Supporting Information for

Designing Lipids for Selective Partitioning Into Liquid Ordered Membrane Domains

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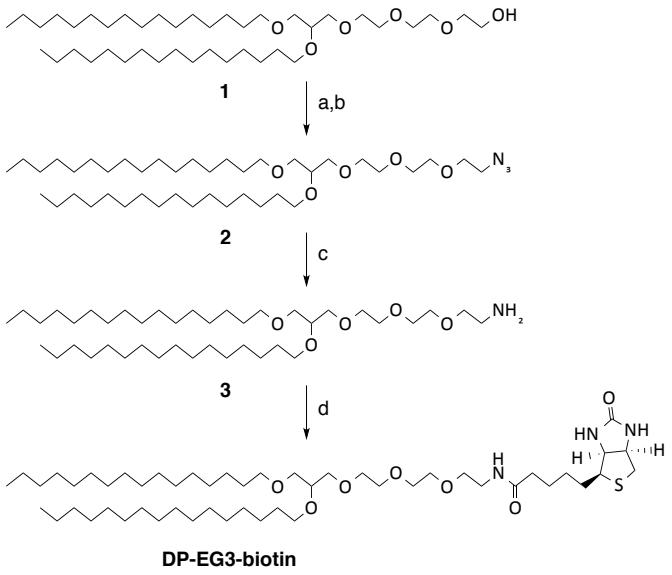
Lipid synthesis

The synthetic route to DP-EG3-biotin starting from 1,2-dipalmityl-glycero-3-triethylene glycol is shown in Scheme S1. DP-EG5-biotin, DP-EG10-biotin, and DP-EG15-biotin were prepared similarly but using pentaethylene glycol to sequentially extend the length of the PEG spacer.



Scheme S1. Synthesis of DP-EG3-biotin starting from 1,2-dipalmitylglycero-3-triethylene glycol (**1**).

9-[2,3-Bis(hexadecyloxy)propyl]-3,6,9-trioxanonyl-1-azide **(2)**. To a cooled (0 °C) solution of **1**[1] (0.66g, 0.98 mmole) and triethylamine (0.36 mL, 2.6 mmole) dissolved in anhydrous tetrahydrofuran (THF) (10 mL) was added methanesulfonyl chloride (0.15

mL, 1.9 mmole) dropwise by syringe and the mixture let stir for 2h. The mesylate of **1** was isolated by liquid extraction using ethyl acetate and water, separating the organics, then drying over $MgSO_4$, followed by filtering and the solvent stripped under vacuum. The isolated waxy material was then taken up in anhydrous dimethylformamide (DMF) (10 mL) and let stir with sodium azide (0.20g, 3.1 mmole) at room temperature overnight. Product **2** was isolated by flash column chromatography (R_f = 0.30, 20% ethyl acetate/hexanes) as a white waxy material (0.58g, 85%).

***9-[2,3-Bis(hexadecyloxy)propyl]-3,6,9-trioxanonyl-1-amine* (3)**. A 1.0M solution of lithium aluminum hydride (LAH) in THF (0.7 mL) was syringed into a mechanically stirred solution of **2** (0.47g, 0.67 mmole) in anhydrous THF (4 mL). The solution was brought to reflux for 15 min., then cooled in an ice bath and quenched with H_2O (0.03 mL), followed by aq. 10% NaOH (0.03 mL), and again with H_2O (0.1 mL). The organics were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to yield **3** (0.41g, 91%) as a white, waxy solid.

9-[2,3-Bis(hexadecyloxy)propyl]-3,6,9-trioxanonyl-1-biotinylamide (DP-EG3-biotin). Compound **3** (0.41g, 0.61 mmole) and biotin-NHS ester[2] (0.27g, 0.79 mmole) were dissolved in a solution of anhydrous DMF (7 mL) and anhydrous THF (3 mL). After stirring overnight at room temperature the solution was extracted with chloroform and water, the organic layer dried over anhydrous $MgSO_4$, filtered, concentrated *in vacuo*, and residual oils were flash column chromatographed (R_f = 0.24, 2% methanol/ethyl acetate) to obtain DP-EG3-biotin as white crystals (0.45 g, 82%). ^{1}H NMR ($CDCl_3$) δ 6.76 (br d, biotin N*H*, 1H), 6.62 (br d, biotin N*H*, 1H), 5.59 (br m, C(O)N*H*, 1H), 4.51 (br m, biotin NHC*H*, 1H), 4.32 (br m, biotin NHC*H*, 1H), 3.64 (m,

CH_2O, 8H), 3.57 (m, CH_2O & CHO, 5H), 3.51 (m, CH_2O, 2H), 3.43 (m, CH_2O & CH_2N, 6H), 3.15 (m, SCH, 1H), 2.90 (m, SCH_2, 1H), 2.75 (d, J = 13 Hz, SCH_2, 1H), 2.24 (dt, J_t = 7.4 Hz, J_d = 2.4 Hz, C(O)CH_2, 2H), 1.75 (m, SCHCH_2, 1H), 1.70 (m, SCHCH_2 & C(O)CH$_2$CH_2, 3H), 1.55 (m, OCH$_2$CH_2, 4H), 1.45 (m, C(O)CH$_2$CH$_2$CH_2, 2H), 1.26 (m, CH_2, 52H), 0.88 (t, J = 7.1 Hz, CH_3, 5H). ^{13}C NMR ($CDCl_3$) δ 173.29, 164.00, 77.88, 71.70, 71.43, 70.86, 70.67, 70.63, 70.43, 70.38, 70.16, 69.99, 61.71, 60.20, 55.60, 40.55, 39.16, 35.97, 31.94, 30.12, 29.73, 29.68, 29.54, 29.38, 28.10, 26.15, 26.11, 25.61, 22.71, 14.15. IR (KBr) 3301.3, 2918.2, 2850.8, 1711.1, 1646.6, 1551.5, 1465.9, 1244.9, 1129.2, 722.0 cm^{-1}. Anal. Calcd. For $C_{51}H_{99}N_3O_7S$: C, 68.18; H, 11.11; N, 4.68; S, 3.57. Found C, 68.45; H, 11.02; N, 4.75; S, 3.45.

