Electronic Supplementary Information

Orientation of Lipid Domains in Giant Vesicles Using an Electric Field

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General. Aqueous solutions were prepared from deionized water (Barnstead Type D4700 NANOpure Analytical Deionization System with ORGANICfree cartridge) registering >18.0 MΩ-cm resistance. Phospholipids were purchased from Avanti Polar Lipids (Alabaster, AL). The lipid dye β-BODIPY® 530/550 C_5-HPC was purchased from Invitrogen (Carlsbad, CA). All solvents and chemicals were purchased from either Fisher Scientific (Pittsburg, PA) or Sigma-Aldrich (St. Louis, MO). Synthetic procedure and characterization of 1,2-distearyl-rac-glycero-3-triethyleneglycyliminodiacetic acid (DSIDA) can be found in the literature.[1]Tris-HCl (10 mM) buffer was adjusted to pH 7.4 using 10 mMTris-base. The osmolarity of the buffer was adjusted to match the osmolarity of the resultant vesicle suspension using a few volume % of 2M glucose. The conductivity of the buffer was measured to be 847 μS/cm, measured using an Oakton Acorn CON 6 conductivity meter (Vernon Hills, IL).

Giant unilamellar vesicles (GUV).GUVs were formed by electroformation according to published protocols,[2] at approximately 70 °C to exceed the highest expected gel transition temperature of the lipid species. For counter-ion binding experiments, vesicles were electroformed in sucrose solution that contained 200 μM EDTA to minimize presence of metal

ions bound to the membrane surface. The osmolarity of the sucrose solution was adjusted to ~350mOsm, as measured on a Fiske One-Ten Osmometer (Norwood, MA).

Glass surface functionality. Microscope cover slips were thoroughly washed with 3% Hellmanex detergent, rinsed liberally with DI water, and then dried at 100 ºC for 1hr. The cover slips were then cleaned in a Piranha bath (25% H_2O_2/H_2SO_4) for 30 minutes. The cover slips were then rinsed with DI water and dried at 100 ºC for 1 hr. Surfacefunctionalizationof the cover slip with the cholesterol tether (Figure S2) followed a general silane treatment and surface grafting procedures (thorough description of the synthesis, characterization, and surface coating of the cholesterol tether molecule is currently under preparation).In brief, the cover slips werefirst immersed in a 20 µM solution of (3-glycidoxypropyl)-trimethoxysilane in tetrahydrofuran (THF) for 24 hrs. After this time, the cover slips were rinsed with THF and dried under a nitrogen stream. The glycidoxy-functionalized surface was then immersed in a THF solution containing the cholesterol tether (3 mM) and 2-(2-amino ethoxy) ethanol (AEE) (30 mM) solution for 2 hrs. The cover slips were then rinsed with THF, dried under a nitrogen stream, and stored under argon.

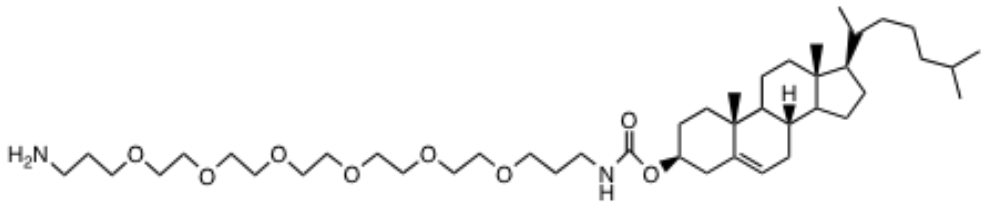

Figure S1. Structure of cholesterol tether molecule.

