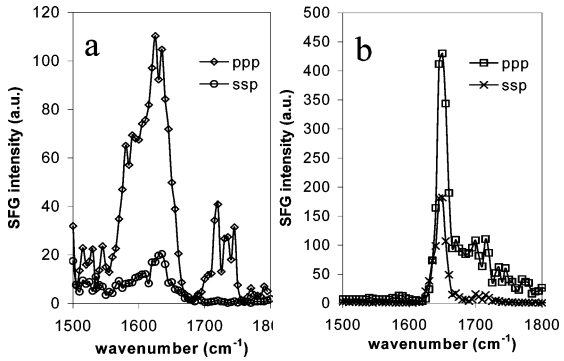

Figure 2. SFG amide I spectra of interfacial G$\beta_1\gamma_2$ adsorbed onto a POPG/POPG bilayer. (a) 250 μg of the soluble form of G$\beta_1\gamma_2$ was first injected into the subphase (~2 mL) of the bilayer. Only relatively weak signals indicative of β−sheet were observed. (b) After the spectra in panel a were collected, 50 μg geranylgeranylated G$\beta_1\gamma_2$ was injected into the subphase and stronger SFG signals indicative of α-helix structure were observed.

Results from wild-type and soluble G$\beta_1\gamma_2$ subunits were compared to evaluate the anchoring role of the geranylgeranyl group on membrane. SFG spectra are shown in Figure 2. The same bilayer was used in both experiments. Even at a relatively high concentration (125 μg/mL), soluble G$\beta_1\gamma_2$ generated relatively weaker signals, with peak centered at 1630 cm^{-1}. The injection of geranylgeranylated G$\beta_1\gamma_2$ to the membrane bilayer subphase resulted in significantly stronger SFG signals from these subunits inserted into the membrane, despite 5-fold lower G$\beta_1\gamma_2$ concentration (25 μg/mL) used. The peak center shifted from 1630 (for soluble G$\beta_1\gamma_2$) to 1650 cm^{-1} (for G$\beta_1\gamma_2$ with the geranylgeranyl group), indicating that the SFG amide I signals were contributed from different secondary structures.[8] Our interpretation is that without the geranylgeranyl group, G$\beta_1\gamma_2$ associates at best weakly with the surface, and when it does the β−propeller faces the membrane surface. At this orientation the helical domains orient more or less parallel to the surface. Therefore, the β−propeller of Gβ_1 should be able to generate some SFG amide signals due to its interaction with the surface despite its native semi-centrosymmetry, but the α-helices

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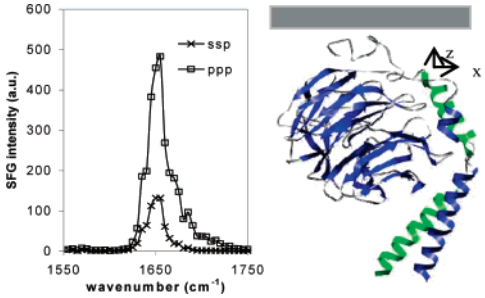

Figure 3. (Left) SFG amide I spectra of interfacial G$\beta\gamma$ (25 μg/mL) adsorbed onto a POPC/POPC bilayer; (right) G$\beta\gamma$ orientation deduced based on the SFG intensity ratio. It tilts −35° against the surface normal from the "zero" position as defined in Figure 1.

lying down generate no or extremely weak ssp and ppp SFG signals. In contrast, wild-type G$\beta_1\gamma_2$ is anchored to the membrane via the geranylgeranyl group with the β−propeller more or less perpendicular to the surface. Under this circumstance, the helical domains are no longer parallel to the surface and hence contribute the dominant spectral features. Furthermore, the β−propeller does not substantially interact with the membrane and its signal is minimal. The difference in spectral intensities and amide I frequencies for the two types of G$\beta_1\gamma_2$ reflects the dual purpose of the geranylgeranyl group: increasing the G$\beta_1\gamma_2$ membrane affinity and dictating the G$\beta_1\gamma_2$ membrane orientation.

