

Supporting Information

The tethered patch formation process is shown in Figures S1, schematically, and S2, by microscopy. These figures are reproduced from reference 1 (Chung, M., Lowe, R. D., Chan, Y-H. M., Ganesan, P. V., Boxer, S. G. *J. Struct. Biol.* **2009**, 168, 190-9) and Figure S3 is reproduced from reference 2 (Chan, Y-H. M., vanLengerich, B., Boxer, S. G. *Biointerphases* **2008**, 3(2), p. FA17-FA21) to supplement the description in the text.

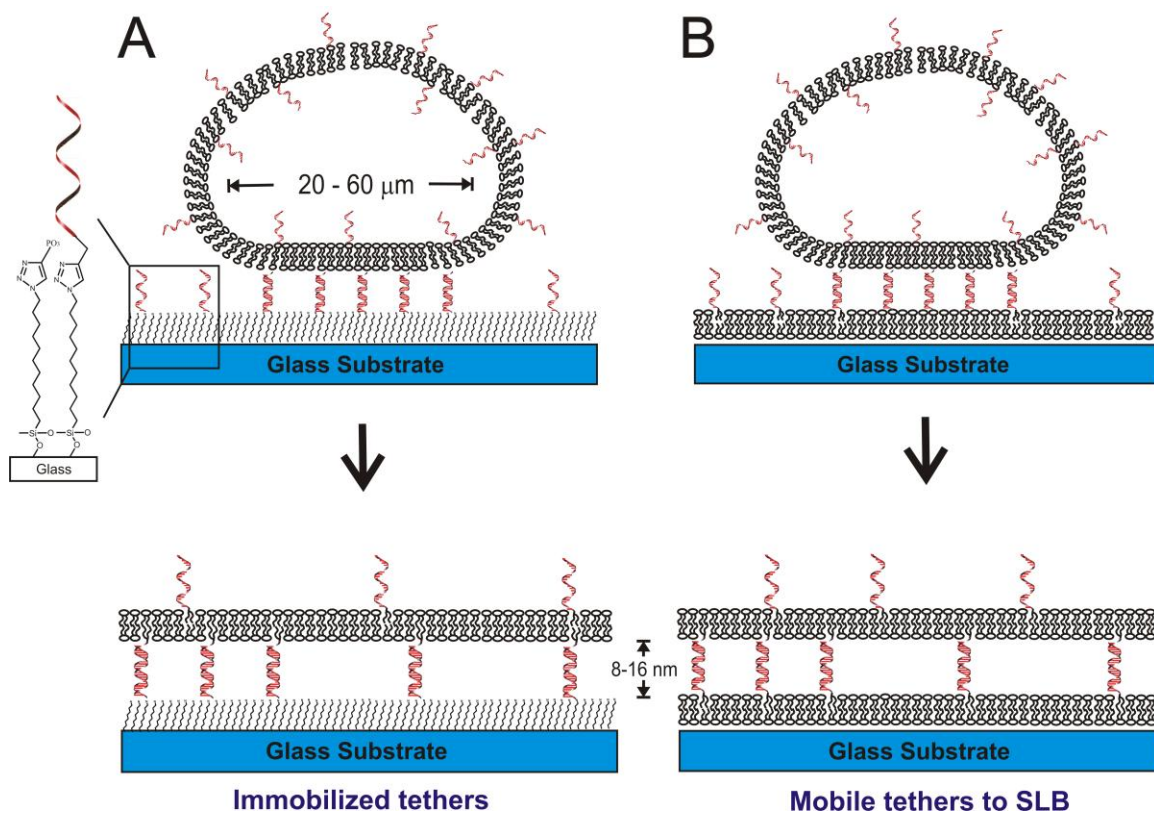


Figure S1: Schematic diagram of DNA-tethered membrane formation by GUV rupture onto (A) self-assembled alkylsilane monolayers, inset for details; and (B) supported lipid bilayers. The GUV membrane contains DNA-linked lipids, which mediate vesicle binding and rupture. In (A), the glass substrate is functionalized with an azide-terminated alkyl-siloxane monolayer to which

complementary DNAs are immobilized via the click reaction. The distance from the substrate is controllable by adjusting the DNA length, about 8 nm for 24mer and 16 nm for 48mer DNA. In (B), a supported lipid bilayer presenting DNA is first formed on the glass substrate, and then exposed to GUVs presenting the complementary DNA to form a DNA-tethered membrane. In both cases, tethered membrane patches whose area is approximately that of the original GUVs are formed. Membrane patches formed with immobilized tethers are stable irrespective of lipid composition, while those formed with mobile tethers are unstable when formed by Egg PC; compositions that lead to stable membrane patches are described in the text.

