Electronic Supplementary Information

Tailoring Liquid Crystals as Vehicles for Encapsulation and Enzyme-Triggered Release

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Materials 4-Cyano-4'-pentylbiphenyl (5CB), 1,6-diphenyl-1,3,5-hexatriene (DPH), HPLC acetone, chloroform (HPLC grade), HPLC dichloromethane (DCM), triethylamine, lipase from *Candida antarctica*, dimethyloctadecyl[3-(trimethoxysilyl)propyl]ammonium chloride (DMOAP), hydrogen peroxide, Nile Red (NR) were purchased from Merck. Lauric acid (LA) and tetraethylene glycol were purchased from TCI India and oxalyl chloride was purchased from Avra chemicals. Deionization of distilled water was performed using a Merck Millipore system.

Preparation of DMOAP treated glass slides The cleaning and DMOAP treatment of glass slides were performed using procedures reported earlier.[1] Glass slides were immersed in piranha solution (70:30, sulfuric acid:hydrogen peroxide) for 1 h at 80 °C. The glass slides were then washed with ample deionized water followed by ethanol. The glass slides were then purged with a stream of nitrogen gas and kept in the oven for at least 3 h at 100 °C. Next, in order to make the surface of glass slides hydrophobic, the slides were treated with a hydrophobic organosilane such as DMOAP. The piranha-cleaned glass slides were then immersed in a 0.1% (v/v) solution of DMOAP in deionized water for 30 min. Following this, the slides were washed with a generous amount of water, purged with nitrogen, and kept in the oven at 100 °C for at least 3 h before use.

Preparation of doped LC films 5 mg of 5CB was taken in 4 microcentrifuge tubes (MCTs). Calculated volumes of 1 mg/mL chloroform solution of tetra(ethylene glycol) mono-*n*-laurate (PEG-4-L) and LA were added into the MCTs so as to reach 0.1, 0.5, 1, and 3 % (w/w) of PEG-4-L or LA in 5CB. The MCTs containing the chloroform solutions of 5CB doped with PEG-4-L or LA were then placed in a desiccator which was connected to a high vacuum for 3-5 h.

Optical characterization of LC films The optical characterization of LC films was performed using a polarizing optical microscope (POM) as described previously.[1] 0.2 µL of 5CB or 5CB/PEG-4-L and 5CB/LA mixtures was pipetted onto the gold grid placed on a piece of DMOAP-treated glass slide. The glass slides which did not show a homeotropic alignment of LC in the air were not used for further experiments. The 5CB or surfactant-doped 5CB films were then placed under a Zeiss Scope.A1 POM in transmission mode under crossed polarizers. To examine the LC ordering at the aqueous interface of doped 5CB films, the DMOAP-treated glass slide containing the Au grid filled with mixed 5CB films was immersed in an optical well containing 2 mL PBS (pH 7.4). In the experiments with lipase, silicone isolators of diameter and depth, 4.5 and 1.6 mm, respectively, were used in place of the optical wells.

Preparation of PEG-4-L micelles A calculated amount of the PEG-4-L was weighed in a round bottom flask and dissolved in 0.2 mL acetone. Then required volume of PBS buffer pH 7.4 was added to the flask and the solution was kept for stirring for 24 h to facilitate the removal of acetone. Following this, the volume of the solution was then adjusted to result in PEG-4-L micelles of 5 mM concentration.

Steady-state fluorescence measurements For the determination of critical micellar concentration (CMC), surfactant solutions with concentrations ranging from 0.1-30 mM were prepared using a surfactant stock solution of 50 mM. 1 µL of 10 mM DPH was added to each of the surfactant solutions. 0.2 mL of the surfactant solution was added to a 10/2 mm pathlength cuvette and the fluorescence emission spectrum was recorded using excitation and emission slit widths of 1 and 1.5 nm, respectively. The spectra were recorded using Shimadzu RF-6000 and Fluormax-4 (Horiba Jobin Yvon) spectrophotometers.

Determination of critical micellar concentration (CMC) The CMC of the surfactant was determined using a previously reported procedure with slight modifications.[2] Using a 50 mM stock solution of PEG-4-L micelles, aqueous solutions of the surfactant were prepared with concentrations varying from 0.1 mM to 30 mM. A weighed amount of DPH was dissolved in THF to reach a concentration of 10 mM. 1 µL of the DPH stock solution was added to the 0.5 mL aqueous solutions of the surfactant. The solutions were then kept in dark for 2-3 h to reverse any photoisomerization of DPH. The fluorescence emission spectra of the aqueous solutions were recorded at an excitation wavelength of 358 nm using excitation and emission slit widths of 1.5 and 1 nm, respectively using Shimadzu RF-6000 fluorescence spectrophotometer. The peak intensity of the emission spectra at 430 nm was then plotted as a function of the surfactant concentration. A straight line was drawn to connect the intensity values in the low concentration regime (slowly increasing). Another line was drawn through the intensities obtained at a high concentration regime (rapidly increasing). The intersection point of the two lines was noted as the CMC. The same procedure was repeated at least 3 times and the concentration at the intersection point was found to be ~3 mM.

