SUPPLEMENTAL FIGURES for

Structured clustering of the glycosphingolipid GM1 is required for membrane curvature induced by cholera toxin

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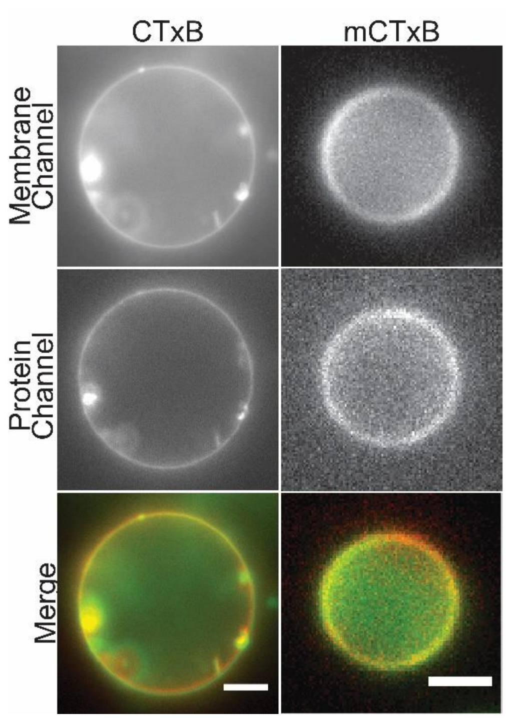

Figure S1: Binding of CTxB, but not mCTxB, to GUVs induces the formation of tubular invaginations. GUVs composed of POPC and 0.3 mol% GM1 were labeled with 0.3 mol% DiI or DiD and incubated for 20 min in the presence of either 1.7nM CTxB or 1.7nM mCTxB prior to imaging with wide-field fluorescence microscopy. Scale bars, 5 μm.

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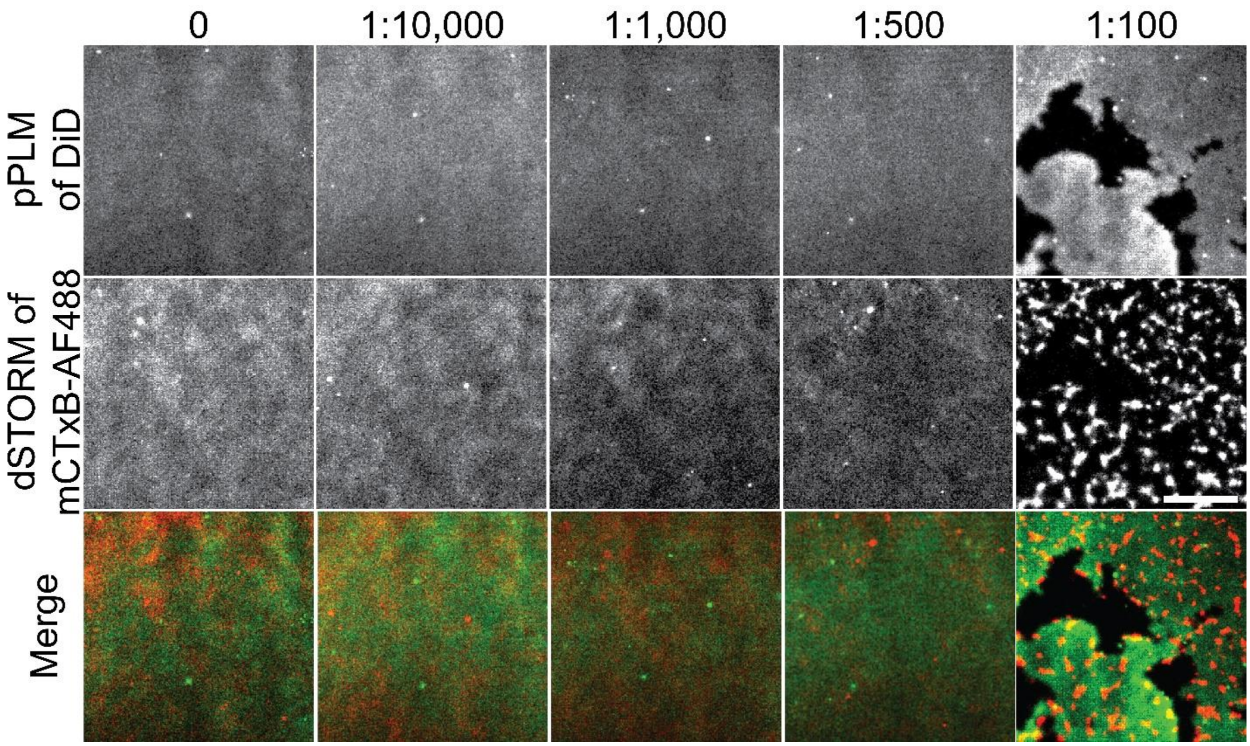

