Soft Matter

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Cite this: DOI: 10.1039/c0xx00000x

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A Comparison of Detergent Action on Supported Lipid Monolayers and Bilayers

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

1. Detailed Experimental Methods.

1.1. Materials. 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), and GM1 Ganglioside were purchased from Avanti Polar Lipids (Birmingham, AL). Texas Red 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (TR) and 1,2 dihexadecanoyl-sn-glycero-3-phospho ethanolamine, triethylammonium salt (NBD-PE) were obtained from Molecular Probes (Eugene, Or). Triton X-100 for Molecular Biology, Cholera Toxin B subunit FITC (CTB) and Octadecyltrichloro-silane (OTS), 90+% were purchased from Sigma Aldrich (Saint Louis, MO). Toluene, Hydrogen peroxide, Chloroform, and Acetone were from Fisher Scientific (Fair Lawn, NJ); Sulfuric acid was obtained from EMD Chemicals. All chemicals were used without further purification. Dulbecco Phosphate Buffered Saline 1X (DPBS) without Calcium chloride and without Magnesium chloride was purchased from Gibco (Grand Island, NY, USA).

1.2. Sample Preparations.

1.2.1. Substrate preparation. The substrates (No. 1 Corning glass coverslips) were cleaned for 5 min with piranha etch, a 4:1 mixture of sulfuric acid and hydrogen peroxide heated to 100 ^{o}C, to remove any organic residue on the surface. *(Caution: this mixture reacts violently with organic materials and must be handled with extreme care).* The substrates were then washed copiously with deionized (18 mΩ-cm) water and dried under a stream of N_2. All cleaned, oxidized substrates were used within 1 day of the pretreatment.

1.2.2. Preparation of Patterned Lipid Monolayer/Bilayer Surfaces. Patterned lipid morphologies displaying pre-defined spatial regions of lipid monolayers and bilayers were prepared via previously reported vesicle fusion onto patterned wettability substrates.

UV Photolithography of Octadecyltrichorosilane Monolayers. We begin with the preparation of patterned wettability substrates by first derivatizing glass substrates using *n*-octadecyltricholorosilane (OTS). All freshly oxidized, coverglass substrates were immersed in a 50 ml self-assembly solution consisting of 2.5 mM octadecyltrichlorosilane (CH3(CH2)17SiCl3, OTS) (90% Aldrich) solution in anhydrous hexadecane (99% Sigma-Aldrich)[1]. The substrates were allowed to incubate with the self-assembly solution for approximately 45 min. All silanization reactions were carried out in glass containers under nominally dry ambient conditions (relative humidity < 20%). After removal from the self-assembly solution, the film-covered wafers were washed extensively with chloroform under ultrasonic conditions to remove all excess reactants. Silanized samples were used within a few days of preparation. Contact angles of OTS derivatized samples were at least 110° by water corresponding to low surface energy (~21 D/cm) of the surface[2].

Spatial patterning of *n*-octadecyltrichlorosilane (OTS) covered substrates was achieved using shortwavelength UV radiation[3, 4]. In particular, spatially directed photoillumination of monolayer samples was achieved using a physical mask and an ozone-generating UV lamp[5]. The masks displaying patterns of chrome over quartz substrate were either acquired from Photoscience, Inc. (Torrance, CA) or produced at the UC Davis Microfabrication Facility. On selective masks, the edges of the chrome were deliberately roughened by over-exposure of the protective photoresist layer prior to etching of the chrome. UV radiation was produced using a medium pressure Hg-discharge grid lamp (UVP, Inc., Upland, CA) in a quartz envelope and maintained in a closed chamber in a chemical hood. The samples were placed in contact with the photomask and positioned approximately 0.5-2 mm from the light source depending on the illumination geometry. (*Caution: direct exposure to short-wavelength UV light (187 nm-254 nm) must be avoided, and appropriate eyewear must be worn*). Care must be taken in venting the ozone by operating grid lamps under chemical hoods. The breathing of ozone in high concentrations is dangerous. Ozone concentration in excess of 0.1 ppm can cause irritation.) The exposure period was approximately 40-60 min depending on the exposure geometry (sample-lamp distance) and the age of the lamp. Following the exposure, the mask was separated from the

substrate surface, samples rinsed thoroughly using water, chloroform, and ethanol, and dried with nitrogen. Patterned OTS samples were used within 24 h of preparation.