Preparation of LC droplets LC in water microdroplets were prepared using a slight modification of the procedure reported earlier.[3] 1 µL of PEG-4-L/5CB or LA/5CB was emulsified in 100 µL 10 mM PBS by vortex mixing for 1 min. For experiments with the aqueous surfactant, 1 µL of 5CB was vortexed with 100 µL aqueous surfactant or surfactant incubated with lipase. The size of the droplets was measured using ImageJ software. The droplets were found to be polydisperse with diameters ranging from 2-50 µm. 2.5 µL of the resulting emulsion was added to a glass microscopic slide and polarized optical micrographs were captured using a 20x objective. For the UV-visible and fluorescence spectroscopy, 1 µL of 5CB was emulsified in 200 µL of the aqueous surfactant with or without lipase.

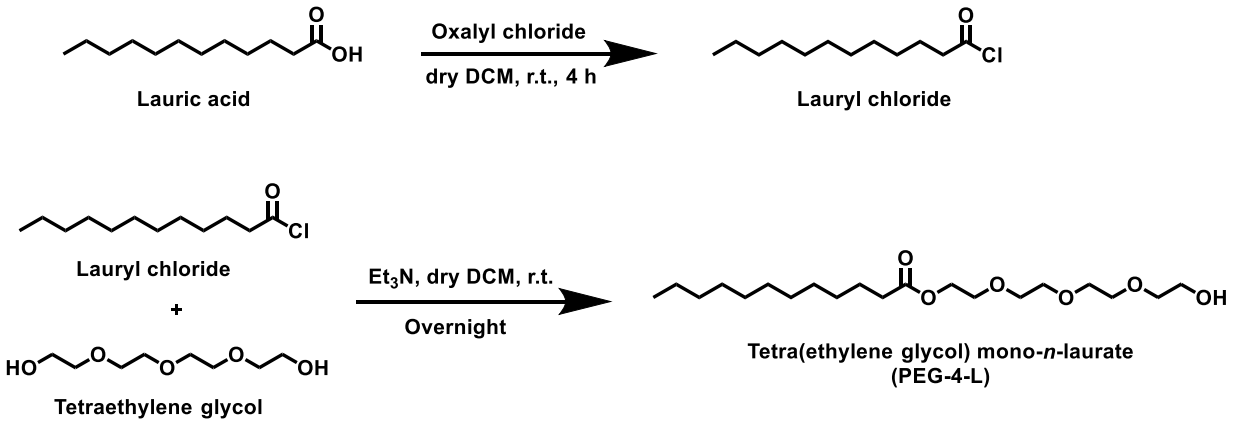
Dye encapsulation experiments A calculated amount of PEG-4-L was weighed in a round bottom flask to reach a concentration of 10 mM in 5 mL PBS. To this, the required volume of 1 mg/mL Nile Red (NR) in HPLC acetone was added to make 100 µM of the dye, keeping dye:surfactant ratio as 1:100. The solution was then kept on stirring for 24 h to remove acetone. The encapsulation of the dye in the surfactant micelles was confirmed by recording the absorption spectra of the

surfactant micelles. The absorption spectra shown in Fig. S12 for the NR-encapsulated surfactant micelles and surfactant-decorated LC droplets show an absorption peak at 536 nm. The emission spectra were then recorded by exciting the sample at 536 nm (absorption maxima obtained from the UV spectra). The intensities of the emission spectra of NR at 630 nm were then plotted versus time for both the samples using OriginPro 2021b.

Fourier Transform Infrared (FT-IR) Spectroscopy Fourier transform infrared (FT-IR) spectra were recorded on Perkin-Elmer Spectrum Two in the range of 600 to 4000 cm^{-1}.

NMR Spectroscopy ^{1}H NMR and ^{13}C NMR of the surfactant were recorded in Bruker Biospin Switzerland Avance-iii 400 and 100 MHz spectrometers, respectively. For recording the samples, methanol-d_4 was used as a solvent.

Synthesis of tetra(ethylene glycol) mono-*n*-laurate (PEG-4-L)



Scheme S1. Synthetic route of PEG-4-L.