Epifluorescence top view

Confocal reconstruction side view

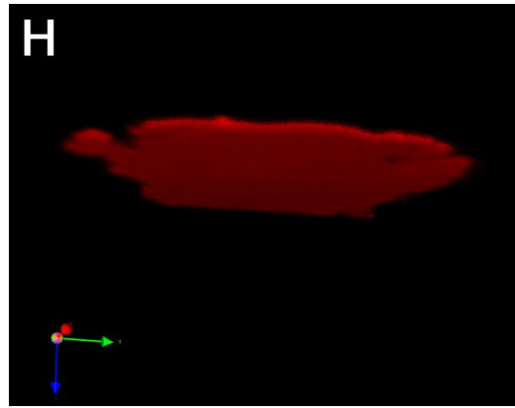
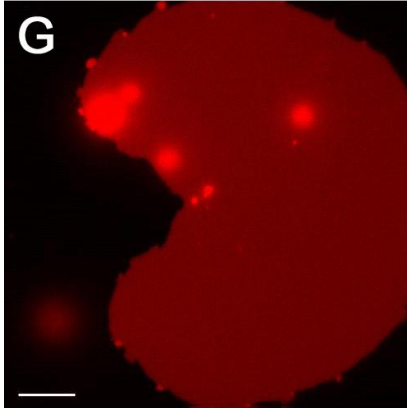
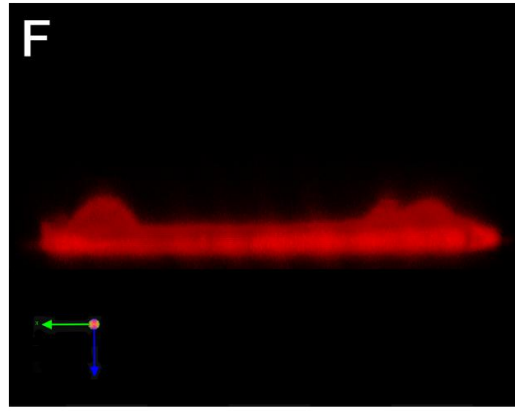
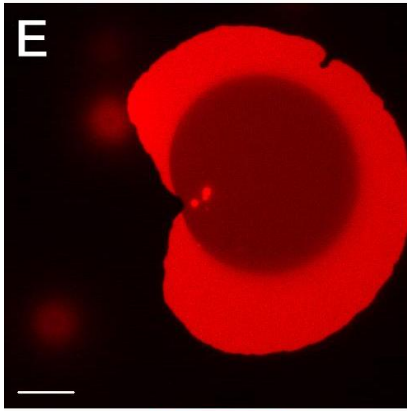
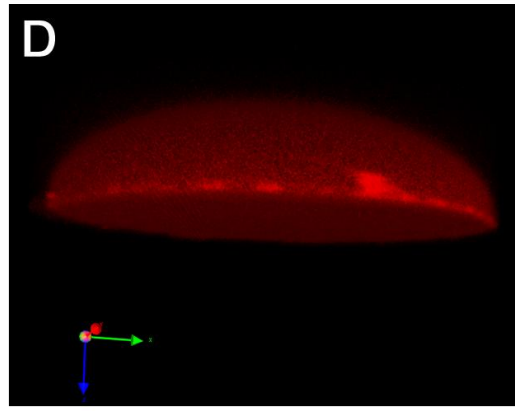
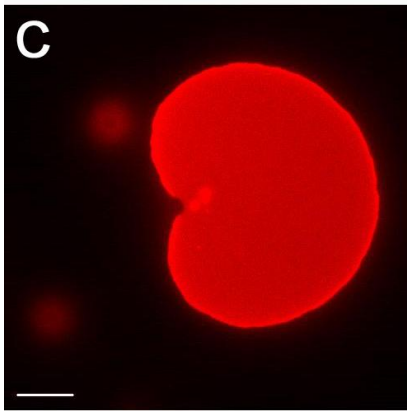
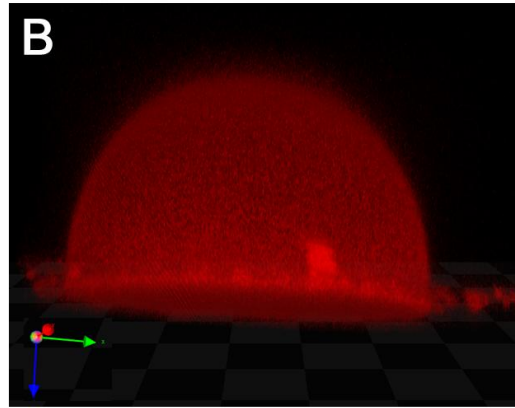
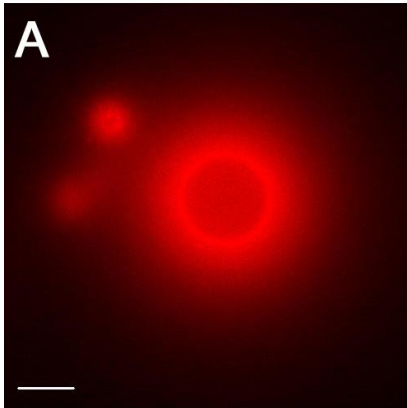


