Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

SUPPLEMENTARY INFORMATION

Effect of electrostatic interaction on the leaflet-specific diffusion in a supported lipid bilayer revealed by fluorescence lifetime correlation analysis

Takuhiro Otosu and Shoichi Yamaguchi*

Department of Applied Chemistry, Graduate School of Science and Engineering, Saitama University, 255 Shimo-Okubo, Sakura, Saitama 338-8570, Japan

Analytical details of 2D FLCS and FLCS analyses.

We performed 2D FLCS analysis to determine the fluorescence lifetimes of fluorescent lipids in the proximal and distal leaflets of a supported lipid bilayer. Obtained leaflet-specific fluorescence decay curves were then used to calculate leaflet-specific fluorescence auto- as well as cross-correlation curves. Here, precise description of 2D FLCS and FLCS analyses are described.

2D FLCS analysis

In measurements, two temporal information, that is, macrotime (T) and microtime (t), are obtained from each detected photon. Macrotime (T) is the 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 (excitation-detection delay time) (Fig. S1a). Microtime resolution is 4.1 ps/ch and the total channels available are 4096. In 2D FLCS analysis, a 2D emission-delay correlation map (M (ΔT, t', t'')) at arbitrary ΔT is calculated from the photon data, where t' and t'' correspond to the microtimes of 1st and 2nd photons in photon pair, respectively.[S1-S4] Before the calculation, the microtime channels (resolution) were reduced to 256 (65 ps/ch) by binning 16 adjacent data points into a single channel, and the photon data with reduced microtime were used for constructing the 2D map. In this study, the maps at ΔT = 100–180 μs and 300–400 μs were constructed from the photon data of DOPC/DOTAP SLB and the maps at ΔT = 100–280 μs and 400–620 μs were constructed for the analysis of DOPC/DOPS SLB. Because the map contains both correlated and uncorrelated photon pairs, the uncorrelated signals are subtracted to obtain the map for the correlated signals (M_{cor} (ΔT, t', t'')). To perform this, 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. For the analysis of both DOPC/DOTAP SLB and DOPC/DOPS SLB, the map at ΔT = 5–5.2 (s) was calculated and used for the subtraction.

After that, 2D inverse Laplace transform is performed on M_{cor} (ΔT, t', t'') with the help of 2D maximum-entropy method (2D MEM) to identify the independent lifetime species and to determine their fluorescence lifetime distributions (and the corresponding fluorescence decay curves). 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:

$$M_{\mathrm{cor}}(\Delta T; t_i', t_j'') = \sum_{k=1}^{L}\sum_{l=1}^{L} \tilde{M}(\Delta T; \tau_k', \tau_l'') \exp(-t_i'/\tau_k') \exp(-t_j''/\tau_l''), \quad \text{(S1)}$$

$$\tilde{M}(\Delta T; \tau_k', \tau_l'') = \sum_{i=1}^{n} a_i(\tau_k') a_i(\tau_l''), \quad \text{(S2)}$$

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 $\tilde{M}^0(\Delta T; \tau', \tau'')$ to calculate a simulated 2D emission-delay correlation map and compare it with an experimental one.

$$M_{\mathrm{cor}}^0(\Delta T; t_i', t_j'') = \sum_{k=1}^{L}\sum_{l=1}^{L} \tilde{M}^0(\Delta T; \tau_k', \tau_l'') \exp(-t_i'/\tau_k') \exp(-t_j''/\tau_l''), \quad \text{(S3)}$$

$$\tilde{M}^0(\Delta T; \tau_k', \tau_l'') = \sum_{i=1}^{n} a_i{}^0(\tau_k') a_i{}^0(\tau_l''). \quad \text{(S4)}$$

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

$$\chi^2 = \frac{1}{K^2 - 1} \sum_{i=1}^{K}\sum_{j=1}^{K} \frac{\left\{M_{\mathrm{cor}}^0(\Delta T; t_i', t_j'') - M_{\mathrm{cor}}(\Delta T; t_i', t_j'')\right\}^2}{M(\Delta T; t_i', t_j'')}, \quad \text{(S5)}$$

$$S = \sum_{i=1}^{n}\sum_{k=1}^{L} \left\{ a_i{}^0(\tau_k) - m_i(\tau_k) - a_i{}^0(\tau_k) \ln \frac{a_i{}^0(\tau_k)}{m_i(\tau_k)} \right\}. \quad \text{(S6)}$$

In Eq. (S6), $m_i(\tau)$ is a prior knowledge of $a_i{}^0(\tau)$. In this work, we set a constant value for $m_i(\tau)$. The optimum $\tilde{M}^0(\Delta T; \tau', \tau'')$ that minimizes the following Q value is then searched and determined,

$$Q = \chi^2 - \frac{2S}{\eta}, \quad \text{(S7)}$$

where η is the regularizing constant.