Synthesis of PEG-4-L This was synthesized according to the previously reported method with some modifications.[4] In a 50 mL round bottom flask, oxalyl chloride (25 mmol, 2.1 mL) was added to the lauric acid solution (1 g, 5 mmol) in 20 mL dry DCM. After stirring the reaction mixture for 4 h at room temperature, the mixture was completely dried under a vacuum. Then, this acyl chloride was dissolved in 2 mL dry DCM and added dropwise to the solution containing tetraethylene glycol (17.4 mmol, 3.0 mL) and triethylamine (5 mmol, 0.70 mL) in 20 mL dry DCM at 0 ºC under an inert atmosphere. The reaction mixture was stirred overnight. After completion of the reaction, the mixture was washed with 1 N HCl and water. The organic layer was separated and concentrated under a rotatory evaporator. The crude product was purified by column chromatography using hexane:ethyl acetate (1:9) as an eluent to afford the product as a pale-yellow oil (yield 74%). ^{1}H NMR (400 MHz, methanol-d_4): δ 4.23 (2H, t, J = 4.0 Hz), 3.73-3.57 (14H, m), 2.35 (2H, t, J = 8.0 Hz), 1.65-1.61 (2H, m), 1.37-1.32 (16H, m), 0.94-0.91 (3H, m). ^{13}C NMR (100 MHz, methanol-d_4): δ 173.75, 72.35, 70.29, 70.20, 70.14, 70.05, 68.80, 63.19, 60.86, 33.64, 31.80, 29.50, 29.38, 29.23, 29.18, 28.92, 28.88, 24.72, 22.48, 13.35. FT-IR (cm^{-1}): 3389.83, 2924.78, 2855.84, 1735.58, 1647.71, 1457.22, 1106.78.

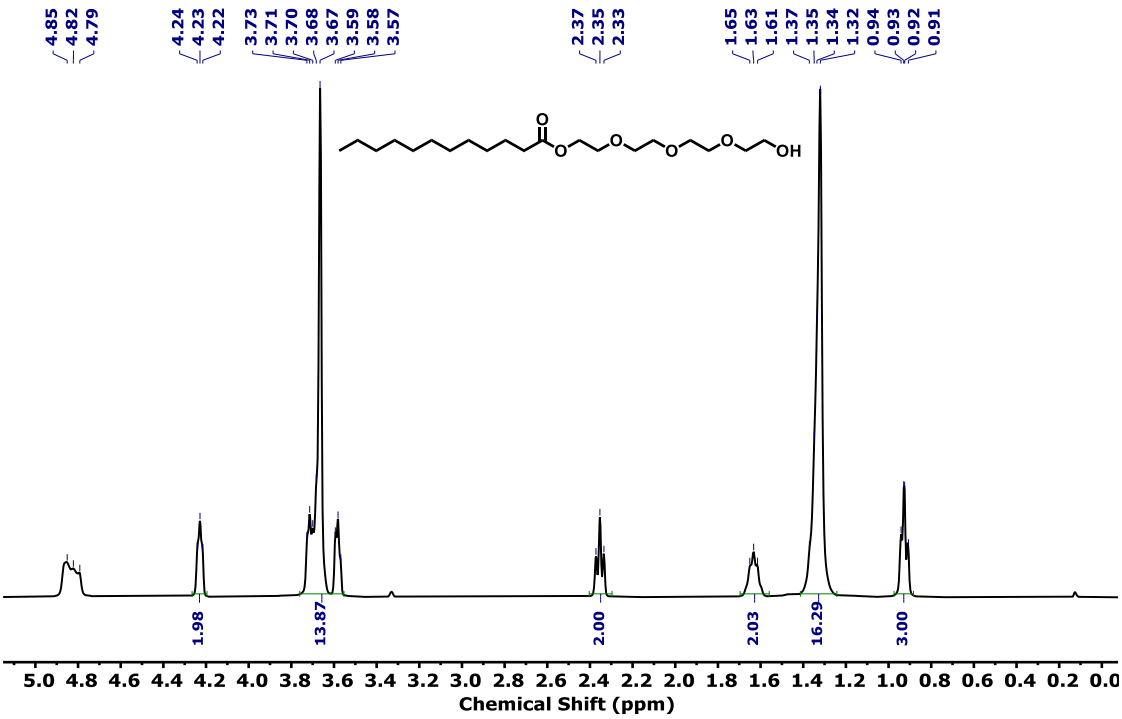


Fig. S1 The ^{1}H NMR spectrum (400 MHz, methanol-d_4) of PEG-4-L.

