Supporting Information

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SI Text

SI Materials and Methods. Fatty acids and fatty acid derivatives were obtained from Nu-Chek Prep (Elysian, MN). Fluorescent dyes were obtained from Invitrogen (Carlsbad, CA). Dithiothreitol, 3-mercaptopropionic acid, 3-mercapto-1-propanol, 1-mercapto-2-propanol, and 3-mercapto-1,2,4-triazole were purchased from Sigma-Aldrich (St. Louis, MO). Oleate vesicles were prepared by resuspending a dried film of oleic acid in 0.2 M Na-bicine (Sigma-Aldrich, St. Louis, MO) containing 2 mM HPTS at pH 8.5, to a final concentration of 10 mM oleic acid. The vesicle suspension was vortexed briefly and then tumbled overnight. Dilutions of vesicles were made using buffers containing fatty acids above the critical aggregate concentration (cac; approximately 80 μM for oleic acid), to avoid vesicle dissolution. Large (approximately 4 μm in diameter) monodisperse multilamellar vesicles were prepared by extrusion and large-pore dialysis as described (1). Briefly, extrusion of polydisperse vesicles through 5-μm-diameter pores eliminates vesicles larger than 5 μm in diameter. Dialysis of extruded vesicles against 3-μm-pore-size polycarbonate membranes then eliminates vesicles smaller than 3 μm in diameter, leaving behind a roughly monodisperse population of vesicles with a mean diameter of approximately 4 μm. Oleate vesicles in 0.2 M glycinamide hydrochloride were prepared and dialyzed using the same method. Oleate vesicles containing 1-hydroxypyrene (Sigma-Aldrich, St. Louis, MO) in the membrane were prepared by co-dissolving 1-hydroxypyrene (20 mol%), oleic acid, and Rh-DHPE (1 mol%) in a chloroform solution, followed by rotary evaporation and resuspension in buffer (0.2 M Na-bicine, pH 8.5, 15 mM DTT). Oleate vesicles containing di-L-cysteine were prepared by resuspension of 10 mM oleic acid in 0.2 M Na-bicine (pH 8.5) containing 2 mM HPTS and 20 mM di-L-cysteine. Di-L-cysteine (cysteinylcysteine) was prepared using F-moc solid phase synthesis on a microwave peptide synthesizer as described (2).

The methods for studying vesicle growth have been previously described (3). In the current study, vesicle growth experiments were performed in a buffer solution containing DTT, 3-mercaptopropionic acid, 3-mercapto-1-propanol, 1-mercapto-2-propanol, 3-mercapto-1,2,4-triazole, or di-L-cysteine. To prepare fatty acid micelle solutions for vesicle growth, fatty acids were dissolved in one equivalent of NaOH (final pH > 10), vortexed briefly, and agitated overnight under argon (4). Large (approximately 4 μm in diameter) multilamellar oleate vesicles (containing 2 mM HPTS) were prepared by large-pore dialysis, diluted 1:10 with the same buffer containing 0.8 mM oleic acid (to a final concentration of approximately 1 mM oleic acid), and stored in a microcentrifuge tube. To observe vesicle growth, five equivalents of oleate micelles were added to pre-formed vesicles, mixed, and then quickly pipetted into a disposable hemocytometer (Incyto, South Korea). The addition of smaller quantities (one equivalent) of oleate micelles was performed using the same method. Vesicles with encapsulated fluorescent dyes were imaged using a Nikon TE2000S inverted epifluorescence microscope with extra long working distance (ELWD) objective lenses (Nikon, Japan). The illumination source was a metal halide lamp (EXFO, Canada) with a 480 ± 20 nm (for HPTS), 546 ± 5 nm (for Rh-DHPE), or 360 ± 20 nm (for UV) optical filter (Chroma, Rockingham, VT). The illumination intensity was controlled using a set of two neutral density filters on the microscope. The images and movies were recorded using a digital camera (Hamamatsu Photonics, Japan) and post-processed using Phylum Live software (Improvision, Lexington, MA). All images were cropped using Photoshop CS4 (Adobe Systems, San Jose, CA), with linear adjustments of brightness and contrast.