Figure S2: Antibody-induced crosslinking of mCTxB fails to induce membrane budding. Planar lipid bilayers containing 0.3 mol% GM1 were labeled with 8.6 nM mCTxB followed by either buffer alone or buffer containing the indicated dilutions of anti-CTxB antibody. They were subsequently imaged by pPLM (A-E) and dSTORM (D-F). Note that the clustering of the mCTxB was readily observed at an anti-CTxB dilution at 1:100 (J). Despite this, no membrane bending was detected under these conditions, although the edge of the supported bilayer was readily apparent (*black area*) (E). Scale bar, 2 μm.

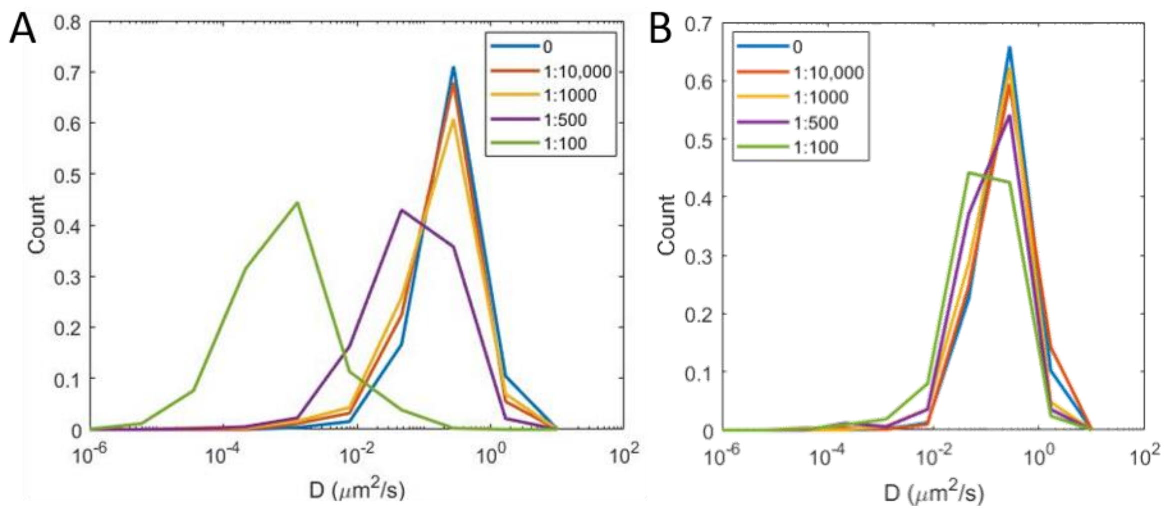

Figure S3: The diffusion of mCTxB, but not DiI, is slowed in the presence of increasing concentrations of anti-CTxB antibody. Histograms show the distribution of diffusion coefficients measured by single-particle tracking in planar supported lipid bilayers for mCTxB (A) or DiI (B) as a function of the indicated dilution of anti-CTxB antibody.

