Supporting Information

Multiplexed Lipid Bilayers on Silica Microspheres for Analytical Screening Applications

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Supporting Figure S1: Time stability of individual and mulitplex sets. The stability of the multiplexed sample over time is shown by comparison of individual and multiplexed samples.

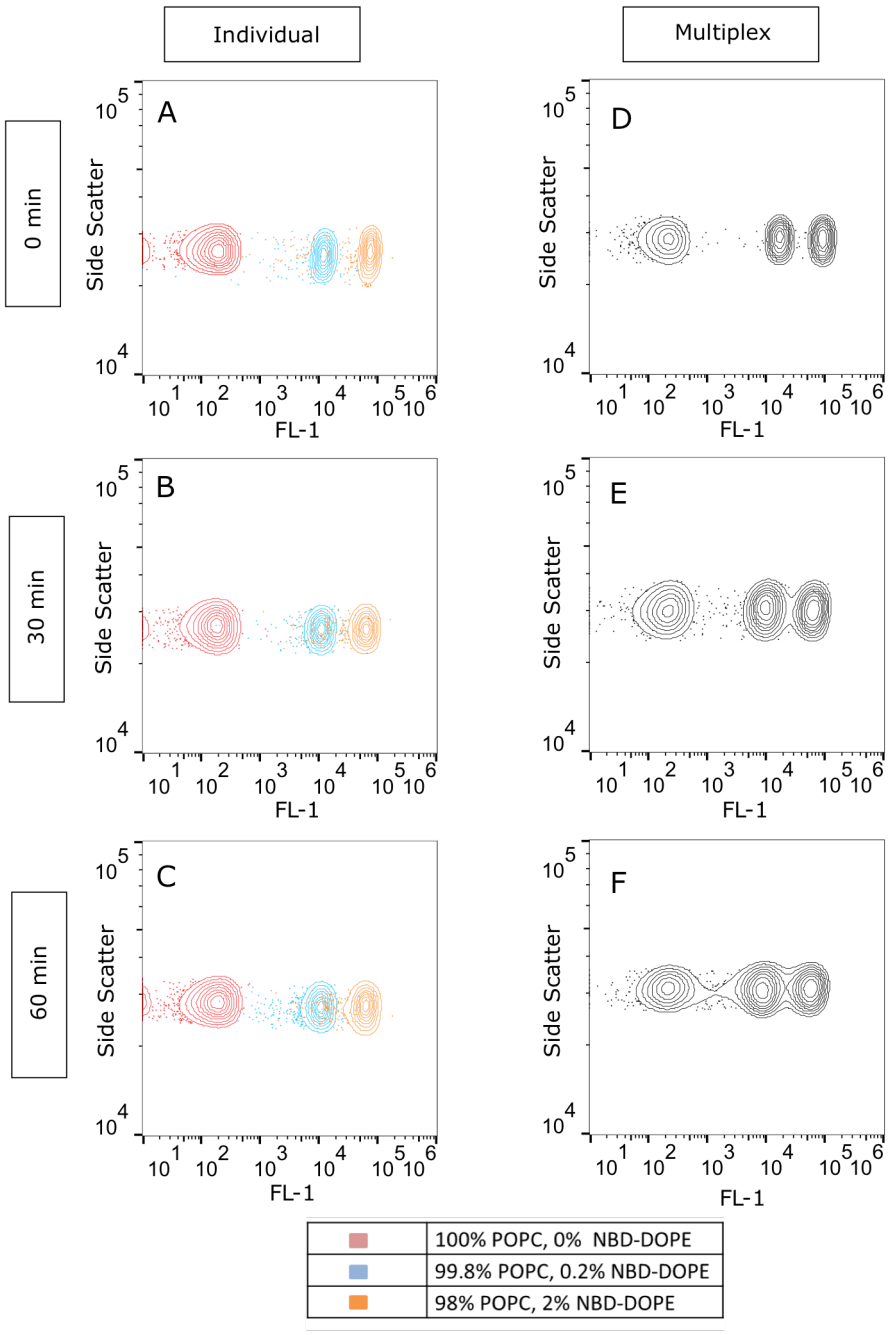


Figure S1. Side Scatter vs FL-1 channel (Laser 488, Filter 533/30nm band pass) of individual and multiplexed samples containing: 0% NBD-DOPE, 0.2% NBD-DOPE and 2% NBD-DPE. **A)** Individual samples at time 0. Here, the results of three individual samples are overlaid in a single bivariate plot, color coded according to the legend given on the right side of the figure. **B)** Individual samples after 30 minutes. **C**) Individual samples after 1 hour. **D)** Multiplex at time 0. **E)** Multiplex after 30 minutes. **F)** Multiplex after 1 hour.