All NMR spectra were recorded at 293 K with a Varian 400 MHz NMR spectrometer (Oxford AS-400) equipped with a 5 mm broadband PFG (z-gradient) probe. DTT and *trans*-4,5-dihydroxy-1,2-dithiane (Sigma-Aldrich, St. Louis, MO) at concentrations of 15–20 mM were added to sonicated vesicle suspensions prior to NMR measurements. Oleate vesicles were prepared without buffer by mixing 15 mM oleate with 7.5 mM NaOD (pH approximately 8.5, in 100% D_2O for STD experiments and in 10% D_2O for waterLOGSY experiments). All 1D STD spectra were recorded with 512 scans. Selective saturation of the vinyl protons of oleic acid hydrophobic chain at 5.3 ppm was performed by applying a series of 40 Gaussian-shaped pulses (50 ms; 1 ms delay between pulses) for a total saturation time of 2.04 s. Subtraction of saturated spectra from off resonance spectra (−4 ppm) was performed by phase cycling. Suppression of vesicle signals was achieved by relaxation filtering prior to detection. Water suppression was achieved by the excitation sculpting method.

WaterLOGSY spectra were recorded with 512 scans. The first water selective 180° refocusing *reburp* pulse of 32 ms was generated from a Pbox of VNMRJ 2.1A. For solvent selection echo, two pulsed field gradients (PFGs) at a pulse length of 2.5 ms and pulse power of 2.5 G/cm were first applied. During the mixing time (1.2 s), a weak rectangular PFG with a power level of 0.02 G/cm was applied. Subsequently, a short gradient recovery time of 2 ms was applied. Water suppression was achieved with an excitation sculpting sequence. The two water selective 180° pulses for solvent suppression echo were applied for a 4.9 ms pulse length. The four PFGs for solvent suppression echo had a pulse length of 0.8 ms and strength of 12 G/cm, respectively. Data were collected with the proton carrier set at 4.75 ppm, with a spectral width of 10,000 Hz, an acquisition time of 0.8 s, and a relaxation delay of 3.8 s. A T1ρ filter was applied to eliminate fast-relaxing vesicle signals.

1. Zhu TF, Szostak JW (2009) Preparation of large monodisperse vesicles. *PLoS ONE* 4:e5009.
2. Erdelyi M, Gogoll A (2002) Rapid microwave-assisted solid phase peptide synthesis. *Synthesis-Stuttgart*:1592–1596.
3. Zhu TF, Szostak JW (2009) Coupled growth and division of model protocell membranes. *J Am Chem Soc* 131:5705–5713.
4. Hanczyc MM, Fujikawa SM, Szostak JW (2003) Experimental models of primitive cellular compartments: Encapsulation, growth, and division. *Science* 302:618–622.

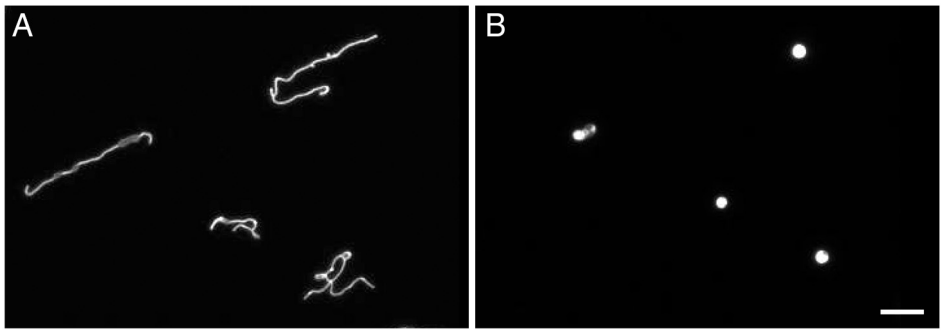


Fig. S1. Long thread-like oleate vesicles round up into large spherical vesicles. (*A*) Oleate vesicles (containing 2 mM HPTS, in 0.2 M Na-bicine, pH 8.5) 30 min after the addition of five equivalents of oleate micelles. (*B*) The long thread-like oleate vesicles rapidly (in approximately 5 s) round up into large spherical vesicles under intense illumination (Movie S1). Scale bar, 20 μm.