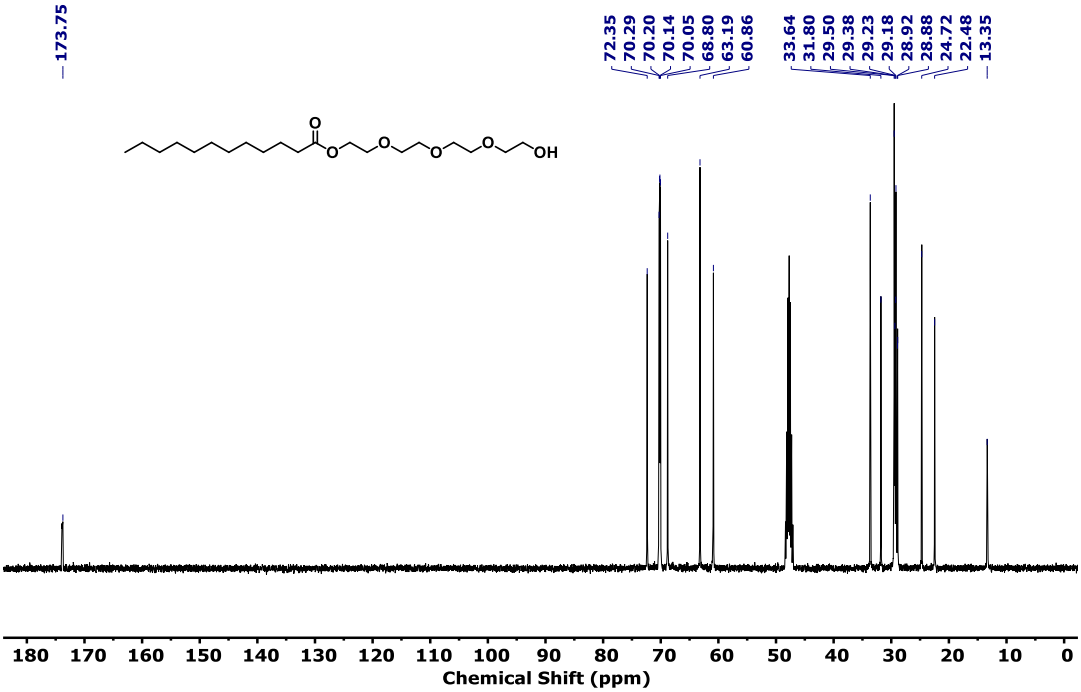


Fig. S2 The ^{13}C NMR spectrum (100 MHz, methanol-d_4) of PEG-4-L.

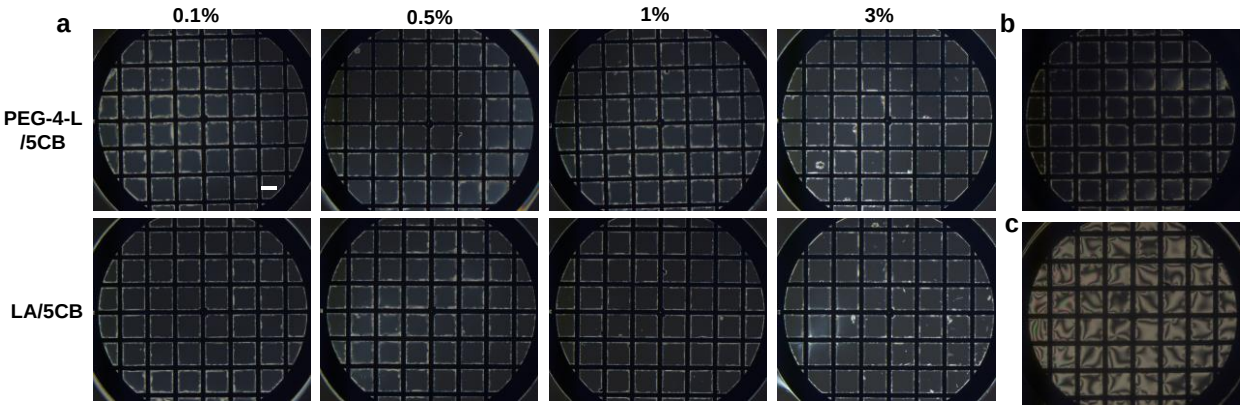


Fig. S3 (a) Polarized optical micrographs of PEG-4-L/5CB and LA/5CB doped films at LC/air interfaces. At all the concentrations (0.1, 0.5, 1, 3%) investigated, the LC film shows a homeotropic anchoring as observed with (b) pure 5CB at LC/air interface. (c) Planar/tilted alignment of LC observed with pure 5CB at the aqueous interface. The scale bar is 200 µm.

