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Electronic Supplementary Information

Peripheral Adsorption of Polylysine on One Leaflet of a Lipid Bilayer Reduces the Lipid Diffusion of both Leaflets

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Detailed descriptions of 2D FLCS and FLCS analyses.

We performed two-dimensional fluorescence lifetime correlation spectroscopy (2D FLCS) analysis to determine the fluorescence decay curves of fluorescent lipids in the inner as well as outer leaflets of GUV.[1,2] Then, we subsequently performed fluorescence lifetime correlation spectroscopy (FLCS) analysis to calculate leaflet-specific fluorescence intensity-correlation curves.[3] In this section, analytical details of 2D FLCS and FLCS are described as follows.

2D FLCS. By using a pulse laser as an excitation light source, two temporal information are obtained from each detected photon. Macrotime (T) is the absolute detection time of photon from the start of the experiment whereas microtime (t) is the emission delay time of the photon detection with respect to the corresponding excitation pulse. In 2D FLCS analysis, a 2D emission-delay correlation map ($M(\Delta T, t', t'')$) at arbitrary ΔT is constructed using this information.[1,2] In the map, t' and t'' correspond to microtimes of 1st and 2nd photons in photon pairs, respectively, and each photon in the photon pair is temporally separated by ΔT. Because the map contains both correlated and uncorrelated photon pairs, the uncorrelated signals are subtracted to obtain the map for the correlated signals ($M_{\mathrm{cor}}(\Delta T, t', t'')$). For that purpose, a 2D emission-delay correlation map at longer ΔT is calculated as a representative of the uncorrelated signals because the correlation is completely lost at that ΔT. In this study, the map at ΔT = 2–2.1 s was calculated and used for the subtraction.

To identify the independent lifetime species and to determine their fluorescence lifetime distributions (and the corresponding fluorescence decay curves), 2D inverse Laplace transform is performed on $M_{\mathrm{cor}}(\Delta T, t', t'')$ with the help of maximum-entropy method (2D MEM). Because $M_{\mathrm{cor}}(\Delta T, t', t'')$ is described with the sum of the single-molecule correlation, it can be represented by the following equations:

$$M_{\mathrm{cor}}(\Delta T; t_i', t_j'') = \sum_{k=1}^{L}\sum_{l=1}^{L} \widetilde{M}(\Delta T; \tau_k', \tau_l'') \exp(-t_i'/\tau_k') \exp(-t_j''/\tau_l''), \quad \text{(S3)}$$

$$\widetilde{M}(\Delta T; \tau_k', \tau_l'') = \sum_{i=1}^{n} a_i(\tau_k') a_i(\tau_l''), \quad \text{(S4)}$$

where L is the number of data points along the lifetime (τ) scale, $a_i(\tau)$ is the independent lifetime distribution of species i, and n is the number of the independent species. In 2D MEM analysis, we set a trial 2D lifetime correlation map $\widetilde{M}^0(\Delta T;\tau',\tau'')$ to calculate a simulated 2D emission-delay correlation map and compare it with an experimental one.

$$M^0_{\mathrm{cor}}(\Delta T;t'_i,t''_j)=\sum_{k=1}^{L}\sum_{l=1}^{L}\widetilde{M}^0(\Delta T;\tau'_k,\tau''_l)\exp(-t'_i/\tau'_k)\exp(-t''_j/\tau''_l)\,, \tag{S5}$$

$$\widetilde{M}^0(\Delta T;\tau'_k,\tau''_l)=\sum_{i=1}^{n}a_i{}^0(\tau'_k)a_i{}^0(\tau''_l)\,. \tag{S6}$$

The fitting error (χ^2) and the entropy (S) of the 2D lifetime correlation map can be defined as:

$$\chi^2=\frac{1}{K^2-1}\sum_{i=1}^{K}\sum_{j=1}^{K}\frac{\left\{M^0_{\mathrm{cor}}(\Delta T;t'_i,t''_j)-M_{\mathrm{cor}}(\Delta T;t'_i,t''_j)\right\}^2}{M(\Delta T;t'_i,t''_j)}\,, \tag{S7}$$

$$S=\sum_{i=1}^{n}\sum_{k=1}^{L}\left\{a_i{}^0(\tau_k)-m_i(\tau_k)-a_i{}^0(\tau_k)\ln\frac{a_i{}^0(\tau_k)}{m_i(\tau_k)}\right\}. \tag{S8}$$