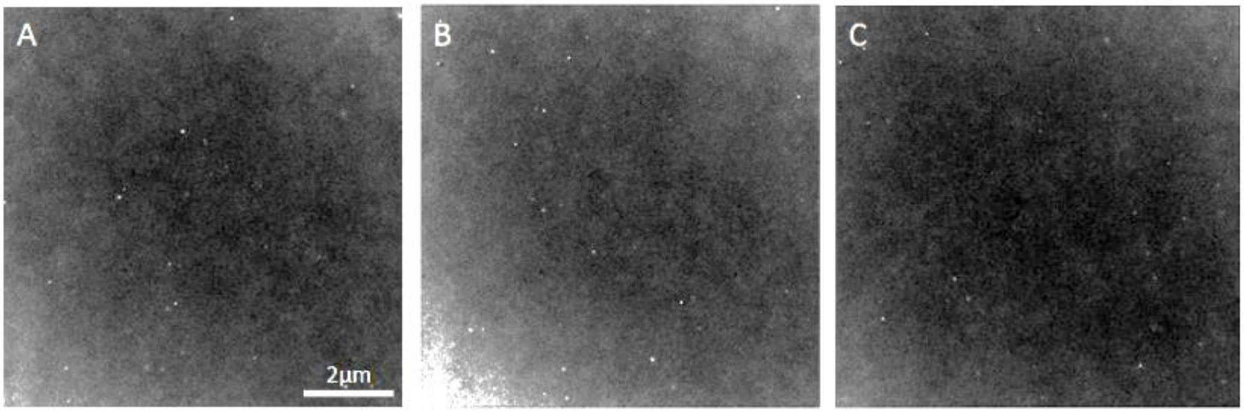

Figure S4: Generic crosslinking of lipids does not induce membrane budding. Planar POPC SLBs containing either (A) 0.3 mol%, (B) 1 mol%, or (C) 3 mol% DPPE-Biotin were labeled with 4.3 nM streptavidin 90 min prior to imaging via pPLM. Scale bar, 2 μm.