Vesicle fusion on Patterned Surfaces. Supported phospholipid bilayers were formed primarily using previously reported vesicle fusion and rupture method[6, 7]. Briefly, small unilamellar vesicles (SUVs) were prepared using vesicle extrusion methods [8]. Typically, a desired amount of lipid or lipid mixtures suspended in chloroform or chloroform/methanol mixtures were mixed in a glass vial. Dye concentrations were 1 mol % for stocks containing Texas. The solvent phase was then evaporated under a stream of nitrogen and subsequently evacuated for at least 1 h in a vacuum dessicator. The dried lipid mixture was then suspended in Millipore water and kept at 4°C to be rehydrated overnight. The total lipid concentration was 2 mg/ml. The desired amount of hydrated aqueous solution was then sonicated and passed through a Avanti Mini-Extruder (Avanti, Alabaster, AL) using 0.1 um polycarbonate membrane filters (Avanti, Alabaster, AL) 21 times at a desired temperature (typically 10 °C above the transition temperature). One part of the resulting SUV solutions was diluted with one part of PBS and kept above the T_m until used. Vesicles were used within a few hours of extrusion. Vesicle spreading was carried out by placing either patterned OTS substrates over a 40µL SUV solution drop placed at the bottom of a plastic petri dish. The samples were allowed to incubate for 10-20 min to ensure equilibrium coverage. The Petri dish was then filled with water and transferred to a large reservoir of water in which the substrate was shaken gently to remove excess lipids. Supported bilayer samples prepared in this way were then stored in deionized water or PBS buffer for further characterization.

1.3. Triton X - 100 Treatment.

Triton X-100 treatment was carried out at room temperature to examine the detergent solubility on monolayer and bilayer. The samples comprising POPC-TR or DMPC-TR were treated with non-ionic detergent Triton X-100 (density, 1.07 g/mL; mol Wt., 625; CMC, 0.24 mM) at different concentrations spanning CMC including 0.07, 0.17, 0.34, 0.51, 3.4, and 17 mM in water. After 5 to 10 minutes incubation in Triton x-100, selected samples were rinse in water for backfilling experiment.

1.4. Gm1-Cholera Toxin Binding Assay.

To determine the distribution of Gm1 in patterned bilayer / monolayer samples, a Gm1-CTB binding assay was performed as previously described[9]. Commercially acquired FITC-labeled CTB (sub-unit MW: 12 kDa) was diluted to 0.5 mg/mL in PBS (which contains 20% protein to give a protein concentration of 0.1 mg/mL). 10 uL of CTB solution was then introduced into 5 mL aqueous PBS phase of the micropatterned bilayer. The final CTB concentration was 416 x 10^{-9} M for B_5 sub-units. After approximately 45 min of incubation, the sample was rinsed several times with 1X PBS.

1.5. Epifluorescence Microscopy.

A Nikon Eclipse TE2000-S inverted fluorescence microscope (Technical Instruments, Burlingame, CA) equipped with a Roper Cool Snap camera (Technical Instruments) and a Hg lamp as the light source was used to visualize all fluorescent samples. Filter cubes were used to filter absorption and emission to the source and the CCD camera, respectively. Typically, images were taken using either a Plan Fluor 10X (NA, 0.25) or a Plan Fluor, ELWD, 20X (NA 0.45) objective (Nikon,Japan). Images were stored and processed using Matlab6 and a simple PCI software (Compix, Inc., Cranberry Township, PA) augmented with a quantitative dynamic intensity analysis module. Excitation and emission maxima for the probes used were 583/601 nm for TR-DHPE. Fluorescence images were taken every 2 mins at 0.4 sec to 0.6 sec exposure time.

