Supporting Online Material for

Endotoxin-Induced Structural Transformations in Liquid Crystalline Droplets

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Materials and Methods

Materials. Endotoxin (from \textit{E.coli} O127:B8), lipid A, and sodium dodecylsulfate (SDS) were purchased from Sigma-Aldrich (St. Louis, MO). 1,2-Dilauroyl-	extit{sn}-glycero-3-phosphatidylcholine (DLPC) and 1,2-dioleoyl-	extit{sn}-glycero-3-phosphatidylcholine (DOPC) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Octadecyltrichlorosilane (OTS), methanol, methylene chloride, sulfuric acid, hydrogen peroxide (30% w/v), and heptane were obtained from Fisher Scientific (Pittsburgh, PA). Ethanol was obtained from Pharmco-Aaper (Brookfield, CT). The 4’-pentyl-4-cyanobiphenyl (5CB) was obtained from EM Sciences (New York, NY). \textit{Limulus} amoebocyte lysate (LAL) reagent water was purchased from Associates of Cape Cod, Inc. (East Falmouth, MA). EndoTrap® Red Equilibration Buffer (described below as PBS buffer; 10mM Na$_2$HPO$_4$/NaH$_2$PO$_4$, 80mM NaCl, pH7.4; certified to contain a concentration of endotoxin of less than 0.02 EU/mL (~2pg/mL)) was purchased from Hyglos GmbH (Regensburg, Germany). Neptune pipette tips (no detectable endotoxin) were purchased from Continental Lab Product, Inc (San Diego, CA). Polystyrene tubes (certificated nonpyrogenic tubes) were purchased from Becton Dickinson Labware (Franklin Lakes, NJ). Fisher’s Finest Premium Grade glass microscope slides and cover glass were obtained from Fisher Scientific (Pittsburgh, PA). Gold specimen grids (20\textmu m thickness, 50\textmu m wide bars, and 283\textmu m grid spacing) were obtained from Electron Microscopy Sciences (Fort Washington, PA).

Preparation of LC Emulsions. The emulsions were formed by sequential sonication and vortex mixing of 2\textmu L of nematic 5CB in 1mL of LAL reagent water or PBS at 25°C. Twelve cycles comprising 10 sec of vortex mixing (at 2500rpm) and 10 sec of sonication yielded milky white
emulsions. The LC droplets were confirmed to exhibit bipolar configurations following sonication. The size distributions of the LC emulsion droplets were determined from optical micrographs using Image J (NIH, Bethesda, MD) software. The majority of the LC emulsion droplets possessed diameters that ranged from 4 μm to 8 μm, and were observed visually to be stable against coalescence at least for 3 hours. The LC emulsions were used within 3 hours of preparation.

**Preparation of Aqueous Dispersion of Endotoxin or Lipid A.** Powdered endotoxin or lipid A was dissolved in LAL reagent water at room temperature. After addition of the endotoxin or lipid A powder to obtain a concentration of either 1mg/mL or 20μg/mL, each solution was mixed by vortexing at 2500 rpm for 4 minutes. After dilution, each solution was vortexed for an additional 45 seconds. Concentrations of endotoxin were verified by a kinetic turbidimetric LAL assay (Charles River Laboratories International, Inc.; Wilmington, MA) performed in the Waisman Clinical Biomanufacturing Facility Laboratory, University of Wisconsin, Madison.

**Preparation of Aqueous Dispersions of DLPC, DOPC and SDS.** Briefly, DLPC or DOPC were dissolved in chloroform and dispensed into glass vials. The phospholipid-containing chloroform solution was evaporated under a stream of N₂, and the vial containing lipid was then placed under vacuum for at least 2 hours. The dried lipid was resuspended in aqueous solution and subsequently sonicated using a probe ultrasonicator which resulted in a clear solution (60 Sonic Dismembrator from Fisher Scientific (Pittsburgh, PA); 3x at 15W for 5 min each in a water bath). The phospholipid solution was then extruded through a 0.22μm pore filter (Millipore; Billerica, MA) before use.
Preparation of Planar LC Films. Detailed descriptions of procedures used to prepare planar LC films (stabilized by hosting the LC within metallic grids) can be found in our past publications (S1,S2). Briefly, glass microscope slides were cleaned according to published procedures and coated with OTS. The quality of the OTS layer was assessed by checking the alignment of 5CB confined between two OTS-coated glass slides. Any surface not causing homeotropic anchoring (perpendicular alignment) of 5CB was discarded. Gold specimen grids that were cleaned sequentially in methylene chloride, ethanol, and methanol were placed onto the surface of OTS-coated glass slides. Approximately 1µL of 5CB was dispensed onto each grid and then excess LC was removed by contacting a capillary tube with the droplet of 5CB. As described in our previous publications, the thickness of the LC film is set by the thickness of the metallic grid (S1,S2). Each LC-filled grid was equilibrated at ambient temperature and subsequently immersed into the aqueous solution of interest at 25°C.