Supporting Figure S2: Cholera toxin B subunit-Alexa 647 does not have specific interactions with the NBD-DOPE multiplex label. Bivariate plots of Cholera toxin B subunit B and NBD-DOPE indicate that no concentration-dependent interaction occurs between CTxB and the labeled lipid used for multiplexing.

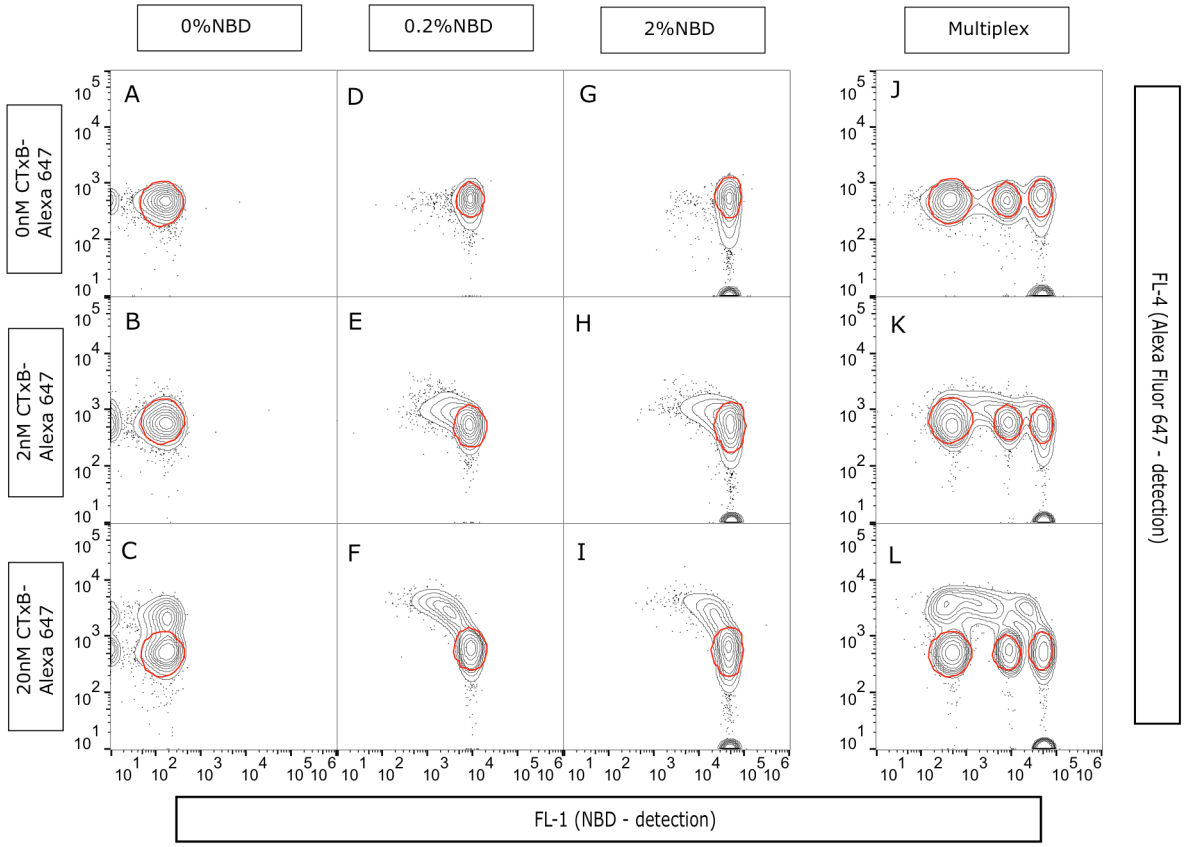
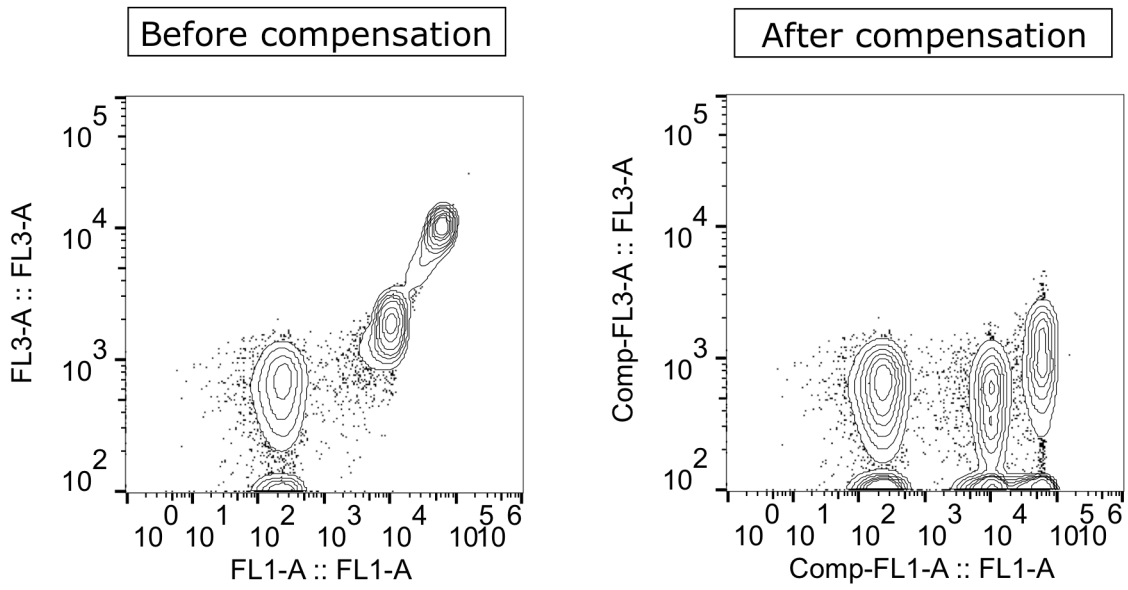