1.6 Fluorescence Recovery after Photobleaching (FRAP).

Membrane at room temperature was measured using the popular microscopy based FRAP technique. Specifically, we used microscopy-based fluorescence photobleach recovery measurements by adapting the circular spot photobleaching method[10, 11]. Here, a circular region of the fluorescent bilayer sample, ~ 30-50 um diameter, was illuminated at high power continuously at the excitation wavelength for the fluorophore through a 60X (NA, 0.70) objective for ~ 2min. The exposure bleaches a dark spot on the bilayer caused by the photoexcitation of the fluorophore followed by an irreversible chemical transformation effected by its reaction with oxygen dissolved in the ambient buffer. After photobleaching, the illumination path was replaced by low power observation beam through a 10X objective to record wide-field images of fluorescence recovery in the bleached area at 5 or 30 sec time intervals. The subsequent lateral motion of unperturbed fluorophore-lipids from the unbleached background into the bleached spot (and vice versa) is recorded in the recovery profiles.

It has been previously established that the precise shape of the recovery curve can be used to qualitatively characterize the nature of the fluorophore motion[11]. Furthermore, for diffusion-like motions, the measurements of the time required for the fluorescence intensity to recover halfway ($t_{½}$) between its immediate post-bleach value and its long-time asymptotic value was used to estimate the diffusion coefficient, D, a measure of the fluidity of the lipid environment[12]. Specifically, we adapted a method reported by Axelrod and co-workers which approximates the solution of two-dimensional lateral diffusion equation using a modified Bessel function. Here, the experimental fluorescence intensity versus time data are replotted as reduced intensity versus time. The reduced intensity is given by I (red) = [I (∞) - I(t)]/ [I (∞) - I(0)] where I(t), I(o), and I(∞) correspond to fluorescence intensity at time t after photobleaching, immediately after photobleaching, and long-time asymptotic recovery values, respectively. The recovery curve is then used to estimate diffusion constant, D, and mobile fraction. Note that the exposure time (~ 2 min) required in objective-based photobleaching introduces some, bilayer fluidity-dependent inaccuracies in the measurements of diffusion constants for probe lipids. A stand-

alone code is available for general use and can be obtained by requesting the corresponding author.

1.7. Imaging Ellipsometry

Imaging ellipsometric measurements were performed using a commercial system (Elli 2000, Nanofilm, Göttingen, Germany) at 60° incidence angle at 532 nm using frequency-doubled Nd:YAG laser. The ellipsometer employed the typical PCSA (polarizer-compensator-sample-analyzer) nulling-con_guration in which a linear polarizer (P) and a quarter-wave plate (C) yields an elliptically polarized incident beam. Upon reflection from the sample (S), the beam is gathered via an analyzer (A) and imaged onto a sensitive CCD camera with a 768 × 572 pixel resolution through a long working distance 10X objective. The samples were incubated with Triton X-100 using a home-built fluid cell. Raw spatially-resolved ellipsometric data (Δ and Ψ) were analyzed using classical electromagnetic theory and a 4-phase parallel-slab model to deduce spatial maps of optical thicknesses following our previous reports.[9] The sample model was approximated by semi-infinite slabs of silicon (n = 3.875 + 0.018i) and water (n = 1.33 + 0i) separated by ~2 nm native silicon oxide layer (n = 1.5 + 0i) and the lipid bilayer slab (n = 1.47 + 0i) of variable thickness.

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Electronic Supplementary Material (ESI) for Soft Matter