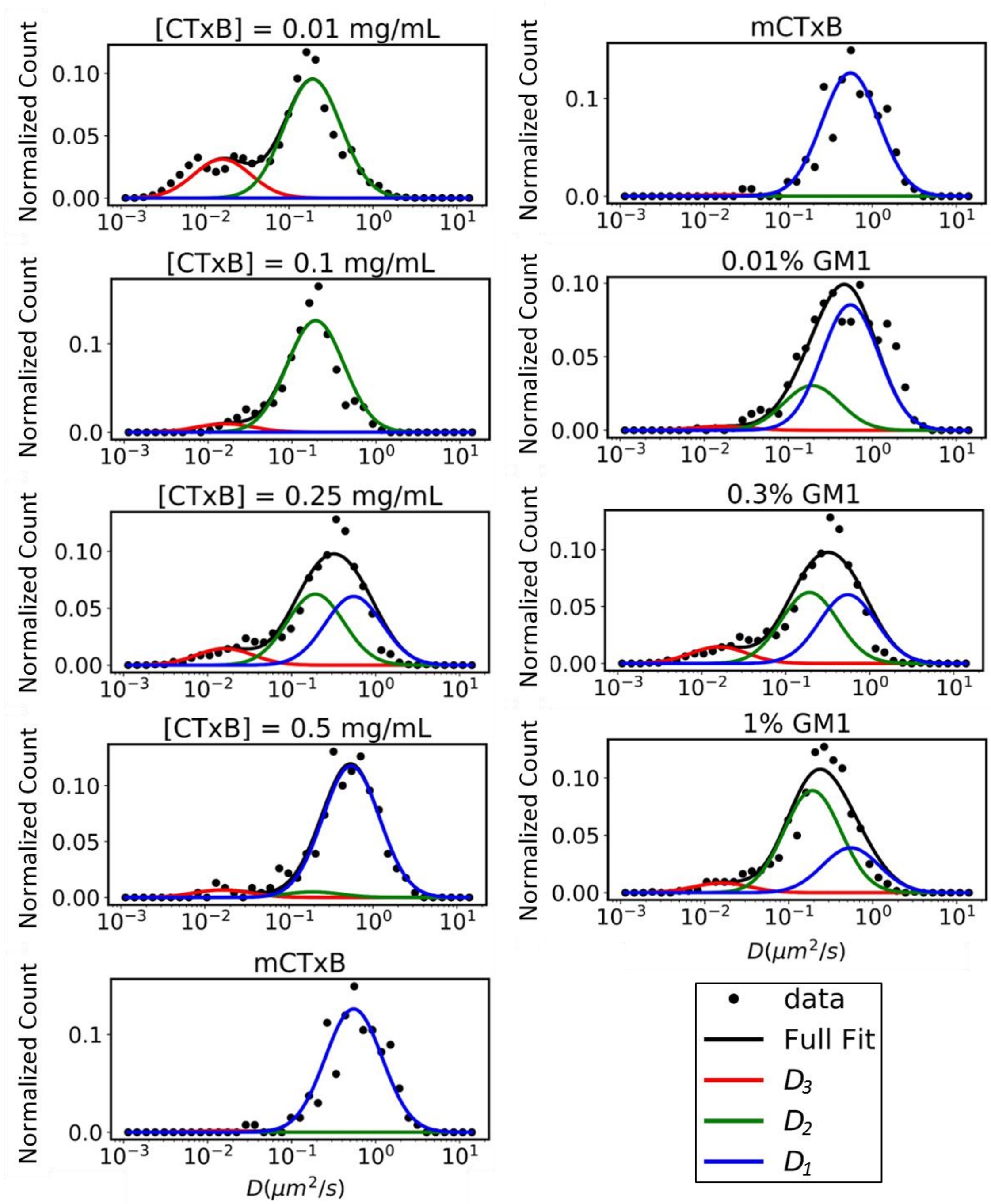

Figure S5: Fits to histograms of *D* for CTxB reveal three distinct subpopulations of diffusing species with different *D* values, whereas the histogram for mCTxB is consistent with the presence of a single population. Each histogram of *D* was fit to Eq. 1 (*black line*), allowing the amplitude of each fitting parameters to vary across experiments. The *D* values corresponding to each subpopulation (D_1, D_2, and D_3) were held constant for all experimental fits. Note that the plot for mCTxB is shown in both columns to allow for better comparison across experimental conditions.

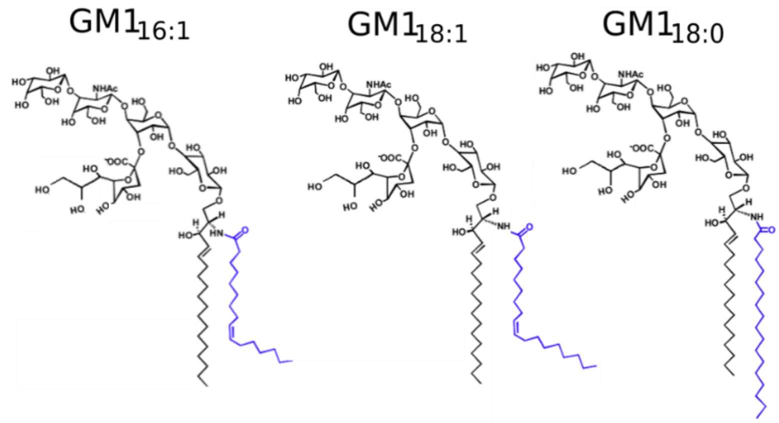

Figure S6: Chemical structure of custom-made GM1$_{16:1}$, GM1$_{18:1}$, and GM1$_{18:0}$ (32)**.** Note that commercially available ovine GM1 is predominantly composed of GM1$_{18:0}$.

