### **Supplemental Information 1**



Fig. SI-1: Domain Area Distribution: Histogram of domain areas in Figs. 1F, 1L, and 2A. With increasing cooling rate, both average domain size and dispersion decrease.

### Supplemental Information 2: GalCer:DLPC 10:90 and 20:80

Fast Cooled ~0.1C/min, 10% GalCer, 90% DLPC



Fast Cooled ~0.1C/min, 20% GalCer, 80% DLPC



Slow Cooled ~0.05C/min, 20% GalCer, 80% DLPC



Figure SI-2: Ellipsomertric contrast images (430x645µm) of two lipid mixtures (10% GalCer / 90% DLPC and 20% GalCer 80% DLPC) at two cooling rates (0.1 °C/min and 0.05 °C/min). Each concentration and cooling rate shows two representative images.

# **Supplemental Information 3: Mixture Comparisons**

Fast Cooled ~0.1C/min 10% GalCer, 90% DLPC



Total Area: 14200 um<sup>2</sup>

Area %: 6%

Ave Dom 118±47 um<sup>2</sup>

109 spots

Slow Cooled ~0.05C/min 10% GalCer, 90% DLPC



Total Area: 13300 um² Area %:

Ave Dom 170±118 um<sup>2</sup>

65 Spots





, Total Area: 33400 um<sup>2</sup>

> Area %: 14%

Ave Dom 427±223 um<sup>2</sup>

77 spots

Slow Cooled ~0.05C/min 20% GalCer, 80% DLPC



Total Area: 36600 um<sup>2</sup> Area %:

15%

Ave Dom 1590±385 um<sup>2</sup> 22 Spots Fast Cooled ~0.1C/min 35% GalCer, 65% DLPC



Total Area: 49900 um<sup>2</sup>

Area %: 23%

Ave Dom 277±338 um<sup>2</sup>

180 Spots

Slow Cooled ~0.05C/min 35% GalCer, 65% DLPC



Total Area: 52400 um<sup>2</sup>

Area %: 24%

Ave Dom 1690±648 um<sup>2</sup>

31 Spots

Figure SI-3: Ellipsomertric contrast images (430x645µm) of three lipid mixtures (10% GalCer / 90% DLPC, 20% GalCer 80% DLPC, and 35% GalCer / 65% DLPC) at two cooling rates (0.1 °C/min and 0.05 °C/min. A comparison of the images illustrates four trends: 1) Domain area increases with GalCer concentration. 2) Domain area increases with longer cooling rates for a given mixture. 3) Number of domains increases as cooling rate increase for all mixtures. 4) Total domain area increases with increasing GalCer concentration.

## Supplemental Information 4: GalCer:DLPC 20:80 Heating/Cooling



Figure SI-4: Ellipsomertric image snapshots from a series of images captured during a sample heating (A-F) followed by a (0.07°C/min) cooling (G-L) cycle of a 20% GalCer / 80% DLPC lipid mixture. The contrast images are 430x645µm. As the sample is heated, defects heal over (A-C) and domains melt becoming continuous with the fluid membrane (A-F). Upon a second cooling at 0.07°C/min, domains and defects in the membrane are first observable at 36.6 °C and 31.5 °C respectively. Both continue to grow after initial formation to form fractal-like morphologies. A movie of this process can be seen in Supplemental Information (SI).

### **Supplemental Information 5: Calorimetry**





The differential scanning calorimetry (DSC) results using a Setaram C 80 calorimeter are consistent with the ellipsometry data. The DSC melting peak at 38-39 °C is very broad and of complex shape, indicating a complex transition that begins above 32 °C and is fully complete above 54 °C. The onset temperature of 43 ± 2 °C in a cooling cycle indicates the beginning of crystallization with its maximum rate at 38-39 °C, in agreement with the formation of pin-hole defects, seen in the ellipsometric experiment starting at 39 °C. The total enthalpy of transition is  $25 \pm 2 \text{ kJ}$  per mole of GalCer.