A comparison of detergent action on supported lipid monolayers and bilayers

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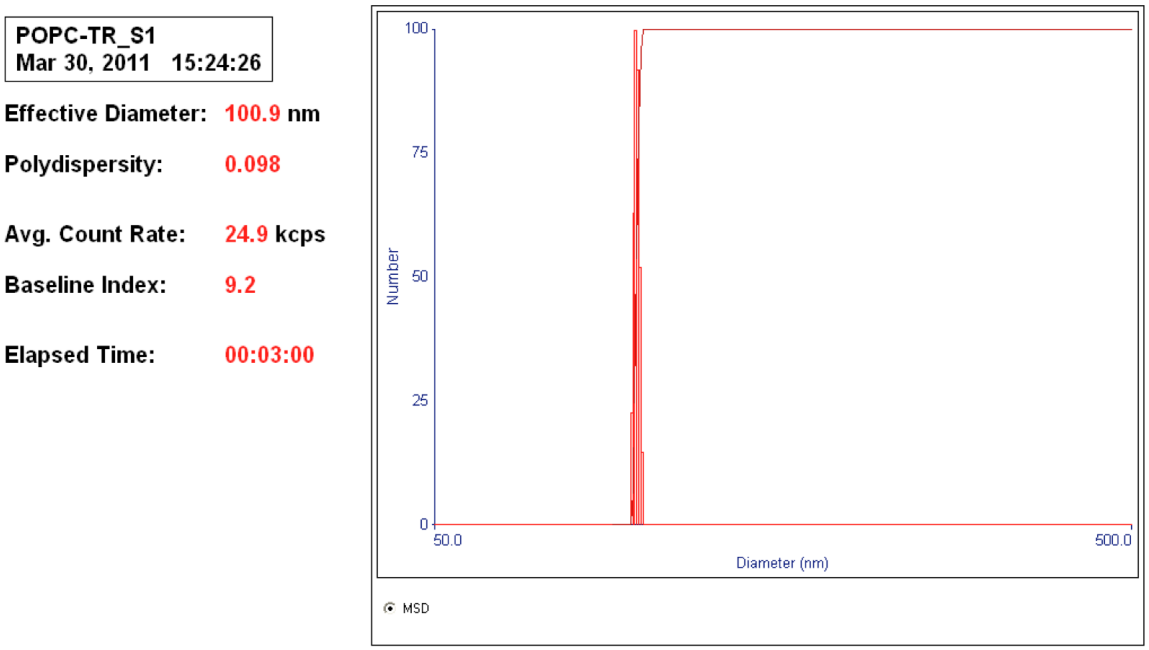

FigureESI 1: Dynamic light scattering characterization of average size of TR-DHPE doped POPC vesicles obtained by extrusion.

