Supporting Information for "*Bulk Self-Assembly of Giant, Unilamellar Vesicles*"

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Scale bar represents 10 μm for all images unless otherwise noted.

Video S1. Oleic acid could self-assemble into GUVs. A video scrolling through a sample under phase contrast microscopy where 5 mM oleic acid and 5 mM NaOH is dispersed in dilute HCl (final pH 8.3).

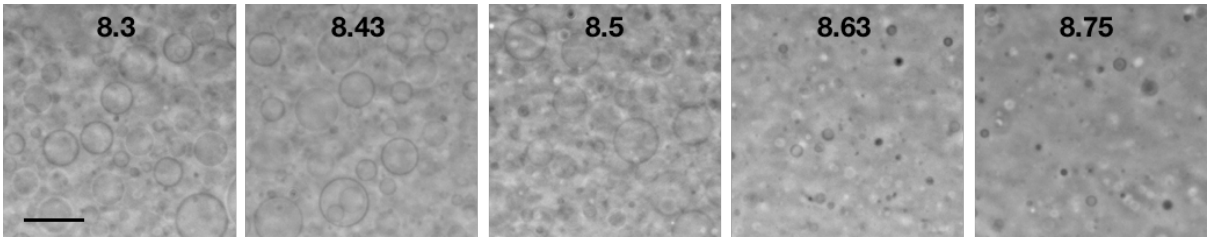

Figure S2. The morphology that oleic acid self-assembled into depended on the pH of the buffer (labelled above). 5 mM oleic acid and 50 mM Na-bicine were used.

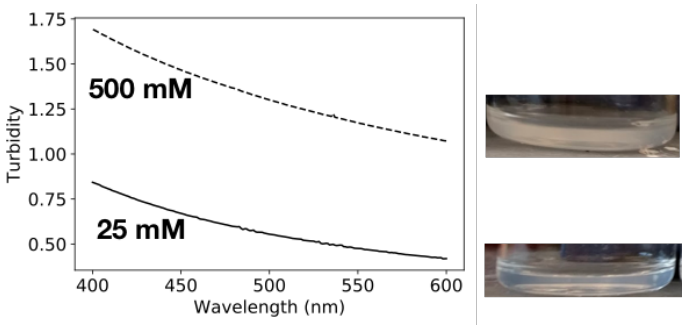

Figure S3. GUV samples (for example, vesicles made with 25 mM Na-bicine) looked almost transparent, and their bulk turbidity (measured on a Cary 60 UV-Vis spectrophotometer) was much lower than the turbidity of MLV samples (for example, vesicles made with 500 mM Na-bicine).