Here, we performed a global 2D-MEM analysis on the 2D emission-delay correlation maps constructed above. In global 2D-MEM analysis, a 2D lifetime correlation map can be described as follows:

$$\tilde{M}(\Delta T; \tau_k', \tau_l'') = \sum_{i=1}^{n}\sum_{j=1}^{n} g_{ij}(\Delta T) a_i(\tau_k') a_i(\tau_l''). \quad \text{(S8)}$$

In Eq. (S8), $g_{ij}(\Delta T)$ is the autocorrelation ($i = j$) or the cross-correlation ($i \neq j$) between species i and j. In 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 $\alpha_i(\tau)$ for all species.

In this study, the microtime range from 0 to 5.7 ns (corresponding to 87 × 87 channels) and from 0 to 6.5 ns (100 × 100 channels) were selected from the maps of DOPC/DOTAP SLB and DOPC/DOPS SLB, respectively. 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. In the analysis, the number of the independent species (n) is fixed. The analysis was then repeated by increasing n until the simulated 2D emission-delay correlation map satisfies an experimental one as judged from the chi-square value and the residuals. In this work, $n = 2$ was enough to describe all 2D maps irrespective of pH and the lipid composition, each of which corresponds to lipids in the proximal or distal leaflets of SLB. To obtain the leaflet-specific fluorescence decay curve, Laplace transform is performed on $a_i(\tau)$.

FLCS analysis

In FLCS analysis, one calculates a filter value of each species (k) at microtime channel i ($f_i^{(k)}$, $1 \leq i \leq N$) as follows, using the intensity-normalized fluorescence decay curve of each species ($p_i^{(k)}$) and the ensemble-averaged decay curve I_i that is calculated from all detected photons: (N is the maximum microtime channel used for the analysis.)

$$\mathbf{F} = [\mathbf{M}^T \cdot \mathrm{diag}(\mathbf{I})^{-1} \cdot \mathbf{M}]^{-1} \cdot \mathbf{M}^T \cdot \mathrm{diag}(\mathbf{I})^{-1}. \quad \text{(S9)}$$

In Eq. (S9), $\mathbf{F}$ is a $k \times N$ matrix with elements $f_i^{(k)}$, $\mathbf{M}$ is a $k \times N$ matrix with elements $p_i^{(k)}$, and $\mathbf{I}$ is a column vector with elements I_i. The leaflet-specific fluorescence decay curves obtained by 2D FLCS analysis were used for $p_i^{(k)}$. Filter values were calculated with a microtime range from 0 ns < t < 5.7 ns ($1 \leq i \leq 1392$) and from 0 ns < t < 6.5 ns ($1 \leq i \leq 1600$) for DOPC/DOTAP SLB and DOPC/DOPS SLB, respectively. The filter values were used to calculate the species-specific auto- and cross-

correlation curves by using Eq. 1 in the maintext.

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

References

S1. T. Otosu and S. Yamaguchi, *J Chem Phys*, 2019, **151**, 025102.

S2. K. Ishii and T. Tahara, *Chem Phys Lett*, 2012, **519-20**, 130-133.

S3. K. Ishii and T. Tahara, *J Phys Chem B*, 2013, **117**, 11414-11422.

S4. K. Ishii and T. Tahara, *J Phys Chem B*, 2013, **117**, 11423-11432.