Figure S2. Bivariate contour plots (FL-4 vs. FL-1). Individual samples: 0 mol% NBD-DOPE: **A)** Plus 0 nM CTxB-Alexa 647. **B)** Plus 2 nM CTxB-Alexa 647. **C)** Plus 20 nM CTxB-Alexa 647. 0.2 mol% NBD-DOPE: **D)** Plus 0 nM CTxB-Alexa 647. **E)** Plus 2 nM CTxB-Alexa 647. **F)** Plus 20 nM CTxB-Alexa 647. 2 mol% NBD-DOPE: **G)** Plus 0 nM CTxB-Alexa 647. **H)** Plus 2 nM CTxB-Alexa 647. **I)** Plus 20 nM CTxB-Alexa 647. Multiplexed samples: **J)** Plus 0 nM CTxB-Alexa 647.**K)** Plus 2 nM CTxB-Alexa 647. **L)** Plus 20 nM CTxB-Alexa 647.

Supporting Figure S3: Compensation details for biotin-streptavidin assays. Details of the compensation matrix applied to the biotin-streptavidin assay are presented.



	FL1-A::FL1-A	FL2-A::FL2-A	FL3-A::FL3-A	FL4-A::FL4-A
FL1-A::FL1-A	100	0	15.8	0
FL2-A::FL2-A	0	100	0	0
FL3-A::FL3-A	0	0	100	0
FL4-A::FL4-A	0	0	0	100

Figure S3. Bivariate plots before and after compensation and compensation matrix used in the biotin-streptavidin assay. The left panel is a bivariate plot of FL-3 versus FL-1 signal intensity, and the right panel is the same data following application of compensation.

Supporting Figure S4: Interaction of Biotin-DOPE and Streptavidin-PE/Cy5 in the individual sample and multiplex approaches. Bivariate plots of streptavidin-biotin assay are shown for individual and multiplex samples.

