Supporting Information

Coupled Growth and Division of Model Protocell Membranes

Ting F. Zhu and Jack W. Szostak

This file includes Text S1 with References, Figures S1 to S6, and Movies S1 to S3.

Text S1

Vesicle Extrusion through Porous Rock

For extrusion to occur by flowing suspended vesicles through a porous rock, the rock must not have any large pores. Since the resistance to a laminar flow through a pore, as described by the Hagen-Poiseuille law, is inversely proportional to the fourth power of the radius of the pore, larger pores would have much less resistance and allow virtually all of the fluid volume to flow through. For instance, a large pore with 1 mm diameter is 10^{10} times less resistant to flow than a 2-μm-diameter pore, and would allow most of the vesicles to flow through without being extruded. To test this theory, we compared the extrusion of large (~4 μm in diameter) oleate vesicles (containing 2 mM HPTS) through membrane filters (with 2-μm-diameter pores) with or without a punctured 1-mm-diameter pore. Using the vesicle counting assay, we found that such a 1-mm-diameter pore completely prevented vesicle division from occurring. For the same reason, flowing vesicles through an open channel (e.g., a stream) would allow virtually all of the vesicles to escape extrusion.

Another important constraint is that the pressure gradient required for extrusion is immense. In the laboratory extrusion procedure,[1] the pressure gradient applied across the membrane filter is estimated to be greater than 100 atm/cm (the membrane filter used is only ~10 μm in thickness). As the required pressure difference increases with the thickness of the porous rock, the rock must either be very thin (e.g., of several μm), or subjected to a high pressure difference (100 atm for 1 cm thickness) to allow for extrusion. Moreover, for multiple cycles of growth and division to occur, the vesicle suspension must be repeatedly recycled through the porous rock. It is unlikely for all these stringent conditions to be satisfied in a natural environment, and thus unlikely for an analogous vesicle extrusion process to occur in a prebiotic scenario on the early Earth.

Model for Vesicle Shape Transformations

Assuming that vesicle elongation results from increased surface area while vesicle volume is conserved, and that a cylindrical shape is most favorable, the lengths of the thread-like vesicles formed after growth are easily calculated. Here we show that the predicted vesicle length agrees with the experimentally determined lengths.

Suppose that a spherical vesicle has a radius of r_1. Its surface area is

$$S_1 = 4\pi r_1^2 \qquad\qquad [1]$$

and its volume is

$$V_1 = 4/3\,\pi\,r_1^3 \qquad\qquad [2]$$

Suppose that after micelle addition, this spherical vesicle has elongated to a thread-like vesicle (simplified as a cylinder, including the branched vesicles) with a radius (or average radius of all branches in a branched vesicle) of r_2 and length (or total length of all branches for a branched vesicle) of l. Neglecting the cylindrical end caps, its surface area is approximately

$$S_2 = 2\pi r_2 l \tag{3}$$

and its volume is

$$V_2 = \pi r_2^2 l \tag{4}$$

Consider that the encapsulated volume of a vesicle is unchanged,

$$V_1 = V_2 \tag{5}$$

From [2], [4], and [5], we get

$$4/3\, \pi r_1^3 = \pi r_2^2 l \tag{6}$$

Multiply both sides by $12\pi r_1$, we get

$$16\, \pi^2 r_1^4 = 12\, \pi^2 r_2^2 l\, r_1 \tag{7}$$

That is

$$(4\pi r_1^2)^2 = 3(2\pi r_2 l)^2\, r_1 /l \tag{8}$$

Substitute $4\pi r_1^2$ with S_1, and $2\pi r_2 l$ with S_2, we get

$l/r_1 = 3(S_2/S_1)^2$ [9]

