GM_1 Clustering Inhibits Cholera Toxin Binding in Supported Phospholipid Membranes

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Supplemental Materials

Partitioning of GM_1 between Membrane Leaflets

The partitioning of GM_1 between the upper and lower leaflets of the bilayer should be considered when supported lipid bilayers are formed on solid substrates via vesicle fusion. Therefore, a set of experiments based on work by Parikh and his colleagues was performed to determine the distribution of GM_1 between the bilayer leaflets under the conditions employed herein.[1] The central idea is that the partitioning of GM_1 ligands between the bilayer leaflets can be determined by comparing the relative extent of dye-labeled CTB binding to supported POPC bilayers and monolayers. This needs to be done at sufficiently low concentrations of GM_1 so that CTB molecules will not be close packed on the surface under saturation conditions.

Photochemically patterned n-octadecylsiloxane (OTS) monolayers were employed to create surfaces patterned with both phospholipid monolayers and bilayer. First, uniformly self-assembled monolayers of OTS were formed on glass substrates. These OTS monolayers were patterned by using deep ultraviolet radiation and a photomask to produce periodic arrays of hydrophilic domains separated by hydrophobic surroundings using previously established

methods.[1] Small unilamellar POPC vesicles in PBS (pH 7.2, 150 mM NaCl, 0.2 mM sodium azide) containing 0.5 mol% GM_1 and 0.1 mol% Texas Red DHPE were then immediately introduced over the substrate for bilayer/monolayer formation. After 1 h incubation, excess vesicles were rinsed away with PBS. Lipid bilayers were formed on the hydrophilic regions, while lipid monolayers were formed in regions where the OTS was not exposed to UV radiation. Figure S1 demonstrates the formation of POPC monolayer and bilayer regions. As can be seen, the fluorescence intensity from a representative bilayer region is approximately twice as high as that from a monolayer region (15,200 vs. 8100, the background level is ~ 800). Therefore, the intensity ratio is (15,200 - 800)/(8100 - 800) = 1.97. Also, a lipid-depleted boundary region can be observed between the monolayer and bilayer regions.

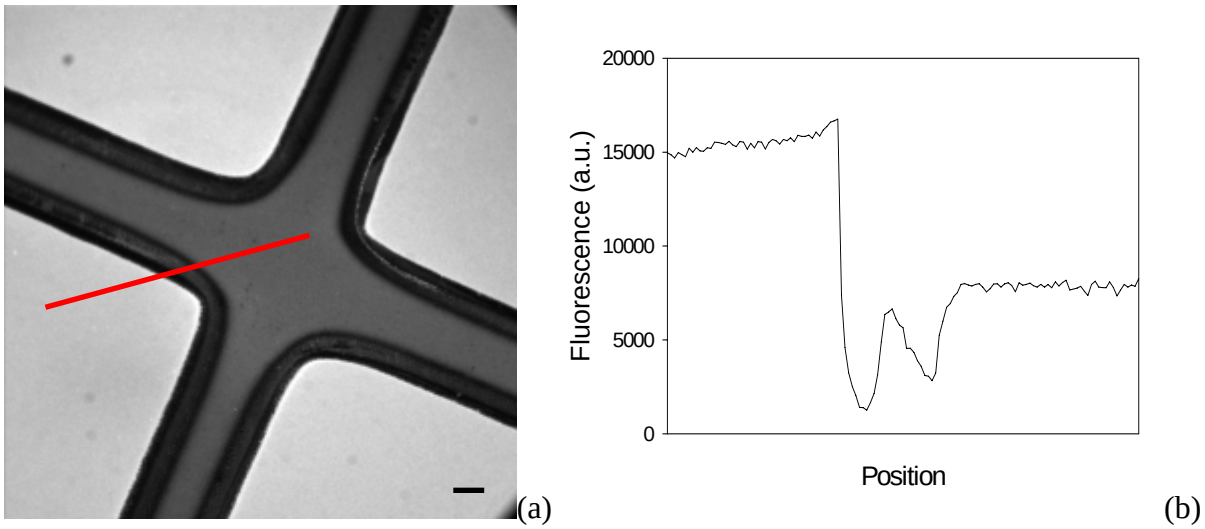

Figure S1. *(a) Epifluorescence image of patterned POPC bilayers and monolayers. The scale bar in the lower right-hand portion of the image is 200 µm. (b) A line scan of fluorescence intensity across the portion of the image shown in red. The POPC membranes contained 0.1 mol% Texas Red DHPE.*

A PBS solution containing 270 nM CTB was utilized for saturation binding studies. The protein was incubated over the substrate for 2 h, which is sufficient for CTB-GM_1 interactions to reach equilibrium. Figure S2a shows the epifluorescence image over patterned monolayer and bilayer regions. A linescan across the image (from the line shown in red) is

provided in Figure S2b. The bright lines occur because of substantial non-specific protein adsorption in the lipid-depleted region between monolayers and bilayers. On either side of this region, however, the fluorescence intensity is virtually identical. Such a result is consistent with the notion of essentially equal partitioning of GM_1 in the two leaflets of the POPC bilayer.

