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Supporting Material

A Novel Fluorescent Probe That Senses the Physical State of Lipid Bilayers

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Supporting Information for

A Novel Fluorescent Probe That Senses the Physical State of Lipid Bilayers

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Figure S1. Fluorescence excitation and emission spectra of NBD-R595 in aqueous buffer

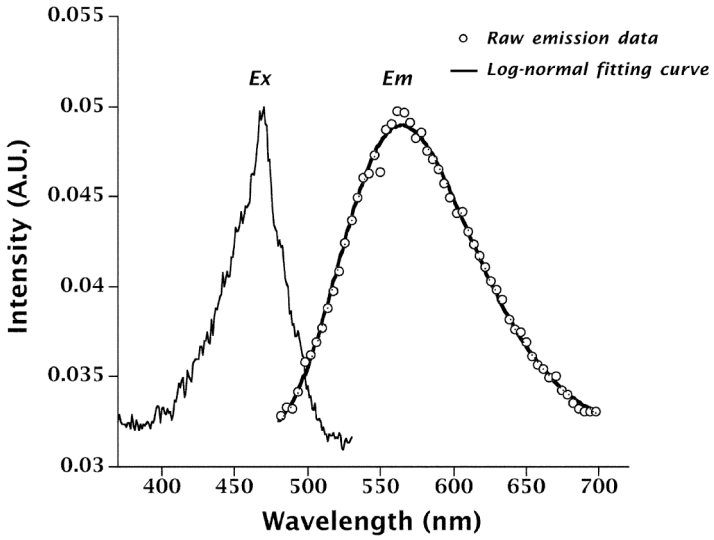


Figure S1. Excitation and emission spectra of NBD-R595 (0.92 μM) in DPBS (pH 7.2) at 35°C. Emission intensities are depicted as open circles. The solid line overlapped with emission spectrum is a log-normal fitting curve. The excitation spectrum was collected for the emission wavelength at 565 nm, and the emission spectrum was collected with excitation wavelength of 470 nm. The peak positions were 470 nm (excitation spectrum) and 565 nm (emission spectrum).

Figure S2. Estimation of the critical micelle concentration of NBD-R595

The critical micelle concentration (cmc) of a non-fluorescent LPS (Kdo_2-Lipid A) has been studied by using dynamic light scattering technique (1). The light scattering data showed that the cmc changes as a function of temperature; cmc is lower at higher temperature (1). Taking advantage of this property, the cmc of fluorescent LPS (NBD-R595) could be estimated by temperature-dependent changes in the emission spectra (Figure S2), because aggregation of NBD-R595 leads to an increased

fluorescence quenching. If a concentration of NBD-R595 is above cmc at high temperature but below it at low temperature, decrease in temperature causes increase in fluorescence emission. In contrast, if a concentration of NBD-R595 is always below cmc at both high and low temperatures, decrease in temperature does not change fluorescence emission. We tested two concentrations of NBD-R595, 92 nM and 198 nM, by monitoring emission at 5 and 37°C (Figure S2). The emission intensities of 92 nM NBD-R595 at 5° and 37°C were absolutely the same (Figure S2 panel a), whereas the intensity of 198 nM NBD-R595 at 37°C was ca. 25% smaller than at 5°C (Figure S2 panel b). These data suggest that the cmc of NBD-R595 at 37°C is 92 nM < cmc < 198 nM, and that the aggregation does not generate the 610 nm emission peak.