Under similar conditions of micelle addition, the efficiency of micelle incorporation is ~50%, as measured in previous studies.[2] Therefore, when 5 equivalents of oleate micelles are added to a vesicle suspension containing ~1 mM oleic acid, the increase of surface area should be ~3.5-fold. In our experiment, as measured in Figure 2A, the average surface area increase was ~3.7-fold (assuming that all membrane layers increase by the same proportion after reaching the steady state), which corresponds to a efficiency of micelle incorporation of 54%. According to formula [9], a spherical vesicle with an original radius of 2 μm, after a surface area increase of 3.7-fold ($S_2/S_1 = 3.7$), should have a length of ~82 μm. In comparison, the vesicle shown in Figure 2A is ~94 μm in length. The vesicles in Figure 1B average 96±15 μm (n = 10) in length (branched vesicles were measured by summing up the total lengths of all branches using Phylum Live software).

The estimation of the volume and surface area of a single vesicle before and after growth from high-resolution images (not shown in figures; available upon request) provided further evidence for volume conservation and surface area increase. Before growth, the vesicle diameter measured ~3.1 μm, which corresponds to a surface area of ~30.2 μm^2 and a volume of ~15.6 μm^3. After growth, the thread-like vesicle length measured ~81.1 μm and the width measured 0.49±0.02 μm (this scale is close to the resolution limit of optical microscopy, and results in larger measurement error), which corresponds to an estimated surface area of ~124.8±5.1 μm^2 (~4-fold increase from that before growth) and an estimated volume of ~15.3±0.6 μm^3.

Critical Shear Rate for Vesicle Division

We can simplify the scenario of wind blowing over the surface of a prebiotic pond as a case of simple shear in laminar flow in water (a Newtonian fluid), where the shear rate can be expressed as a gradient of velocity. We assume that the velocity at the fluid surface is the same as the speed of a typical wind at 5 m/s (~11 mph), and the fluid at the bottom of the pond is static. The shear rate in such a body of fluid is a function of its depth: 5 sec^{-1} for a depth of 1 m, 50 sec^{-1} for a depth of 0.1 m, or 500 sec^{-1} for a depth of 1 cm. Thus the closer to the shore, the higher the shear rate. In reality, the presence of waves, vortices, and turbulent flow may produce complex flow fields with a wide range of shear rates.

We determined that the critical shear rate for thread-like multilamellar vesicles to divide was 15 sec^{-1}, while spherical multilamellar vesicles, prepared with the same fatty acid and buffer, remained undisrupted under the same or higher shear rates of up to 1,500 sec^{-1} (the maximum shear rate measurable by our instrument). This suggests that the division of thread-like multilamellar vesicles is unlikely to be caused by simple membrane rupture, but rather through more complex mechanism (e.g., vesicle pearling under Plateau-Rayleigh instability, as previously observed with tubular phospholipid vesicles under surface tension induced by a laser tweezer[3]). The mechanism of the division for thread-like multilamellar vesicles under modest shear forces remains largely unclear and will require in-depth future studies. We also found that large unilamellar vesicles were very fragile. On exposure to gentle fluid shear (190 sec^{-1}), some of the vesicles were disrupted and a significant fraction of the encapsulated contents were released into the solution (Figure S6). In addition, some of the originally unilamellar vesicles transformed into multilamellar vesicles, as observed by confocal

microscopy (Figure 5H). How this occurred remains unclear, but may involve partial membrane fragmentation and reorganization in response to shear forces. In contrast, multilamellar vesicles can divide under a much high shear rates ($> 1,500$ sec^{-1}) without disruption or loss of contents. In a prebiotic, wind-agitated pond, the shear rate induced by wind could easily exceed 15 sec^{-1}, and might often reach much higher values. Vesicles that could divide robustly under a wide range of shear rates without membrane disruption would therefore have an advantage in terms of decreased loss of contents (including genetic polymers) to the environment.

References

(1)   Hanczyc, M. M.; Fujikawa, S. M.; Szostak, J. W. *Science* **2003**, *302*, 618-622.

