Supporting Information

Formation and Spatio-Temporal Evolution of Periodic Structures in Lipid Bilayers

Sharon Rozovsky, Yoshihisa Kaizuka and Jay T. Groves

Department of Chemistry, University of California, Berkeley, California 94720

Experimental Section

Materials All reagents and solvents were at least analytical grade and were used as supplied. Cholesterol, 1,2-Dioleoyl-*sn*-Glycero-3-Phosphocholine (DOPC), (2S,3R,4E)-2-acylaminooctadec-4-ene-3-hydroxy-1-Phosphocholine (Sphingomyelin from Egg, Chicken), 1,2-Dioleoyl-3-Trimethylammonium-Propane (Chloride Salt) (DOTAP), 1,2-Dimyristoyl-*sn*-Glycero-3-Phosphocholine (DMPC), and 1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl)(16:0 NBD PE) were purchased from Avanti Polar Lipids (Alabaster, AL). Texas Red 1,2-dipalmitoyl-*sn*-glycero-3- phosphoethanolamine (TR-DPPE) and cholesteryl 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a- diaza- *s*-indacene-3-dodecanoate (cholesteryl BODIPY) were purchased from Molecular Probes (Eugene, OR). 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) was purchased from Sigma-Aldrich (St. Louis, MO).

Giant unilamellar vesicles (GUVs) preparation A lipid mixture in chloroform was evaporated in a small round bottom flask and dried overnight under vacuum. The dry film was resuspended in a prewarmed 0.5M sucrose solution and left to incubate in a 50 °C bath for several hours. More details can be found in the original methodology report.[1,2] All samples were used fresh on the day of preparation.

Small unilamellar vesicles (SUVs) preparation A lipid mixture was mixed in a chloroform solvent (1 mg/ml) and dried under nitrogen. Subsequently the mixture was

hydrated, sonicated and left at room temperature for a few hours to swell. The vesicles were extruded with a Lipex extruder, Northern Lipids (Vancouver, Canada) using a 100 nm polycarbonate filter.

Glass-supported planar bilayer Glass-supported planar bilayer were formed by fusion of a 10 μL SUVs solution (0.4 mg/ml) in 20 mM Hepes, 150 mM NaCl buffer to a round 25 mm glass coverslip. Following a short incubation the supported bilayer was rinsed in distilled water. Membrane fluidity was established by FRAP as described before.[2] Molecular Probes Attofluor cell chamber was used for visualization.

Sample Preparation GUVs under osmotic pressure were brought in contact with supported lipid bilayer immersed in distilled water at 25 °C. Frequently, the GUVs rupture and create a second bilayer which rapidly form new vesicles in a variety of shapes and contact conditions. The newly formed vesicles gradually evolved to hemispherical shapes, reducing in the process the area of contact with the supported lipid bilayer. This process ensures that GUVs interior solution is likely to be the same as that of the surrounding solution and hence the osmotic pressure is assumed to be small. In general, the interior solution of the GUVs is not a dominant factor in determining pattern formation as GUVs formed in ultra pure water (or formed in a variety of different sugar solutions) also display superstructures. Changes in surface area-to-volume ratio during shape transformation are due to lipid intake from the supported lipid bilayer (as monitored through transfer of a fluorescent lipids from the supported bilayer to the adhered vesicle) or the formation of transient pores. The rate of lipid mixing is greatly reduced by minimizing the percent of charged lipids.

Controls established that charged lipids in the GUVs are not necessary for pattern formation. Pure electrostatic repulsion is unlikely to be a dominant driving force since domain patterns are observed in high ionic strength solutions (40 mM NaCl) as well. Though, their frequency in is greatly reduced and their stability is diminished. Domain patterns are not unique to the lipid compositions used in the reported work but have also been observed in several other ternary compositions.

Epifluorescence microscopy Nikon Corporation (Tokyo, Japan) TE300 and TE2000 inverted fluorescence microscopes were employed with 100x oil immersion objective (adjustable aperture set to NA=1.3). Pictures were taken with a Hamamatsu charge-coupled device camera Hamamastsu C4742-98 (Hamamatsu, Japan). Images were acquired with SIMPLE PCI (Compix, Cranberry Township, PA) and analyzed by Metamorph (Universal Imaging Corporation, Downingtown, PA) and MATLAB (MathWorks, Natick, MA).