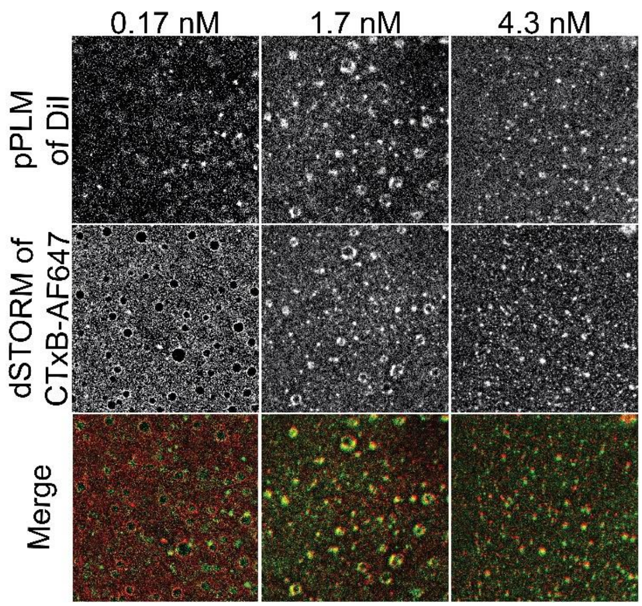

Figure S7: Increasing the ratio of GM1 to CTxB results in increased membrane curvature in model membranes containing cholesterol. The GM1:CTxB ratio was altered by labeling planar supported bilayers containing 0.3 mol% GM1 and 30 mol% cholesterol with either 0.17 nM, 1.7 nM, or 4.3 nM CTxB. Samples were then imaged by pPLM and dSTORM. Merged images show areas where clustered CTxB (*red*) colocalizes with sites of induced curvature (*green*). The presence of cholesterol had no significant effect on the membrane curvature created by CTxB. Scale bar, 2 µm.

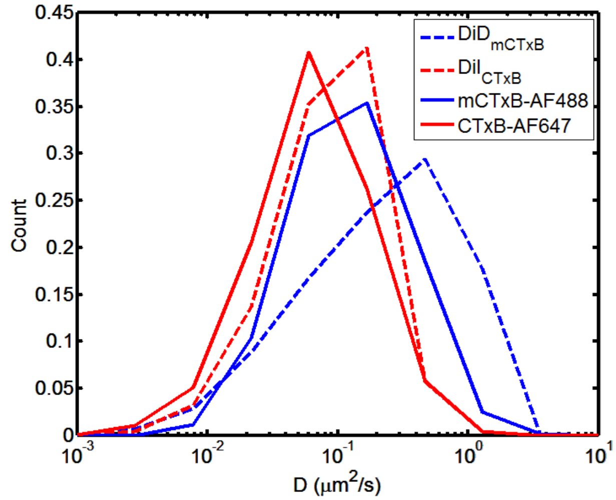

Figure S8: CTxB diffuses more slowly than mCTxB on live cells. Histograms of single-molecule diffusion coefficients obtained in live COS-7 cells are shown for CTxB, mCTxB, and DiI or DiD in the presence of CTxB or mCTxB, respectively.

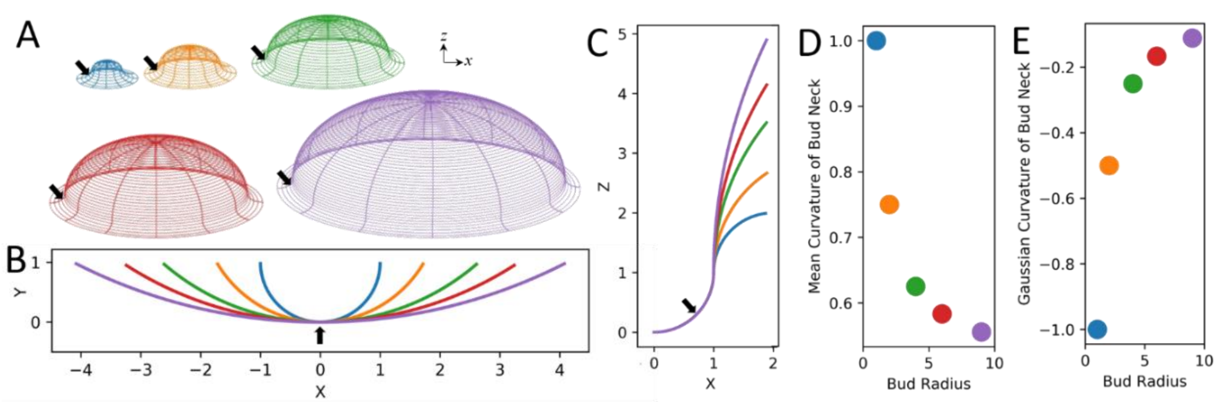

Figure S9: The necks of membrane buds provide one dimension of negative curvature. A model of the membrane buds of increasing radii are shown (A). The bud necks are highlighted (*black arrows*). The decreasing positive curvature in the X-Y plane (B) and consistently negative curvature in the X-Z plane (C) results in the mean curvature at the bud neck decreasing as the bud radius grows (D). In contrast, the Gaussian curvature increases to zero as a function of increasing bud radius (E). Additionally, a larger membrane bud would provide more membrane area on the neck to accommodate more CTxB.