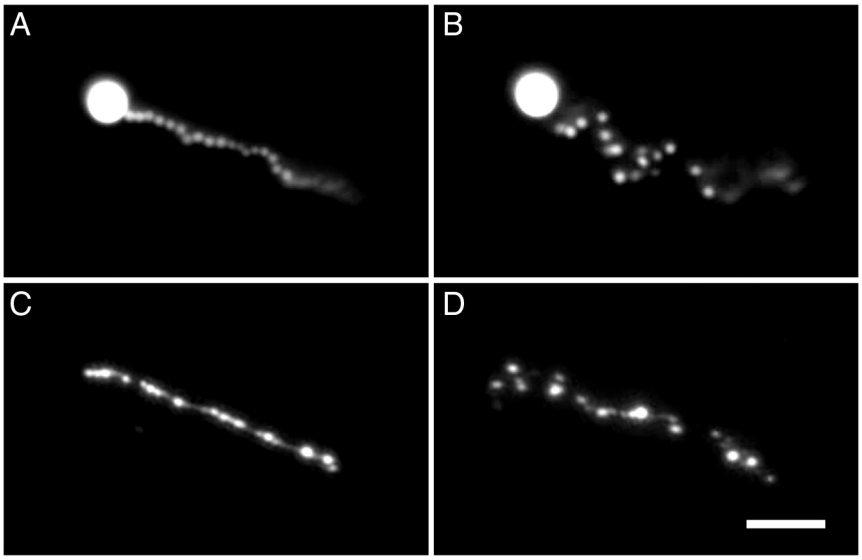


Fig. S2. Vesicle pearling and division under various conditions. (*A* and *B*) An oleate vesicle (containing 2 mM HPTS, in 0.2 M Na-bicine, pH 8.5, 10 mM DTT) 30 min after the addition of one equivalent of oleate micelles. Under intense illumination (for 2 s and 9 s, respectively), the filamentous portion of the vesicle went through pearling and division (Movie S3). (*C* and *D*) A decanoate:decanol (2:1) vesicle (in 0.2 M Na-bicine, pH 8.5, 30 min after the addition of two equivalents of decanoate micelles and one equivalent of decanol emulsion) went through pearling and division under intense illumination (for 3 s and 10 s, respectively). Scale bar, 10 μm.