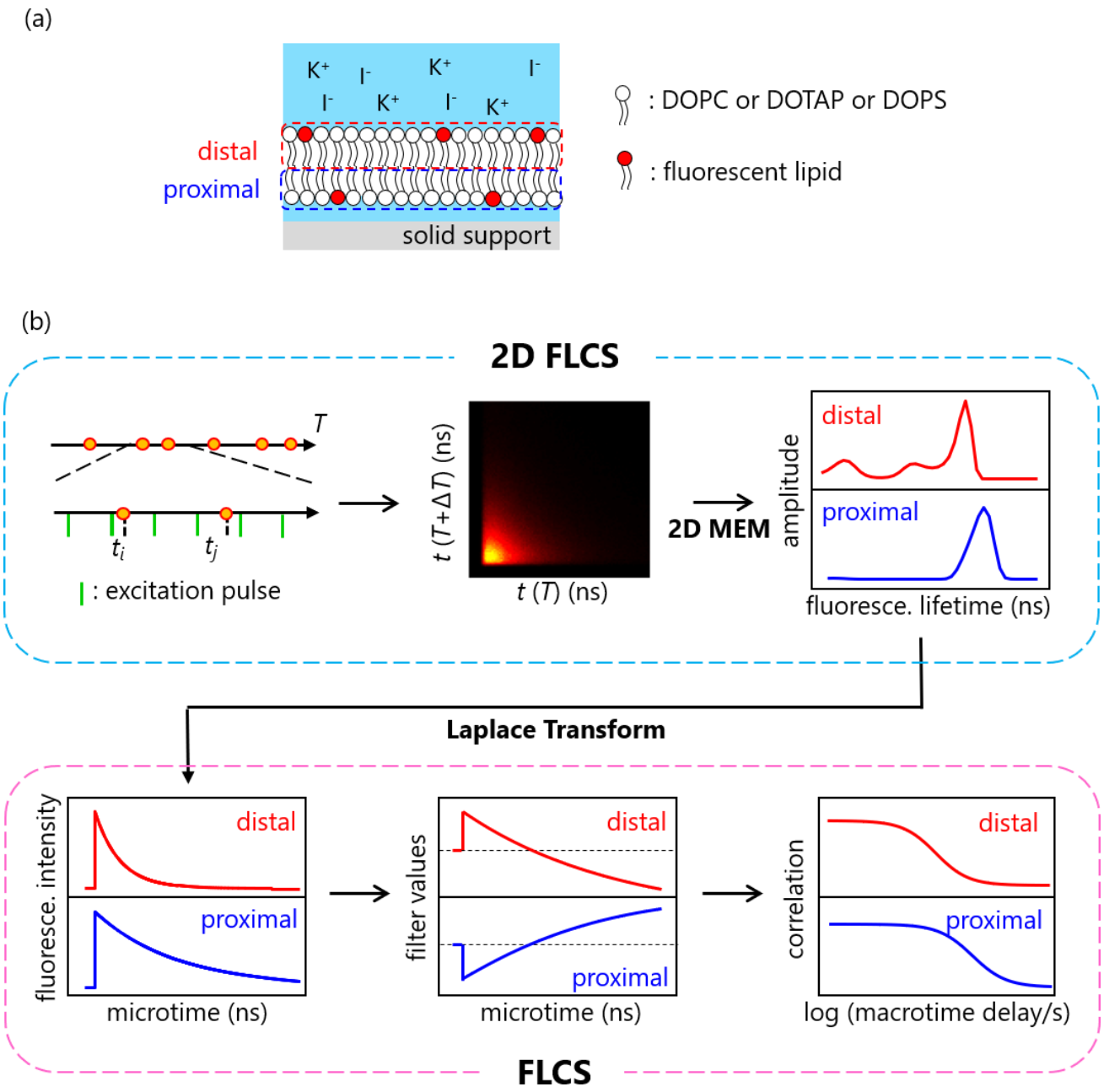

Fig. S1. (a) Schematic image of the sample used in this study. (b) Illustrated procedure of the leaflet-specific lipid diffusion by means of 2D FLCS and FLCS.