15-[2,3-Bis(hexadecyloxy)propyl]-3,6,9,12,15-pentaoxapentadecyl-1-biotinylamide (DP-EG5-biotin). Product was isolated by flash column chromatography on silica gel (R_f = 0.31, 20% methanol/ethyl acetate) as a white crystalline powder. ^{1}H NMR ($CDCl_3$) δ 6.58 (br m, biotin NH, 1H), 6.02 (br s, biotin NH, 1H), 5.10 (br s, C(O)NH, 1H), 4.52 (br m, biotin NHCH, 1H), 4.34 (br m, biotin NHCH, 1H), 3.65 (m, CH_2O, 16H), 3.57 (m, CH_2O & CHO, 6H), 3.51 (m, CH_2O, 2H), 3.44 (m, CH_2O & CH_2N, 5H), 3.16 (m, SCH, 1H), 2.92 (dd, J_{d1} = 10 Hz, J_{d2} = 13 Hz, SCH_2, 1H), 2.75 (d, J = 13 Hz, SCH_2, 1H), 2.24 (m, C(O)CH_2, 2H), 1.76 (m, SCHCH_2, 1H), 1.70 (m, SCHCH_2 & C(O)CH$_2$CH_2, 3H), 1.56 (m, OCH$_2$CH_2, 4H), 1.46 (m, C(O)CH$_2$CH$_2$CH_2, 2H), 1.26 (m, CH_2, 52H), 0.89 (t, J = 7.1 Hz, CH_3, 6H). ^{13}C NMR ($CDCl_3$) δ 173.15, 163.60, 77.88, 71.68, 71.43, 70.83, 70.80, 70.61, 70.57, 70.55, 70.51, 70.45, 70.39, 70.12, 69.95, 61.74, 60.15, 55.43, 40.53, 39.15, 35.89, 31.92, 30.11, 29.70, 29.66, 29.52, 29.35, 28.10, 26.14, 26.10, 25.52, 22.68, 14.11. IR (KBr) 3292.9, 2918.2, 2851.0, 1709.6, 1645.9, 1554.1,

1466.9, 1353.4, 1245.7, 1125.9, 721.9 cm^{-1}. Anal. Calcd. For $C_{55}H_{107}N_3O_9S \bullet ½ H_2O$: C, 66.36; H, 10.93; N, 4.22. Found: C, 66.31; H, 10.93; N, 4.31.

30-[2,3-Bis(hexadecyloxy)propyl]-3,6,9,12,15,18,21,24,27,30-decaoxatriacontyl-1-biotinylamide (DP-EG10-biotin). Product was isolated by flash column chromatography on silica gel (R_f = 0.26, 2% methanol/chloroform) as a white crystalline powder. ^1H NMR ($CDCl_3$) δ 6.65 (br m, biotin N*H*, 1H), 6.16 (br s, biotin N*H*, 1H), 5.122 (br s, C(O)N*H*, 1H), 4.51 (t, J = 6.0 Hz, biotin NHC*H*, 1H), 4.33 (t, J = 5.6 Hz, biotin NHC*H*, 1H), 3.68 – 3.61 (m, CH_2O, 35H), 3.59 – 3.48 (m, CH_2O & C*H*O, 9H), 3.47 – 3.40 (m, CH_2O, 5H), 3.16 (m, SC*H*, 1H), 2.92 (dd, J_{d1} = 4.9 Hz, J_{d2} = 12.7 Hz, SCH_2, 1H), 2.75 (d, J = 12.7 Hz, SCH_2, 1H), 2.24 (m, C(O)CH_2, 2H), 1.80 – 1.62 (m, SCHCH_2 & C(O)CH_2CH_2, 4H), 1.55 (m, OCH_2CH_2, 4H), 1.46 (m, C(O)CH_2CH_2CH_2, 2H), 1.26 (m, CH_2, 52H), 0.89 (t, J = 6.9 Hz, CH_3, 6H). ^{13}C NMR ($CDCl_3$) δ 173.14, 163.40, 77.87, 71.66, 71.41, 70.84, 70.80, 70.61, 70.59, 70.56, 70.53, 70.47, 70.42, 70.12, 69.93, 61.74, 60.10, 55.37, 40.53, 39.15, 35.84, 31.92, 30.11, 29.70, 29.68, 29.66, 29.52, 29.36, 28.09, 26.14, 26.10, 25.51, 22.69, 14.12. IR (KBr) 3292.3, 2918.1, 2851.1, 1708.7, 1645.4, 1554.7, 1467.3, 1246.8, 1117.7 cm^{-1}. Anal. Calcd. For $C_{65}H_{127}N_3O_{14}S$: C, 64.69; H, 10.61; N, 3.48; S, 2.66. Found: C, 64.93; H, 10.75; N, 3.67; S, 2.36.

45-[2,3-Bis(hexadecyloxy)propyl]-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45-pentadecaoxapentatetracontyl-1-biotinylamide (DP-EG15-biotin). Product was isolated by flash column chromatography (R_f = 0.29, 10% methanol/chloroform) as a white crystalline powder. ^1H NMR ($CDCl_3$) δ 6.50 (br m, biotin N*H*, 1H), 6.10 – 5.60 (br m, biotin N*H*, 1H), 5.25 – 4.85 (br m, C(O)N*H*, 1H), 4.51 (br m, biotin NHC*H*, 1H), 4.32 (br m, biotin NHC*H*, 1H), 3.68 – 3.61 (m, CH_2O, 55H), 3.60 – 3.48 (m, CH_2O & C*H*O, 9H),

3.47 – 3.40 (m, CH_2O & CH_2N, 5H), 3.16 (m, SCH, 1H), 2.92 (m, SCH_2, 1H), 2.75 (d, J = 12.7 Hz, SCH_2, 1H), 2.23 (m, C(O)CH_2, 2H), 1.80 – 1.62 (m, SCHCH_2 & C(O)CH$_2$CH_2, 5H), 1.55 (m, OCH$_2$CH_2, 4H), 1.46 (m, C(O)CH$_2$CH$_2$CH_2, 2H), 1.26 (m, CH_2, 52H), 0.89 (t, J = 6.9 Hz, CH_3, 6H). ^{13}C NMR ($CDCl_3$) δ 173.15, 163.39, 77.86, 71.66, 71.40, 70.84, 70.80, 70.74, 70.61, 70.59, 70.56, 70.46, 70.42, 70.11, 69.94, 61.73, 60.10, 55.37, 40.53, 39.15, 35.82, 31.92, 30.11, 29.71, 29.66, 29.52, 29.36, 28.09, 26.14, 26.09, 25.51, 22.68, 14.12. IR (KBr) 3294.1, 2918.2, 2851.4, 1705.3, 1644.8, 1553.6, 1466.9, 1350.8, 1248.8, 1114.5, 951.1 cm^{-1}. Anal. Calcd. For $C_{75}H_{147}N_3O_{19}S$: C, 63.12; H, 10.38; N, 2.94; S, 2.25. Found: C, 63.53; H, 10.94; N, 3.08; S, 1.97.