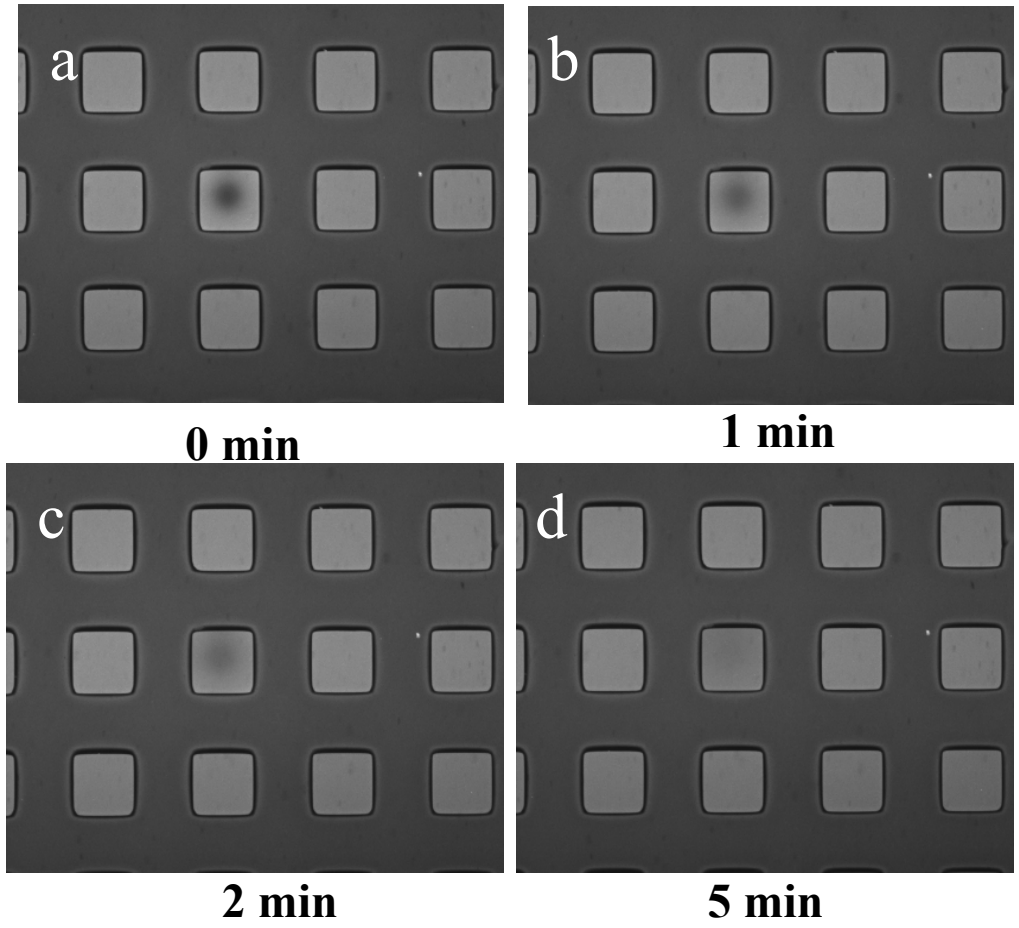

Figure ESI 2: Selected frames from fluoresence photobleaching recovery image sequence for fluidity of the bilayer patch. The lipid composition is 1% Texas red DHPE, 99% POPC. The set of images (a-d) confirm fluidity in the corralled bilayer. Diffusion coefficient D for bilayer for the present case is ~0.6 x 10^{-8} cm^2/s. Between multiple samples (n=5), this value varied averaging at 1.2 ± 0.6 x 10^{-8} cm^2/s

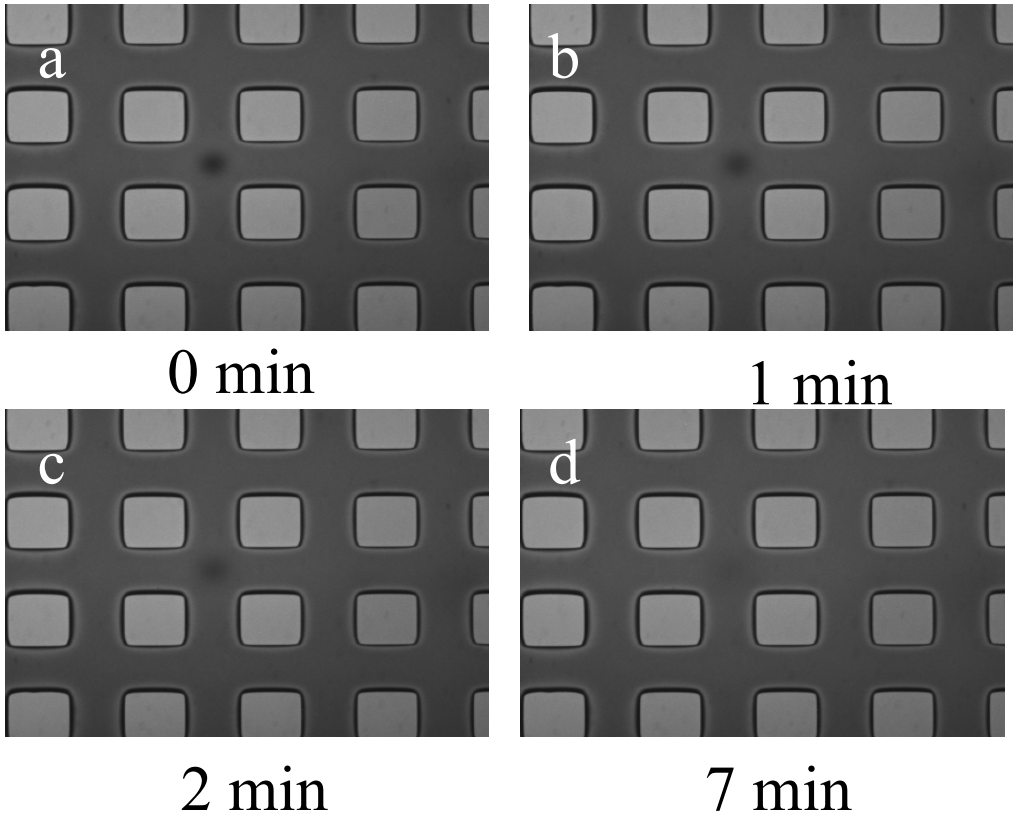

Figure ESI 3: Selected frames from fluoresence photobleaching recovery image sequence for fluidity of monolayer. Lipid here is 1% Texas red DHPE, 99% POPC. The set of images (a-d) confirm fluidity of the monolayer. Probe diffusion coefficient, D, for monolayer is 0.3 x 10^{-8} cm^2/s for this sample. Between multiple samples (n=5), this value varied averaging at 0.6 ± 0.4 x 10^{-8} cm^2/s