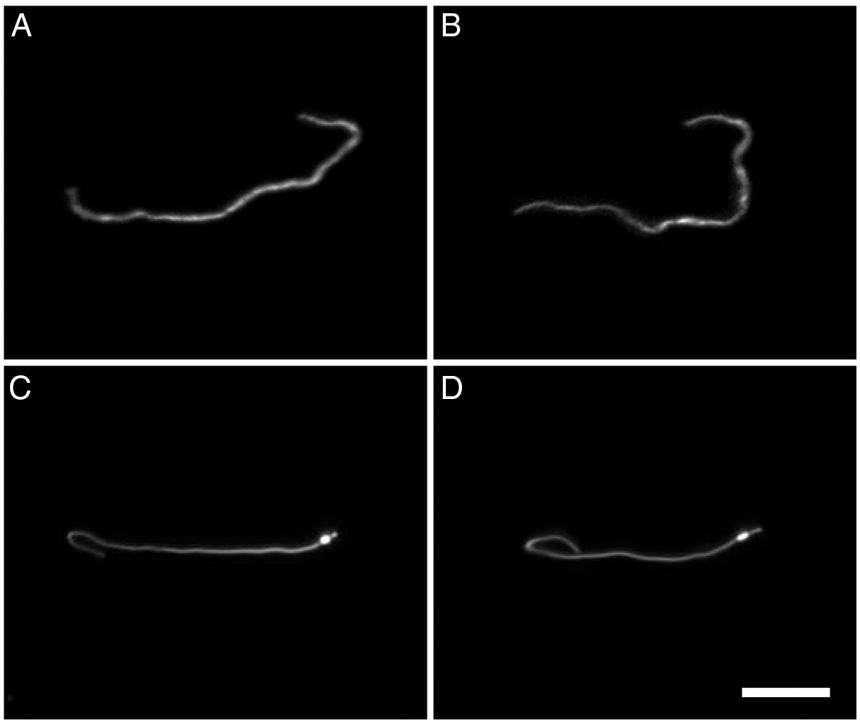


Fig. S3. Rh-DHPE labeled oleate vesicles and HPTS labeled POPC vesicles. (*A* and *B*) A long thread-like oleate vesicle labeled with a membrane-localized dye (0.5 mol% Rh-DHPE, in 0.2 M Na-bicine, pH 8.5, 10 mM DTT, 30 min after the addition of five equivalents of oleate micelles), under intense illumination for 10 s, did not go through pearling and division. (*C* and *D*) A long thread-like POPC vesicle (containing 2 mM HPTS, in 0.2 M Na-bicine, 0.5 M sucrose, pH 8.5, 10 mM DTT), under intense illumination for 10 s, did not go through pearling and division. Scale bar, 20 μm.

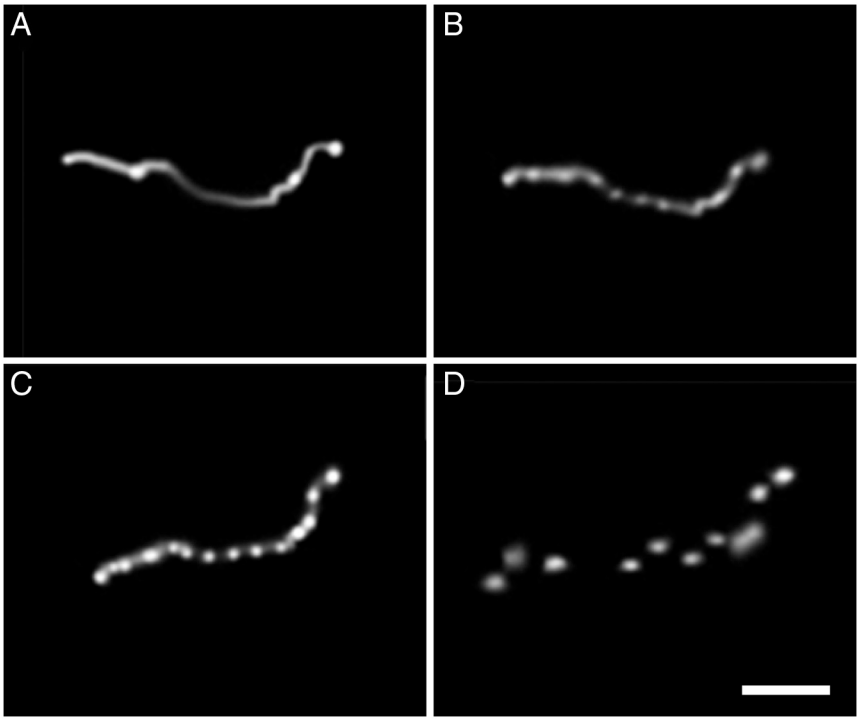
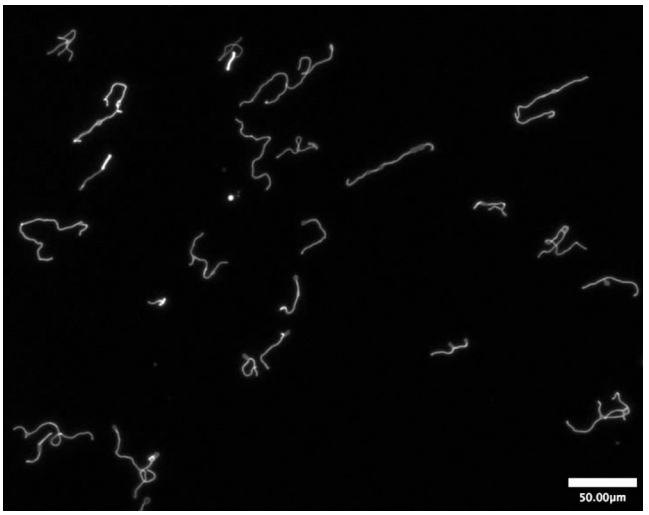
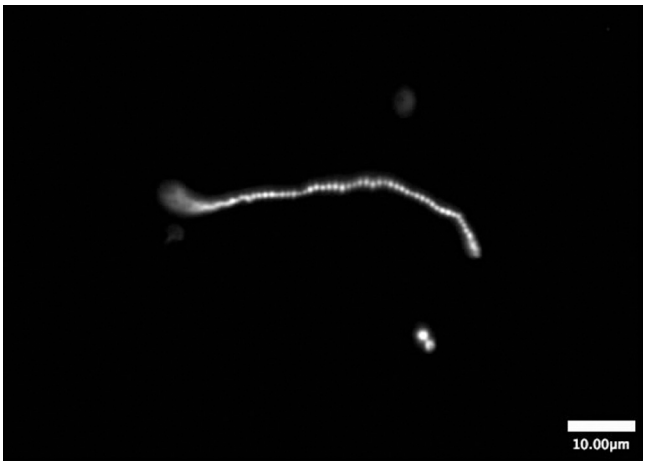