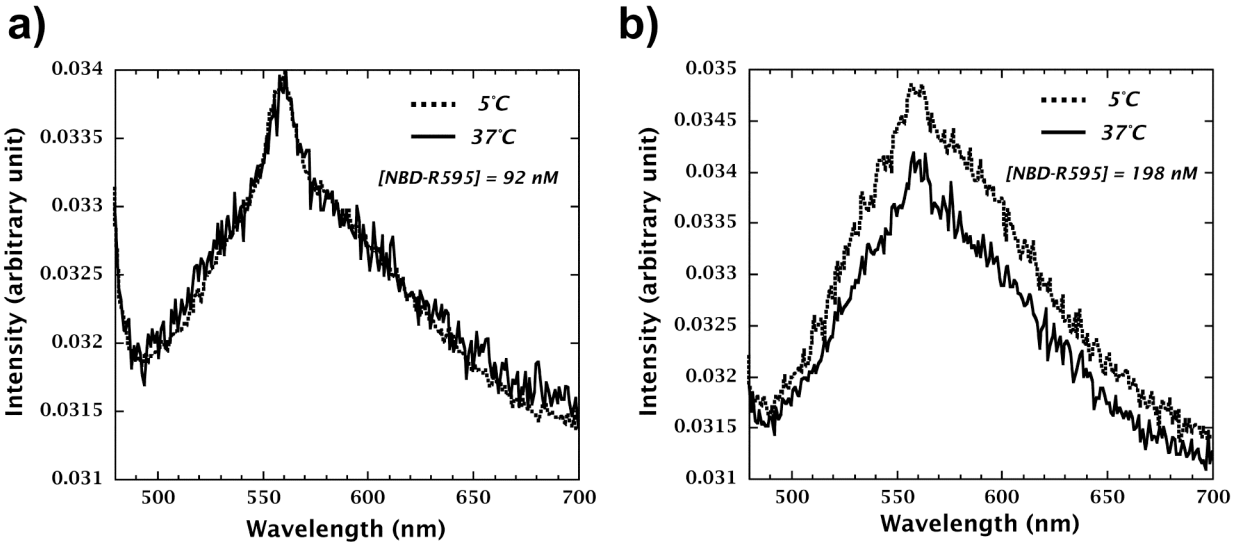
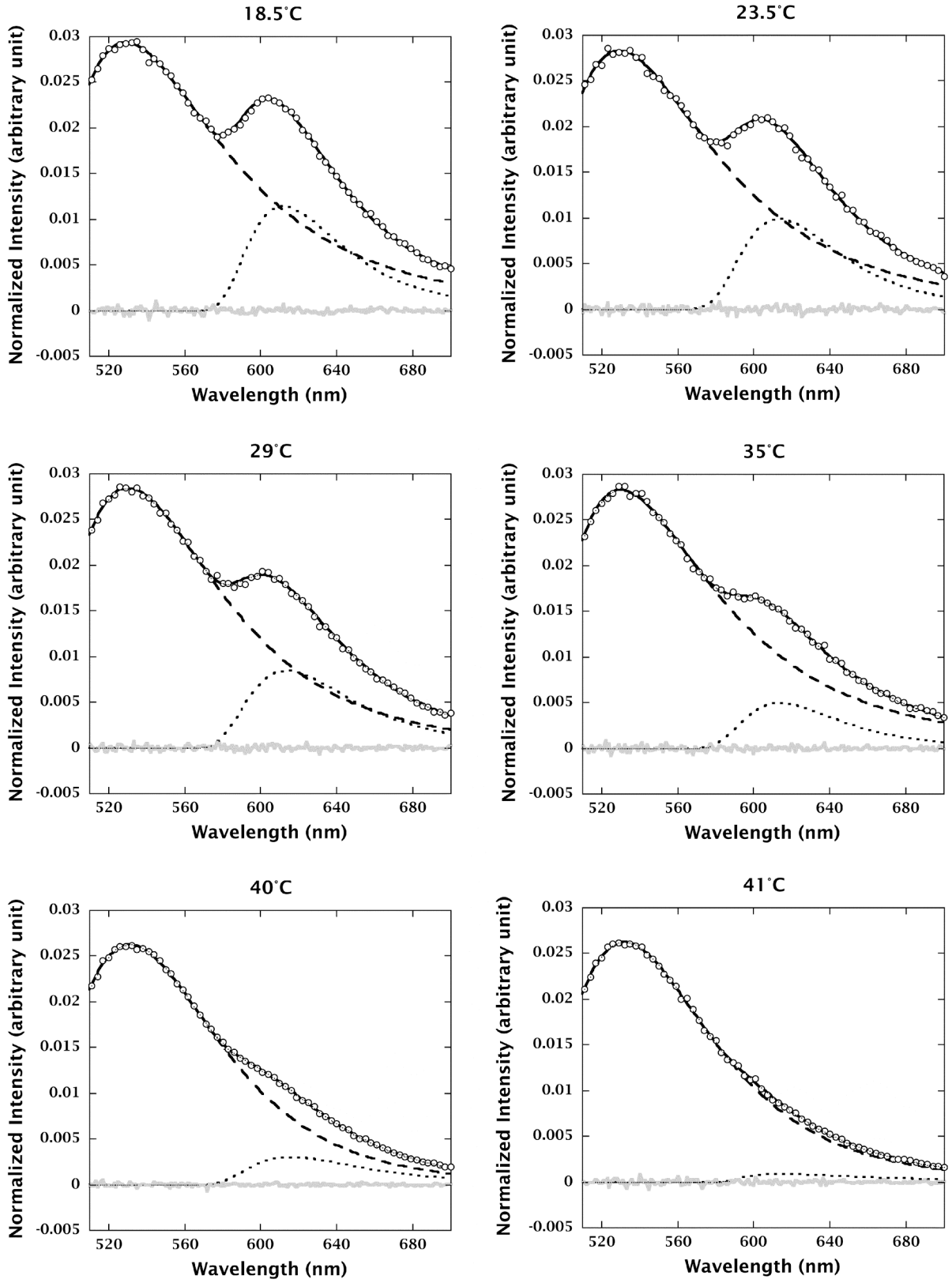