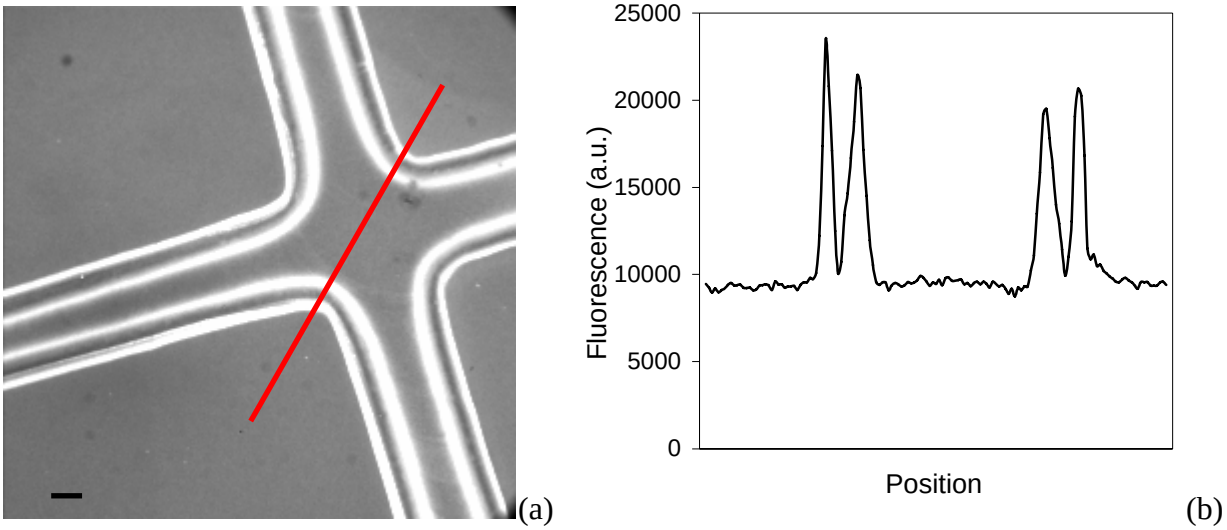

Figure S2. *(a) Epifluorescence image of dye-labeled CTB binding to 0.5 mol% GM_1 in both POPC bilayers and monolayers. The scale bar in the lower left-hand portion of the image is 200 μm. (b) A line scan of fluorescence intensity across the portion of the image shown in red.*

Binding curves

Figure S3 shows the binding isotherms for CTB as a function of GM_1 concentration ranging from 0.02 to 10.0 mol% in POPC bilayers. The data were taken under the identical conditions described for Figure 3. The corresponding values abstracted for K_d, K_H, and n are provided in Table 1.

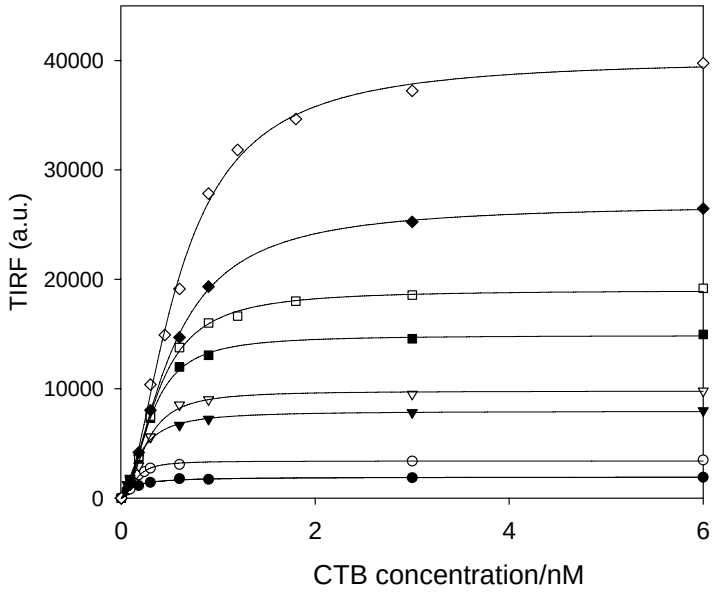

Figure S3. *TIR fluorescence intensity vs. bulk CTB concentration in supported POPC membranes containing 10.0 (open diamond), 5.0 (solid diamond), 2.0 (open square), 1.0 (solid square), 0.5 (open triangle down), 0.1 (solid triangle down), 0.05 (open circle), and 0.02 (solid circle) mol% GM_1. The solid lines are fits to the data with the Hill-Waud equation.*

AFM Studies of GM_1 Clustering and Histograms

Figure S4 shows AFM images of POPC bilayers with increasing concentrations of GM_1 from 0.0 to 10.0 mol%. The data were taken under the identical conditions as described in Figure 5.