Fig. S4. Vesicle pearling and division with equal concentrations of HPTS inside and outside the vesicles. (*A*) An oleate vesicle with 2 mM HPTS inside and outside the membranes (labeled by 1 mol% Rh-DHPE for imaging, in 0.2 M Na-bicine, pH 8.5, 10 mM DTT) 30 min after the addition of five equivalents of oleate micelles. (*B–D*) Under intense illumination (for 2 s, 7 s, and 11 s, respectively), the long thread-like vesicle went through pearling and division. Scale bar, 10 μm.



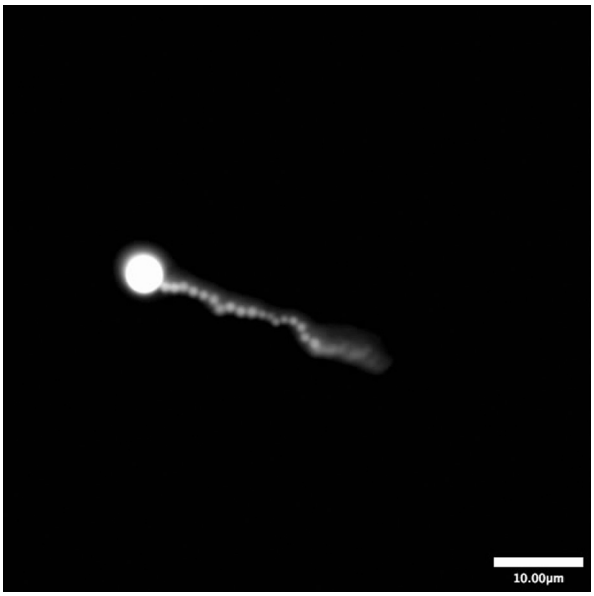
Movie S1. This real-time movie shows that long thread-like oleate vesicles (containing 2 mM HPTS, in 0.2 M Na-bicine, pH 8.5, 30 min after the addition of five equivalents of oleate micelles) rapidly (in approximately 5 s) round up into large spherical vesicles under intense illumination (QuickTime; 9 FPS; 7 MB). Scale bar, 50 μm.