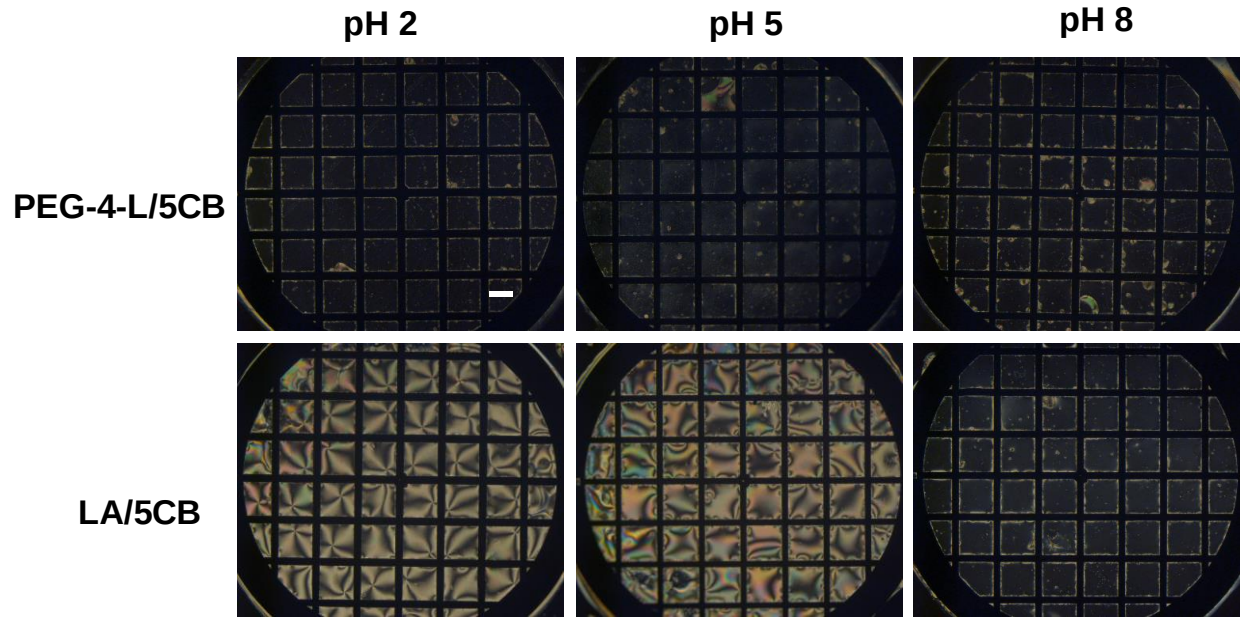


Fig. S4 Polarized optical micrographs of LC films doped with 0.5% PEG-4-L and LA in 5CB in contact with aqueous PBS at pH 2, 5, and 8. The homeotropic orientation of LC was retained at all pH in the case of 0.5% PEG-4-L/5CB. The scale bar is 200 µm.

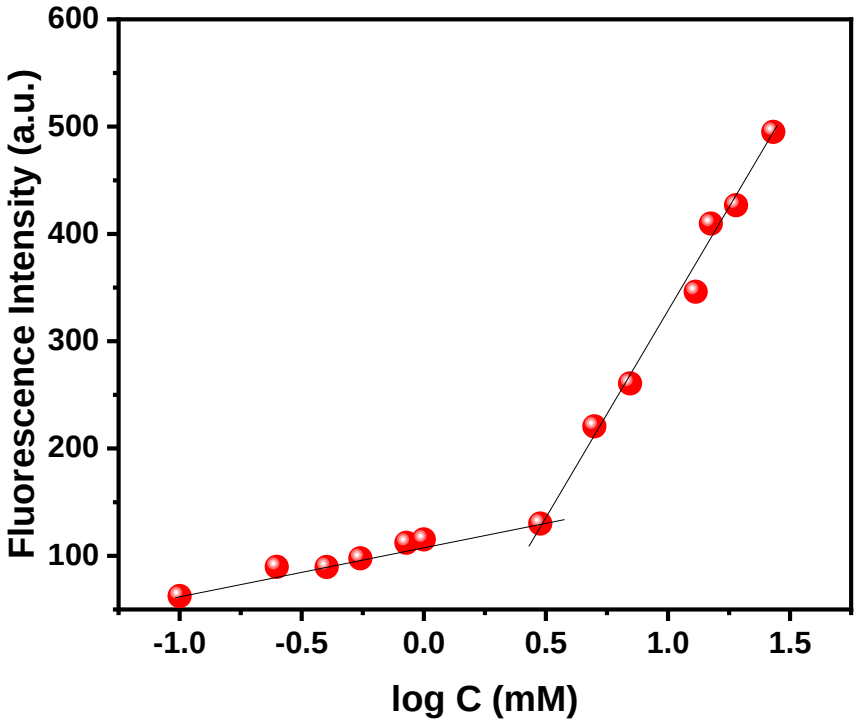


Fig. S5 Plot showing the fluorescence intensities of DPH versus log (concentration in mM) of the surfactant. The intersection of two lines drawn through the intensities obtained at low and high concentrations of the surfactant gives the CMC of PEG-4-L. The intensities (at the emission wavelength of 430 nm) in the plot were obtained by exciting the sample at 358 nm.

