Electronic Supplementary Material (ESI) for Soft Matter

Supporting Information

Tubulation in surface-supported flat unilamellar phospholipid vesicles prepared from biotin headgroup-conjugated lipids.

Methods

Liposome preparation: A 3 µl droplet of a lipid-dye suspension[1] (10 mg/ml), containing 1,2 dioleoyl-3-trimethylammonium-propane (chloride salt) 18:1 TAP DOTAP (20 % w/w) (Avanti Polar Lipids) , soy bean polar lipid extract (78 % w/w) (Avanti Polar Lipids), 1,2-dioleoyl-*sn*-glycero-3 phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) 18:1 Liss Rhod-PE (1% w/w) (Avanti Polar Lipids) and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(cap biotinyl) (sodium salt) 18:1 Biotinyl Cap PE (1% w/w) (Avanti Polar Lipids) was dehydrated for 15 min on a cover slip in an evacuated desiccator. The dry lipid film was subsequently rehydrated with 0.5 ml of 10 mM HEPES buffer (containing 100 mM NaCl, pH 7.8 adjusted with NaOH, Sigma) for 10 min to allow formation of MLVs. MLV samples were transferred into an observation chamber with a SiO_2 cover slip at the bottom, which contained 5 ml of 10mM HEPES buffer. Deionised water was obtained from a Milli-Q system (Millipore).

Surface Fabrication: Glass cover slips (Menzel Gläser) were pre-cleaned by sonication in a megasonic bath, followed by microwave oxygen plasma treatment at 250 W for 2 min (Tepla Plasma Batch System 300, AMO GmbH). SiO2 was deposited onto the cleaned glass substrate by reactive sputtering, using a MS 150 Sputter system (FHR Anlagenbau GmbH), to a final film thickness of 84 nm. Surface quality was confirmed by contact angle measurements (contact angle with water between 0° and 5°).

Microscopy imaging: A confocal laser scanning microscopy scanning (Leica TCS SP2 RS), with a HCX PL APO CS 40x/NA 1.25-0.75 oil objective was used for fluorescence imaging. Texas Red DHPE was excited at 594 nm by a He/Ne laser. The emitted light was collected by a photomultiplier tube in the range

between 600–670 nm. The length of the tubes was recorded with a Hamamatsu C6157 3CCD color camera with in-house programmed acquisition software. A mercury arc lamp with a 540/40 nm band pass filter was used as excitation source for fluorescence imaging with the camera, emission light above 600 nm was used for imaging.

Results

When multilamellar vesicles (MLV) with biotin headgroup-conjugated lipids in a buffer droplet of 10 mM HEPES were placed onto silicon dioxide (SiO_2) substrates in the absence of Ca^{2+}, the vesicles spread instantly as a double bilayer. Tubulation occurs immediately (Figure SI-1), and the tubes remain during the entire observation time. In contrast to the Ca^{2+}-dependent tubulation, nanotubes do not retract during the spreading process (SI movie).

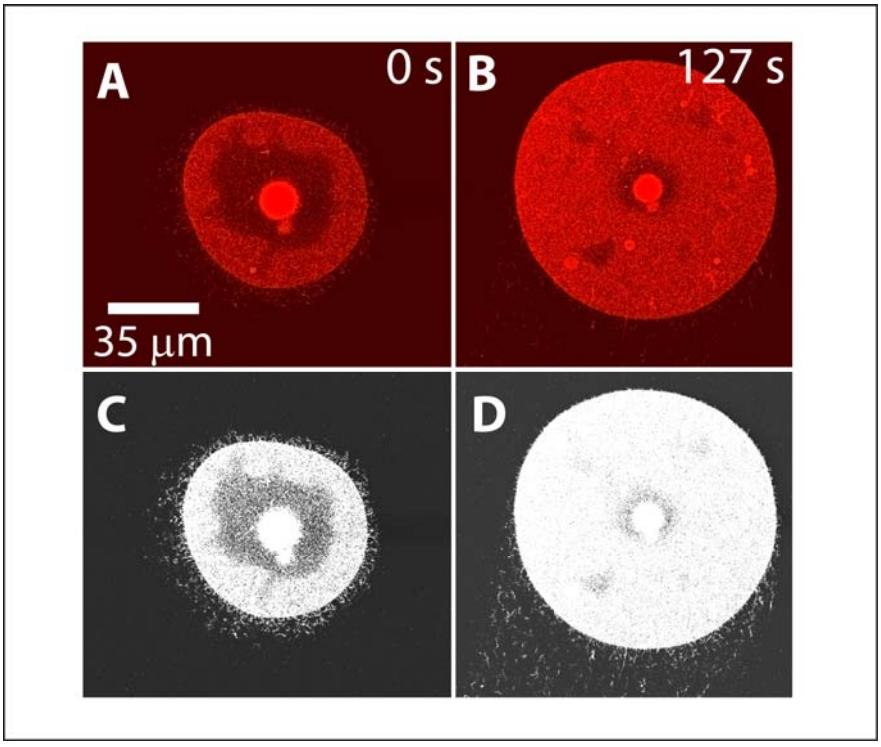

Figure SI-1. **Tubulation in biotinylated, surface supported flat unilamellar phospholipid vesicles.** (A-B) False colored fluorescence micrograph time series of a flat giant unilamellar vesicle (FGUV) with biotin

containing lipids which is displaying transient tubulation over time while circularly spreading on SiO_2. (C-D) Black and white images of (A-B), respectively, to enhance the appearance of the tubes.

Supporting Information References:

1. Karlsson, M.; Nolkrantz, K.; Davidson, M. J.; Stromberg, A.; Ryttsen, F.; Akerman, B.; Orwar, O., Electroinjection of colloid particles and biopolymers into single unilamellar liposomes and cells for bioanalytical applications. *Analytical Chemistry* **2000,** *72* (23), 5857-5862.