Shown in Figure 3a are the SFG amide I spectra of POPC:POPC bilayer-associated G$\beta_1\gamma_2$ collected with ssp and ppp polarization combinations. Both spectra can be fitted with a single peak around 1655 cm^{-1} and a small nonresonant background, confirming that the signals are mainly contributed by G$\beta_1\gamma_2$ helical structure on the neutral bilayer. We have shown in a previous publication that for an α-helix the ppp and ssp spectral intensity ratio is a function of the helical orientation and the orientation of an α-helix can thus be determined experimentally.[8d] The orientation of the helical domain of G$\beta_1\gamma_2$ can therefore be determined with helical domains being the dominant signal contributor. Multiple crystal structures of G$\beta\gamma$, both alone and in complex with proteins, have revealed a consistent conformation and fixed orientation of its helices with respect to the propeller domain.[3,4] The four helices in G$\beta_1\gamma_2$ form a coiled-coil structure, stabilized by many hydrophobic contacts. The coiled coil is in turn fixed to the side of the β-propeller, although the extreme N-termini of Gβ_1 and Gγ_2 have exhibited some limited dynamic flexibility among the crystal structures. It is therefore reasonable to determine the overall orientation on the basis of the orientation of the helical domains. The hyperpolarizability of G$\beta_1\gamma_2$ is deduced as described previously (see Supporting Information). Figure 1 shows the G$\beta_1\gamma_2$ zero position defined in the molecular $x-y-z$ coordinate system. For simplicity, it is assumed that G$\beta_1\gamma_2$ can only freely rotate about the y-axis. The choice of y-axis as the rotation axis and such a molecular coordinate system are based on the orientational change previously hypothesized.[4] From the measured ppp and ssp intensity ratio, the orientation of the G$\beta_1\gamma_2$ is found to be about −35° around the y-axis (Figure 3).

To further investigate how the membrane charge composition affects G$\beta_1\gamma_2$ adsorption, G$\beta_1\gamma_2$ in lipid bilayers with inner POPC leaflets and outer leaflets of various lipid compositions were systematically studied, similar to the system investigated in ref 5. Figure 4 displays the SFG spectra of these G$\beta_1\gamma_2$ adsorbed in bilayers with POPC:POPG (1:1 w/w) and pure POPG as the outer leaflets, respectively. Similar spectral features were observed from G$\beta_1\gamma_2$ regardless of the lipid composition, indicating that the

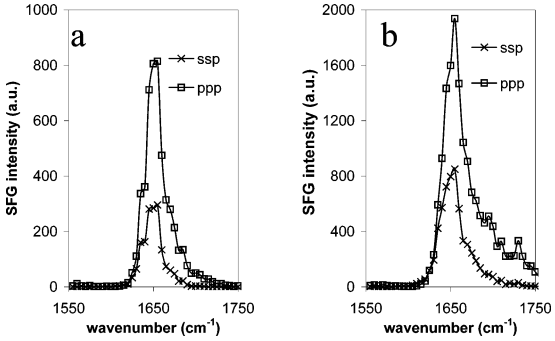

Figure 4. SFG amide I spectra of interfacial G$\beta\gamma$ (25 μg/mL) adsorbed onto lipid bilayers with different lipid composition. POPC was used as the proximal leaflet, while POPC:POPG (1:1 weight ratio) (a) and POPG (b) were used as the distal leaflet.

α-helices remain the dominant source of signal, while the spectral intensity increased with the increase in POPG percentage. The ppp and ssp spectral intensity ratio also changed significantly. Such intensity ratio changes indicate that the surface charges modulate the overall G$\beta_1\gamma_2$ molecular orientation, or the tertiary structure of G$\beta\gamma$, such as the relative orientation of the four helical domains due to the stronger charged lipid−G$\beta_1\gamma_2$ interactions.

In summary, we successfully observed the in situ insertion of the geranylgeranyl group of G$\beta_1\gamma_2$ into a membrane bilayer, deduced the detailed orientation of G$\beta_1\gamma_2$ in a POPC bilayer, and demonstrated that different lipid compositions in membrane affect orientations of G$\beta_1\gamma_2$. SFG is a powerful technique that has been applied to study biologically relevant questions recently.[9] Here we demonstrated that it can determine orientation of a peripheral membrane protein in situ, providing vital structural information orthogonal to crystal structures that will greatly facilitate understanding the interactions of proteins at the membrane surface, in particular between heterotrimeric G proteins and their effector targets.

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Supporting Information Available: SFG experiment and G protein orientation deduction. This material is available free of charge via the Internet at http://pubs.acs.org.

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