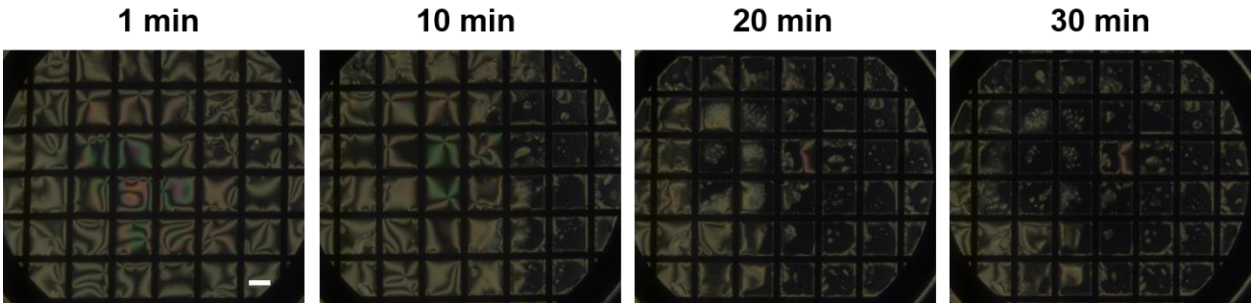


Fig. S6 Dynamic ordering transition of LC upon addition of a pre-incubated mixture of 0.5 mM PEG-4-L and 1 µM deactivated lipase. The lipase was deactivated by heating at 80 °C for 4 h. The incubation time was 2 h. The scale bar is 200 µm.

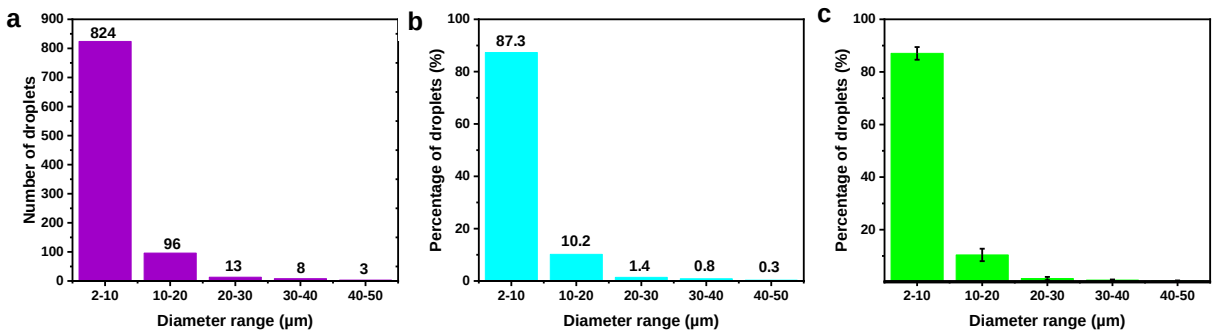


Fig. S7 Histograms showing the (a) total number and (b) the percentage of droplets in each diameter range of 944 PEG-4-L-decorated LC droplets from 3 representative polarized optical micrographs. (c) The average and standard deviation of the percentage of droplet population in each diameter range was calculated from 3 representative optical micrographs. The concentration of PEG-4-L was 0.5 mM.

