

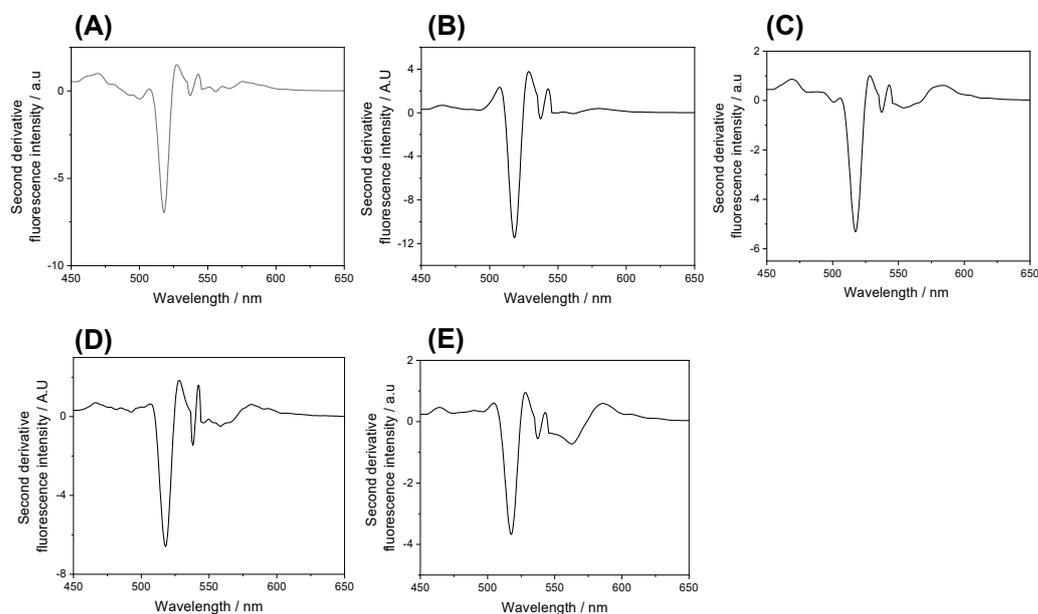
# Characterization of Phase Separated Planar Lipid Bilayer Membrane by Fluorescence Ratio Imaging and Scanning Probe Microscope

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The two states of LipiORDER in (POPC/SM/CHOL) SUVs and these peak wavelengths were confirmed with the second derivative fluorescence spectra. The second derivative fluorescence spectra in Supporting Figure 1 were obtained by the Origin graph software (Lightstone, Tokyo, Japan) under following conditions: Savitzky-Golay algorithm, a quadratic polynomial of 15 points, and 0.5 nm interval. The spectra show that the two peaks could be found at 505~515 nm and 560~585 nm ( $L_d$  phase) in (B) (37/32/31), (C) (49/29/22), (D) (60/26/14) and (E) (100/0/0), and only one peak in (A) (POPC/SM/CHOL: 20/40/40), which is from  $L_o$  phase. Hence, the fluorescence ratio value derived from  $L_o$  and  $L_d$  phase is effective for phase state analysis.



**Supporting Figure 1.** Second derivative fluorescence spectrum of LipiORDER in (POPC/SM/CHOL) SUVs.

Molar ratio (%) of lipid composition (POPC/SM/CHOL): (A) (20/40/40), (B) (37/32/31), (C) (49/29/22), (D) (60/26/14) and (E) (100/0/0).