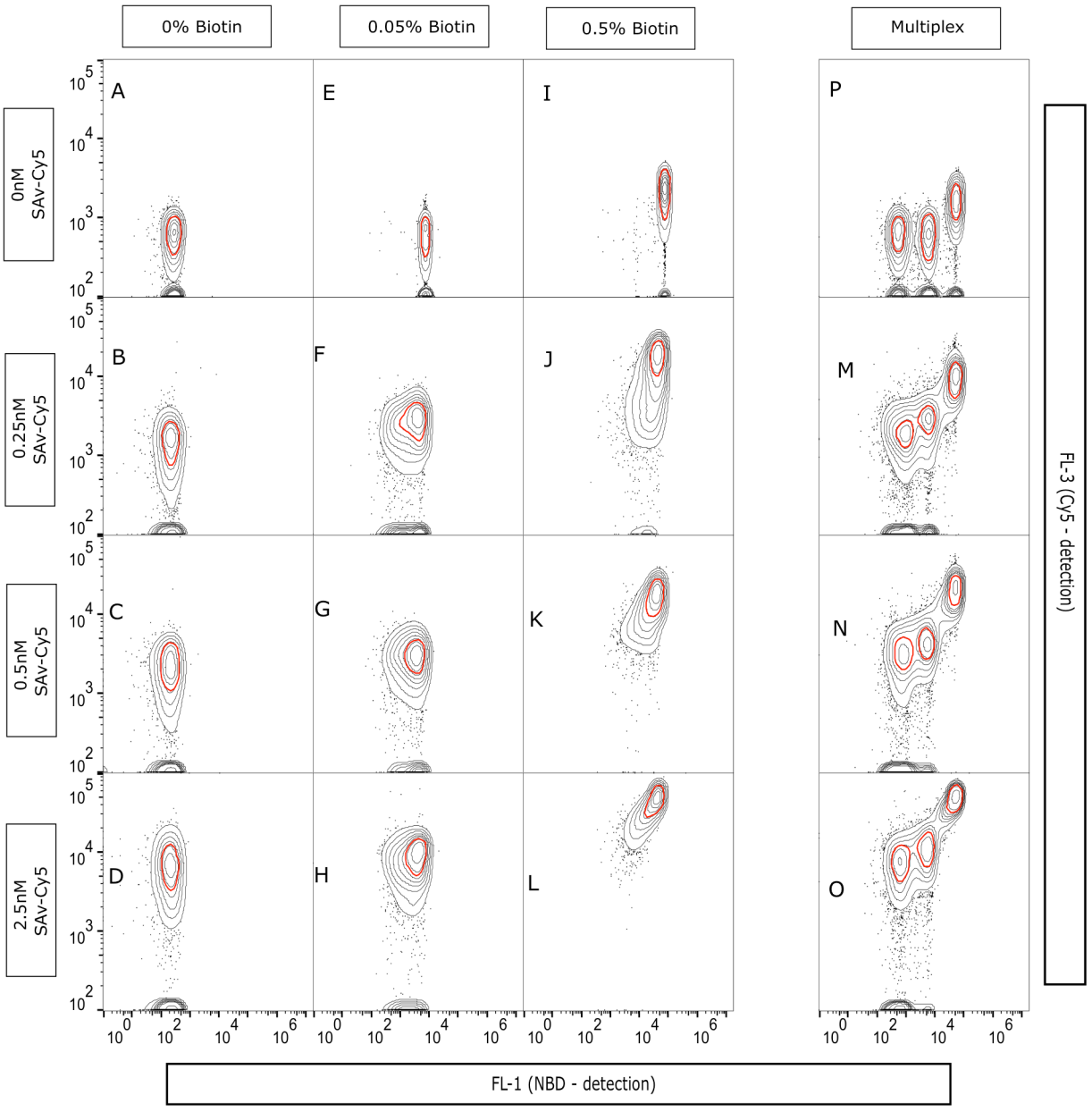


Figure S4. Bivariate contour plots (compensated FL-3 vs. FL-1). Individual samples: 0 mol% Biotin: **A)** Plus 0 nM SAv-Cy5. **B)** Plus 0.05 nM SAv-Cy5. **C)** Plus 0.25 nM SAv-Cy5. **D)** Plus 0.5 nM SAv-Cy5. **E)** Plus 2.5 nM SAv-Cy5. 0.05 mol% Biotin: **F)** Plus 0 nM SAv-Cy5. **G)** Plus 0.05 nM SAv-Cy5. **H)** Plus 0.25 nM SAv-Cy5. **I)** Plus 0.5 nM SAv-Cy5. **J)** Plus 2.5 nM SAv-Cy5. 0.5 mol% Biotin: **K)** Plus 0 nM SAv-Cy5. **L)** Plus 0.05 nM SAv-Cy5. **M)** Plus 0.25 nM SAv-Cy5. **N)** Plus 0.5 nM