Supporting Information for

A new pathway of formation of radial nematic droplets within a lipid-laden aqueous-liquid crystal interface

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Experimental Section

Materials: L- α- Phosphatidylcholine (PC), oleoyl-L-α-Lyso phosphatidic acid sodium salt (LPA), hexadecyltrimethylammonium bromide (CTAB), lipopolysaccharides (from E.coli 0111:B4), N,N'-Bis(2,5-di-tert-butylphenyl)-3,4,9,10-perylenedicarboximide (BTBP), N,N-dimethyl-N-octadecyl-3-aminopropyltrimethoxysilyl chloride (DMOAP), and sodium dodecylsulfate (SDS) were purchased from Sigma-Aldrich (St. Louis, MO). 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC) and 1-Palmitoyl-2-{12-[(7-nitro-2-1,3-bezoxadiazol-4-yl) amino] dodecanoyl-sn-glycero-3-phosphocholine (NBD PC) were purchased from Avanti Polar Lipids Inc. Phospholipase A_2 from naja mossambica mossambica was purchased from Sigma-Aldrich (St. Louis, MO). Sulfuric acid, chloroform and hydrogen peroxide (30% w/v) were purchased from Merck (Mumbai, India). Ethanol was obtained from Jebsen & Jenssen GmbH and Co., Germany (s d. fine–chem limited). 4-cyano-4'-pentylbiphenyl (5CB) was obtained from Sigma-Aldrich (St. Louis, MO). Deionization of a distilled water (DI water) source was performed using a Milli-Q-system (Millipore, bedford, MA). Fisher's Finest Premium Grade glass microscopic slides and cover glass were obtained from Fischer Scientific (Pittsburgh, PA). Gold specimen grids (20 μm thickness, 50 μm wide bars, 283 μm grid spacing) were obtained from Electron Microscopy Sciences (Fort Washington, PA).

Preparation of liquid crystal films in TEM grids:

Glass slides were cleaned with piranha solution (70:30 (% v/v) H_2SO_4:H_2O_2) for 1h at 100 °C according to the published procedures.[1] These cleaned glass slides were then dipped into 0.1%

(v/v) DMOAP solutions in DI for 5 min at room temperature and rinsed with DI to remove the unreacted DMOAP from the surface. The DMOAP coated glass slides were dried under a stream of nitrogen gas and kept in oven at 100 °C for 3 h.

Cleaned gold specimen grids were placed on DMOAP-coated glass slides. The grids were filled with approximately 0.2 μL of 5CB and the excess of LC was removed with the help of syringe to produce a planar interface.

Preparation of Vesicles:

Vesicles were prepared according to the published procedures.[2] Briefly, the lipids were dissolved in chloroform (0.5 mL) and dispensed into round bottomed flask. Prior to re-suspension, the chloroform was evaporated from the flask under vacuum for at least 2 h until it formed a thin film along the inner walls of the flask. The lipid film formed in the flask was then placed under a stream of nitrogen for 30 min. The dried lipid was then hydrated in the aqueous solution (DI water) for at least 1h and vortexed for 1 min. This results in the cloudy solution indicative of large multilamellar vesicles. Subsequent sonication of lipid suspension using a probe ultrasonicator (1 x 15 min at 25 W) resulted in a clear solution. Vesicles size was determined by DAWN8$^+$ dynamic light scattering instrument (see Figure S9). Prior to dynamic light scattering measurements, the solution of PC vesicles was filtered twice using 0.22 μm filter. The vesicles were used within 24 h of their preparation.

Formation of liquid crystal droplets with radial defects:

The DMOAP coated glass surface supported LC-filled grid was submerged into glass wells containing 3 mL of distilled water. 1 mL of PC lipid solution was added to the aqueous solution to make a final concentration of 0.5 mg/mL. The LC-filled grid in PC solution was then allowed to incubate for 48 h. Paraffin was placed over the top of the glass well to prevent evaporation of water.

Optical characterization of liquid crystal droplets with radial defects:

The orientational ordering of liquid crystal droplets were determined using under Nikon Eclipse LV100POL Polarizing Microscope using an objective power of 20X with cross polars. Orthoscopic examinations were performed with the source light intensity set to 50% of full

illumination and the aperture set to 0.45 in order to collimate the incident light. All the images were captured using a Q-imaging camera.