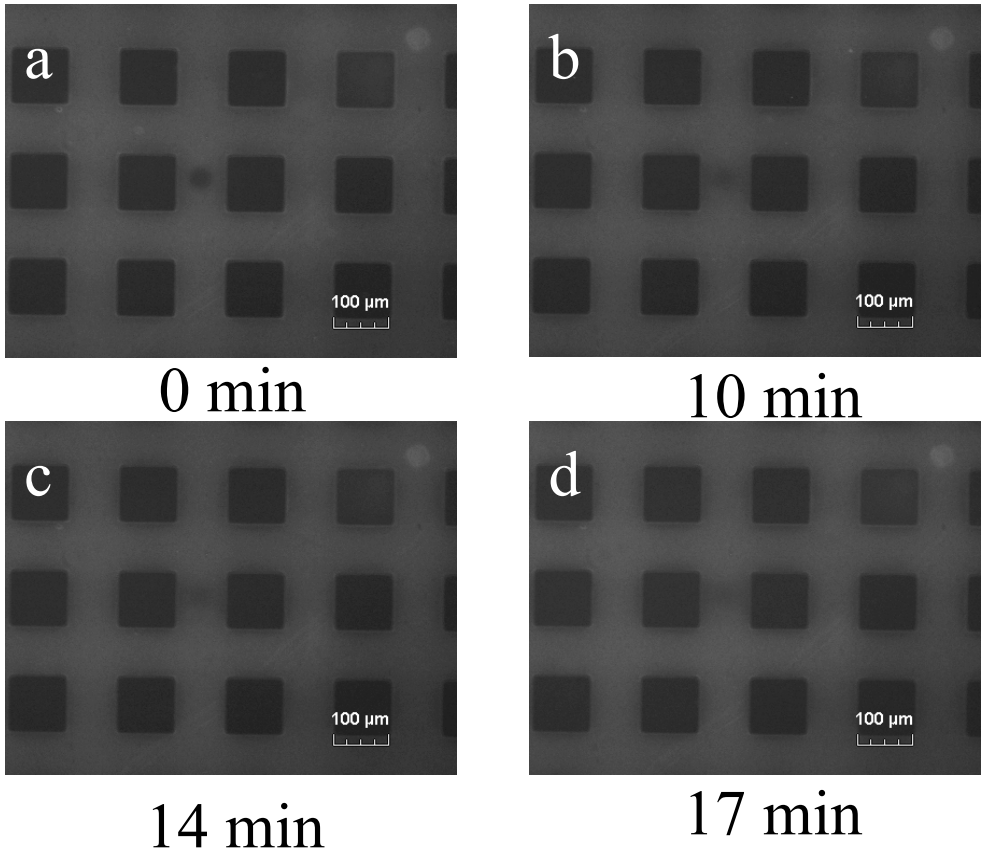

Figure ESI4: Fluidity of Monolayer after triton X 100 treatment at 0.34 mM concentration. Templated membrane array was exposed to Triton X 100, which resulted in removal of bilayer, leaving the monolayer intact. Sequence of images from (a-d) confirm the fluidity of monolayer left behind. Lipid here is 1% Texas red DHPE, 99% POPC. Monolayer fluidity measures at $0.5 10^{-8}$ cm^2/s for this sample.