SAv-Cy5. **O)** Plus 2.5 nM SAv-Cy5. Multiplexed samples: **P)** Plus 0 nM SAv-Cy5. Q) Plus 0.05 nM SAv-Cy5. **R)** Plus 0.25 nM SAv-Cy5. **S)** Plus 0.5 nM SAv-Cy5. **T)** Plus 2.5 nM SAv-Cy5.

Supporting Figure S5: Streptavidin-PE/Cy5 does not have specific interactions with the NBD-DOPE multiplex label. Bivariate plots of Streptavidin-PE/Cy5 and NBD-DOPE indicate that no concentration-dependent interaction occurs between streptavidin and the labeled lipid used for multiplexing.

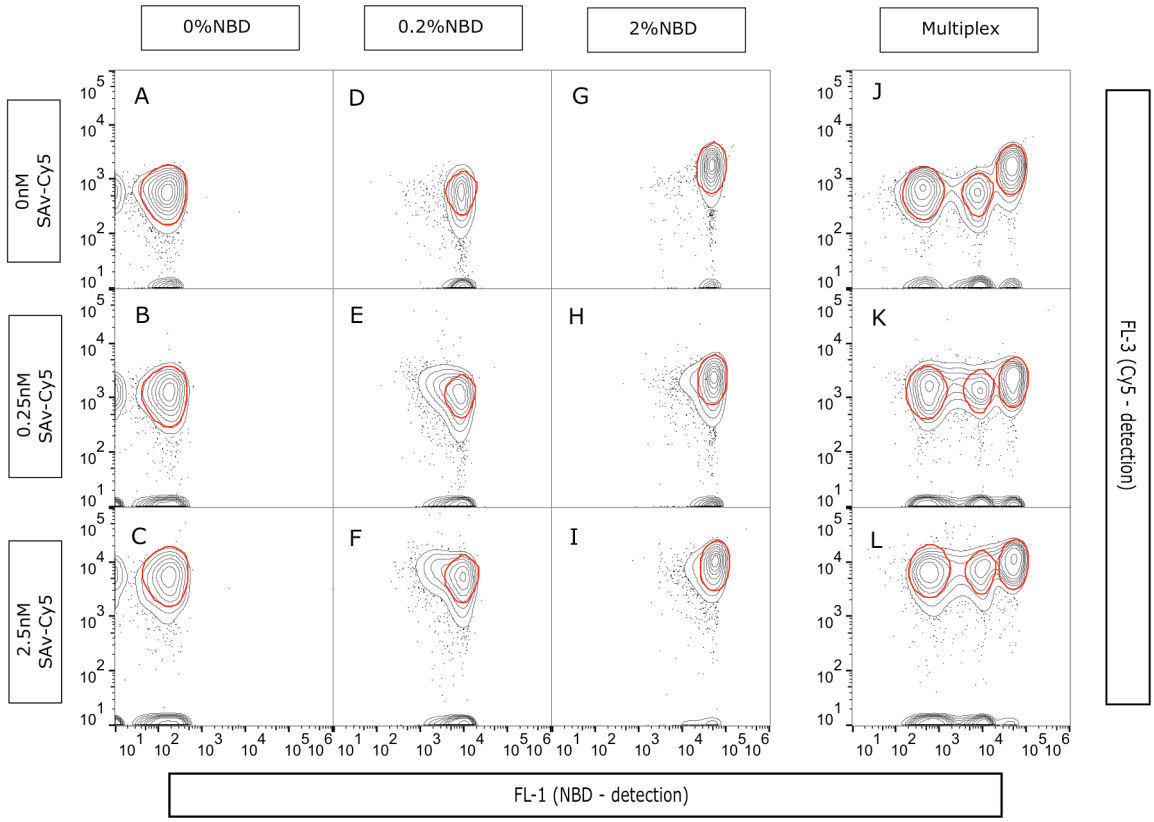
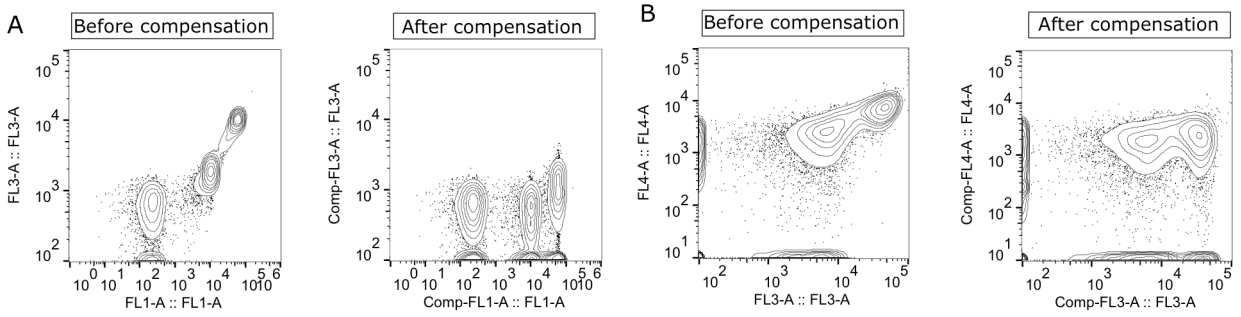