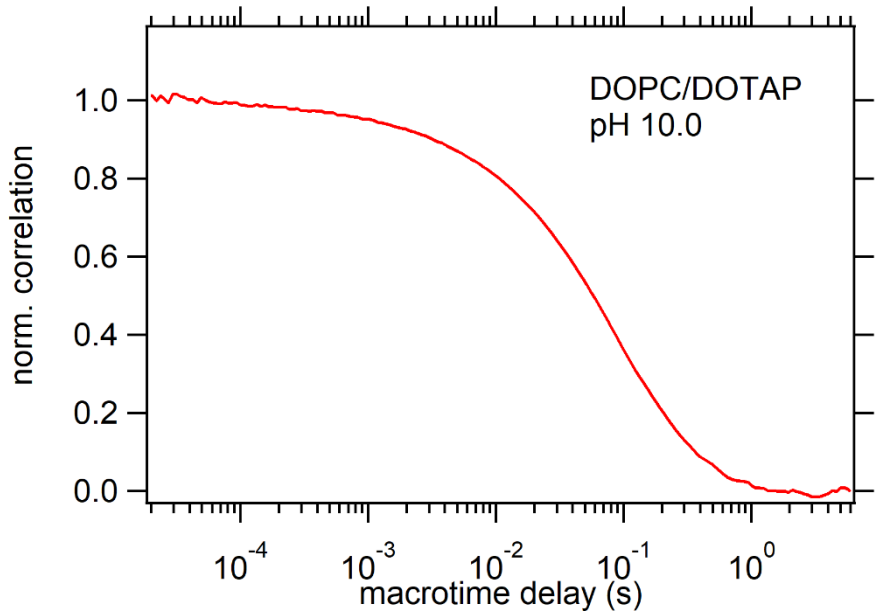

Fig. S2. Ordinary fluorescence intensity-correlation curve of fluorescent lipids in a supported DOPC/DOTAP bilayer. Data were measured at pH 10.0.