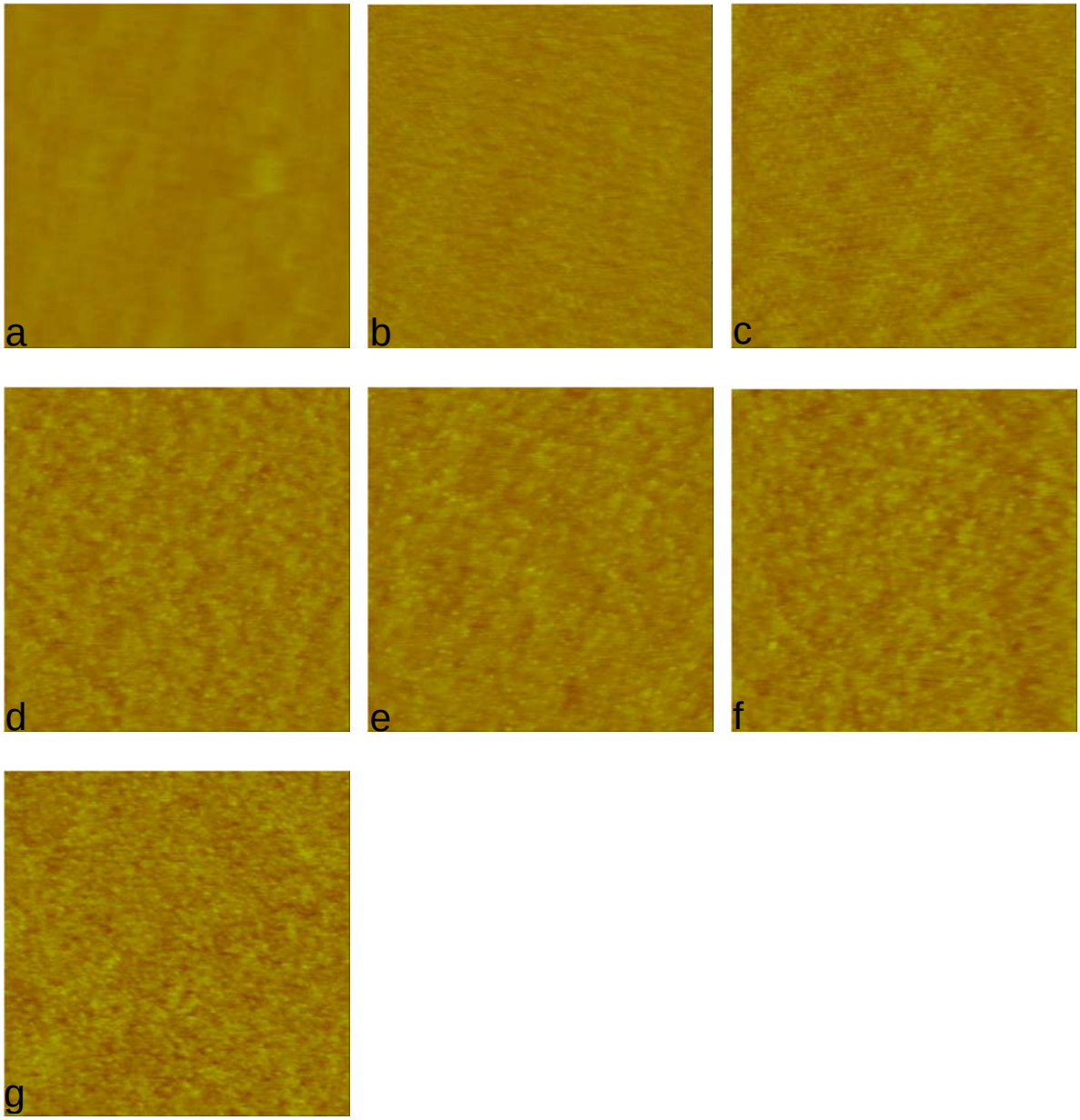

Figure S4. *AFM images of POPC bilayers containing: (a) 0.0 mol%, (b) 0.1 mol%, (c) 0.5 mol%, (d) 1.0 mol%, (e) 3.0 mol%, (f) 5.0 mol%, and (g) 10.0 mol% GM_1, respectively. Each image is 1.0 µm × 1.0 µm.*

Figure S5 shows histograms for the apparent domain diameters of GM_1 within POPC bilayers for 0.1 to 10.0 mol% GM_1. The data were taken under the identical conditions described in Figure 6.