(2)   Chen, I. A.; Szostak, J. W. *Biophys. J.* **2004**, *87*, 988-998.

(3)   Bar-Ziv, R.; Moses, E. *Phys. Rev. Lett.* **1994**, *73*, 1392-1395.
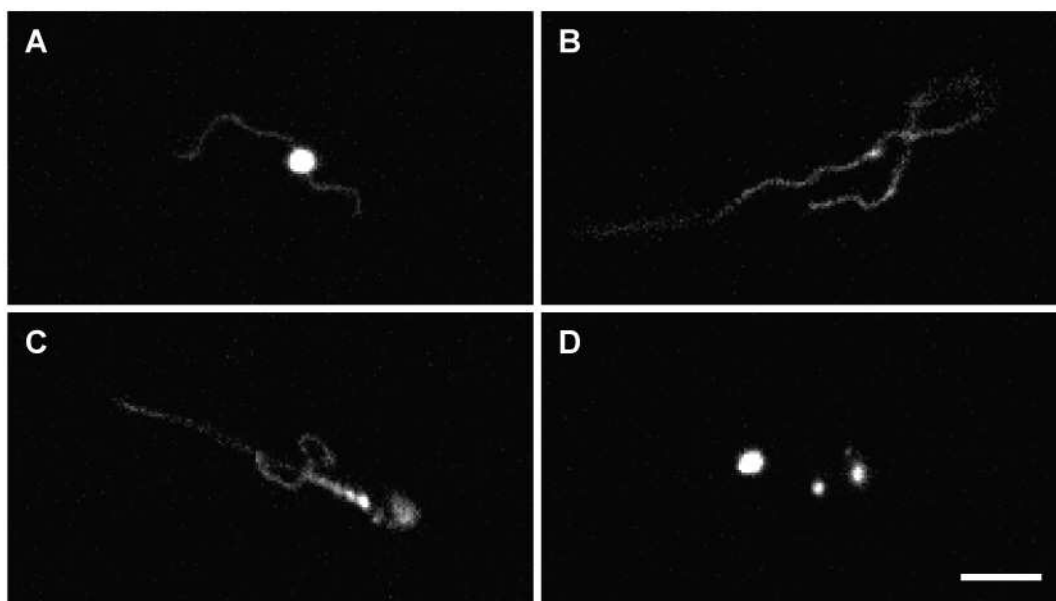
Figures S1 to S6