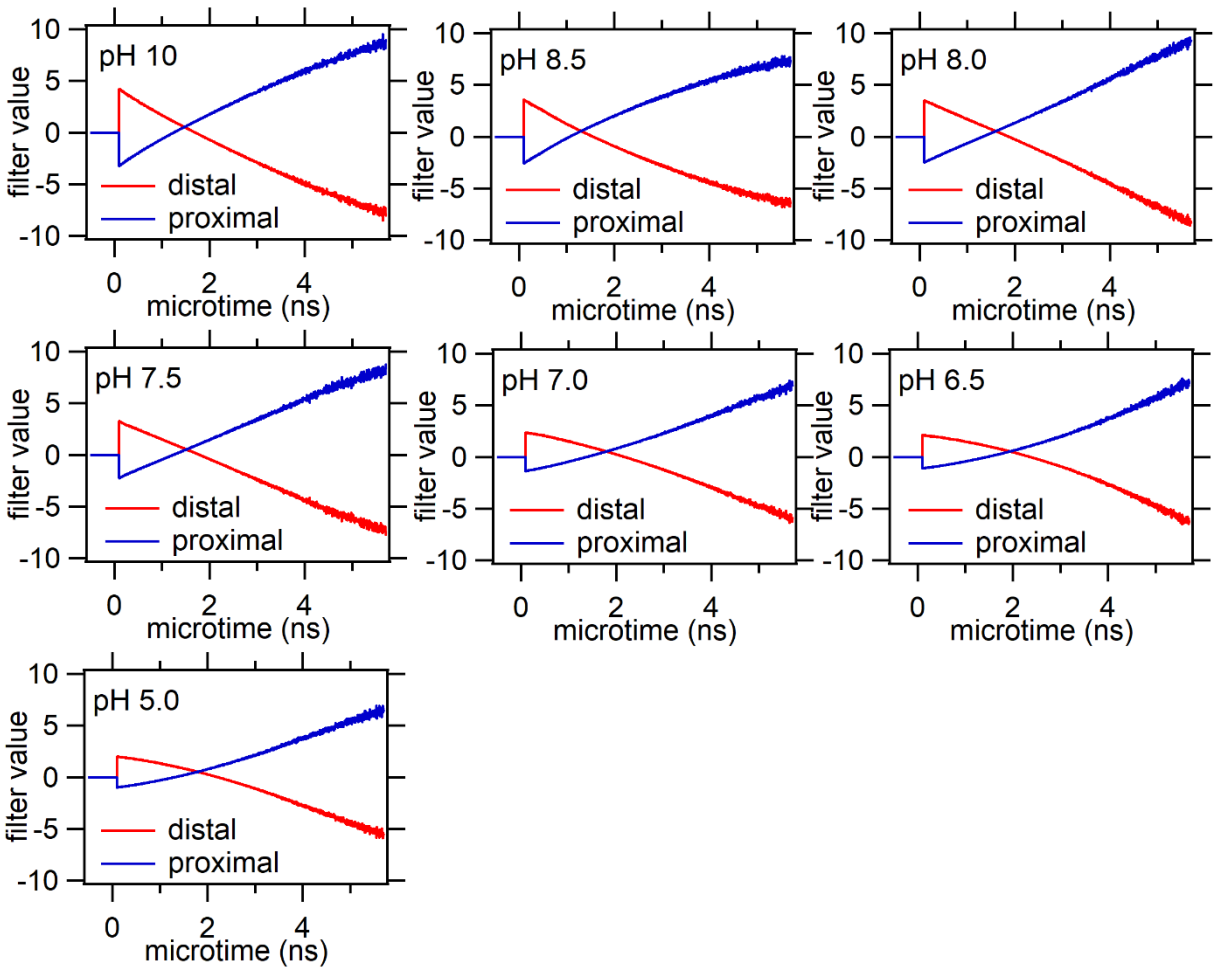

Fig. S3. Filter values corresponding to the distal (red) and proximal (blue) leaflets of a supported DOPC/DOTAP bilayer measured at various pH.