Estimation of the Magnitude of FRET Contribution to Streptavidin Intensity

Fluorescence resonance energy transfer (FRET) is a possibility from the FITC-labeled streptavidin to BODIPY 530/550 HPC in the membrane. This FRET process could alter the fluorescence efficiency of streptavidin in the L_d domains containing BODIPY versus that in the unlabeled L_o domains. The Förster radius for this fluorophore pair is not available, but using similar fluorophores (Atto Tec website) we estimated it to be ~60Å. The average FITC on streptavidin is estimated to be ~30Å from the membrane surface. Thus, lipids on the outer membrane leaflet within a 52Å radius are within the Förster radius of a FITC on streptavidin. This radius occupies an area of 85 nm^2 with approximately 100 DPhPC lipids. With a BODIPY 530/550 concentration of 0.3% only 0.3 of the FITCs on streptavidin have a BODIPY 530/550 within the Förster radius. Since the Förster radius corresponds to a quenching fraction of 0.5 we estimate less than 20% quenching of the FITC on streptavidin by BODIPY 530/550.

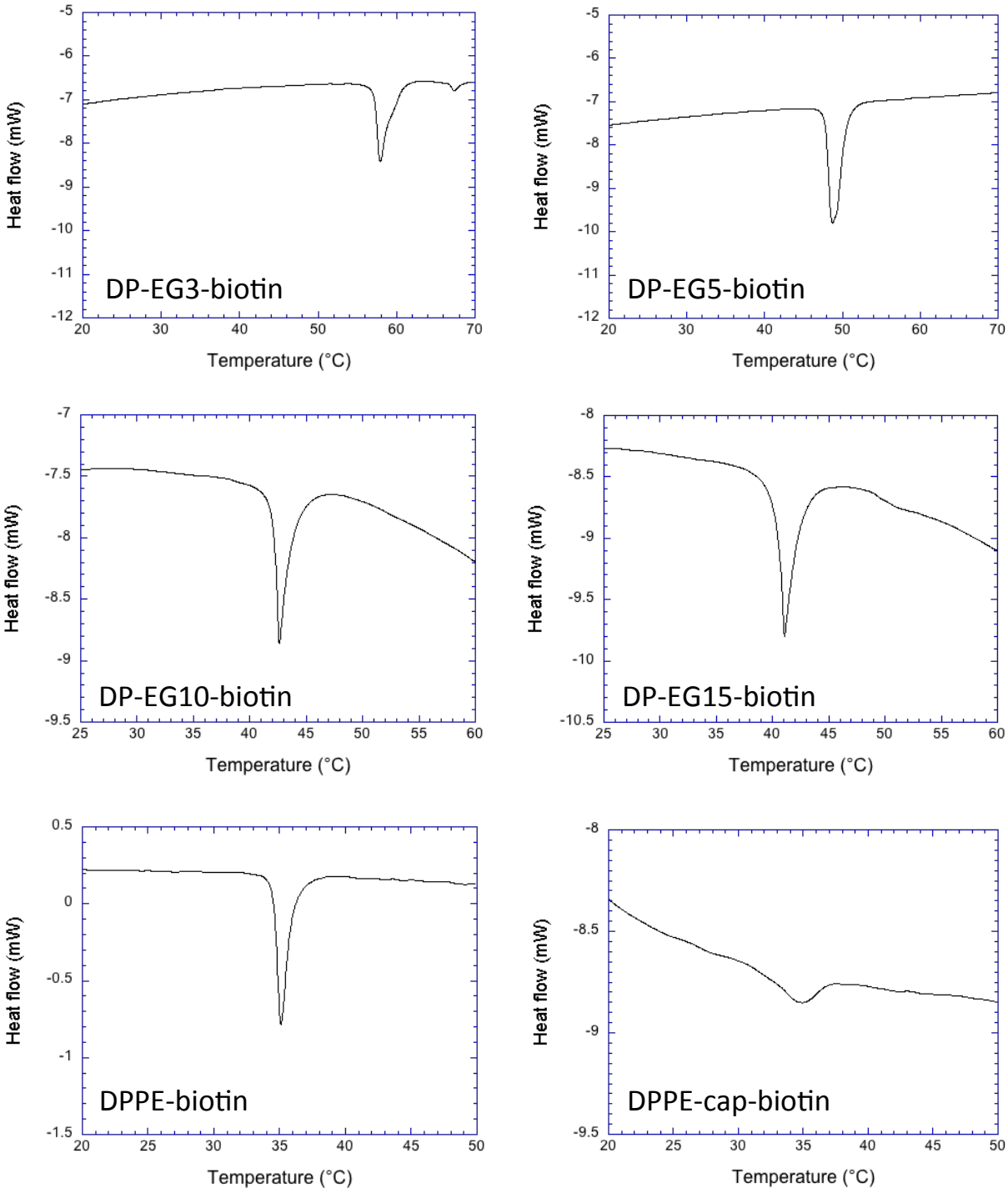


Figure S1. Differential scanning calorimetry measurements of biotinylated lipids.

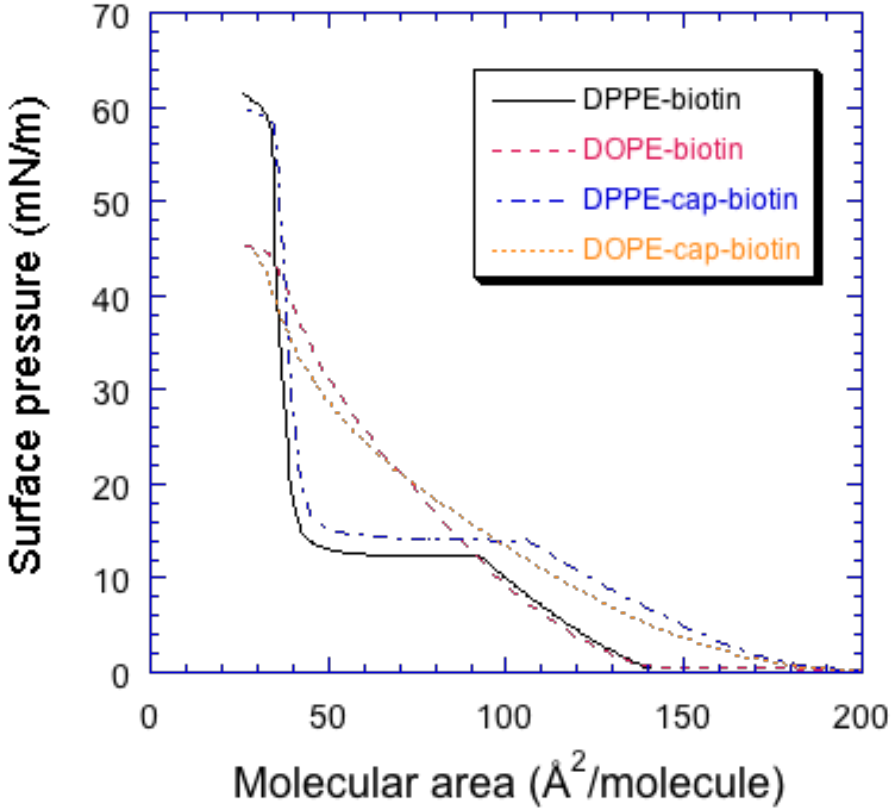


Figure S2. Pressure-area isotherms of biotinylated PE lipids measured at 20 °C on pure water subphase.