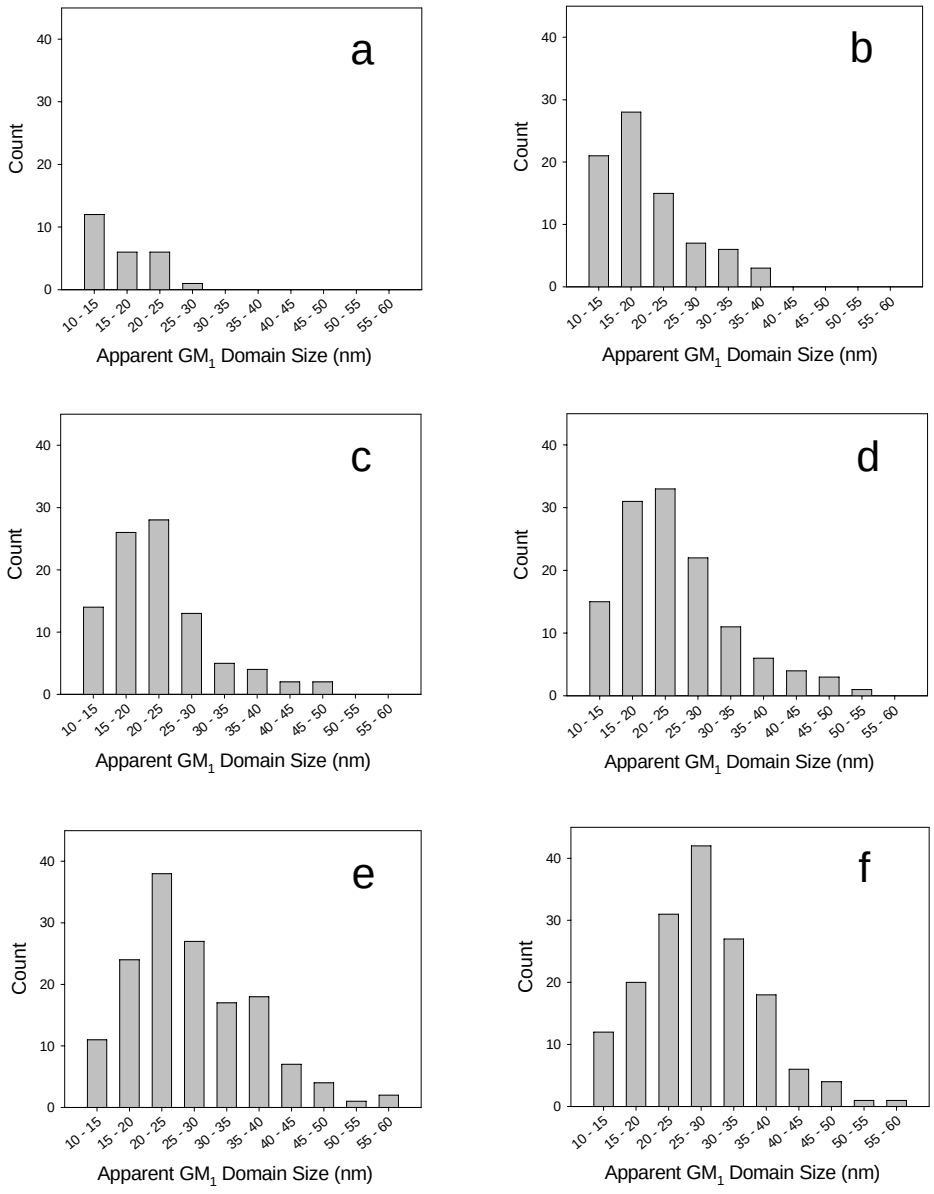

Figure S5. *Histograms for the size distribution of GM_1 domains within POPC bilayers: (a) 0.1 mol%, (b) 0.5 mol%, (c) 1.0 mol%, (d) 3.0 mol%, (e) 5.0 mol%, and (f) 10.0 mol% GM_1/POPC.*

References:

(1) Howland, M. C.; Sapuri-Butti, A. R.; Dixit, S. S.; Dattelbaum, A. M.; Shreve, A. P.; Parikh, A. N. *J. Am. Chem. Soc.* **2005**, *127*, 6752-6765.