Fluorescence Imaging:

The LC-filled grid was incubated under lipid solution containing 1 μM NBD-PC for 48 h. After incubation the solution was then exchanged with distilled water thrice to remove the excess of NBD-PC from the bulk solution. The LC-filled grid supported on DMOAP coated surfaces was then removed from the solution and placed over a glass slide. Few drops of distilled water was poured over it and covered with a cover slip keeping a distance of 0.2 cm from the sample. Fluorescence imaging was performed with Zeiss (Scope. A1) fluorescence microscope. The samples were viewed using a fluorescence filter cube with a 460 nm excitation filter and a 534 nm emission filter. Images were obtained with Axio cam camera.

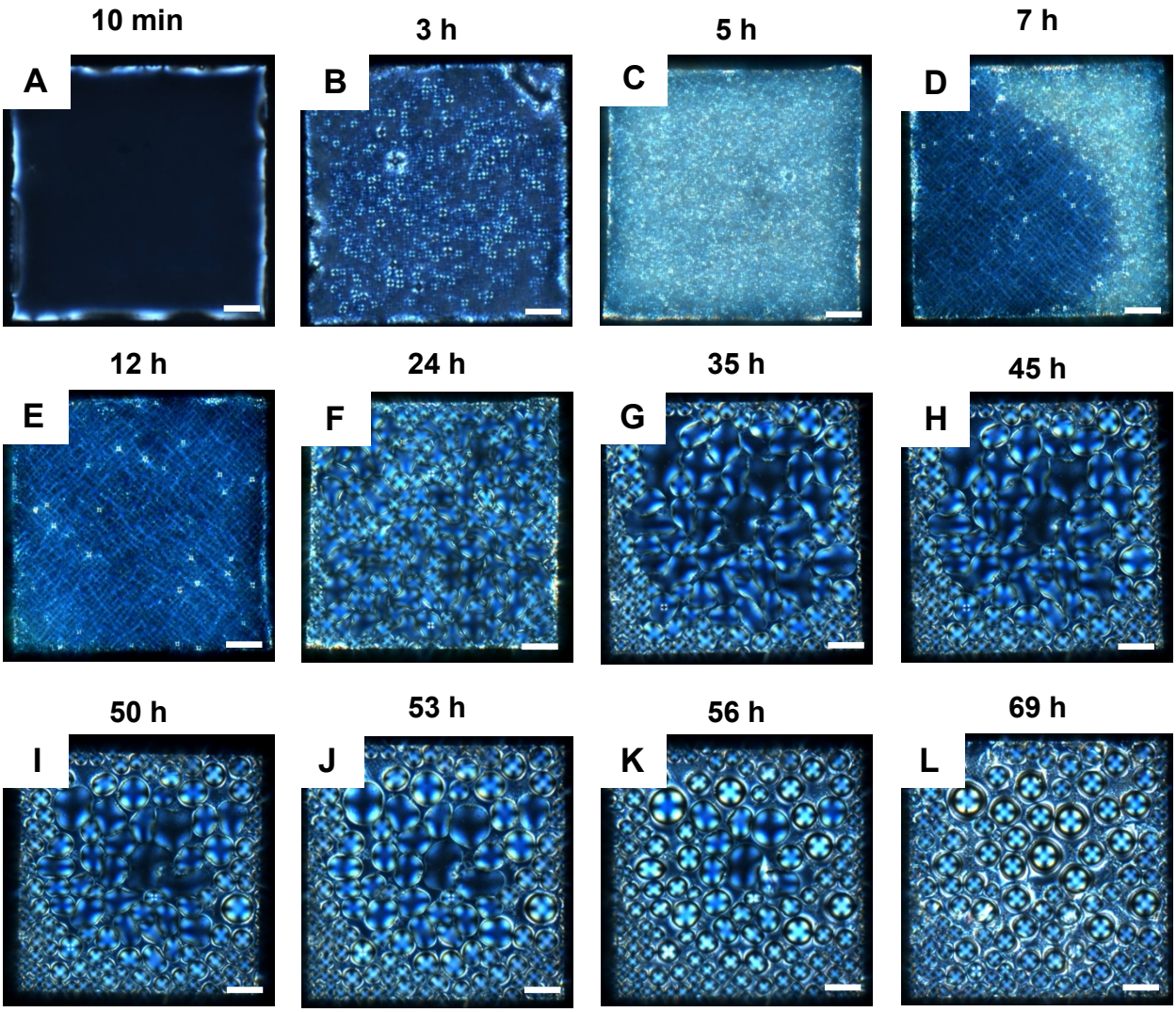

Figure S1. Time-dependent polarized optical micrographs (A-L) of aqueous-5CB interfaces within TEM grids supported on DMOAP coated glass slides upon exposure to 0.5 mg/mL phosphatidylcholine (PC). Scale bar = 40 μm.

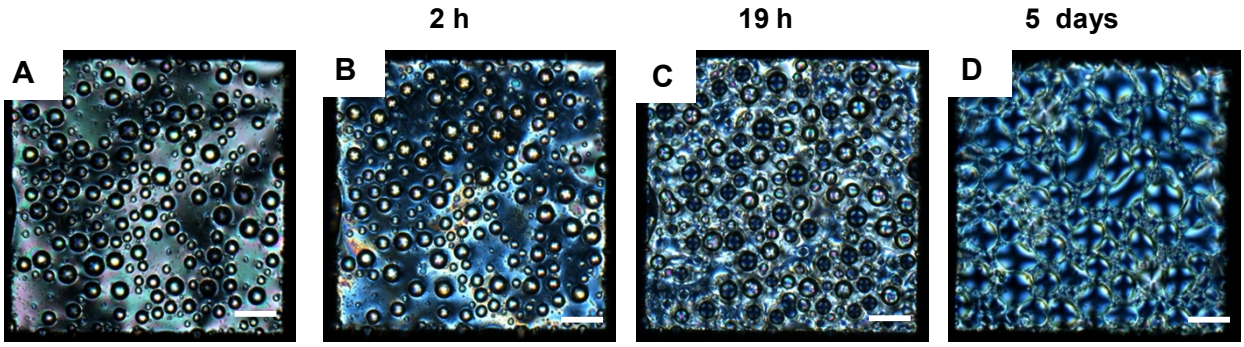

Figure S2. A) Polarized optical images of 5CB within TEM grids supported on DMOAP coated glass slides in contact with water for 3 days. (B-D) represents the time lapse polarized micrographs of aqueous–5CB interface after exchanging the water with dispersion of PC vesicles. It shows the reorganization of water droplets to liquid crystal droplets radial LC ordering. Scale bar = 40 μm.