In eq S8, $m_i(\tau)$ is a prior knowledge of $a_i{}^0(\tau)$. In this work, we set a constant value for $m_i(\tau)$. The optimum $\widetilde{M}^0(\Delta T;\tau',\tau'')$ that minimizes the following Q value is then searched and determined,

$$Q=\chi^2-\frac{2S}{\eta}\,, \tag{S9}$$

where η is the regularizing constant.

In this study, we performed a global 2D-MEM analysis using a set of 2D emission-delay correlation maps calculated at different ΔTs. The maps at ΔT = 100–250 μs and ΔT = 300–450 μs were used for the analysis. In a global 2D-MEM analysis, a 2D lifetime correlation map can be described as follows:

$$\widetilde{M}(\Delta T;\tau'_k,\tau''_l)=\sum_{i=1}^{n}\sum_{j=1}^{n}g_{ij}(\Delta T)a_i(\tau'_k)a_i(\tau''_l)\,. \tag{S10}$$

In eq S10, $g_{ij}(\Delta T)$ is the autocorrelation ($i=j$) or the cross-correlation ($i\neq j$) between species i and j. In a global 2D-MEM analysis, we fixed the autocorrelation of the shortest ΔT ($g_{ii\,(}\Delta T)$) to unity and that of the cross-correlation to 0. 2D emission-delay correlation maps are then globally analyzed by using common $a_i(\tau)$.

In the measurement, microtime information was recorded for every detected photon with the microtime resolution of 3.1 ps/ch. Total channels available are 4096. The channels (resolution) were reduced to 256 (49 ps/ch) by binning 16 adjacent data points into a single channel, and the photon data with reduced microtime were used for constructing 2D emission-delay correlation maps. For 2D MEM analysis, the microtime range from 0 to 7.7 ns (corresponds to 158 × 158 channels) was selected from those maps. For saving the computation time, the microtime resolutions of the selected 2D emission-delay correlation maps were further reduced to 24 × 24 channels. In this procedure, the binning width was changed logarithmically along the microtime axes to keep the lifetime information as much as possible. Discrete τ values (total 40 τ points) that are equally distributed in a logarithmic scale between 0.07 ns and 9.76 ns were used for determining $a_i(\tau)$ and $g_{ij}(\Delta T)$. Global 2D-MEM analysis was performed by increasing n until the simulated 2D emission-delay correlation map satisfies an experimental one as judged from χ^2 and the residuals. In this experiment, $n = 2$ is sufficient to describe the raw 2D map. Two independent lifetime species correspond to the fluorescent lipids in outer (longer lifetime species) and inner (shorter lifetime species) leaflets of the GUV. To obtain the species-specific fluorescence decay curve, Laplace transform is performed on $a_i(\tau)$.

FLCS. In FLCS analysis, one calculates a filter value of each species (i) at microtime channel j (f_{ij}, $1 \le j \le N$) as follows, using the intensity-normalized fluorescence decay curve of each species (p_{ij}) and the ensemble-averaged decay curve I_j that is calculated from all detected photons:[3] (N is the maximum microtime channel used for the analysis.)

$$\mathbf{F} = [\mathbf{M}^T \cdot \mathrm{diag}(\mathbf{I})^{-1} \cdot \mathbf{M}]^{-1} \cdot \mathbf{M}^T \cdot \mathrm{diag}(\mathbf{I})^{-1}. \quad \text{(S11)}$$