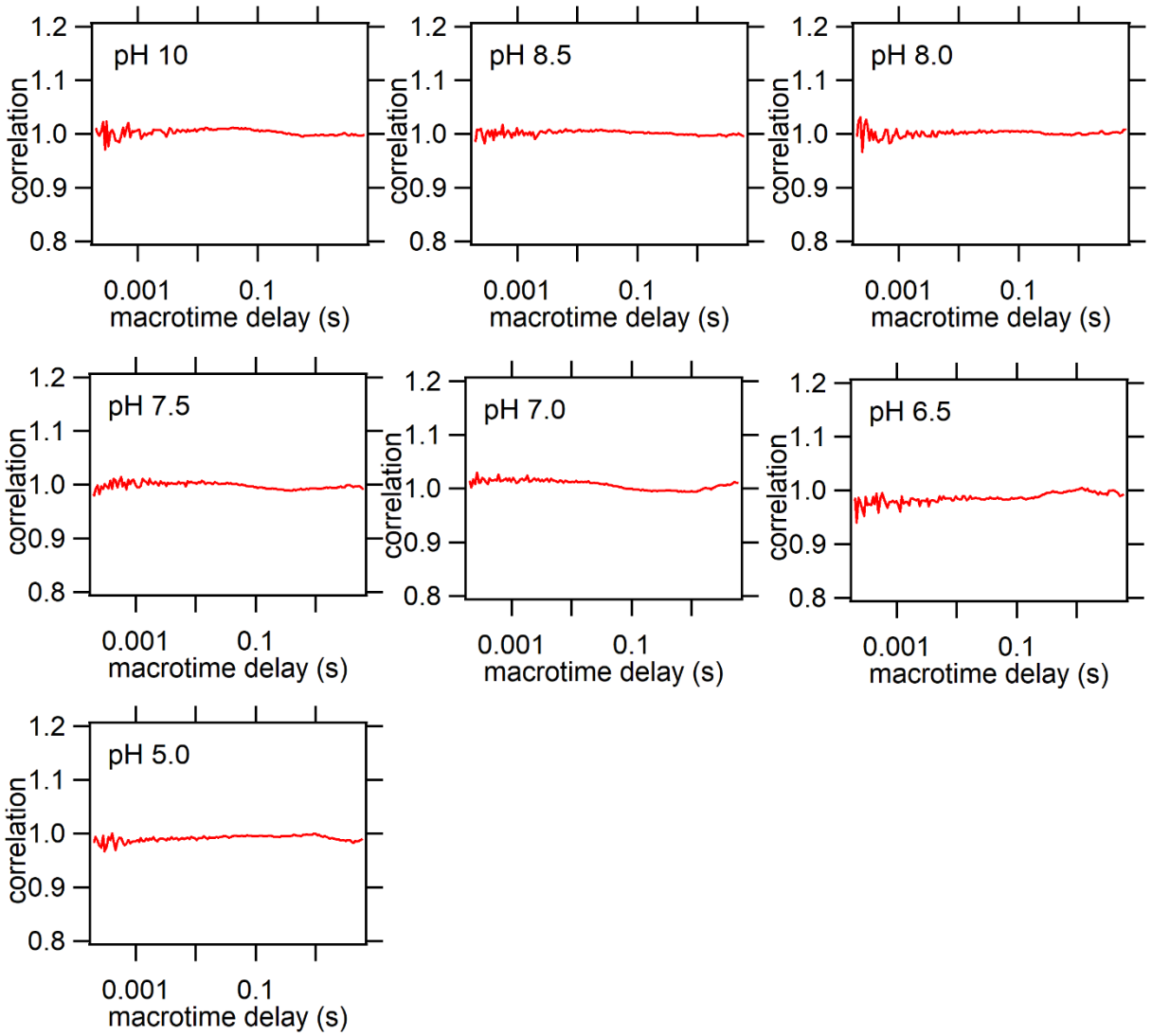

Fig. S4. Leaflet-specific cross-correlation between the distal and proximal leaflets of a supported DOPC/DOTAP bilayer at various pH.

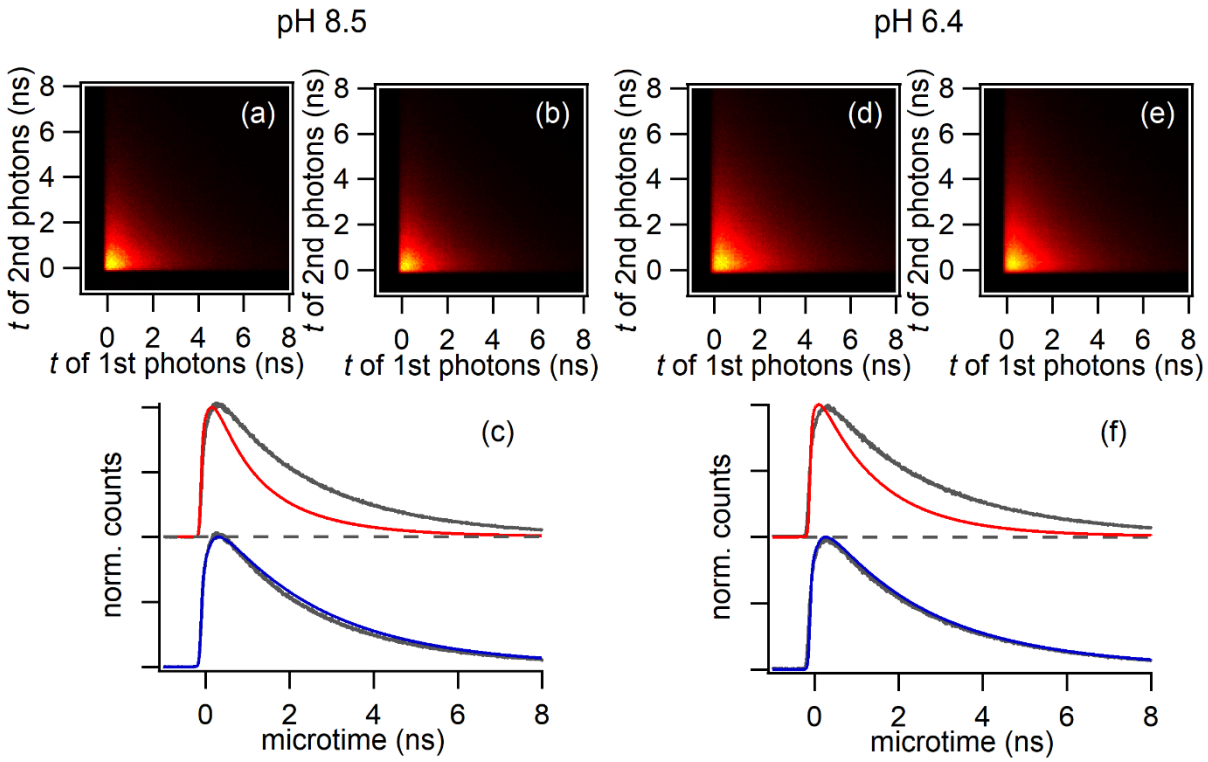

Fig. S5. Two-dimensional (2D) emission-delay correlation maps (a,b,d and e) and the independent fluorescence decay curves (c and f) of fluorescent lipids in a supported DOPC/DOPS bilayer measured at pH 8.5 (a-c) and 6.4 (d-f). The maps were calculated with the macrotime delays of ΔT =100–280 (a and d) and 400–620 (b and e) μs. The independent fluorescence decay curves were calculated by performing a global 2D maximum entropy method analysis on these 2D maps. For comparison, the ensemble-averaged fluorescence decay curve of fluorescent lipids in the absence of potassium iodide is shown with a gray solid line.