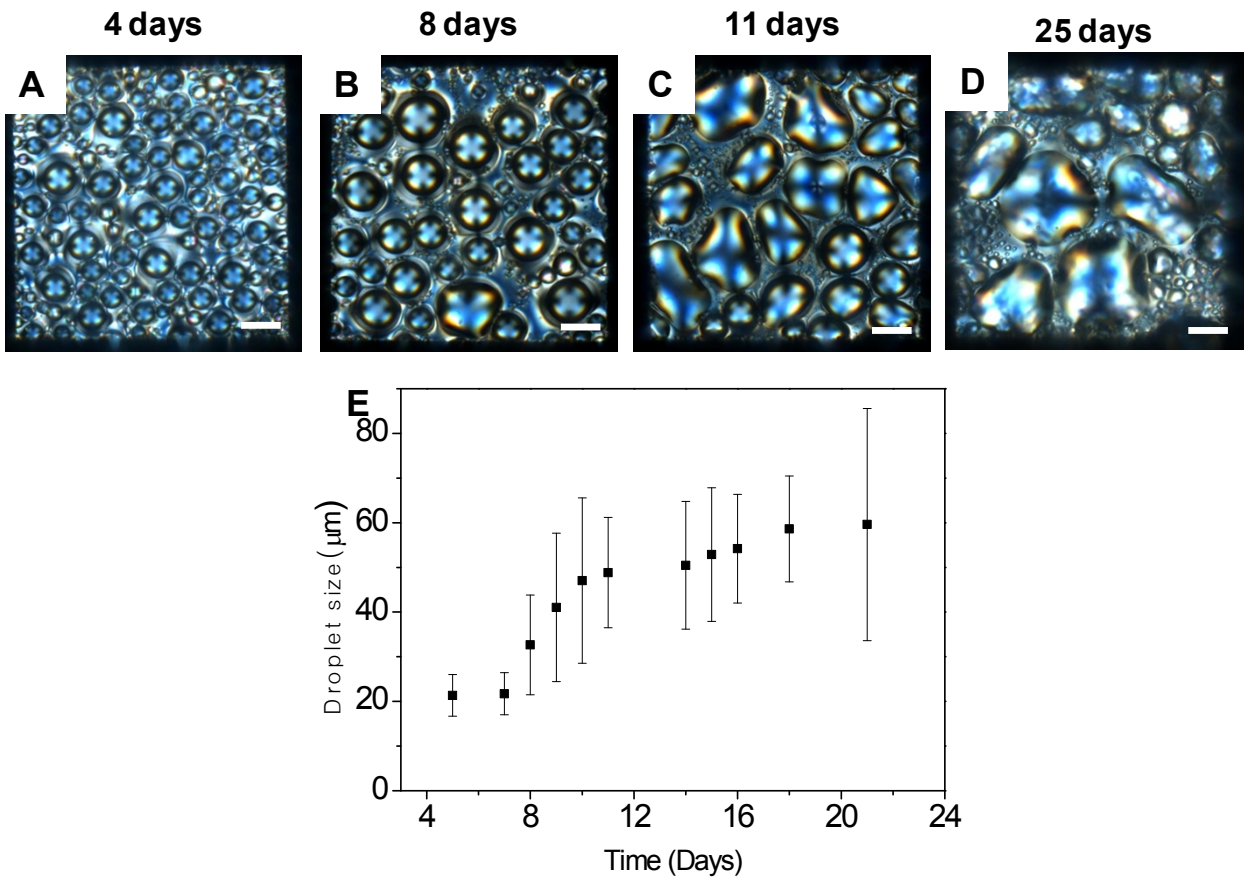

Figure S3. (A-D) Polarized optical images of time-dependent growth of 5CB droplets formed within TEM grids supported on DMOAP coated glass slides in contact with PC (0.5 mg/mL). E) Represents the time-dependent growth of domains (in µm) of 5CB droplets in presence of PC (0.5 mg/mL). Scale bar = 40 µm.

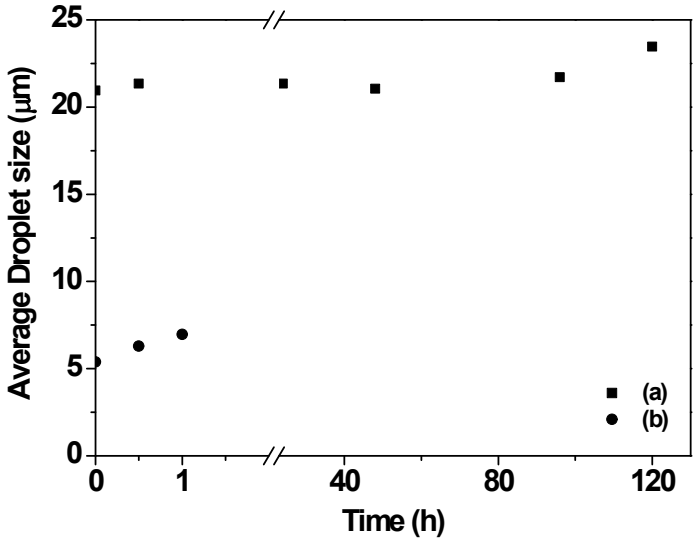

Figure S4. Stability and average size of LC droplets formed a) within TEM grids supported on DMOAP coated glass slides in contact with PC (our approach) b) by sequential sonication and vortexing of LC in surfactant solution (past studies),[3] respectively.

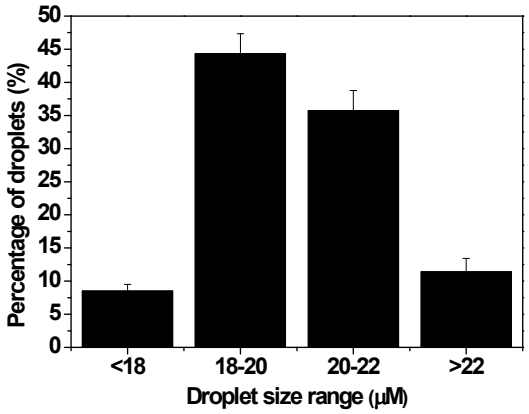

Figure S5. Size distribution of LC droplets formed within TEM grids supported on DMOAP coated glass slides in contact with PC.

