

Supplementary Information

Calcium-dependent and -independent annexin V binding: distinct molecular behaviours at cell membrane interface

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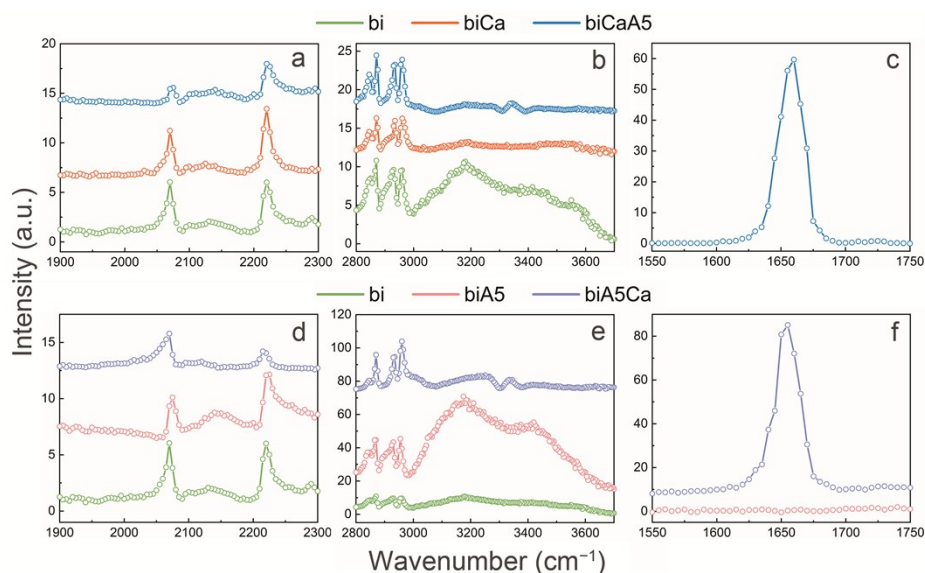


Fig. S1 SFG ppp spectra collected in the C–D (a and d), C–H and O–H (b and e), and amide I (c and f) frequency ranges for the dPPC–DOPC2DOPS1 bilayers in the Ca^{2+} -dependent (a–c) and -independent (d–f) cases. For the Ca^{2+} -dependent case, a bilayer was initially put into contact with the pure water (bi), then the Ca^{2+} (biCa) and finally the A5 (biCaA5) solutions were added, sequentially. For the Ca^{2+} -independent case, a bilayer was initially put into contact with the pure water (bi), then the A5 (biA5) and finally the Ca^{2+} solutions (biA5Ca) were added, sequentially.

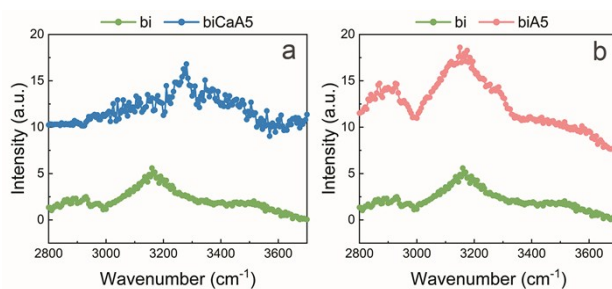


Fig. S2 SFG ssp spectra collected in the C–H and O–H stretching frequency ranges for the dPPC2dDPPS1–dPPC2dDPPS1 bilayers in the Ca^{2+} -dependent (a) and -independent (b) cases. For the Ca^{2+} -dependent case, a bilayer was initially put into contact with the pure water (bi), then the Ca^{2+} and finally the A5 (biCaA5) solutions were added, sequentially. For the Ca^{2+} -independent case, a bilayer was initially put into contact with the pure water (bi) and then the A5 solution (biA5) was added. It should be noted, for the biCaA5 case, no C–H signals were observed, indicating that A5 had no contribution in this case; for the biA5 case, although interfered by the water signals, the C–H signals were observed, indicating that A5 had the certain contribution. However, the A5 contributed C–H signals formed a broad peak, which hardly affected the sharp C–H peaks and the deduced ratio $\chi_{\text{CH}_3}/\chi_{\text{CH}_2}$.

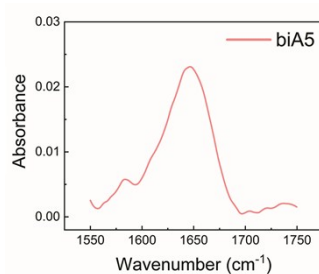


Fig. S3 ATR-FTIR spectrum in the amide I range collected from A5 adsorbed onto the DOPC2DOPS1–DOPC2DOPS1 bilayer without Ca^{2+} (biA5), using the same bilayer without A5 and Ca^{2+} as background. D_2O was used in the ATR experiment to avoid spectral interference caused by H_2O .