Figure S2: Giant vesicle to tethered membrane patch transformation observed by fluorescence microscopy. These images are of stable tethered membranes with immobile tethers (Fig S1A); those with mobile tethers are very similar. The epifluorescence microscopy images, left side, and confocal microscopy images in the corresponding state, right side, are displayed in parallel. While F is exhibited parallel to the surface, B, D and H are 20 degree tilted to show a better view of their 3-dimensional shape. (A and B) When the GUV starts to bind via DNA hybridization, only the bottom part of vesicle is in contact with the surface and shows a ring shape. Meanwhile, the upper part of the vesicle out of the focal plane appears as a cloudy halo around the ring. (C and D) Further binding of DNA from the vesicle to the surface flattens the vesicle asymmetrically. (E and F) Eventually, the upper membrane ruptures. Afterwards, a single bilayer remains (dark area) with some transient double bilayer (bright regions). (G and H) After all of the upper bilayer spreads, a homogeneous tethered membrane is formed. While the epifluorescence microscopy images are taken from the same GUV in about 5 minutes, the confocal microscopy images take much longer to collect and are of different GUVs captured at comparable times. Scale bar of epifluorescence microscopy is 15 μ m.

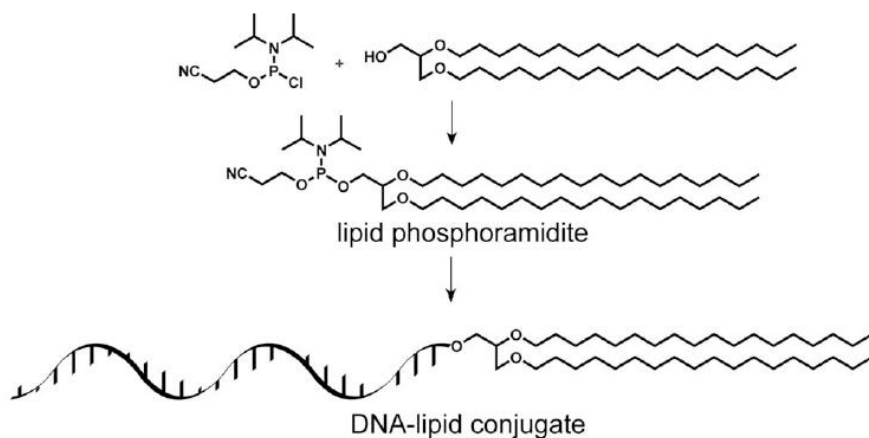


Figure S3: Synthetic scheme of DNA-lipid conjugate.

Table S1: Sequences of nonrepeating (NR) or repeating (Poly A/T) DNA oligonucleotides coupled to lipids and used as DNA hybrid tethers. In this report, the 5 ' end of the DNA is coupled to the lipid anchor. For some experiments, the 3 ' end labeled with Cy5.

	Sequence
NR 24mer	TCG ACA CGG AAA TGT TGA ATA CTA
cNR 24mer	TAG TAT TCA ACA TTT CCG TGT CGA
NR 48mer	TAA CTA CAG AAT TTA TAC TAT CCC GGG TCA CAG CAG AGA AAC AAG ATA
cNR 48mer	TAT CTT GTT TCT CTG CTG TGA CCC GGG ATA GTA TAA ATT CTG TAG TTA
T	TTT TTT TTT TTT TTT TTT TTT TTT
A	AAA AAA AAA AAA AAA AAA AAA AAA