Figure S5. Bivariate contour plots (FL-3 vs FL-1). Individual samples: 0 mol% NBD-DOPE: **A)** Plus 0 nM SAv-Cy5. **B)** Plus 0.25 nM 0nM SAv-Cy5. **C)** Plus 2.5 nM 0nM SAv-Cy5. 0.2 mol% NBD-DOPE: **D)** Plus 0 nM SAv-Cy5. **E)** Plus 0.25 nM SAv-Cy5. **F)** Plus 2.5 nM SAv-Cy5. 2 mol% NBD-DOPE: **G)** Plus 0 nM SAv-Cy5. **H)** Plus 0.25 nM SAv-Cy5. **I)** Plus 2.5 nM SAv-Cy5. Multiplexed samples: **J)** Plus 0 nM SAv-Cy5.**K)** Plus 0.25 nM SAv-Cy5. **L)** Plus 2.5 nM SAv-Cy5.

Supporting Figure S6: Compensation details for cross reactivity assays. Details of the compensation matrix as applied in the cross-reactivity assay are presented.



C

	FL1-A::FL1-A	FL2-A::FL2-A	FL3-A::FL3-A	FL4-A::FL4-A
FL1-A::FL1-A	100	0	16	0
FL2-A::FL2-A	0	100	0	0
FL3-A::FL3-A	0	0	100	15
FL4-A::FL4-A	0	0	0	100

Figure S6. Bivariate plots before and after compensation and compensation matrix used in the cross-reactivity assay seen in**: A)** FL-3 versus FL-1 of the multiplex system with no added protein, before (left) and after (right) compensation. **B)** FL-4 versus FL-3 of the multiplex system plus SAv-Cy5, before (left) and after (right) compensation. **C)** Compensation matrix.

Supporting Figure S7: Cross-reactivity median fluorescence intensities – Median fluorescence intensities in FL-4 and FL-3 in cross-reactivity assay are presented.