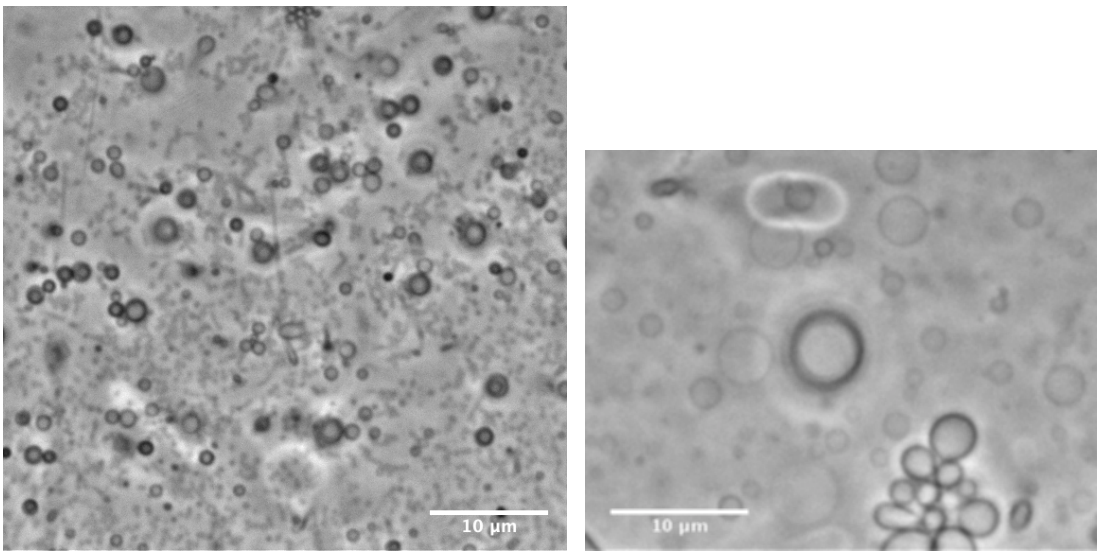

Figure S4. Unclean glass can seed the growth of vesicles that are easily distinguished from the self-assembled GUVs. Left: Leaving a 5 mM oleic acid in 25 mM Na-bicine, pH 8.43 solution on a glass coverslip resulted in growth of high-contrast structures. Right: 5 mM pre-assembled oleic acid GUVs in 50 mM Na-bicine, pH 8.43 incubated on an unwashed glass coverslip also resulted in the growth of high-contrast structures on the coverslip. The bulk-assembled GUVs have much lower contrast and appear much more spherical than the structures grown on the glass.

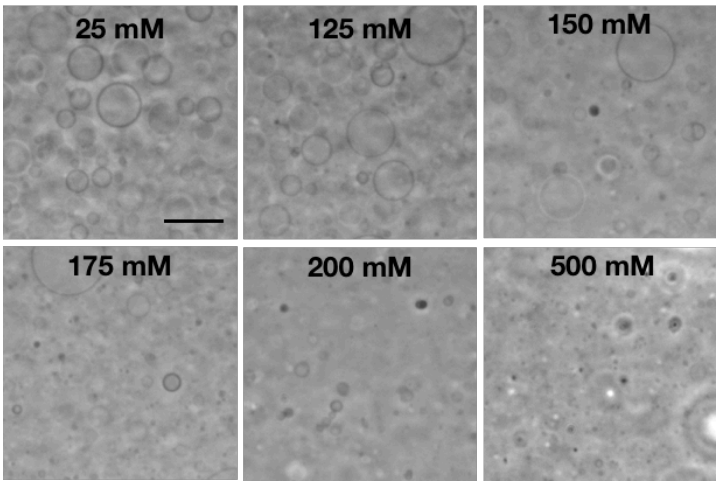

Figure S5. The vesicle morphology that 5 mM oleic acid self-assembled into depended on the total Na-bicine concentration (labelled).

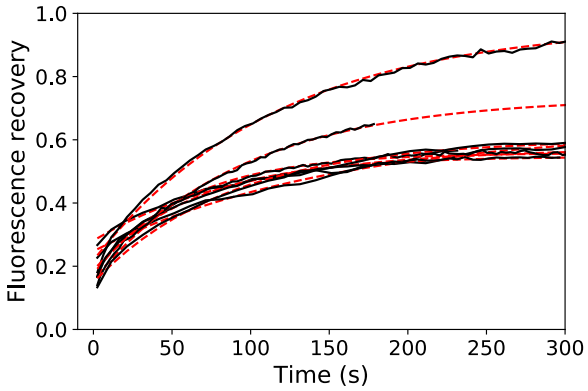

Table 1. FRAP results

Best-fit D	R^2
7.591	0.993
12.315	0.980
11.275	0.997
21.06	0.995
12.507	0.998
20.993	0.994
14.604	0.995

Figure S6. For an oleic-acid supported lipid bilayer, the fluorescence recovery over a 54-µm-diameter bleached region is monitored over time (black). The model from Kang *et al.* (red) is used to fit the data. This model accounts for any fluorescence recovery that occurs during photobleaching. Best-fit D, and the R^2 statistic for each of the fits is shown in **Table 1**.

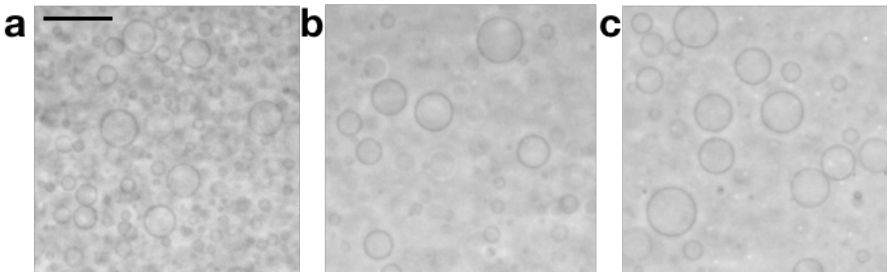

Figure S7. Fatty acids with mixed and varied saturation could also self-assemble into GUVs. Phase contrast images are shown. a) 5 mM linolenic acid in 50 mM Na-bicine pH 8.2, b) 3.5 mM oleic acid and 1.5 mM lauric acid in 25 mM Na-bicine, pH 8.43 c) 4 mM oleic acid and 1 mM myristic acid with 5 mM NaOH and 2.5 mM HCl.