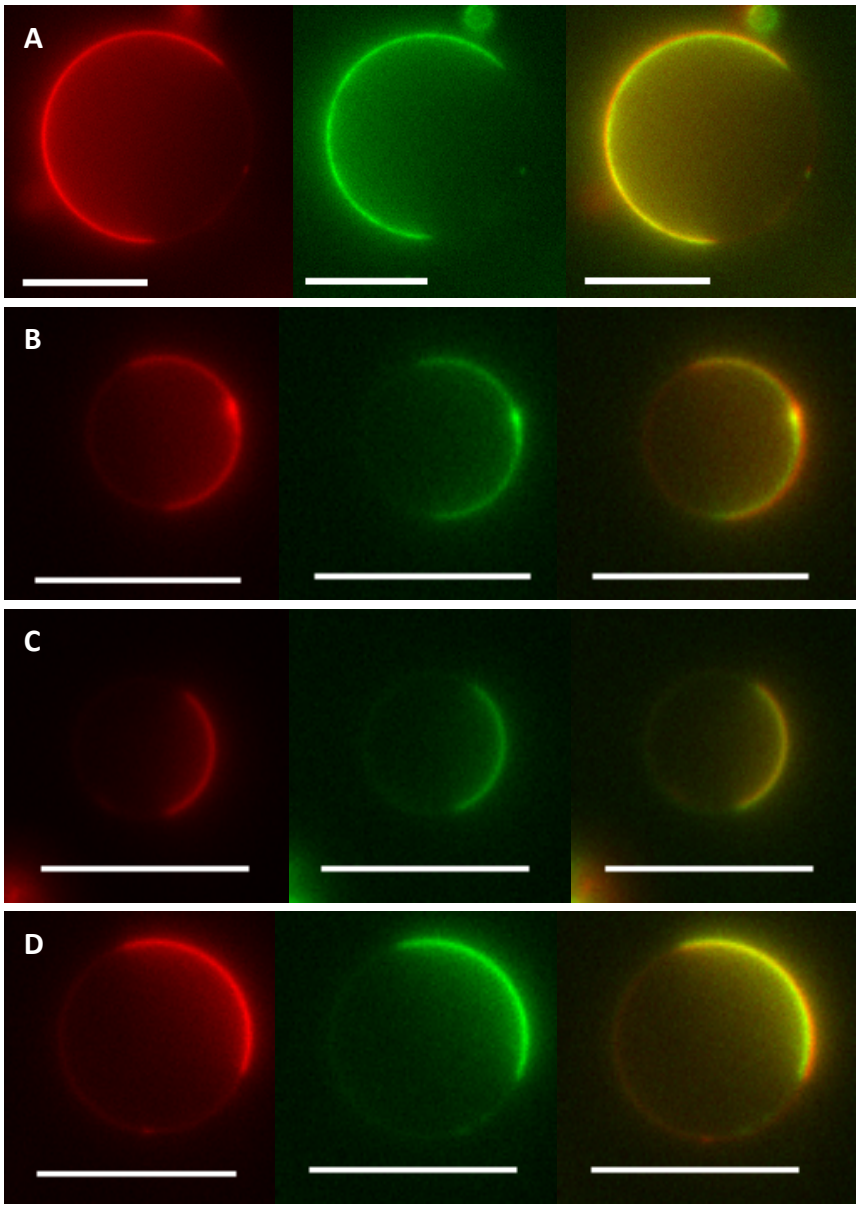


Figure S3. Fluorescence microscopic images of PE-biotin containing GUVs following exposure to 1 µM FITC-streptavidin in the (left) red channel showing the L_d phase, (middle) green channel showing where FITC-streptavidin is bound, and (right) the merged images. GUVs composed of DPhPC/DPPC/ cholesterol/PE-biotin at corresponding ratios of: (A) 38:34:24:4 DPPE-biotin, (B) 38:27:24:11 DPPE-biotin, (C) 18:40:40:2 DOPE-biotin, (D) 14:40:40:6 DOPE-biotin. All membranes contain 0.3% TRITC-DHPE. (Scale bars = 10 µm)

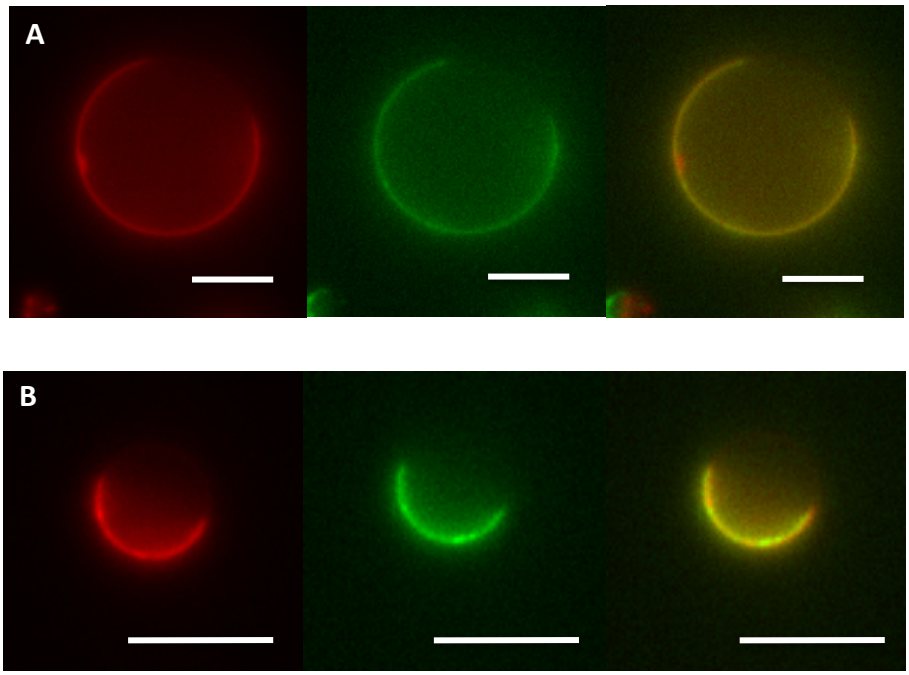


Figure S4. Fluorescence microscopic images of PE-cap-biotin containing GUVs following exposure to 1 µM FITC-streptavidin in the (left) red channel showing the L_d phase, (middle) green channel showing FITC-streptavidin, and (right) the merged images. GUVs composed of DPhPC/DPPC/cholesterol/PE-cap-biotin at corresponding ratios of: (A) 38:34:24:4 DPPE-cap-biotin, and (B) 18:40:40:2 DOPE-biotin. All membranes contain 0.3% TRITC-DHPE. (Scale bars = 10 µm)