In eq S11, $\mathbf{F}$ is a $i \times N$ matrix with elements f_{ij}, $\mathbf{M}$ is a $i \times N$ matrix with elements p_{ij}, and $\mathbf{I}$ is a column vector with elements I_j. Obtained filter values can be used to generate the species-specific fluorescence intensity-correlation function based on the following equation:

$$G_{kl}(\Delta T) = \frac{\sum_{j=1}^{N}\sum_{j'=1}^{N} f_{kj} f_{lj'} \left\langle I_j(T) I_{j'}(T+\Delta T) \right\rangle}{\sum_{j=1}^{N} \left\langle f_{kj} I_j(T) \right\rangle \sum_{j'=1}^{N} \left\langle f_{lj'} I_{j'}(T) \right\rangle}. \quad \text{(S12)}$$

In eq S12, point brackets represent T-averaging. If $k = l$, $G_{kl}(\Delta T)$ corresponds to the autocorrelation of species k whereas $G_{kl}(\Delta T)$ corresponds to the cross-correlation between species k and l when $k \neq l$.

In the actual analysis, p_{ij} is calculated by performing Laplace transform on the independent lifetime distributions obtained by 2D FLCS analysis. Filter values and species-specific fluorescence correlation curves are then calculated by using photon data, I_j, p_{ij}, eq S11, and eq S12 with a microtime range from 0 ns < t < 7.7 ns corresponding to $1 \leq j \leq 2529$. It is noted that species-specific correlation curves are calculated on the photon-by-photon basis as described in the literature.[4,5]

All the calculation in 2D FLCS and FLCS analyses was performed by a code written in C and was run by Igor Pro (WaveMetrics).

Accuracy of determined diffusion coefficients

It has been reported that a diffusion coefficient of a lipid bilayer determined by one-spot (conventional) FCS is not accurate as compared to more sophisticated techniques such as Z-scan FCS and two-focus FCS.[6,7] It is mainly caused by the difficulty in correctly placing the waist of excitation beam to the sample plane. To check the accuracy of the data shown in Figure 3, fluorescence correlation curves were measured from 10 GUVs in the absence of PLL as shown in Figure S2. In each measurement, the axial position of the beam, in other words, the position of the objective was determined by referring the fluorescence intensity. (Maximum intensity is observed when the waist matches with the sample plane.) Each data was then fitted with the theoretical equation (eqn 2), and the standard deviation of the data was calculated. Based on this analysis, the standard deviations of the determined diffusion coefficients are estimated to be ~15% of the average ones, which is slightly larger but comparable to the reported value obtained with Z-scan FCS.[8]

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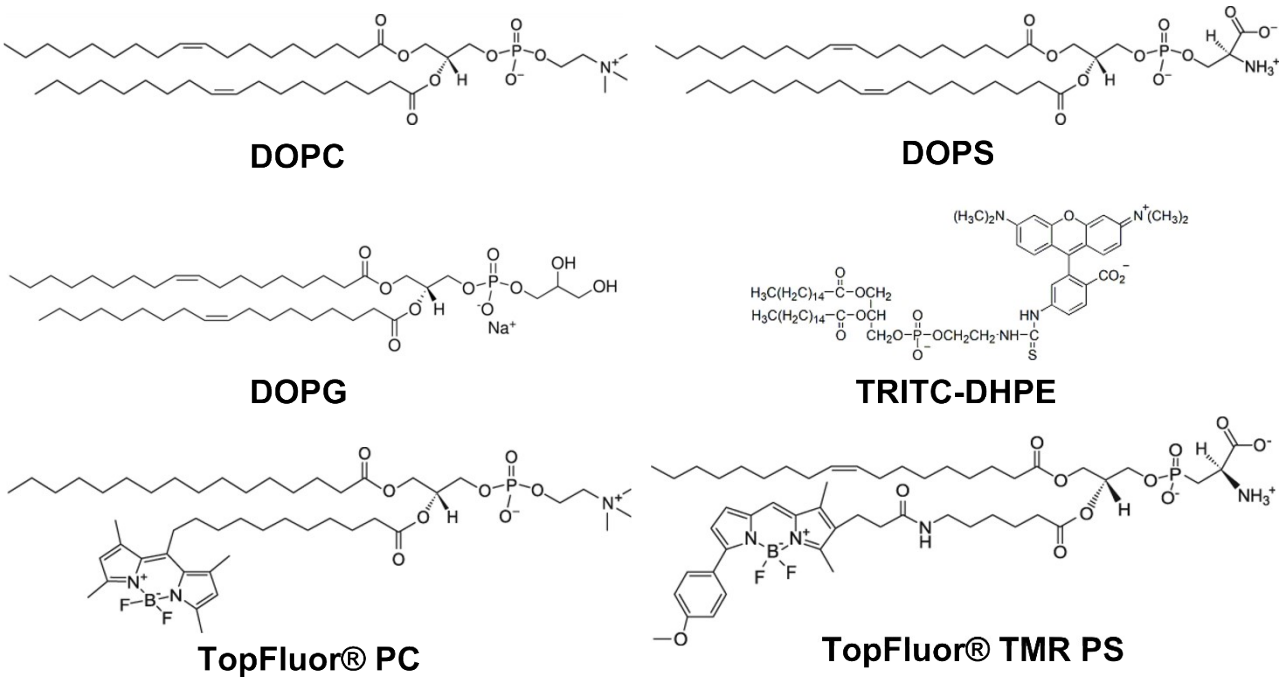