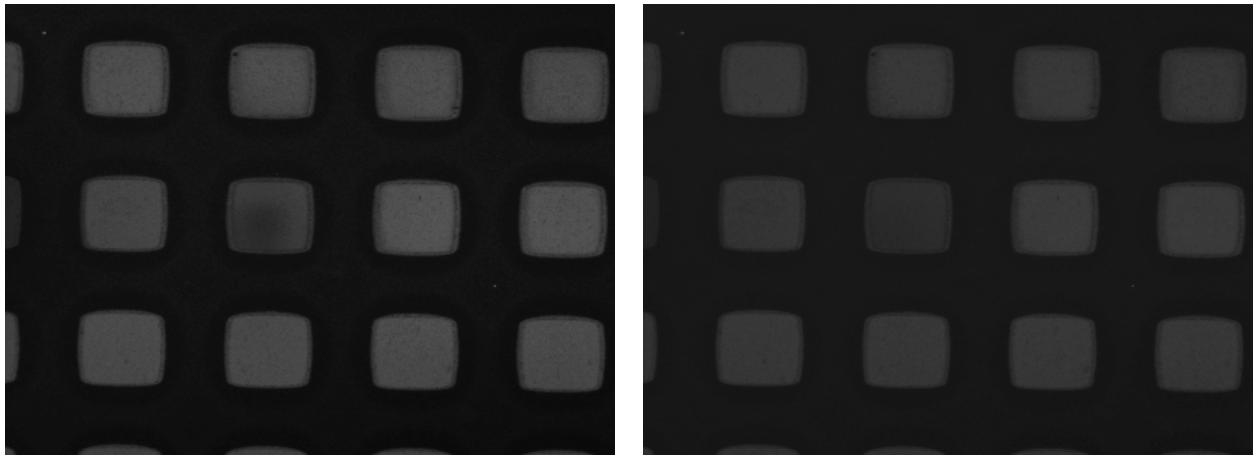

Figure ESI5: Fluidity of Backfilled bilayer: Templated membrane array was exposed to Triton X 100 at 0.34 mM concentration, which resulted in removal of bilayer, leaving the monolayer intact. And this substrate was subsequently backfilled with SUVs consisting of 3% NBD 97% POPC. Lipid here is 3% NBD DHPE, 97% POPC. The images (a) and (b) confirm fluidity (1.3 x 10^{-8} cm^2/s) of the backfilled bilayer.

Figure ESI6: Movie of time-lapse epifluorescence images of real-time measurements of Triton X-100 (0.17 mM) induced changes in fluorescence, 0-475 sec) of patterned lipid mono/bilayer sample consisting of POPC lipid (99 mol%) containing fluorescently-labeled Texas-Red DHPE probe (1 Mol%)] Movie attached separately.

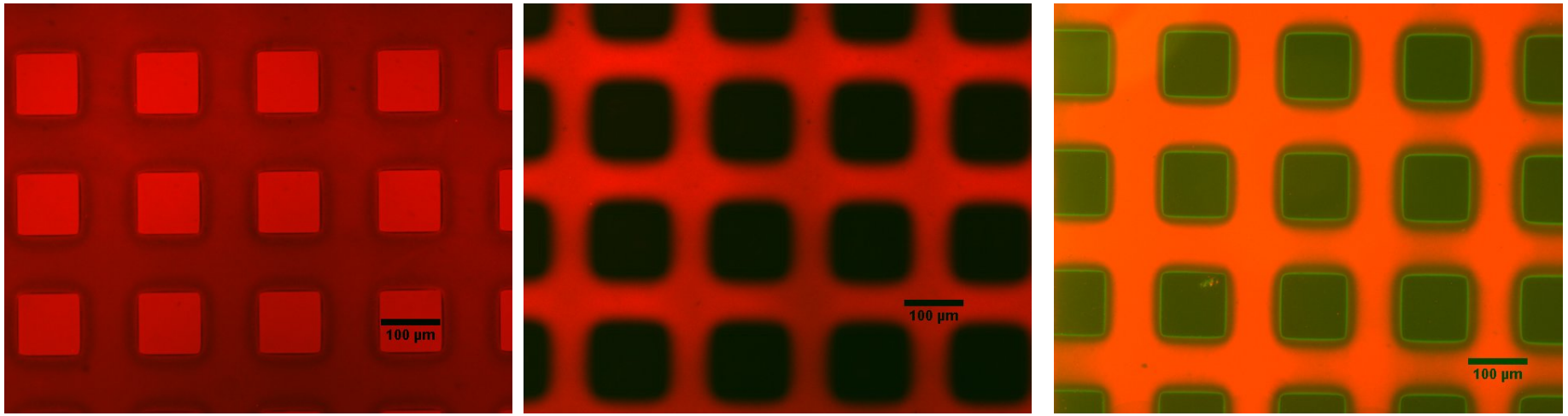

Figure ESI7: Epifluorescence images of (left) lipid monolayer/bilayer construct obtained by fusion of DMPC vesicles doped with 1 mol % Texas-red DHPE with patterned OTS surfaces; (middle) the same obtained after incubation with Triton X-100 (0.34 mM) revealing patterned dissolution of lipid bilayers; and (right) obtained after fusion of POPC/GM1 (99/1 molar ratio) and incubation with FITC-labeled cholera toxin (Concentration 416 x 10^{-9} M)