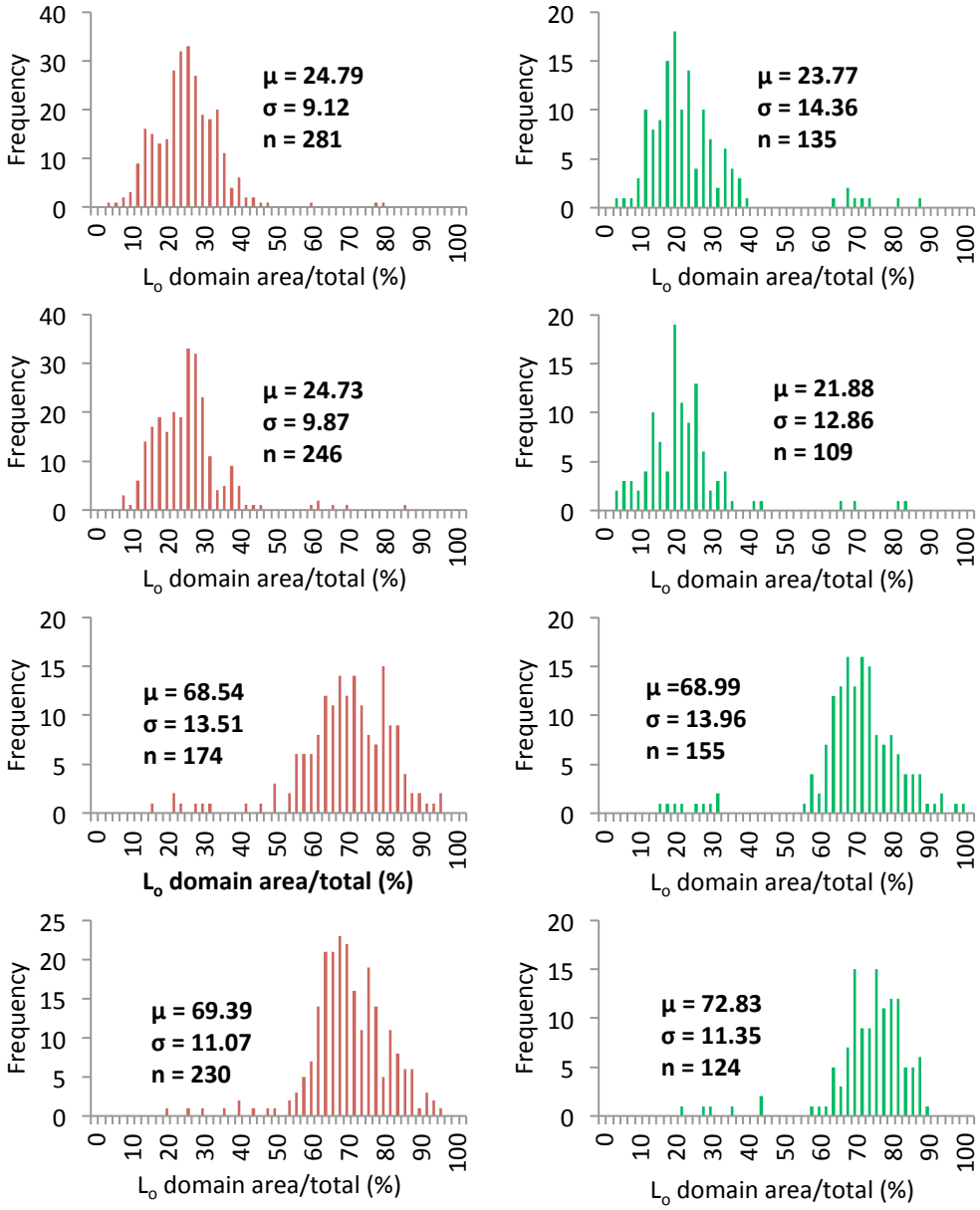


Figure S5. Histogram of L_o domain area over total GUV area for A through D in Figure S3 in the absence (left histograms, in red) and presence (right histograms, in green) of 1 μM FITC-streptavidin. Displayed on each histogram is the mean (μ), standard deviation (σ), and sample size (n)

