Electronic Supplementary Material (ESI) for Soft Matter
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Electronic Supplementary Material

Interaction of sphingomyelinase with sphingomyelin-containing supported membranes

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ESI 1 and ESI 2. Epifluorescence Movies. Time-lapse sequences of fluorescence images revealing gross membrane reorganization induced by the lipolytic activity of enzyme sphingomyelinase on sphingomyelin containing supported lipid bilayers. The movies depict the changes seen over 40 min in an unpatterned (Movie S1a_r and S1r) and patterned (Movie S-2) supported bilayers consisting of DOPC, sphingomyelin, and cholesterol (DOPC:SM:CHOL, 2:2:1 + 1% Rho-DOPE) when exposed to sphingomyelinase (Smase) from B. Cereus at 37 °C. **For unpatterned sample in Movie S-1 Smase concentration was 1 U/ml and for patterned sample, the concentration was 0.5 U/ml.** AVI videos S-1 (S1a_r , 2Dand S1r, 3D renditions) and S-2 attached.

ESI 3. Atomic force microscopy characterization of the initial condition of mixed lipid bilayers. Before SMase treatment, domains too small to be detected by light microscopy (≤ 0.5 mm) are observed by AFM, and are likely to be SM & CH-rich Lo domains (Fig. S-3, panels 1, 2). In addition, larger (1-5 mm) RDE are present, which represent defects in the bilayer (Fig. S-3, panels 3-5). These defects disappear when the sample is heated above 30 °C, but reappear on cooling to RT.

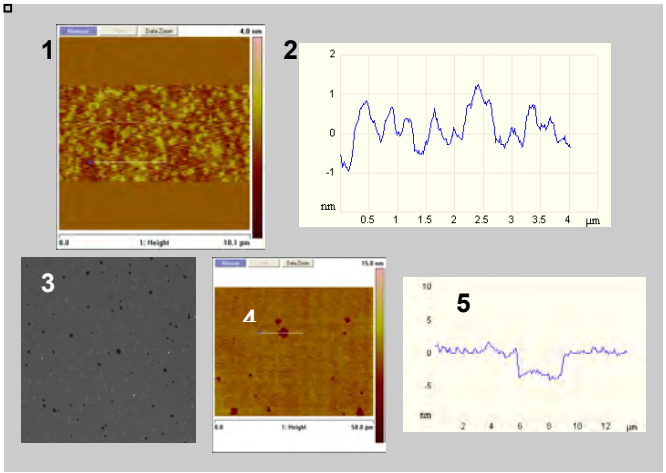

Fig. S-3. (1) AFM image of a freshly prepared planar bilayer composed of PC:SM:CH (2:2:1). (2) An AFM line scan shows the presence of domains (≤ 500 nm in diameter and ~1 nm higher than the bilayer surface). (3) Regions of dye exclusion (RDE), shown in this FM image can also be detected by AFM (panel 4). (5) The AFM line-scan indicates that such RDE existing on freshly prepared bilayers are defects in the bilayer, ranging from 1-5 μm in diameter.

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ESI 4. RDE Stability. After SMase activity, the regions of dye-exclusion (RDE) produced are extremely robust. For instance, following SMase-treatment of a mixed lipid bilayer, the sample was cycled from the temperature at which the enzyme treatment occurred (37 °C) to higher (44 °C) and lower (8 °C) temperatures. No changes were visually apparent in the size or shape of the RDE (Fig. S-4)

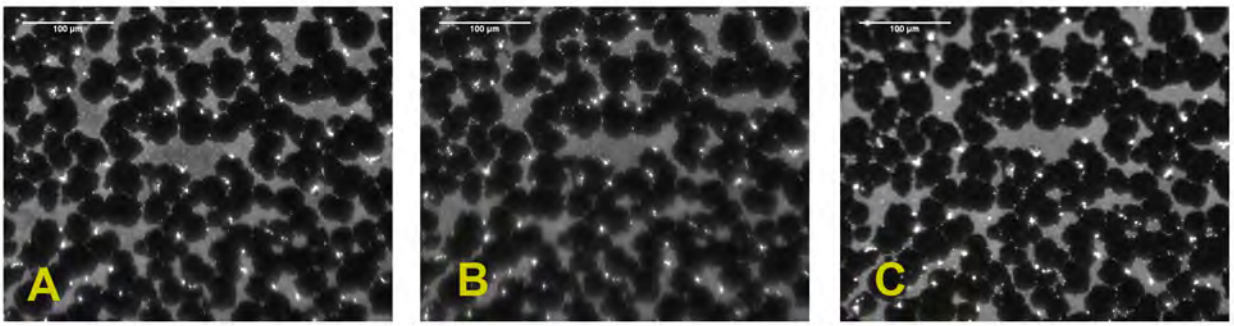

Fig. S-4. Fluorescence micrographs of mixed bilayers composed of PC:SM:CH (2:2:1) (with 1 mol% Rho-DOPE) following a 40-min treatment at 37 ^{o}C with 1 U/mL SMase from *B. Cereus*. Increasing or decreasing the temperature of the sample does not affect the morphology of the sample. Images were taken in this order at (A) 36 ^{o}C; (B) 44 ^{o}C; (C) 8 ^{o}C.

4. Dynamics of Membrane Reorganization. Following the lag phase after the addition of SMase, the small black RDE become surrounded by a bright halo. During Phase II, the halo becomes progressively thicker up to a point (Fig. S-5 A-C). After 15 min, a change occurs. During Phase III, the RDE begin to enlarge exponentially (Fig. S-5 D-F). Eventually, the halo is no longer visible, and the black RDE touch each other (Phase IV), occupying up to 80% of the field of view (Fig. S-5 G-I) (also see Fig. S-6). The increased intensity of the halo may result from some combination of two factors: a different physical environment at the edge regions of the RDE, which can affect quantum yield of the fluorophore, as well as a higher concentration of fluorophore than the bulk.