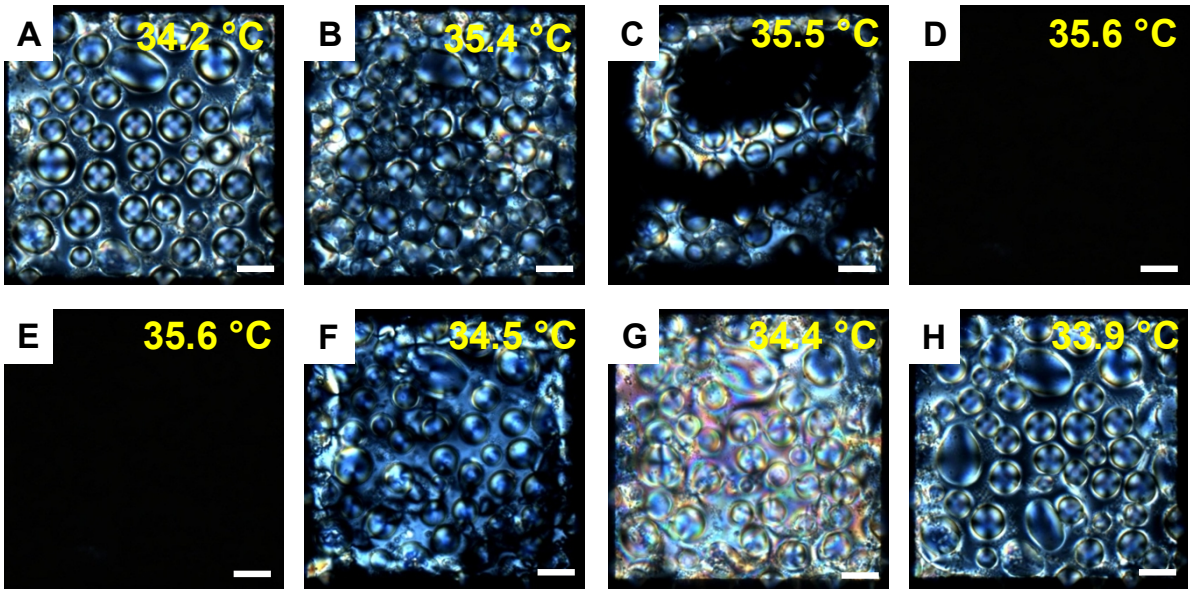

Figure S6. Optical images under crossed polarizers of 5CB within TEM grids supported on DMOAP coated glass slides in contact with aqueous dispersion of PC (0.5 mg/mL) vesicles after incubation for 3 days followed by heating above and below the phase transition temperature (T_{N-I} ~ 35 °C) of 5CB between nematic and isotropic states. The top rows (A-D) and the bottom rows (E-H) show the LC droplet patterns on heating above and cooling below the T_{N-I}, respectively. Scale bar = 40 μm.