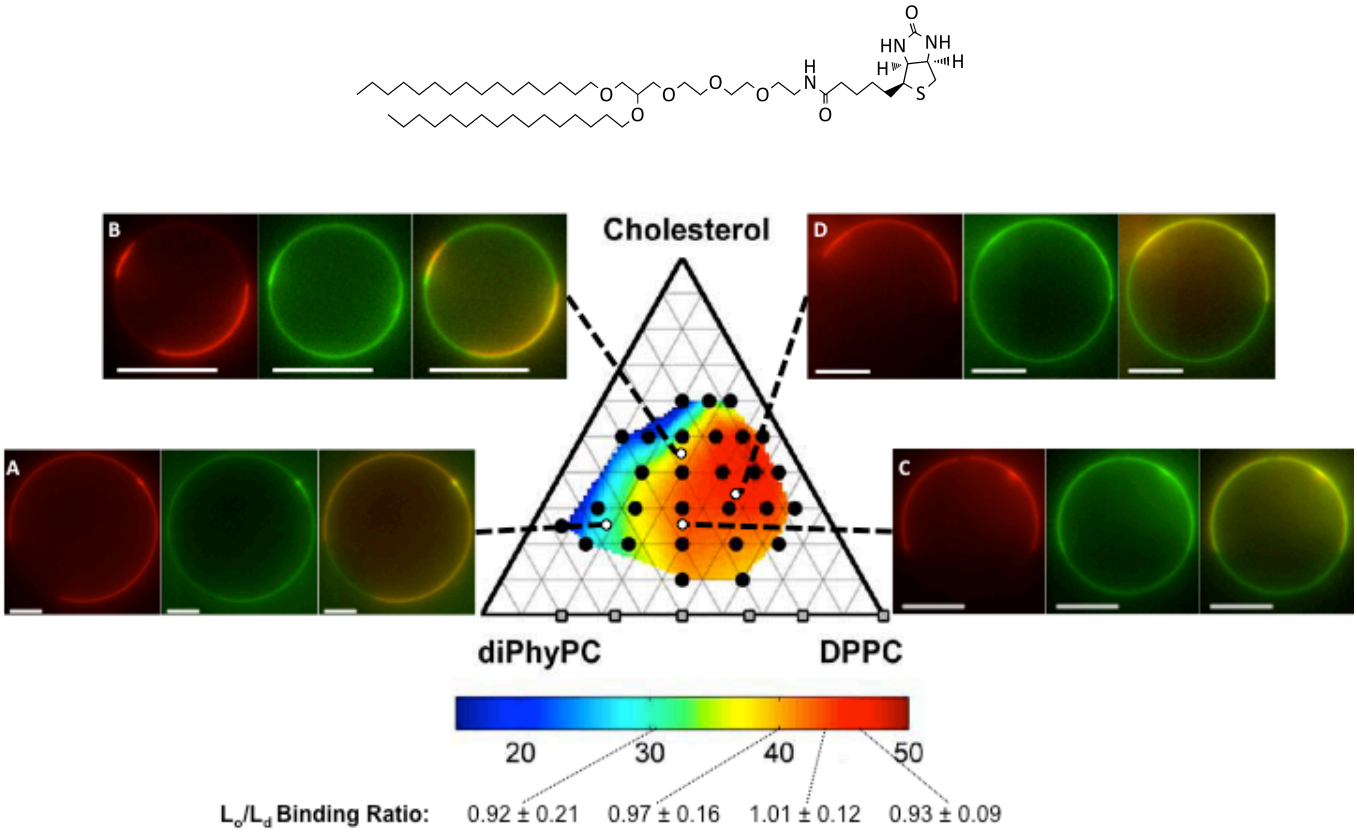


Figure S6. Fluorescence images of GUVs containing DP-EG3-biotin exposed to FITC-streptavidin (1 µM) at the different miscibility transition temperatures in the L_o/L_d coexistence region. GUVs composed of DPhPC/DPPC/cholesterol/DP-EG3-biotin at corresponding ratios of: (A) 56:17:25:2, (B) 28:25:44:3, (C) 38:34:24:4, D) 20:43:32:5. All membranes contain 0.3% BODIPY 530-550 HPC to label the L_d phase. A series of three images for each membrane composition are shown under the (left) red and (middle) green channels along with the (right) merged image. Phase diagram is from reference 42. (Scale bars = 10 µm)

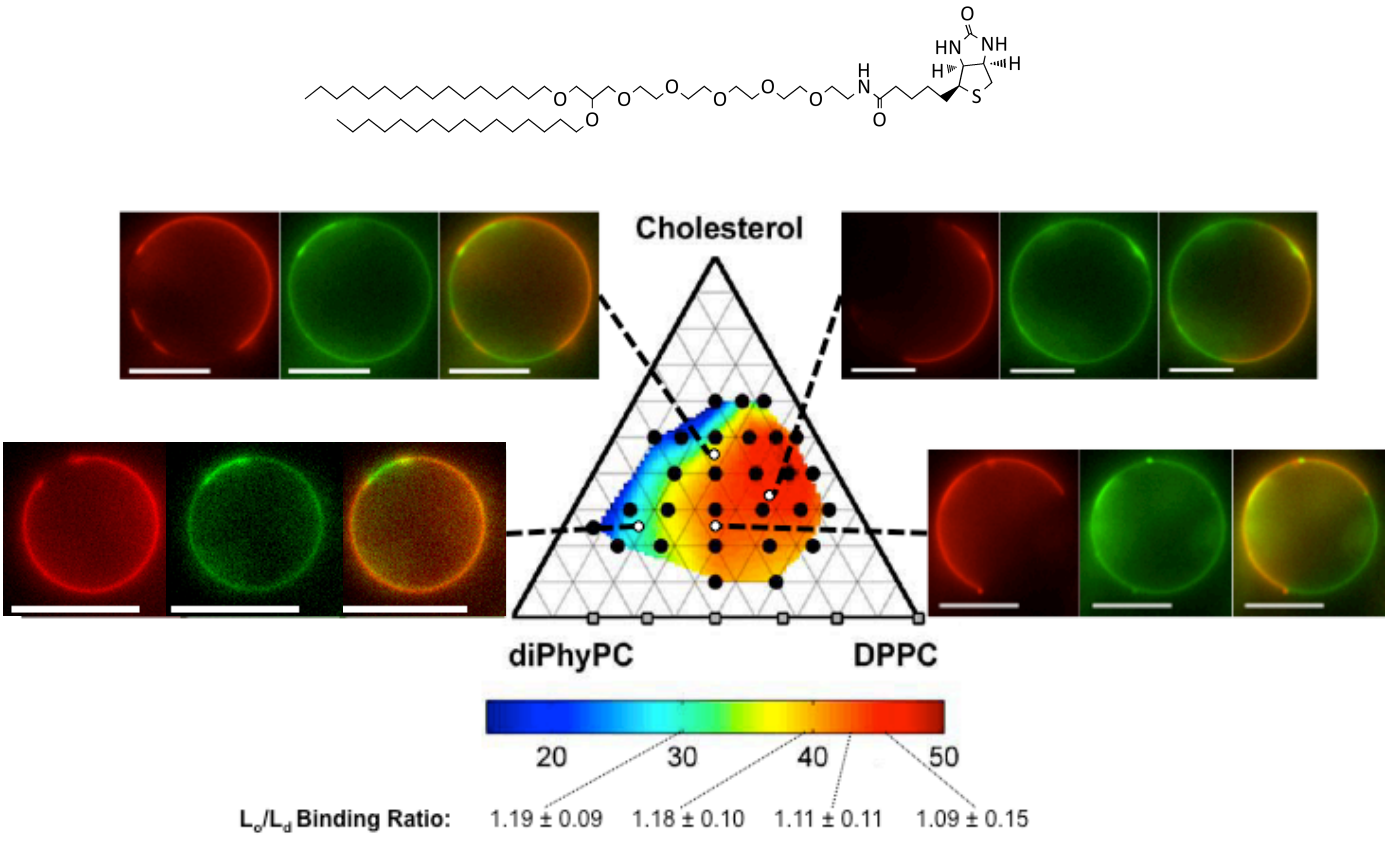


Figure S7. Fluorescence images of GUVs containing DP-EG5-biotin exposed to FITC-streptavidin (1 µM) at the different miscibility transition temperatures in the L_o/L_d coexistence region. GUVs composed of DPhPC/DPPC/cholesterol/DP-EG5-biotin at corresponding ratios of: (A) 56:17:25:2, (B) 28:25:44:3, (C) 38:34:24:4, D) 20:43:32:5. All membranes contain 0.3% BODIPY 530-550 HPC to label the L_d phase. A series of three images for each membrane composition are shown under the (left) red and (middle) green channels along with the (right) merged image. Phase diagram is from reference 42. (Scale bars = 10 µm)