Figure S2. Spectral changes between 5°C and 37°C in emission spectra of NBD-R595 at various concentrations (panel a: 92 nM, panel b: 198 nM) in DPBS (pH 7.2). Emission spectra collected at 5°C and 37°C are drawn as dotted and solid lines, respectively. Both emission spectra shown in panels a and b were collected with excitation wavelength of 470 nm. These data show that NBD-R595s at different concentrations have different dependences upon temperature. Higher concentration of NBD-R595 suffers more decrease in emission intensity accompanying the increase in temperature.

Figure S3. Changes in emission spectra of NBD-R595 in the presence of DPPC LUVs as a function of temperature. The spectra have been decomposed as described in Figure S4.



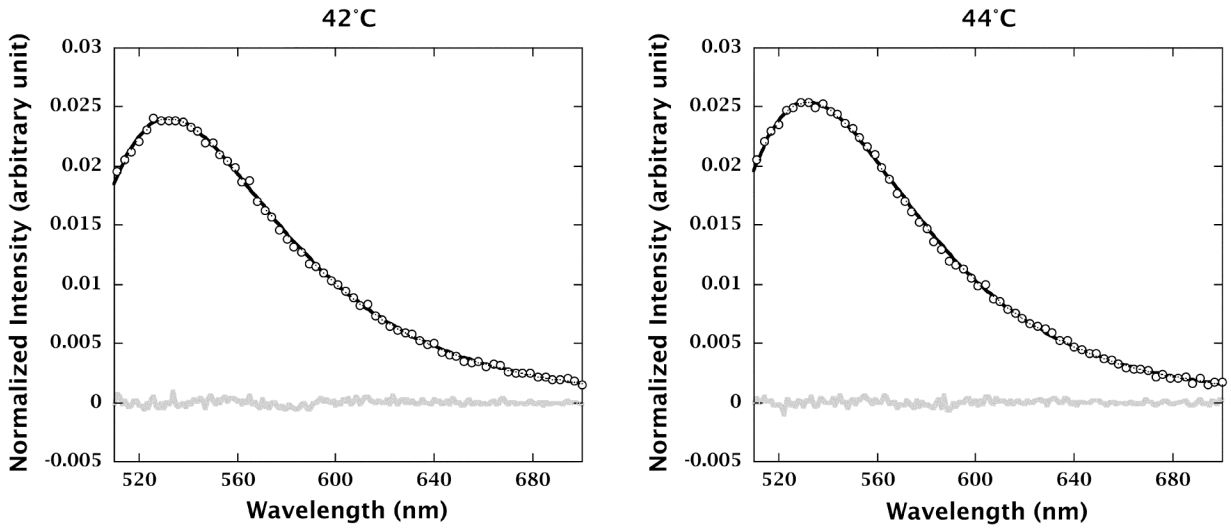


Figure S3. Emission spectra of NBD-R595 (0.92 μM) mixed with DPPC LUVs (326 μM) at different temperatures. These data were used for the analyses shown in Figure 3. Normalized intensity was calculated by subtracting $I_{background}$ from raw values (see Figure S4). Open circles represent the observed spectra of NBD-R595. Broken and dotted lines are log-normal curves for the peaks observed at shorter- and longer-wavelengths, respectively. Solid lines drawn in black and gray are the sum of two log-normal curves, and the residuals between observed data and black solid line, respectively. Excitation wavelength was 470 nm. Intensity of 610 nm emission peak decreased accompanying the increase in temperature, and vanished above T_m (~41°C).