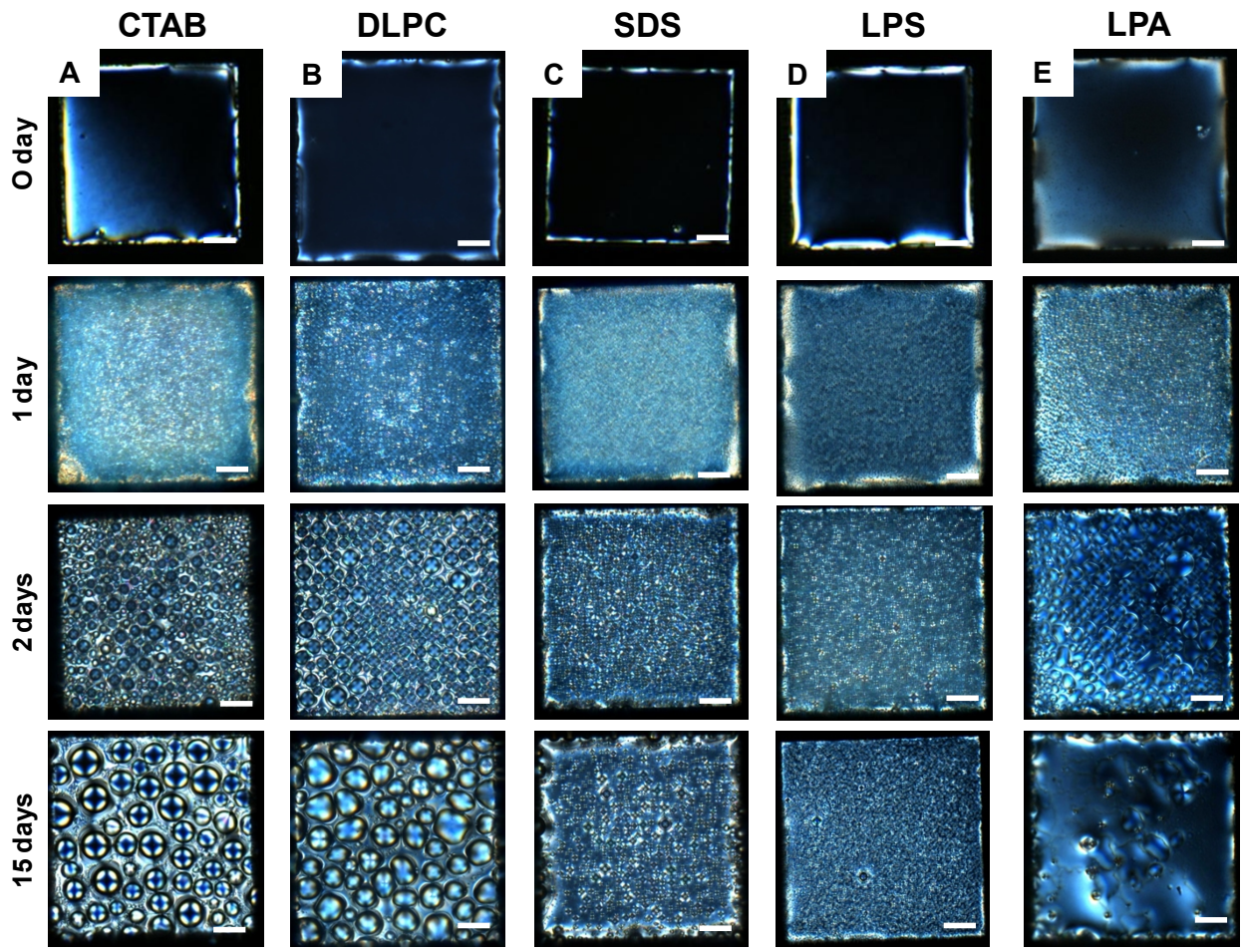

Figure S7. Time-dependent optical images under crossed polarizers of 5CB within TEM grids supported on DMOAP coated glass slides after exposure of aqueous- 5CB interface to A) positively charged hexadecyltrimethyl ammonium bromide (0.1 μM CTAB), B) Zwitterionic 1,2-dilauroyl-sn-glycero-3-phosphocholine (0.5 mg/mL DLPC), C) negatively charged sodium dodecylsulfate (2 μM SDS), D) negatively charged lipopolysaccharides (0.5 mg/mL LPS) and E) negatively charged oleoyl-L-α lysophosphatidic acid sodium salt (40 μM LPA). Scale bar = 40 μm.