Figure S1. Chemical structures of lipids used in this study.

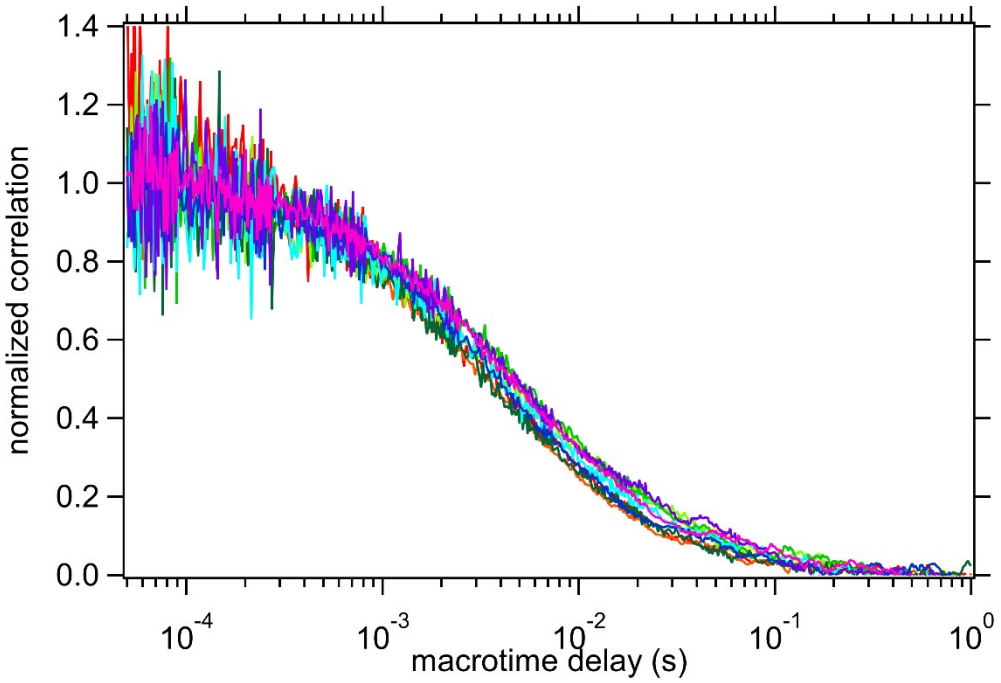

Figure S2. Fluorescence correlation curves of fluorescent lipids (TopFluor TMR PS). The data were measured from 10 GUVs and the calculated correlation curves of each data are shown with different colors.