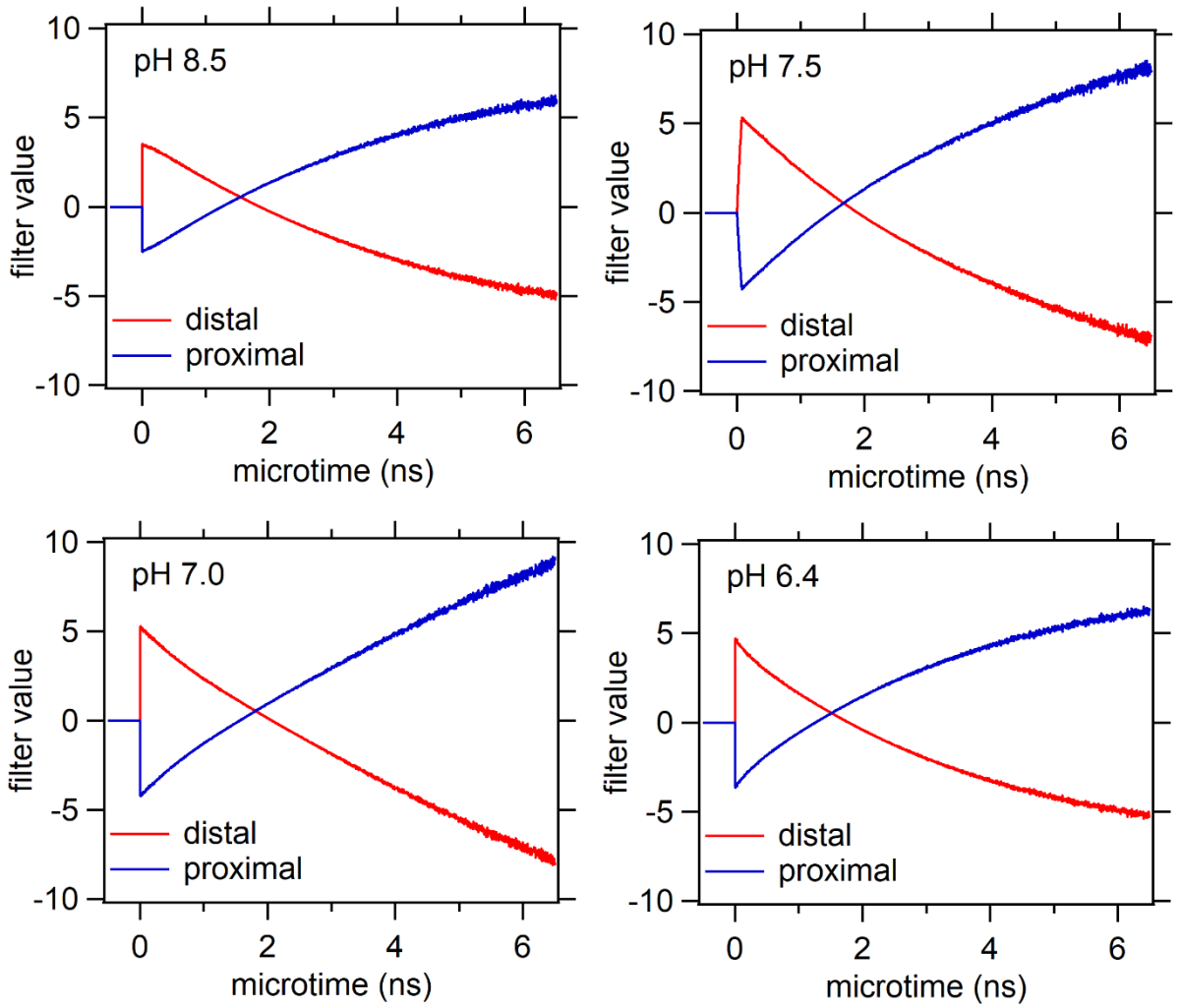

Fig. S6. Filter values corresponding to the distal (red) and proximal (blue) leaflets of a supported DOPC/DOPS bilayer measured at various pH.