Optical Characterization of Liquid Crystal in the Presence of Lipid by Polarized Light Microscopy. A volume of 40 μL of an aqueous dispersion of lipid (in LAL reagent water or PBS) was dispensed onto a cover glass. A volume of LC emulsion, prepared as described above, was added to the lipid dispersion to produce a sample containing the desired number of LC droplets. The configurations of the LCs within the emulsion droplets were determined by observation of the optical appearance of the droplets under an Olympus IX71 inverted microscope (Center Valley, PA) using an objective power of 100x (an oil lens). Bright-field and polarized light micrographs of the LC emulsions were collected with a Hamamatsu 1394 ORCA-ER CCD camera (Bridgewater, NJ) connected to a computer and controlled through SimplePCI imaging software (Compix, Inc., Cranberry Twp., NJ). As described in detail elsewhere, we
characterized LC droplets that were translating with a velocity greater than 1 \( \mu \text{m/s} \) to avoid observation of droplets interacting with the surfaces of the cover slips (S3). The droplets were diffusing (translating and rotating) – thus a radial droplet has an optical appearance that is invariant with time when viewed between crossed-polars whereas the bipolar droplet has a distinct, time-varying optical appearance.

The orientations of the LCs within Au grids were determined by using an Olympus BX60 microscope with crossed-polars (transmission mode). Orthoscopic examinations were performed with the source light intensity set to 50\% of full illumination and the aperture set to 10\% to collimate the incident light. Homeotropic (perpendicular) alignment of LC was determined by insertion of a condenser below the stage and a Bertrand lens above the stage to allow conoscopic examination of the specimen. Observation of an optical pattern consisting of two crossed isogyres confirmed the homeotropic alignment. Images were captured with a microscope-mounted digital camera (Olympus C-4000 Zoom) set to an f-stop of 2.8 and a shutter speed of 1/320s.

**Confocal Microscopy of BODIPY-Labeled Endotoxin Adsorbed to LC Droplets.** Confocal fluorescence imaging was performed using a Leica SP2 confocal system and Leica DMIRE2 inverted microscope at Northwestern University (Illinois). The BODIPY was excited at 488nm using an argon laser, and the detector collected light with wavelengths from 499nm to 634nm. The thickness of the equator focal plane was 40.7 nm. The exposure time required to obtain each confocal section was \( \sim \)6 sec.
Anchoring Energy of Nematic 5CB at Aqueous-Nematic Interface. We determined the magnitude of the anchoring energy of nematic 5CB at the aqueous-nematic interface by preparing planar films of 5CB of known thickness within metallic grids on OTS-treated glass slides (see above for details; the thicknesses of the metallic grids were 5μm and 20μm). We confirmed the thicknesses of the LC films by quantifying the displacement of the focal plane when imaging the two interfaces of each LC film. We measured the optical retardance of the LC films, and calculated the anchoring energy assuming (i) the elastic constant of nematic 5CB to be 5pN, (ii) the anchoring energy per unit area to be described by $W=1/2\sin^2\theta$, and (iii) the anchoring of the 5CB at the OTS-treated glass interface to be homeotropic and strong. From these measurements, we determined the magnitude of the anchoring energy to be $\sim 10\mu J/m^2$.

Figure S1. Polarized light micrographs (crossed-polars) of 20 μm-thick LC films with planar interfaces in contact with aqueous solutions: (A) Endotoxin-free water for 1 day; (B) 1mg/mL endotoxin for less than 2 minutes; (C) 1μg/mL endotoxin for 1 day. The bright appearance of the optical micrographs in A and C indicate that the orientation of the LC is parallel to the aqueous-LC interface in these samples. In contrast, the dark optical appearance in B indicates an orientation of the LC that is perpendicular to the aqueous-LC interface, a result that is confirmed by a conoscopic image (inset, lower right). Scale bars are 300μm.

Characterization of the Kinetic Pathway of the Endotoxin-Induced Ordering Transition. We used videomicroscopy to identify the kinetic pathways underlying the endotoxin-induced
ordering transitions between the bipolar and radial configurations of the LC droplets. The LC droplets dispersed in water were translating and rotating during imaging. Because the LC droplets rotated between the times at which we obtained the crossed polar and bright-field images for each droplet, in Fig. S2 and S3, we rotated the bright field images to align them with the crossed polars images.

First, we performed experiments using high concentrations of endotoxin (50μg/ml) to characterize the kinetic pathway of LC droplets going through a surface-driven ordering transition in the presence of endotoxin (Fig. S2). We note that a concentration of 50μg/ml of endotoxin is sufficient to saturate the interface of the LC droplets in these experiments. Inspection of Fig. S2 reveals that the kinetic pathway observed at high endotoxin concentrations involves transitions states that comprise (i) a disclination loop, (ii) a preradial configuration, and (iii) an escaped-radial configuration. We note also that the kinetic pathway shown in Fig. S2 is similar to past observations by us (using SDS and L-DLPC (S4)) and others (using lecithin (S5)) in which adsorbate-induced changes in the easy axis were observed to drive the ordering transitions of the LC droplets.