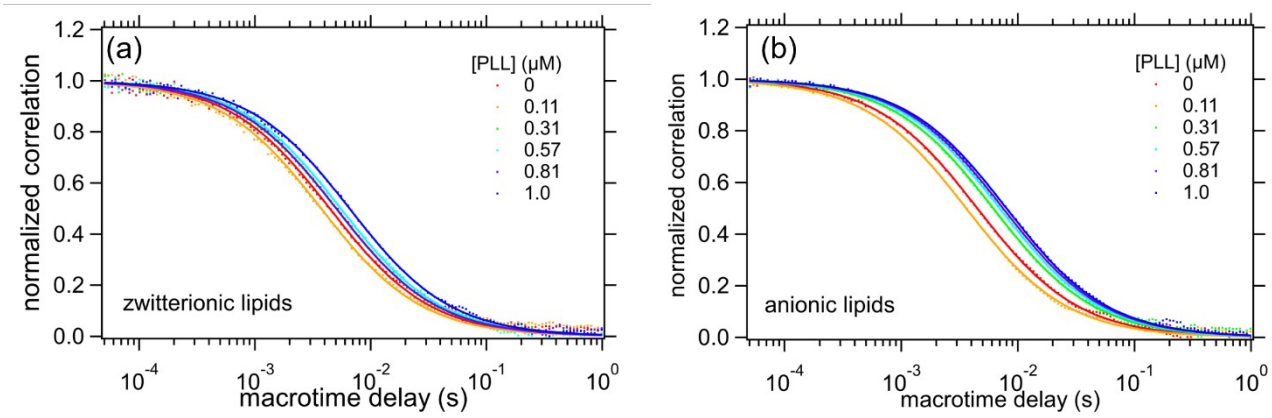

Figure S3. Fluorescence correlation curves of the zwitterionic (a) and anionic (b) lipids (dots) in a giant unilamellar vesicle composed of DOPC and DOPS with the molecular ratio of 4:1 and their fitting results (solid lines). Fitting is performed by using eqn (2).

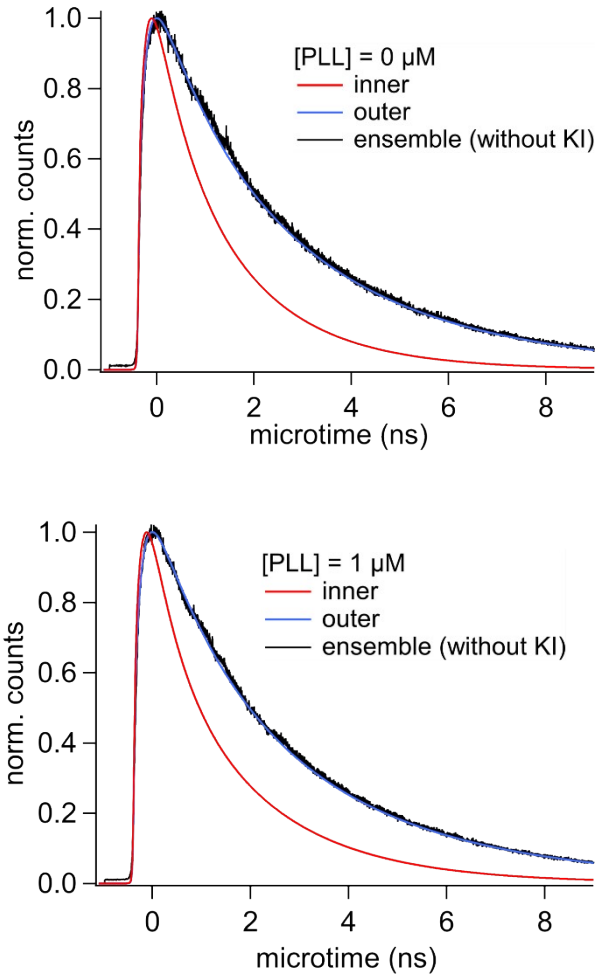

Figure S4. Independent fluorescence decay curves of the inner (red solid line) and the outer (blue solid line) leaflets of the GUV in the absence (upper) and the presence (lower) of 1 µM polylysine. For comparison, the ensemble-averaged (both inner and outer leaflets) fluorescence decay curves in the absence of potassium iodide (KI) are shown.