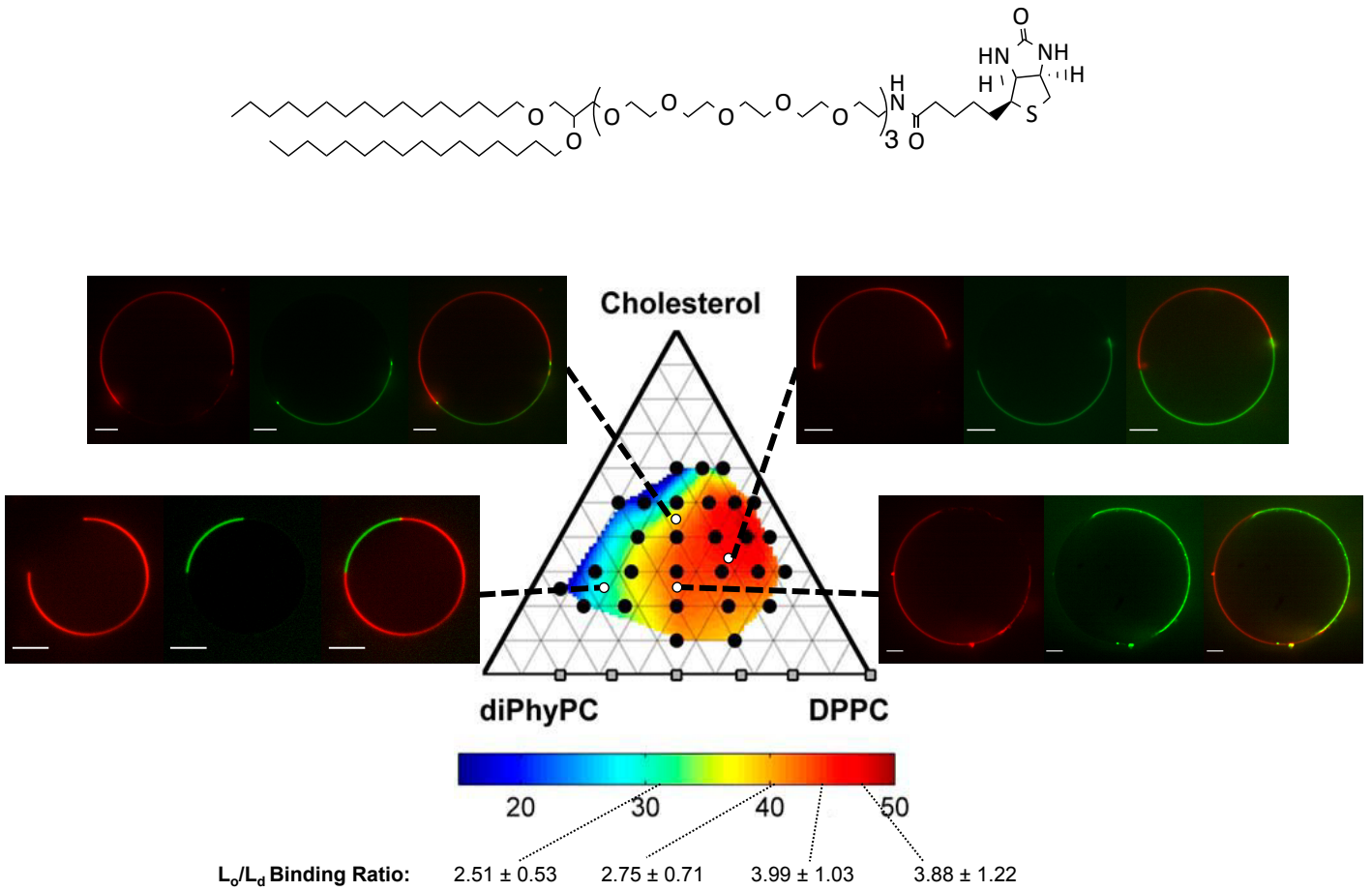


Figure S8. Fluorescence images of GUVs containing DP-EG15-biotin exposed to FITC-streptavidin (1 µM) at the different miscibility transition temperatures in the L_o/L_d coexistence region. GUVs composed of DPhPC/DPPC/cholesterol/DP-EG15-biotin at corresponding ratios of: (A) 56:17:25:2, (B) 28:25:44:3, (C) 38:34:24:4, D) 20:43:32:5. All membranes contain 0.3% BODIPY 530-550 HPC to label the L_d phase. A series of three images for each membrane composition are shown under the (left) red and (middle) green channels along with the (right) merged image. Phase diagram is from reference 42. (Scale bars = 10 µm)