Figure S4. Decomposition of fluorescence spectra

Fluorescence emission spectra were analyzed using log-normal functions of the following form (2):

For $\lambda > \lambda_{max} - \rho\Gamma/(\rho^2 - 1)$,

$$I(\lambda) = I_{background} + I_o \exp[(\ln 2/\ln^2\rho) \times \ln^2\{1 + (\lambda - \lambda_{max})(\rho^2 - 1)/\rho\Gamma\}]$$

while for $\lambda < \lambda_{max} - \rho\Gamma/(\rho^2 - 1)$, $I(\lambda) = I_{background}$

where $I_{background}$ is the intensity of background and I_o is the intensity above the $I_{background}$ at the wavelength of maximum intensity λ_{max}. Γ and ρ are the full width of the spectrum at half-maximum intensity $I_o/2$ and the distribution asymmetry parameter, respectively. Using a linear combination of this analytical model (3), fluorescence spectra were fitted by nonlinear least-squares fitting. The

fittings were carried out with the commercial software package Prism 4 (GraphPad Software, Inc., San Diego, CA).

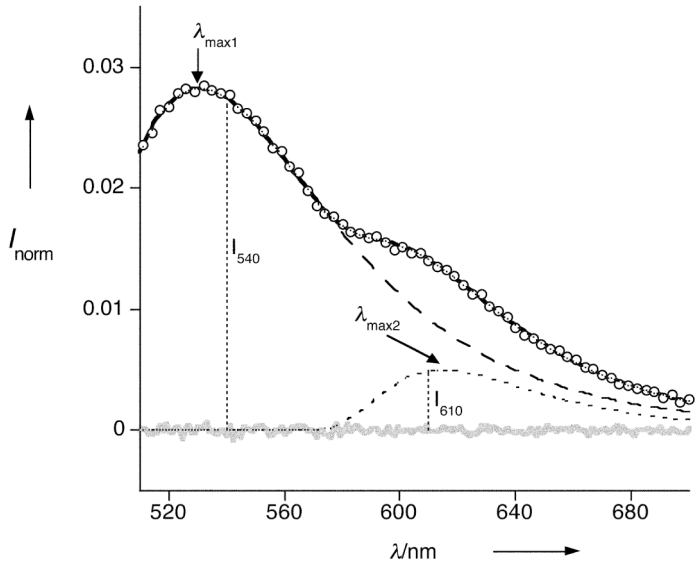


Figure S4. Open circles are observed data. Broken and dotted lines are log-normal curves for the peaks observed at shorter- and longer-wavelength, respectively. Solid lines drawn in black and gray are the sum of two log-normal curves, and the residuals between observed data and black solid line, respectively. Normalized intensity (I_{norm}) was obtained by subtracting $I_{background}$ from raw values. Spectral parameters used in the text are also shown in this figure.