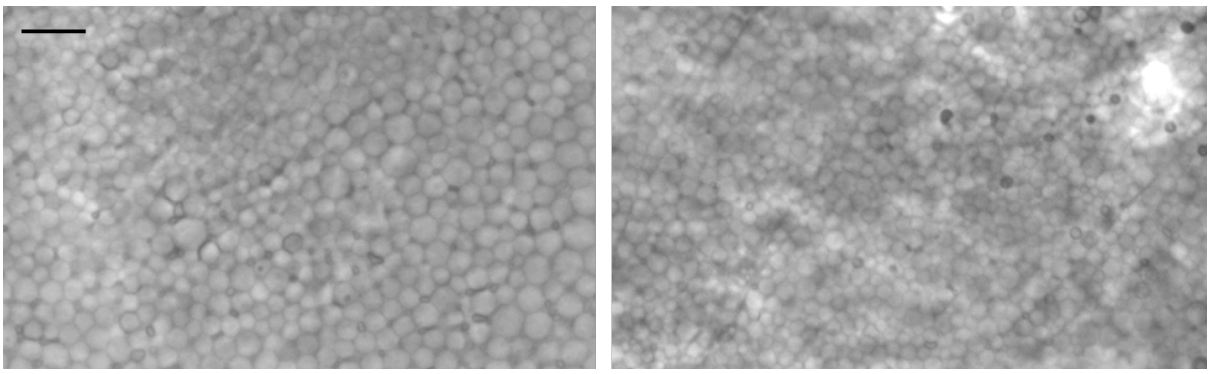

Figure S8. Vesicles were jammed in some parts of a sample containing 20mM oleic acid dispersed in 50 mM bicine, pH 8.23.

Video S9. Oleic acid could self-assemble into a space-filling, and almost jammed state. Phase contrast video scrolling through a sample of 20mM oleate dispersed in 50 mM Na-bicine, pH 8.23.

Video S10. Decanoic acid could self-assemble into a space-filling, and almost jammed state. Video scrolling through a sample of 40mM decanoic acid dispersed in 100 mM PIPES, pH 6.6. The vesicles were space-filling and are almost jammed. There were many spherical vesicles within vesicles.

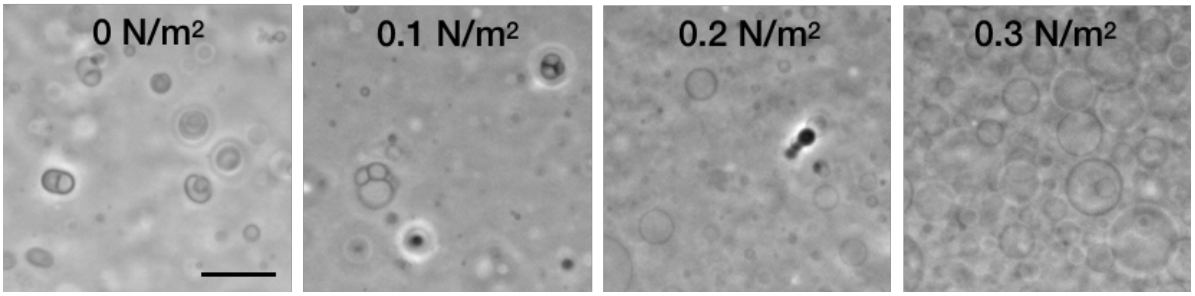

Figure S11. Samples containing 5 mM oleic acid in 50 mM Na-bicine, pH 8.43, were left overnight on orbital shakers. They were exposed to different levels of maximum shear (0 rpm = 0 N/m^2, 40 rpm = 0.1 N/m^2, 60 rpm = 0.2 N/m^2, 80 rpm = 0.3 N/m^2) and self-assembled into different morphologies ranging from quite varied to giant and spherical.

