# Supplementary Information

# Membrane Orientation and Binding Determinants of G Protein-Coupled Receptor Kinase 5 as Assessed by Combined Vibrational Spectroscopic Studies

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**1. Unpolarized ATR-FTIR spectra**

Unpolarized ATR-FTIR was used to compare the surface coverage of GRK5 or GRK51-531 on 9:1 POPC:POPG and pure POPG lipid bilayers. The ATR-FTIR signal of protein is proportional to the number of protein molecules binding to the lipid bilayer surface. Results showed that the ATR-FTIR signals of GRK5 and GRK51-531 are similar on different lipid bilayers, indicating that the surface coverages of GRK5 (Figure S1A) and GRK51-531 (Figure S1B) are not very different on either 9:1 POPC:POPG or POPG lipid bilayers. Supplementary Figure S1C shows the unpolarized ATR-FTIR spectra from GRK5NT (50 and 150 mM NaCl in buffer) and GRK5 (150 mM NaCl in buffer) on a 1:1 POPC:PIP2 lipid bilayer, showing that both GRK5NT and GRK5 can bind to the 1:1 POPC:PIP2 bilayer with a similar surface coverage.

Figure S1.tif

**Figure S1. The unpolarized ATR-FTIR spectra**. Unpolarized ATR-FTIR spectra of 336 nM (A) GRK5 and (B) GRK51-531 on 9:1 POPC:POPG (open square dots) and pure POPG (solid circles) lipid bilayer. (C) Unpolarized ATR-FTIR spectra of 336 nM GRK5NT (50 and 150 mM NaCl in buffer) and GRK5 (150 mM NaCl in buffer) on 1:1 POPC:PIP2 lipid bilayer.

**2. SFG and ATR-FTIR orientation analysis**

We have previously reported the determination of membrane orientation of G-protein by using SFG and ATR-FTIR methods [1,2]. Computer programs were developed to facilitate protein orientation analysis by calculating the SFG signal ratios () and ATR-FTIR dichroic ratio (RATR) at any orientation of a given crystal structure relative to the lipid bilayer [1,2].

Figure S2.tif

**Figure S2. Possible orientation of GRK5 using the 3NYN crystal structure.** Contour plots showing the (A) calculated SFG ratio () and (B) calculated ATR-FTIR dichroic ratio RATR of GRK5 for all unique twist (ψ) and tilt (θ) angles by using the 3NYN crystal structure. The possible orientations of GRK5 on POPG lipid bilayers determined by (C) SFG measurement (=0.93±30%), and (D) ATR-FTIR measurement (dichroic ratio RATR =1.5±15%). The effect of experimental errors (such as uncertainty in the Fresnel coefficients) is accounted for using a coloring scheme based on how well the calculated and experimentally measured quantities agree for each possible orientation, within specified error bars (1,2). If the calculated  ratio does not match the experimental value within error bars, a score of 0 was assigned. A score of 100% indicates an exact match.

Figure S3.tif

**Figure S3. Possible orientation of GRK5 using the 2ACX crystal structure.** Contour plots showing the (A) calculated SFG ratio  and (B) calculated ATR-FTIR dichroic ratio RATR of GRK5 for all unique combinations of the twist (ψ) and tilt (θ) angles using the 2ACX crystal structure. Orientation of GRK5 on a POPG lipid bilayer at which the calculated values best match experimentally measured value for (C) SFG ratio of  (0.93±30%), and (D) the ATR-FTIR dichroic ratio RATR (1.5±15%).

Figure S4.tif

**Figure S4. Possible orientation of GRK51-531 using the 3NYN crystal structure.** Contour plots showing the (A) calculated SFG ratio  and (B) calculated ATR-FTIR dichroic ratio RATR of GRK51-531 for all unique combinations of the twist (ψ) and tilt (θ) angles by using the 3NYN crystal structure with C-terminal residues beyond residue 531 deleted. Orientation of GRK51-531 on POPG lipid bilayers at which the calculated values best match experimentally measured value for (C) SFG ratio of  (1.35±30%), (D) the ATR-FTIR dichroic ratio RATR (1.52±15%), and (E) combination of SFG and ATR-FTIR measurements. (F) The same plot as panel E, but only showing orientation areas with a score ≥ 70% (red). There are two likely orientation ranges (twist, tilt) for GRK51-531: (170-200˚, 30-35˚) and (230-260˚, 45-60˚). (G) The possible orientations of GRK51-531 on a 1:1 POPC:PIP2 lipid bilayer determined by SFG measurement (= 0.87±30%).

Figure S5.tif

**Figure S5. Possible membrane orientations of GRK51-531.** Possible membrane orientations of GRK51-531 on POPG lipid bilayers as determined from SFG and ATR-FTIR experimental measurements using the crystal structure of 3NYN (C-terminus structure was deleted): (A) twist=190˚, tilt=35˚, (B) twist=245˚, tilt=50˚. The plane of the membrane relative to the protein is shown as a blue rectangle.

Figure S6.tif

**Figure S6. Possible orientation of GRK51-531 using the crystal structure of 2ACX.** Calculated possible orientation of GRK51-531 on a POPG lipid bilayer by using the crystal structure of 2ACX for (A) SFG ratio of  (1.35±30%), and (B) the ATR-FTIR dichroic ratio RATR (1.52±15%).

**SUPPLEMENTARY REFERENCES:**

1. Boughton AP, Yang P, Tesmer VM, Ding B, Tesmer JJ, et al. (2011) Heterotrimeric G protein β1γ2 Subunits Change Orientation upon Complex Formation with G Protein-coupled Receptor Kinase 2 (GRK2) on a Model Membrane. Proc Natl Acad Sci U S A 108: E667-E673.
2. Yang P, Boughton AP, Homan KT, Tesmer JJG, Chen Z (2013) Membrane Orientation of Gαiβ1γ2 and Gβ1γ2 Determined via Combined Vibrational Spectroscopic Studies. J Am Chem Soc. 135: 5044-5051.