Samples Composition In figures 1 and 3 of the main text, supplementary movies 1 and 3, and in the table of contents the vesicles are composed of DOPC 34.85%, cholesterol 28%, Egg Sphingomyelin 34.85%, DOTAP 2%, TR-DPPE 0.3%. The supported lipid bilayer composition is DMPC 96%, DOTAP 2%, 16:0 NBD PE 2%.
In figure 2 of the main text and in supplementary movie 2 the vesicle is composed of DOPC 34%, cholesterol 28%, Egg Sphingomyelin 34%, DOTAP 2%, cholesteryl BODIPY 2%. The supported lipid bilayer composition is DMPC 98%, DOTAP 2%. The lipid phase to which cholesteryl BODIPY partitions was established by comparison with the partitioning behavior of TR-DPPE (known to localize in the liquid disordered phase).[3,4]

Calculation of pair distribution function The radial pair distribution function, g(r), was calculated from the spatial coordinates of lipid domains, combining information from a time sequence of 20 images (Movie 3). Each image (4.2×4.2 μm^2) contained about 40 domains; images were acquired 6 seconds apart. This frame rate insured that the lateral positions observed are uncorrelated as the typical time scale of for the domain diffusion is ~1 ms.[5] We identify the domains position by applying a bandpass filter that allows only frequencies spaced between 1/3 and 3/2 of the lattice's fundamental periodicity. Those positions were further refined as the center of mass in the gradient of intensity around the domains. The best fit has domain diameter of 200 nm. The domain locations are used to compute the radial pair distribution function, g(r), for each image using the following equation for a finite rectangular window of spatial dimensions X by Y [6]

$$g(r) = \frac{\eta(r)X^2Y^2}{N(N-1)\delta r[\pi XYr - 2(X+Y)r^2 + r^3]}$$

where $\eta(r)$ is the number of domain pairs with separation distance, δr is set to 10 nm, $r \pm \frac{\delta r}{2}$, ($0 \le r \le X = Y \approx 4.2$ μm), and N is the total number of domains.

The plot of *g(r)* in Figure 3 of the main text is constructed from the average of all frames and renormalized. Using a harmonic approximation for the potential of mean force, an effective spring constant is estimated from the best Gaussian fit to the first peak of *g(r)*. Image analysis procedures were benchmark tested against model image data with known domain positions and comparable noise to confirm accuracy. Our image processing procedure was implemented in home written programs and MATLAB (MathWorks, Natick, MA).

(1) Akashi, K.; Miyata, H.; Itoh, H.; Kinosita, K. *Biophys. J.* **1996**, *71*, 3242-3250.
(2) Wong, A. P.; Groves, J. T. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 14147-14152.
(3) Baumgart, T.; Hess, S. T.; Webb, W. W. *Nature* **2003**, *425*, 821-824.
(4) Kahya, N.; Scherfeld, D.; Bacia, K.; Poolman, B.; Schwille, P. *J. Biol. Chem.* **2003**, *278*, 28109-28115.
(5) Kaizuka, Y.; Groves, J. T. *Biophys. J.* **2004**, *86*, 905-912.
(6) Baksh, M. M.; Jaros, M.; Groves, J. T. *Nature* **2004**, *427*, 139-141.

Supplementary Movie 1

A time series of Epi-fluorescence microscopy images presented in Figure1 of the main text, showing the transition of a stripe superstructure to a hexagonal lattice of circular domains. The vesicle was monitored for 24 min in 6 sec intervals at 25 °C in distilled water shortly after its adhesion to the supported lipid bilayer.

Supplementary Movie 2

A time series of Epi-fluorescence microscopy images presented in Figure 2 of the main text, showing the formation of a stripe superstructure from a circular domain. The vesicle was monitored for 5 min in 1 sec intervals at 25 °C in distilled water shortly after its adhesion to the solid supported lipid bilayer.

Supplementary Movie 3

A time series of Epi-fluorescence microscopy images used for estimating the $g(r)$ presented in Figure 3 of the main text. The time interval between images is 6 sec. Experimental conditions are similar to those detailed above.