Microfluidic device. Themicrofluidic device consists of a three-port glass slide manifold and a cover slip (untreated or functionalized with cholesterol tether) sandwiched between a 250 µm

thick silicone membrane (Figure S2). Prior to bonding the manifold and cover slip to the membrane a *Versa*Laser cutter was used to cut a channel away from the center of the membrane to define the fluidic channel. NanoPort assemblies were glued with epoxy to the fluidic ports on the glass manifold to facilitate interconnection to gas-tight syringes. Liquids and vesicles were precisely pumped into the microchannel using a Harvard Apparatus Pump 33 digitally controlled syringe pump (Holliston, MA). Platinum electrodes placed at the inlet and center ports were used to orient the domains on the GUV's, while the third port downstream serves as the fluidic outlet.

Domain orientation experiments. Prior to introducing the GUVs to the microfluidic channel, the device was hydrated by flowing in a buffer solution at a flow rate of 20 µL/min until the entire channel was filled. After ten minutes, a solution of freshly prepared GUVs was thenadded at a flow rate of 5 µL/min for 20 minutes. The pumps were then turned off and the GUVswere allowed to settle to the substrate surface for 15 minutes. Unbound vesicles were then removed by flushing the channel with buffer solution at 5 µL/min. After removal of the unbound vesicles the flow rate was set to zero and an electric field of 1.6 V/cm was applied across the electrodes using a Mastech HY1503C DC power supply (Kwun Tong, Hong Kong).

Fluorescence imaging. Images were taken on a Zeiss 200M Axiovert inverted microscope (Thornwood, NY) in epifluorescence mode using an Olympus UPlanFl 100x oil immersion objective and captured on an AndoriXON EMCCD camera (South Windsor, CT). The images were acquired using open source µManager software.

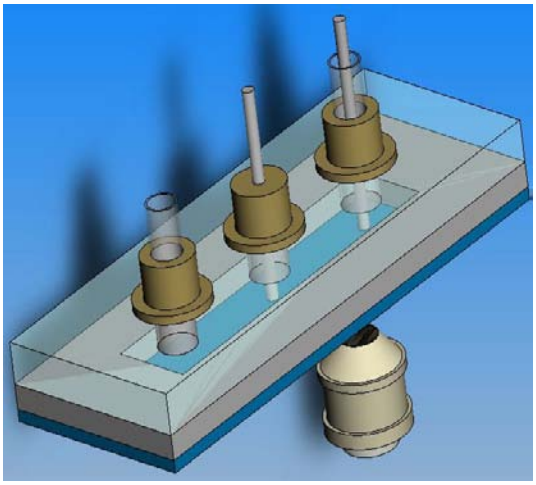

Figure S2. Three portmicrofluidic device consisting of an input port with integrated platinum electrode (far right), the center port contains the second platinum electrode, and the output port (left). Micrograph images of the tethered GUVs are taken between the input and center ports (microscope objective shown below). Figure not drawn to scale.

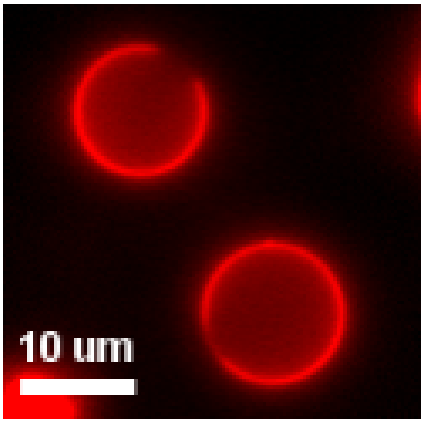

Figure S3. Movie of GUV composed of 15% DSIDA/DPhPC/0.3% β-BODIPY 530/550 HPC in microfluidic device responding to an electric field. The field runs horizontally in the image and was switched several times during the course of the movie.

Figure S4. Movie of GUV composed of 10% DSIDA/DPhPC/0.3% β-BODIPY 530/550 HPC orienting in an electric field of 4 V/cm. Application of the field starts a few seconds after the start of the movie.

References:

1) D. R. Shnek, D. W. Pack, D. Y. Sasaki, F. H. Arnold, *Langmuir* **1994**, *10*, 2382.

2) M. I. Angelova, D. S. Dimitrov, *Farad. Discuss. Chem. Soc.* **1986**, *81*, 303 - 311.