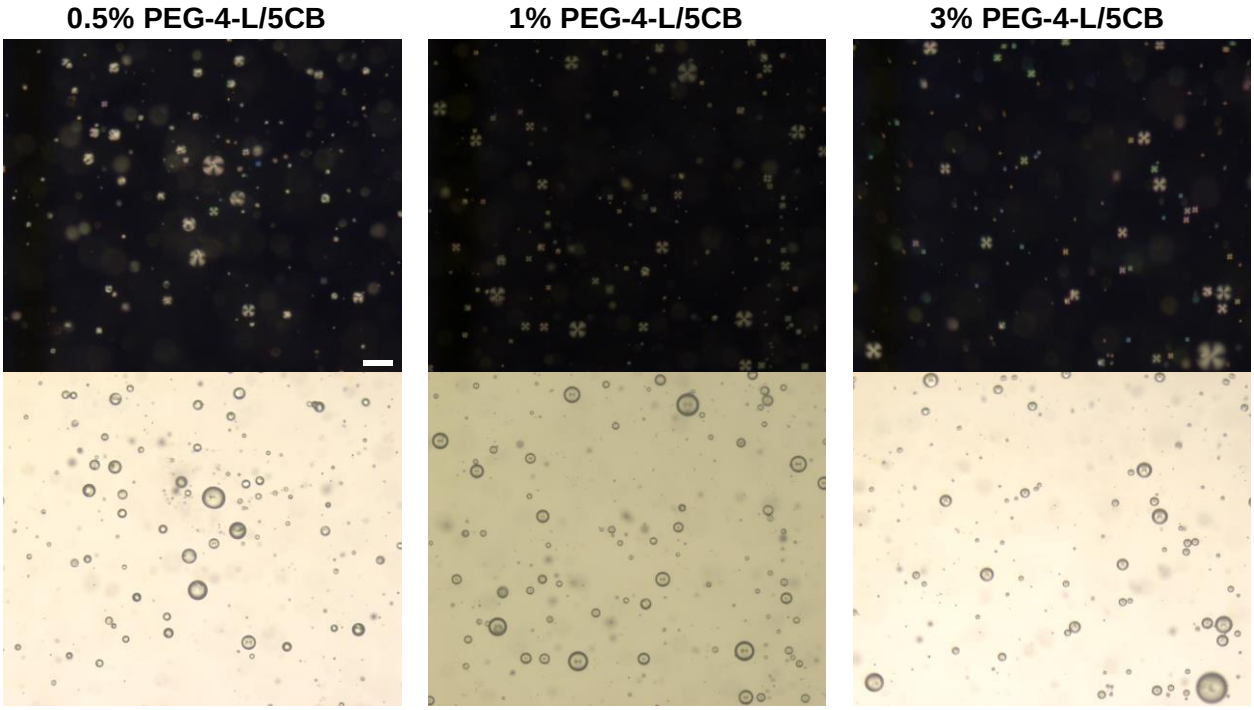


Fig. S8 Polarized light and bright-field micrographs of 0.5, 1, and 3% PEG-4-L/5CB droplets in aqueous PBS (pH 7.4). The upper and lower panels show polarized light and bright-field micrographs, respectively. The scale bar is 50 μm.

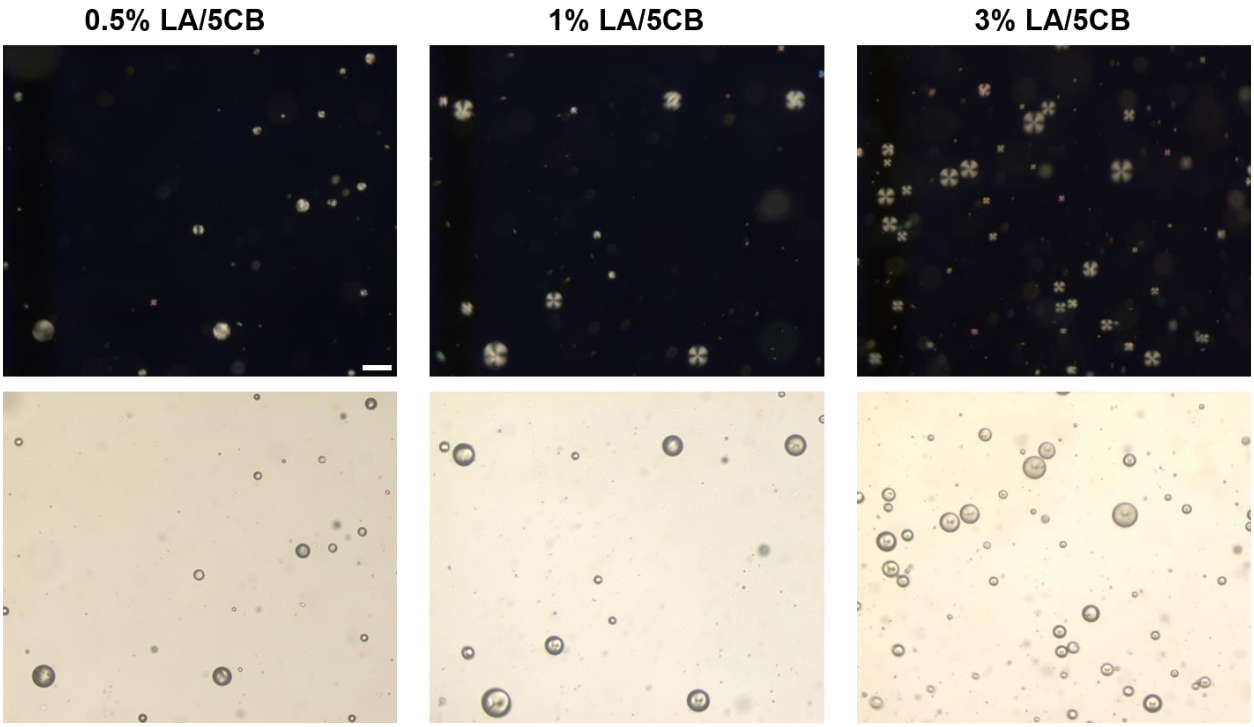


Fig. S9 Polarized light and bright-field micrographs of 0.5, 1, and 3% LA/5CB droplets in aqueous PBS (pH 7.4). The upper and lower panels show polarized light and bright-field micrographs, respectively. The scale bar is 50 µm.