Movie S1 (MOV)



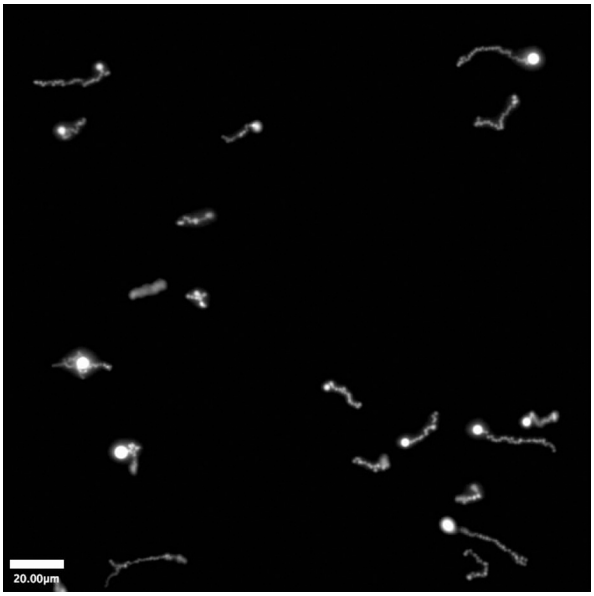
Movie S2. This real-time movie shows that a long thread-like oleate vesicle (containing 2 mM HPTS, in 0.2 M Na-glycinamide, pH 8.5, 10 mM DTT, 30 min after the addition of five equivalents of oleate micelles) went through pearling and division under intense illumination (QuickTime; 9 FPS; 2 MB). Scale bar, 10 μm.

Movie S2 (MOV)



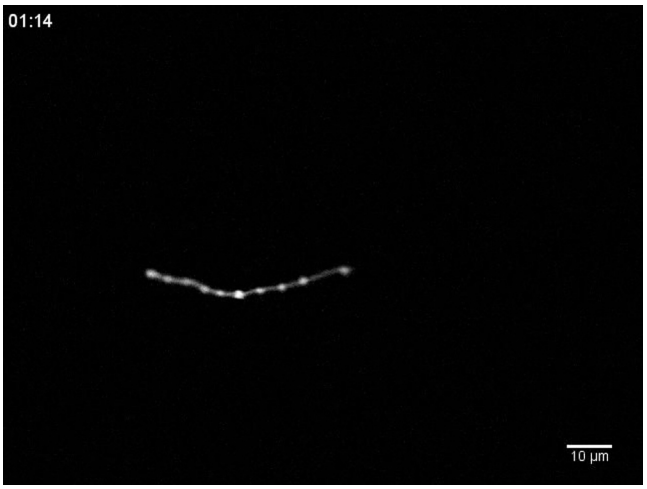
Movie S3. This real-time movie shows that the filamentous portion of an oleate vesicle (containing 2 mM HPTS, in 0.2 M Na-bicine, pH 8.5, 10 mM DTT, 30 min after the addition of one equivalent of oleate micelles), under intense illumination, went through pearling and division (QuickTime; 9 FPS; 1 MB). Scale bar, 10 μm.

Movie S3 (MOV)



Movie S4. This real-time movie shows that the filamentous portions of a group of oleate vesicles (containing 2 mM HPTS, in 0.2 M Na-bicine, pH 8.5, 10 mM DTT, 30 min after the addition of one equivalent of oleate micelles), under intense illumination, went through pearling and division (QuickTime; 9 FPS; 5 MB). Scale bar, 20 μm.

Movie S4 (MOV)



Movie S5. This movie shows that a filamentous oleate vesicle (with 20 mol% 1-hydroxypyrene and 1 mol% Rh-DHPE in the membrane, in 0.2 M Na-bicine, pH 8.5, 15 mM DTT, 30 min after the addition of five equivalents of oleate micelles), under intense illumination, went through pearling and division (AVI; 25 FPS, 60X accelerated, timer on top shows mm:ss; 4 MB). Scale bar, 10 μm.

Movie S5 (AVI)