Note that neither during the early- nor the late-stage evolution of RDE, do new RDEs emerge following the initial nucleation. A high activation energy barrier for nucleation likely blocks the formation of new RDE following the initial stage, and addition to the perimeter of initially formed defects appears to be the dominant pathway.

The progression of SMase- induced RDE enlargement continues until these regions come into contact with one another. This “jamming” of RDE coincides with the slowing and cessation of the membrane reorganization.

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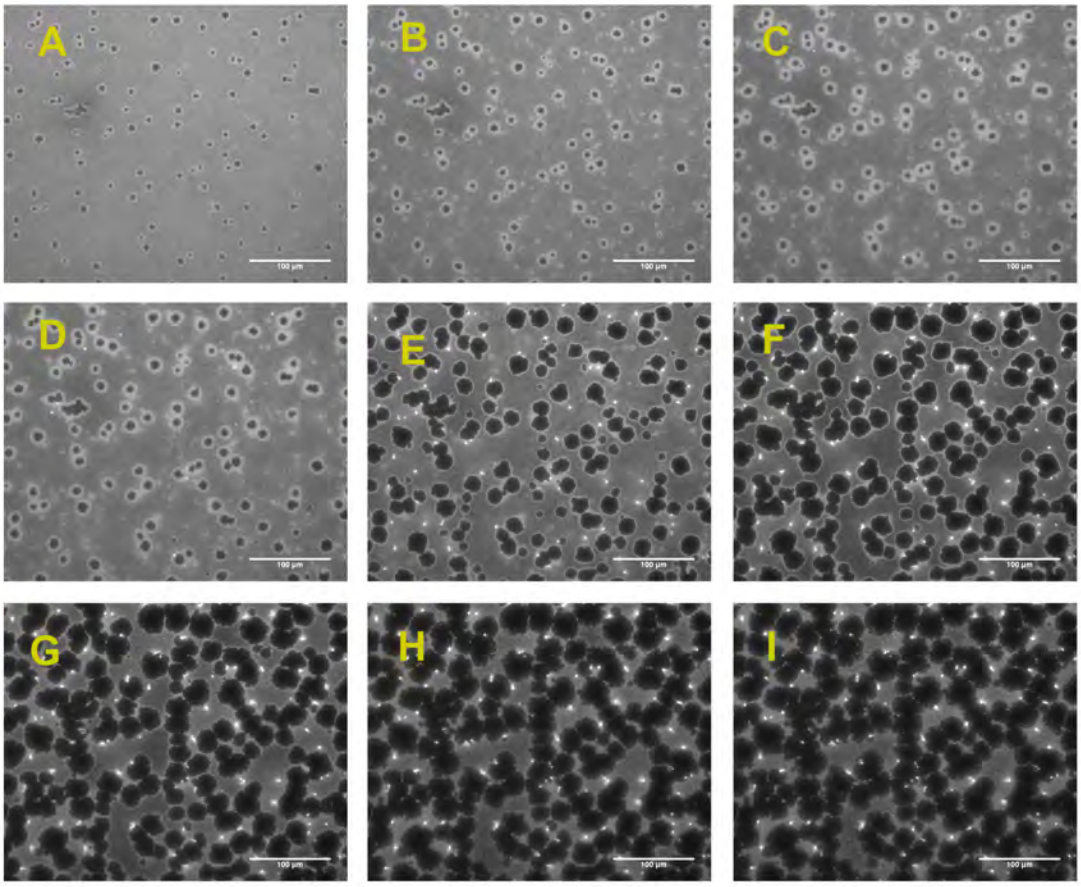

Fig. S-5. Fluorescence micrographs of mixed bilayers composed of PC:SM:CH (2:2:1) (with 1 mol% Rho-DOPE) taken during a 40-min treatment at 37 ^{o}C with 1 U/mL SMase from *B. Cereus*. The images selected to display the progression were recorded at the following time points: (A) 5; (B) 9; (C) 14; (D) 16; (E) 20; (F) 24; (G) 29; (H) 34; and (I) 38 min.

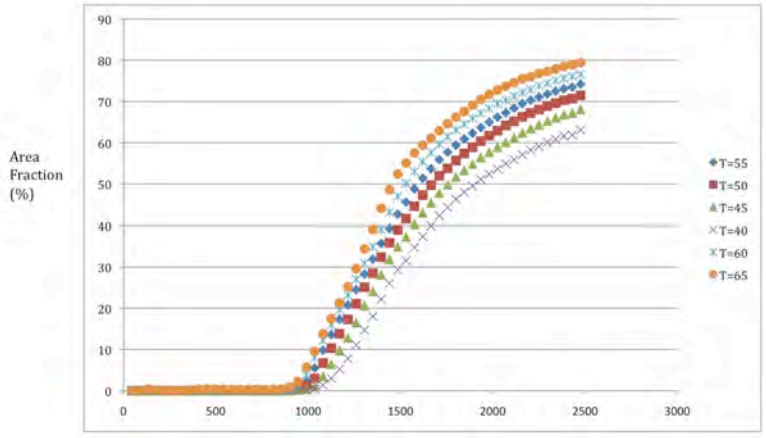

Fig. S-6. Total Area Fraction (% of the field of view) occupied by the RDE as a function of time. The data were obtained from an ImageJ analysis of the movie presented in Fig. S-1 using several different threshold values (listed to the right of the figure). By the time the RDE were no longer changing in shape, they occupied approximately 60 – 80% of the field of view.