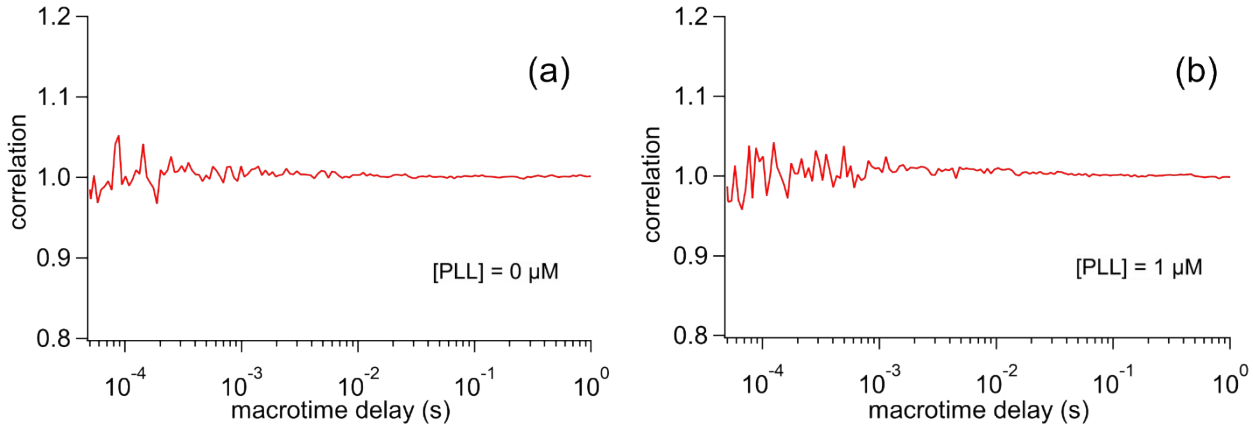

Figure S5. Cross-correlation curves between the signals of the shorter and longer lifetime components of lipids in a giant unilamellar vesicle in the absence (a) and the presence (b) of 1 µM polylysine.

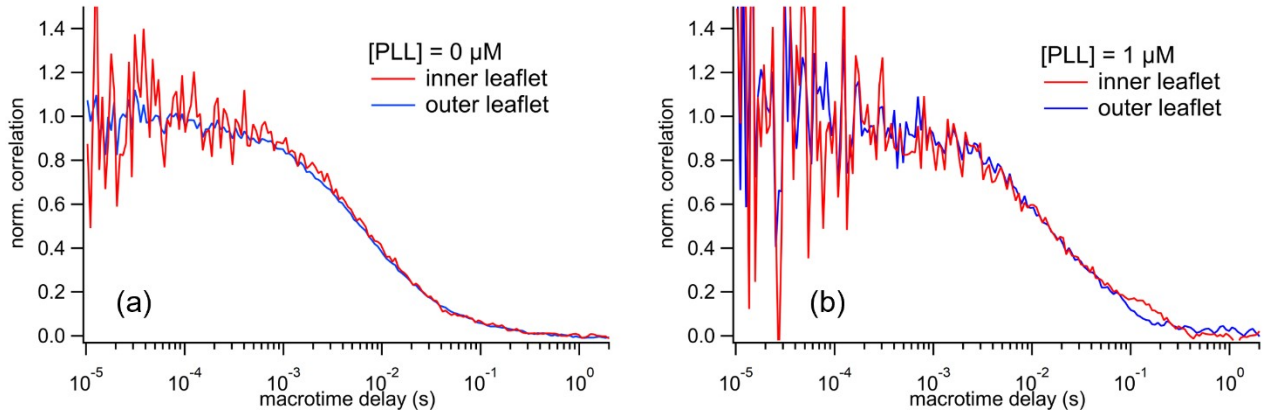

Figure S6. Fluorescence correlation curves of fluorescent lipids in the inner (red solid line) and outer (blue solid line) leaflets of a giant unilamellar vesicle composed of DOPC and DOPG with the molecular ratio of 4:1 in the absence (a) and the presence (b) of 1 μM polylysine. Data are normalized with the correlation amplitude at the macrotime delay of ~100 μs.