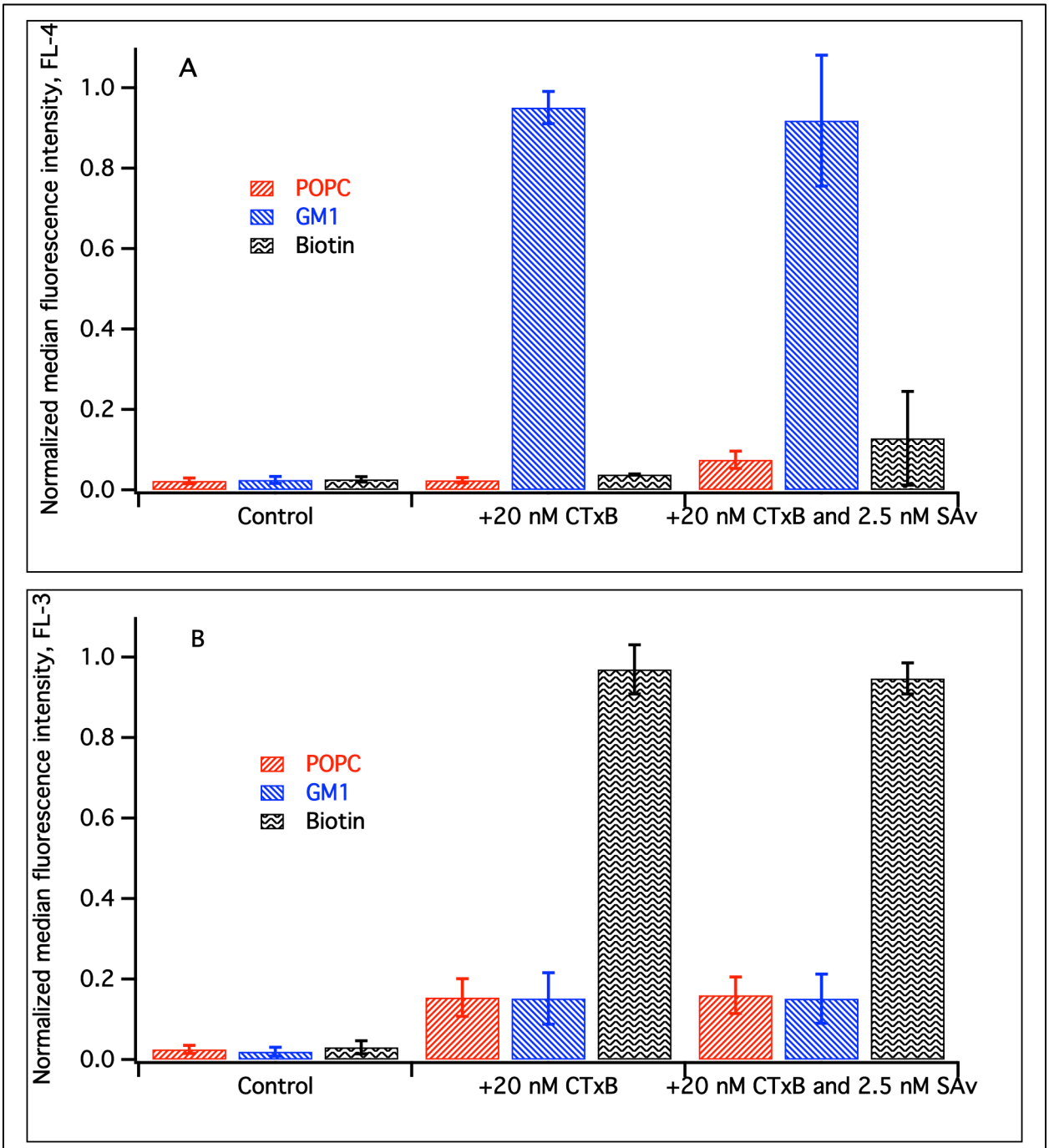


Figure S7. Median fluorescence Intensity in a fluorescently indexed multiplex system with lipids containing: 100% POPC (blue bars); 99.3% POPC, 0.5% GM1, 0.2% NBD-DOPE (orange bars); 97.5% POPC, 0.5% Biotin-DOPE, 2% NBD-DOPE. **A)** FL-4 channel (Laser 640nm, Filter 675/25) after 10-minute incubation with: 20 nM CTxB-Alexa 647, 2.5 nM SAv-Cy5, and 20 nM CTxB-Alexa 647 and 2.5 nM SAv-Cy5. **B)** FL-3 channel (Laser 488nm, Filter 670 LP) after 10-minute incubation with: 20 nM CTxB-Alexa 647, 2.5 nM SAv-Cy5, and 20 nM CTxB-Alexa 647 and 2.5 nM SAv-Cy5. These data are the average of four replica experiments, each normalized to the highest intensity observed in the fluorescence channel for that replicate, and where the data of each replicate correspond to gated populations similar to those of Figure 5 of the main text.