Second, we performed experiments in which we recorded the transition states assumed by the LC droplets during an ordering transition triggered by the presence of 10pg/ml of endotoxin (Fig. S3). Inspection of Fig. S3 reveals that the LC droplets were initially in a bipolar configuration. The onset of the transition was first evidenced by the disappearance of one of the point defects (boojums) at a pole of the droplet. The remaining point defect transformed to a hedgehog corresponding to a preradial configuration. Finally, the point defect migrated from the droplet surface, corresponding to an escaped-radial configuration, to the core of the droplet forming a radial configuration. In contrast to the
surface-driven ordering transition induced by 50μg/ml of endotoxin (Fig. S2), the ordering transition triggered by 10pg/ml did not involve a transition state with a disclination loop, an observation that is consistent with our conclusion that the ordering transition in the LC droplets caused by 10pg/ml of endotoxin is not caused by a change in surface anchoring energy. In summary, these results establish that the kinetic pathway of the surface-driven transition caused by 50μg/ml of endotoxin is different from the kinetic pathway when using 10pg/ml of endotoxin. This result supports our conclusion that the ordering transition induced by 10pg/ml of endotoxin is not due to a change in the surface anchoring energy.

Figure S2. (A-F) Schematic illustrations, (G-L) bright field micrographs, and (M-R) polarized light micrographs (crossed-polars) of 8-μm-diameter 5CB droplets following exposure of the droplets to 50 μg/mL of endotoxin. Initially, the droplets were in a bipolar configuration (A, G, M). As the boundary conditions transitioned from tangential to normal, the two point defects (boojums) at the diametric ends of the bipole disappeared, and a disclination loop appeared near the droplet equator (B, H, N). The disclination loop subsequently moved towards a pole of the droplet (C, I, O) and shrank to a surface point defect (hedgehog) corresponding to a preradial configuration (D, J, P). Finally, the point defect moved from the surface of the droplet, corresponding to an escaped-radial configuration (E, K, Q), to the droplet center forming a radial configuration (F, L, R).
We also place the observations described above into the context of past observations of ordering transitions reported by Volovik et al (S5) and Prichepa et al (S6). First, when endotoxin is present at high concentrations (50 μg/ml), we observed an intermediate state that exhibited the distinct optical signature of a disclination loop (Fig. S2). A kinetic pathway involving a disclination ring has also by reported by Volovik et al (S5). Second, in contrast to the above studies by ourselves (S4) and Volovik et al (S5), we observed LC droplets to transform from bipolar to radial configurations in the presence of 10 pg/ml of endotoxin without exhibiting a disclination line as an intermediate state. We note that Prichepa et al (S6) have...
reported a kinetic pathway for a bipolar-to-radial ordering transition for LC droplets (embedded in a solid polymer matrix) that does not involve an intermediate disclination loop. However, key differences also exist between our observations and those reported by Prischepa *et al.* *(S6)*. In particular, Prischepa *et al.* *(S6)* report that the intermediate states (e.g., escaped-radial) observed during a bipolar-to-radial transition correspond to equilibrium states of the system that can be accessed by varying the concentration of lipid dissolved into the solid, polymeric matrix. In our experiments, we do not observe the intermediate states (e.g., escaped-radial) to be stable equilibrium states of the system that are encountered with increasing endotoxin concentration (in the pg/ml range) in the aqueous solution. In contrast, we observe the droplets to exhibit only transient intermediate states between bipolar and radial configurations, and at equilibrium, with increasing concentration of endotoxin in the pg/ml range, we observe an increasing fraction of radial droplets in solution (see Fig 1L). This point is illustrated in Fig S4, where the fractions of emulsion droplets in the bipolar, preradial/escaped-radial, or radial configurations were recorded each minute after exposure to 10 pg/mL of endotoxin *(Fig. S4)*. Inspection of Fig. S4 reveals that prior to addition of the endotoxin, the majority of the LC droplets were determined to be in a bipolar configuration. In the first minute following addition of the endotoxin, a transient rise in the percentage of droplets exhibiting the preradial/escaped-radial configuration was observed. A maximum in the fraction of preradial/escaped-radial droplets in solution was observed after ~3-4mins. The final state of the system was comprised of radial LC droplets.
**Figure S4.** Percentage of LC droplets that exhibited bipolar (♦), preradial/escaped-radial (■), and radial (▲) configurations following the addition of 10 pg/mL endotoxin. The lines are drawn to guide the eye. Each data point represents the average of 3 independent experiments.

**References for Supporting Information**