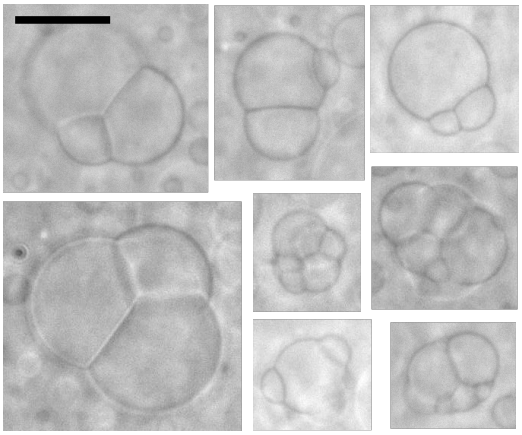

Figure S12. Foam-like intermediates of vesicles appeared prior to GUV formation. Sample shown here is 4 mM oleic acid and 1 mM myristic acid, with 5 mM NaOH and 2.5 mM HCl.

Video S13. Oleic acid GUVs could encapsulate colloidal particles during self-assembly. Video of trapped 400-nm-diameter colloidal particles in 50mM Na-bicine, pH 8.2, with 500 mM sucrose encapsulated and 500 mM glucose in the equiosmolar dilution buffer.

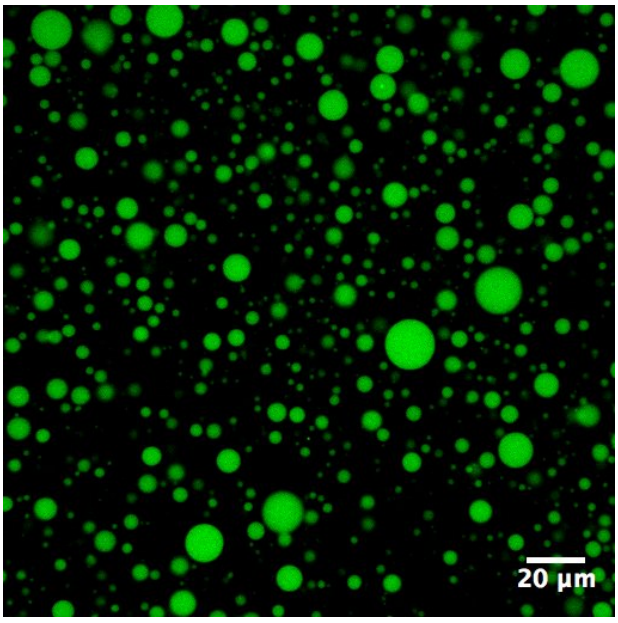

Figure S14. Vesicles survived centrifugation against an Amicon Ultrafree-MC filter for 25 minutes at 500 *g*. Confocal micrograph of 5 mM oleic acid vesicles in 50 mM Na-bicine, pH 8.2 containing 0.2 mM HPTS dye, diluted 1 in 10 into buffer without any dye.

Video S15. and

Video S16. Vesicle solutions appeared unchanged after 2 weeks at room temperature. 5 μL aliquots of a well-mixed 1 mL sample are taken and imaged with phase contrast microscopy at the start (Video S15) and end (Video S16) of a two-week period. The samples are 3.5 mM oleic acid and 1.5 mM lauric acid in 25 mM Na-bicine, pH 8.43.

Video S17. and

Video S18. Oleic acid GUVs could divide upon a sudden increase in surface area. Video of oleic acid vesicles encapsulating 10 μM of fluorescently tagged RNA in 100 mM Na-bicine pH 8.2 and 200 mM sucrose, diluted into equiosmolar 200 mM glucose buffer. Addition of 5 μL of 100 mM oleic acid micelles into a sample trough containing 165 μL of vesicle solution, near the imaging site, resulted in GUV division.

Video S19. Oleic acid GUVs could pearl upon an increase in surface area. Video of vesicles encapsulating 10 μM of fluorescently tagged RNA in 100 mM Na-bicine pH 8.2 and 200 mM sucrose, diluted into equiosmolar 200 mM glucose buffer. Addition of 5 μL of 100 mM oleic acid micelles into a sample trough containing 165 μL of vesicle solution while mixing well resulted in GUV pearling without fission.