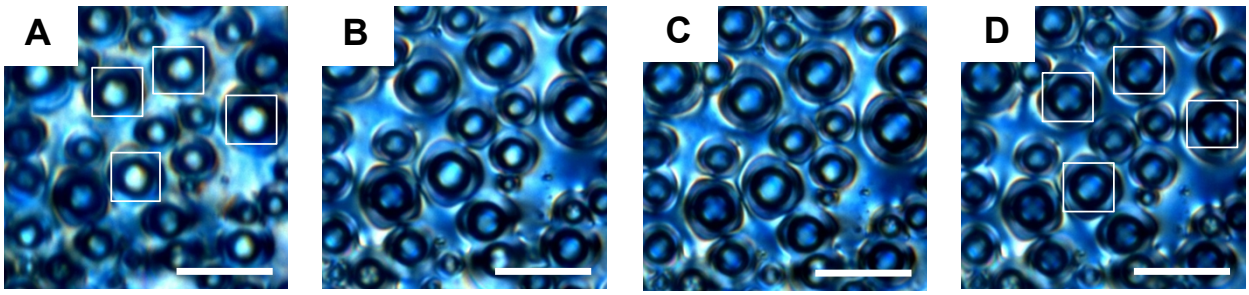

Figure S8. A) Polarized optical image of 5CB droplets covered with DLPC upon exposure of 150 nM PLA_2 containing 10 mM $CaCl_2$ into an aqueous solution of TBS (pH = 8.9). (B-D) represents the time lapse polarized micrographs of aqueous–5CB interface following contact with 100 μg/mL aqueous LPS. It represents the change in anchoring transition of LC droplets from bipolar to radial upon adsorption of LPS. Scale bar = 40 μm.

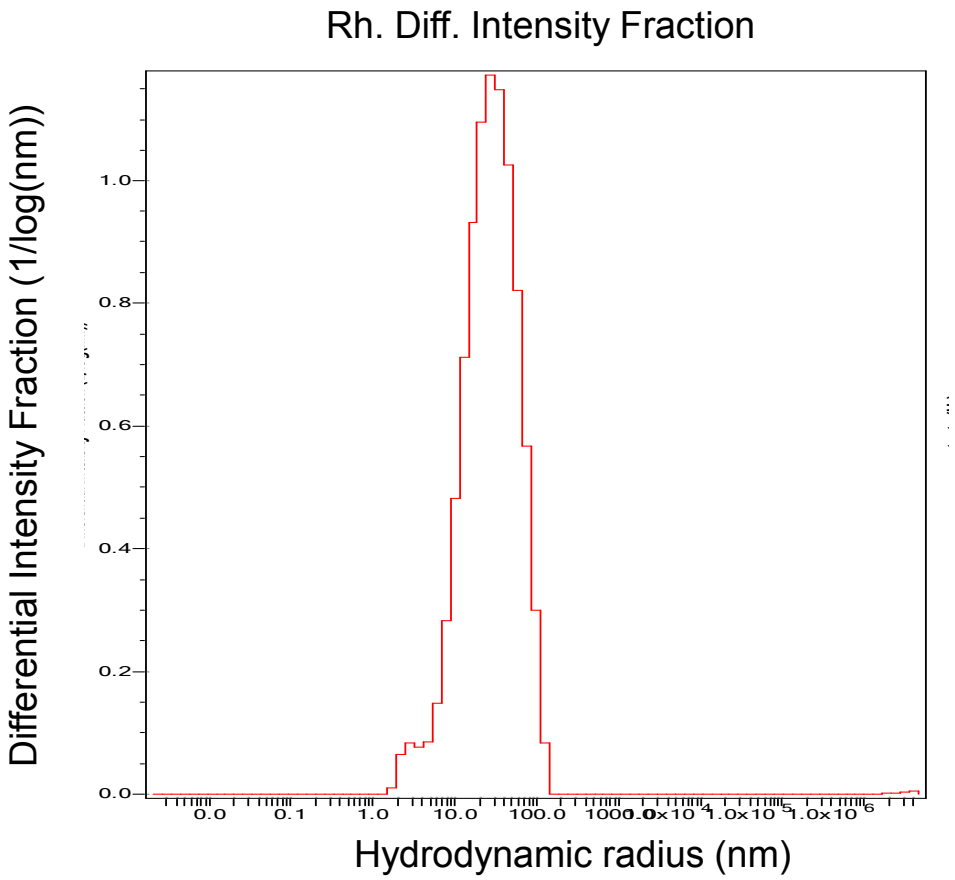

Figure S9. Plot showing the PC vesicle size distribution using dynamic light scattering.

References for Supporting Information

(1) J. M. Brake, N. L. Abbott, *Langmuir,* 2002, **18**, 6101.

(2) J. K. Gupta, J. S. Zimmerman, J. J. de Pablo, F. Caruso, N. L. Abbott, *Langmuir,* 2009, **25**, 9016.

(3) D. S. Miller, N. L. Abbott, *Soft Matter*, 2013, **9**, 374.