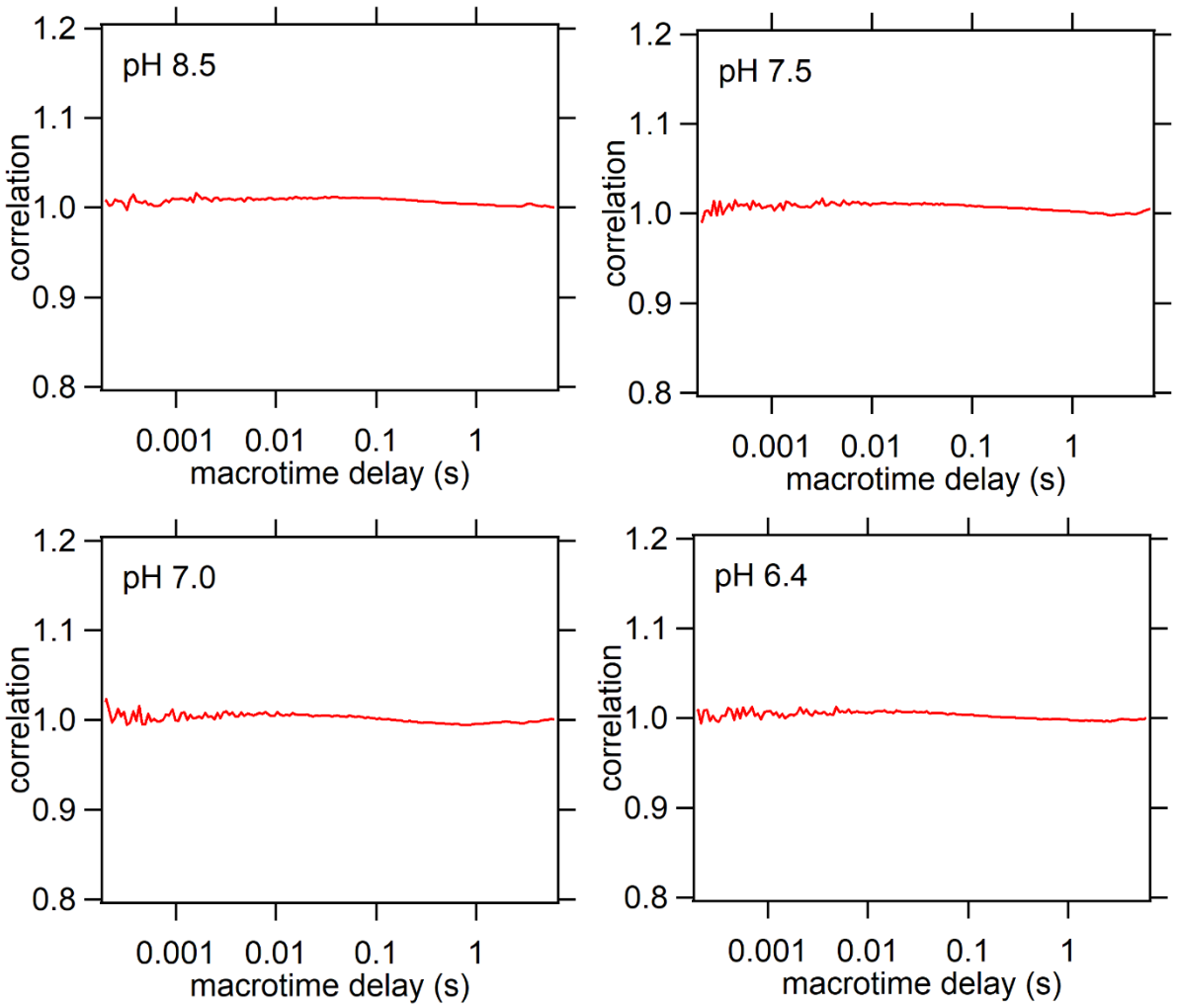

Fig. S7. Leaflet-specific cross-correlation between the distal and proximal leaflets of a supported DOPC/DOPS bilayer at various pH.