Supporting Information: Millimeter-area, free standing, phospholipid bilayers

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Additional Lipid Bilayer Formation Images

Phase separation of 18:61:21 DOPC:DPPC:Cholesterol in n-hexadecane

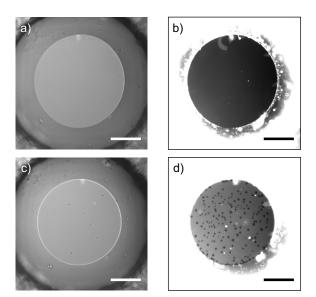


Figure S1: Bilayer of a 18:61:21 molar ratio mixture of DOPC:DPPC:Cholesterol shows uniform fluorescence at (A) 35.5 °C and lipid domains appear when the bilayer is cooled to (B) 33 °C. Left panel is the imaging channel and the right panel is the fluorescence channel. The fluorescence signal comes from the addition of 0.7 mol % Rh-DOPE which preferentially partitions into the more fluid phase. The scale bars represent 0.2 mm.

Phase separation of 60:40 DOPC:DPPC in n-hexadecane

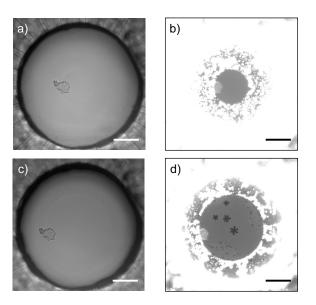


Figure S2: Bilayer of a 60:40 molar ratio mixture of DOPC:DPPC shows uniform fluorescence at (A) 31.0 °CC and lipid domains appear when the bilayer is cooled to (B) 27.1 °CC. Left panel is the imaging channel and the right panel is the fluorescence channel. The fluorescence signal comes from the addition of 0.9 mol% NBD-DOPE which preferentially partitions into the more fluid phase. The scale bars represent 0.2 mm.

DMPC bilayer

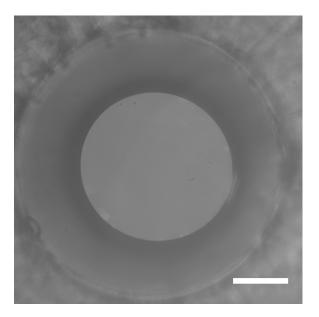


Figure S3: Bilayer of DMPC in squalene at 20 $^{\circ}\mathrm{C}.$ The scale bar represents 0.2 mm.

POPG bilayer

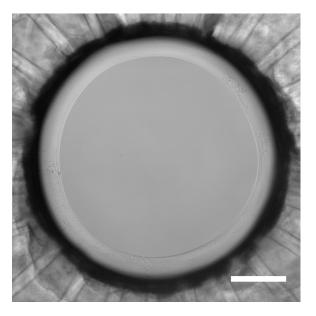


Figure S4: Bilayer of POPG in n-hexadecane at room temperature. See also corresponding video of bilayer formation, Movie S2. The scale bar represents 0.2 mm.

DOPG bilayer

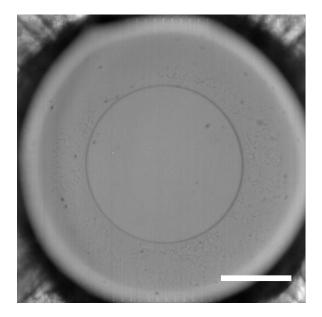


Figure S5: Bilayer of DOPG in n-hexadecane at room temperature. The scale bar represents $0.2~\mathrm{mm}.$

E. coli TLE bilayer

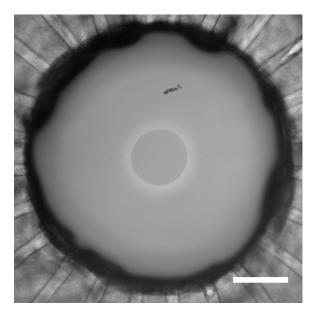


Figure S6: Bilayer of E. coli total lipid extract in n-hexadecane at room temperature. See also corresponding video of bilayer formation, Movie S3. The scale bar represents 0.2 mm.

DOPC bilayer

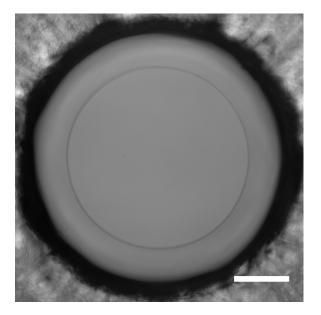


Figure S7: Bilayer of DOPC in n-hexadecane at room temperature. See also corresponding video of bilayer formation, Movie S4. The scale bar represents 0.2 mm.

DPPC bilayer

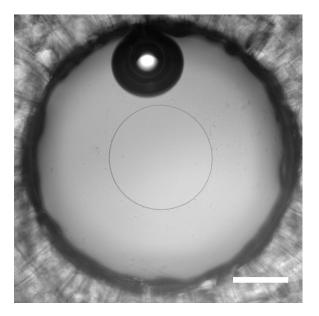


Figure S8: Bilayer of DPPC in n-hexadecane formed at 41°C. The bilayer formed despite the presence of an air bubble trapped above the Plateau border. See also corresponding video of bilayer formation, Movie S5. The scale bar represents 0.2 mm.

Movie files may be accessed from the separate links provided.

Lipid Bilayer Formation Movies

Phase separation of 34:33:33 DOPC:DPPC:Cholesterol in n-hexadecane

Movie S1: Corresponding video to Figure 2 in the main text. Lipid domains appear when a film formed from an 34:33:33 molar ratio mixture of DOPC:DPPC:Cholesterol at 34 °C is cooled to 29 °C. Left panel is the imaging channel and the right panel is the fluorescence channel. The fluorescence signal comes from the addition of 0.7 mol % Rh-DOPE which preferentially partitions into the more fluid phase. Playback is sped up 10x. The scale bars represent 0.1 mm.

POPG bilayer nucleation

Movie S2: Bilayer of POPG in n-hexadecane forming at room temperature. The scale bar represents $0.2~\mathrm{mm}.$

E. coli TLE bilayer nucleation

Movie S3: Bilayer of E. coli TLE in n-hexadecane forming at room temperature. Playback is sped up 30x. The scale bar represents $0.2~\rm{mm}.$

DOPC bilayer nucleation

Movie S4: Bilayer of DOPC in n-hexadecane forming at room temperature. Playback is sped up 20x. The scale bar represents 0.2 mm.

DPPC bilayer nucleation

Movie S5: Bilayer of DPPC in n-hexadecane forming at 41°C. Playback is sped up 50x. The scale bar represents 0.2 mm.