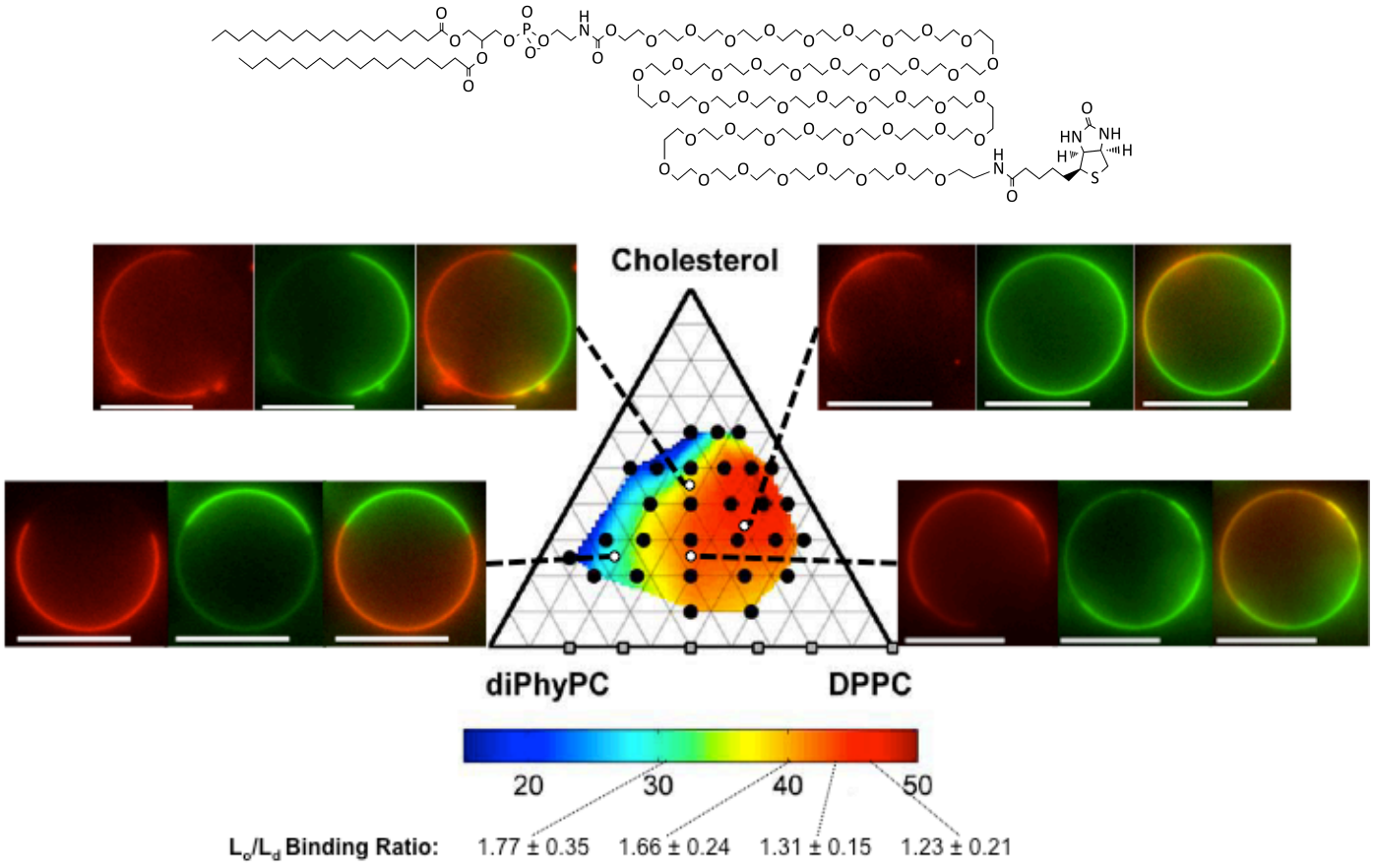


Figure S9. Fluorescence images of GUVs containing DSPE-PEG2000-biotin exposed to FITC-streptavidin (1 µM) at the different miscibility transition temperatures in the L_o/L_d coexistence region. GUVs composed of DPhPC/DPPC/cholesterol/DSPE-PEG2000-biotin at corresponding ratios of: (A) 56:17:25:2, (B) 28:25:44:3, (C) 38:34:24:4, D) 20:43:32:5. All membranes contain 0.3% BODIPY 530-550 HPC to label the L_d phase. A series of three images for each membrane composition are shown under the (left) red and (middle) green channels along with the (right) merged image. Phase diagram is from reference 42. (Scale bars = 10 µm)

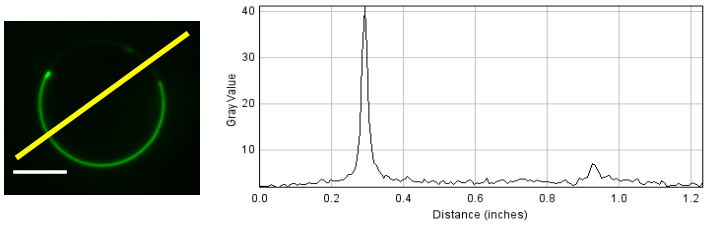


Figure S10. (Right) Intensity profile plot of line drawn through image on left of 20% DPhPC/43% DPPC/ 32% cholesterol/5% DP-EG10-biotin with 0.3% BODIPY 530-550 HPC (membrane composition D, from Figure 3D) in the green channel. Pixel height of 41.1 in L_o phase and 7.1 in L_d phase (bleed through corrected) yielding K_d = 5.8 for this image. Scale bar is 10 µm.

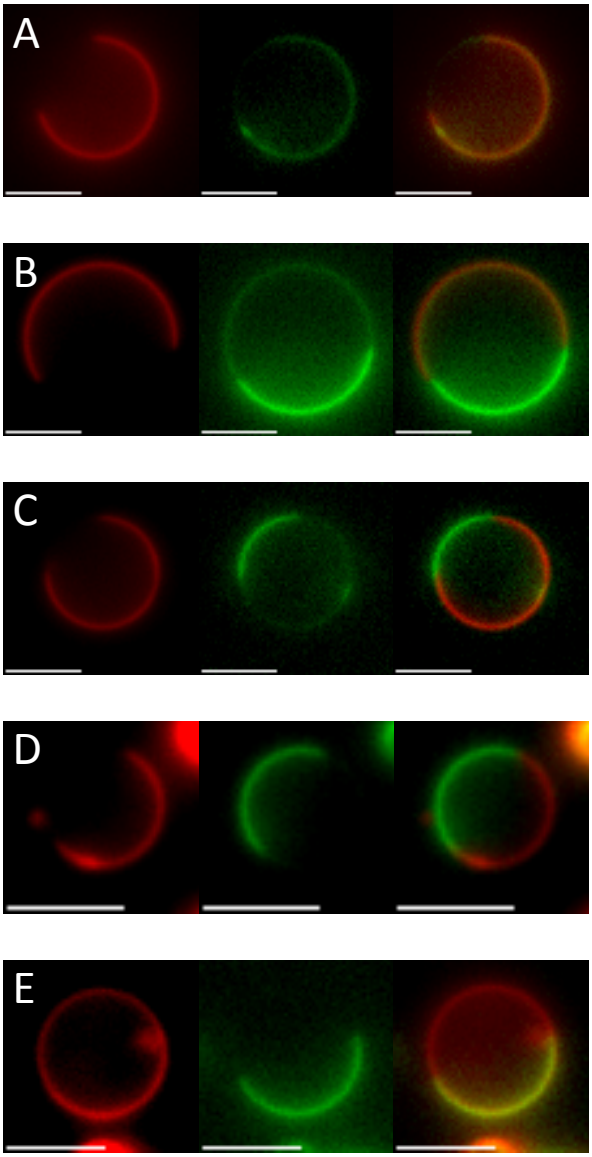


Figure S11. Fluorescence images of GUVs composed of 34% DPPC/38% DPhPC /24% cholesterol/0.2% BODIPY 530-550 HPC and 0.8% of A) DP-EG3-biotin, B) DP-EG5-biotin, C) DP-EG10-biotin, D) DP-EG15-biotin, and E) DSPE-PEG2000-biotin exposed to FITC-streptavidin (1 µM). (Scale bars = 5 µm)