EXPERIMENTAL METHODS

Materials. The lipids, 1,2-dipalmitoyl-d62-*sn*-glycero-3-phosphocholine (dDPPC), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine (sodium salt) (DOPS) and 1,2-dipalmitoyl-d62-*sn*-glycero-3-phospho-L-serine (sodium salt) (dDPPS), were purchased from Avanti Polar Lipids. The lipids were dissolved in chloroform (SINOPHARM, China) at a concentration of 3 mg/mL with the desired mole ratios for further construction of lipid bilayers. Calcium chloride (CaCl₂·2H₂O, 99.99% metals basis) was purchased from Aladdin (China) to prepare the aqueous stock Ca²⁺ solution at a concentration of 1 M. Annexin V (A5) ordered from eBioscience was reconstituted in water at a stock concentration of 1 mg/mL with possible 20 mM phosphate buffer (pH 7.0), 0.02% Tween 80, 130 mM arginine HCl. Low salt concentration in diluted protein solution cannot affect SFG water signals. Deuterium oxide (D₂O, 99.9% D) was purchased from Energy Chemical (China) for the ATR experiment. All chemicals were used as received. Ultrapure water (18.2 MΩ·cm) was obtained from a Millipore water purification system.

Calcium fluoride (CaF₂) right angle prisms (Chengdu Yasi Optoelectronics, China) were soaked in chloroform over 24 h and then washed with ethanol and detergent solution, followed by rinsing several times. Before deposition of a lipid monolayer, the prisms were dried by nitrogen gas and cleaned by oxygen plasma (PDC-MG, Mingheng, China) for 4 min.

Lipid bilayer preparation. Lipid bilayers on CaF₂ prisms were prepared using Langmuir–Blodgett (LB) and Langmuir–Schaefer (LS) method. In brief, for example, a dDPPC lipid monolayer at the surface pressure of 34 mN/m was deposited on a prism using the KN 2003 LB system (KSV NIMA). This monolayer was then put into contact with the other DOPC/DOPS (molar ratio of 2:1) monolayer spread on the water surface at the same surface pressure to form a lipid bilayer. The as-prepared asymmetric lipid bilayer was thus composed of the dDPPC top leaflet (attached to the prism) and the DOPC2DOPS1 bottom leaflet (in contact with the water). Other bilayers with different components were prepared in the same method. The stock Ca²⁺ and A5 solution were injected into the subphase to achieve the final concentrations of 20 mM and 20 μg/mL under stirring, respectively. All experiments were carried out at an ambient temperature of about 22 °C.

SFG measurement and analysis. SFG is a powerful analytical tool capable of probing molecular-level surface/interfacial structures and dynamics with intrinsic surface/interface selectivity *in situ* in real time. Two pulsed laser beams, a frequency-tunable infrared (IR) beam and a frequency-fixed visible beam (532 nm), overlapped at the bilayer interface temporally and spatially to generate a sum frequency signal beam containing the interfacial molecular information. The SFG measurements were conducted using ssp (s polarized signal beam, s polarized visible beam, p polarized IR beam) and ppp polarization combinations.

The reflective SFG output intensity is proportional to the squared modulus of the effective second-order nonlinear susceptibility $\chi_{\text{eff}}^{(2)}$

$$I(\omega) \propto \frac{8\pi^3 \omega^2 \sec^2 \beta}{c^3 n(\omega_1) n(\omega_2) n(\omega)} |\chi_{\text{eff}}^{(2)}|^2 I_1(\omega_1) I_2(\omega_2) \quad (1)$$

where $I_1(\omega_1)$, $I_2(\omega_2)$ and $I(\omega)$ are the intensities of input visible and IR beams and output sum frequency beam with the frequencies of ω_1 , ω_2 and ω , respectively. The reflective index $n(\omega_i)$ corresponds to the beam i in the incident medium at the frequency ω_i . The angle β is the reflected angle of the output beam.

Additionally, the collected SFG spectra, plotted by the normalized SFG signal intensity I_{SFG} versus the input IR beam frequency ω_2 , can be fitted using the following Lorentz equation

$$I_{\text{SFG}} \propto |\chi_{\text{eff}}^{(2)}|^2 = \left| \chi_{\text{NR}}^{(2)} + \sum_q \frac{A_q}{\omega_2 - \omega_q + i\Gamma_q} \right|^2 \quad (2)$$

The nonresonant background $\chi_{\text{NR}}^{(2)}$ is typically a constant for a particular spectrum because of interfacial electron polarization contribution. A_q , ω_q and Γ_q are the signal amplitude, resonant frequency, and damping coefficient (or peak width) of the vibrational mode q , respectively.

ATR-FTIR measurement. The DOPC2DOPS1–DOPC2DOPS1 bilayer was prepared on the ZnSe crystal (Simplex Scientific) for the attenuated total reflection Fourier transform infrared (ATR-FTIR) experiment. The DOPC and DOPS lipids at the molar ratio of 2:1 were dissolved and mixed in chloroform, dried using nitrogen gas, and then placed in vacuum overnight. These lipids was dispersed in D₂O to reach a concentration of 4 mg/mL. The lipid suspension was extruded by using a Mini-Extruder (Avanti Polar Lipids) through a 100 nm polycarbonate membrane filter with 21 passes. The obtained liposome solution was diluted to 1 mg/mL in D₂O for the next-step bilayer preparation. The ZnSe crystal was extensively washed by ethanol, detergent solution, water and D₂O, and then treated by oxygen plasma for 3 min to create a hydrophilic surface. The liposome solution was put into contact with the ZnSe crystal surface, followed by incubation for 2h at the room temperature. After the rinse with D₂O at least 5 times, the background spectrum was collected from the formed bilayer using a Nicolet iS50 FTIR spectrometer. The A5 solution (in D₂O) was added to the

formed bilayer and then rinsed by D₂O for 5 times after reaching equilibrium. The ATR-FTIR spectrum in the amide I range for A5 adsorbed onto the bilayer surface was collected against the bilayer background.