Figure S5. Fluorescence excitation spectra of NBD-R595 for different emission wavelengths in the presence of DPPC LUVs

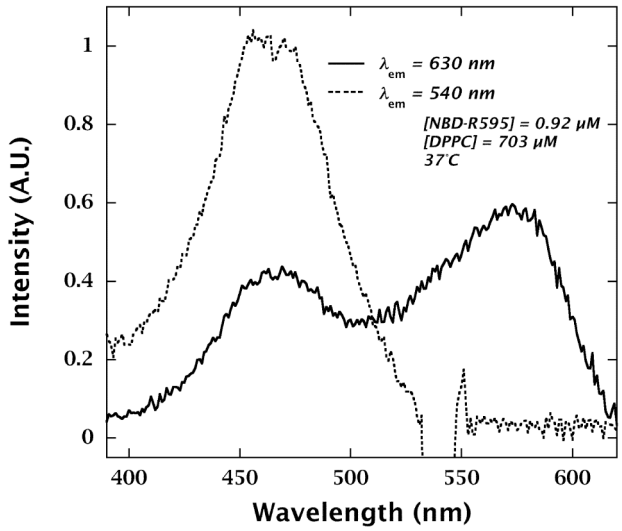


Figure S5. Excitation spectra of NBD-R595 (0.92 μM) in DPBS (pH 7.2) in the presence of DPPC LUVs (703 μM) at 37°C. Excitation spectra for the emission wavelengths at 540 nm and 630 nm are drawn as dotted and solid lines, respectively. Intensities have been corrected to account for the wavelength dependence of the intensity of excitation light, by using the data collected with Rhodamine B and Nile Blue (4). These data show that a broad peak around 570 nm appeared only in the spectrum for 630-nm emission.

Figure S6. UV-Vis absorption spectra of NBD-lysoPPE in the presence of DPPC LUVs

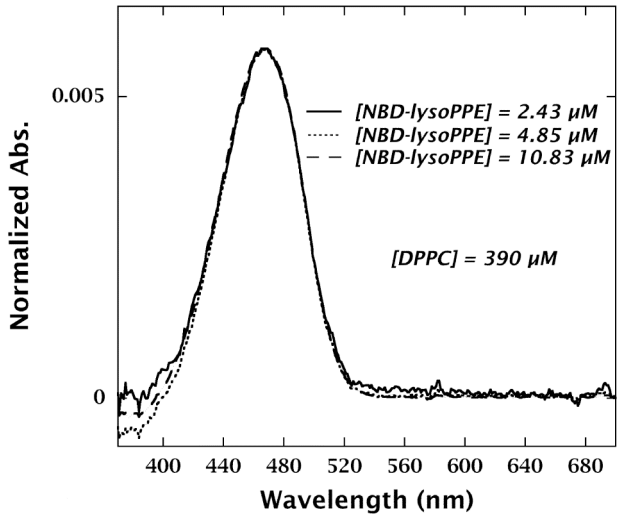


Figure S6. UV/Vis absorption spectra of NBD-lysoPPE at different concentrations in the presence of DPPC LUVs (390 μM) at room temperature. Solid-black, dotted-black and broken-black lines represent the spectra collected for NBD-lysoPPE at 2.43 μM, 4.85 μM and 10.83 μM, respectively. Absorbance has been normalized to account for differences in concentration of the probe. In contrast to NBD-R595, NBD-lysoPPE did not show the 570 nm absorption peak at any concentrations measured.

Calculation of transition temperature depression of the DPPC by NBD-R595 assuming that the probe acts as a simple impurity

The ΔT was calculated using the standard equation for freezing-point depression that is discussed in physical chemistry texts:

$$\Delta T = (R T_m^2/\Delta H)\chi$$

where R is the gas constant, ΔH is the enthalpy change of DPPC's main-transition, and χ is the mole

fraction of DPPC-bound NBD-R595. The T_m and ΔH for main-transition of DPPC LUVs have been reported to be 41.4 ± 0.1°C and 7.5 ± 0.5 kcal/mol, respectively (5), and χ was estimated to be (2.50 ± 0.08) × 10^{-3} from the partition coefficient measurements at 37°C. The ΔT for DPPC T_m was calculated to be ~0.07 K, or about 0.1°C.

References

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