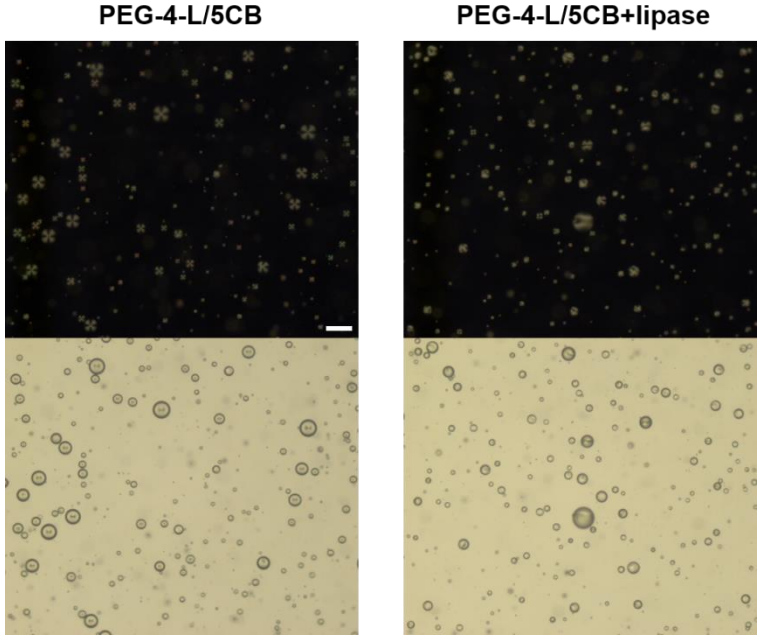


Fig. S10 Polarized light and bright-field micrographs of 1% PEG-4-L/5CB droplets before and after the addition of 1 µM lipase. The radial configuration of droplets in presence of 1% PEG-4-L/5CB changed to intermediate configurations upon the addition of lipase. The upper and lower panels show polarized light and bright-field micrographs, respectively. The scale bar is 50 µm.

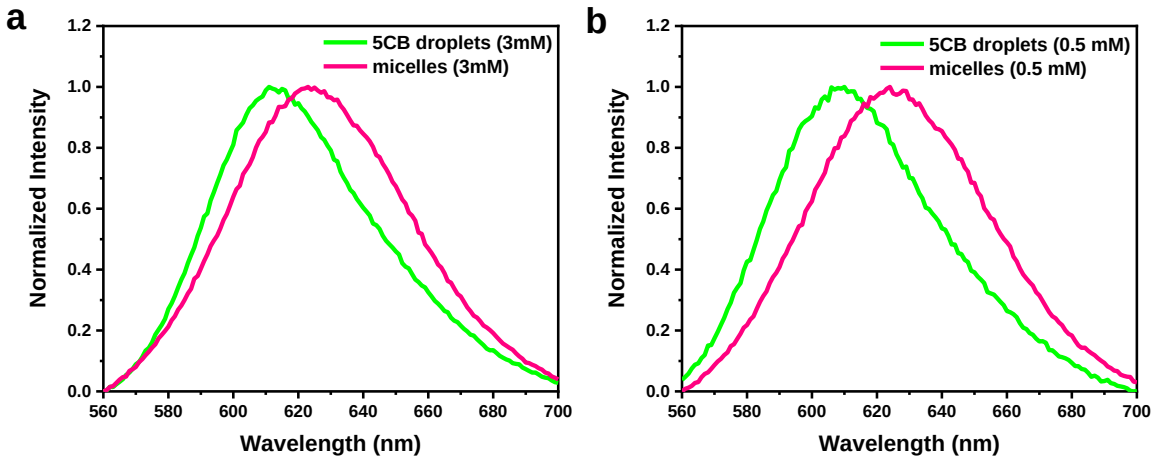


Fig. S11 (a) 3 mM NR-encapsulated surfactant micelles and 5CB droplets decorated with 3 mM surfactant, (b) 0.5 mM NR-encapsulated aqueous surfactant and 5CB droplets decorated with 0.5 mM surfactant. A blue shift is observed in the emission spectra in presence of the 5CB droplets at both concentrations.

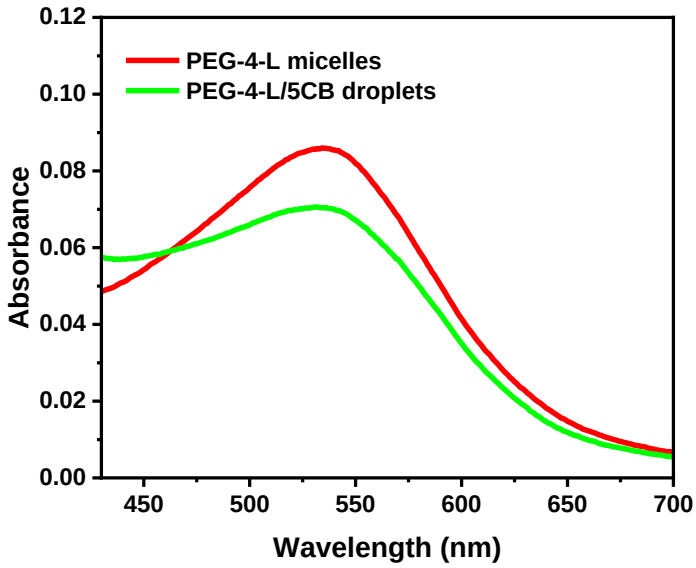


Fig. S12 Absorption spectra of PEG-4-L micelles and PEG-4-L-decorated 5CB droplets. The concentration of PEG-4-L was 5 mM. The appearance of a peak with λ_{max} = 536 nm indicates that the dye is present in a hydrophobic environment.

References

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