Figure S1. An Additional Example of Vesicle Growth and Division

Conditions as in Figure 1, D to H; also shown in Movie S2. (A, B) Oleate vesicle growth at 10 min and 30 min after the addition of 5 equivalents of oleate micelles, respectively. (C, D) Under mild fluid agitation, this thread-like vesicle divided into multiple smaller daughter vesicles. Scale bar, 10 μm.
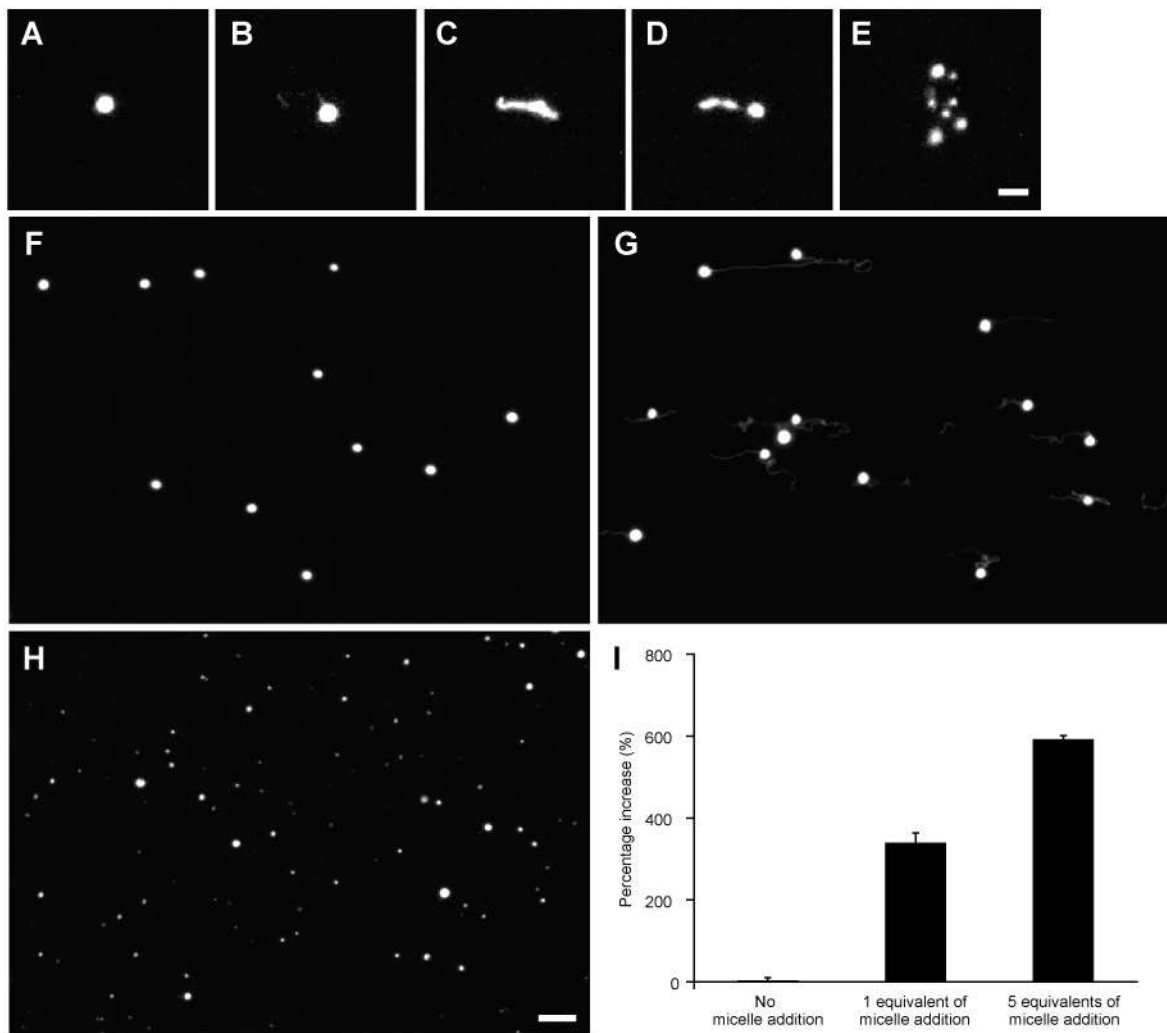
Figure S2. Vesicle Growth and Division After the Addition of Small Quantities of Micelles

(A, B) Oleate vesicle (containing 2 mM HPTS, in 0.2 M Na-bicine, pH 8.5, ~1 mM initial oleic acid) immediately, and 10 min after the addition of 1 equivalent of oleate micelles, respectively. (C-E) In response to mild fluid agitation, this thread-like vesicle divided into multiple smaller daughter vesicles. Scale bar for (A-E), 10 µm. (F) Image for vesicle counting before growth and division. (G) Vesicle shapes at 30 min after the addition of 1 equivalent of oleate micelles (no further shape transformation was observed after 30 min, up to 2 hrs), and

(H) image for vesicle counting after agitation, 30 min after the addition of micelles. (I) Percentage increases in the number of oleate vesicles from a baseline count after brief agitation, 30 min after the addition of 0, 1, and 5 equivalents of oleate micelles, respectively, suggesting that the efficiency of vesicle growth and division is influenced by the amount of fatty acid micelles added, n = 3. Scale bar for (F-H), 20 μm.
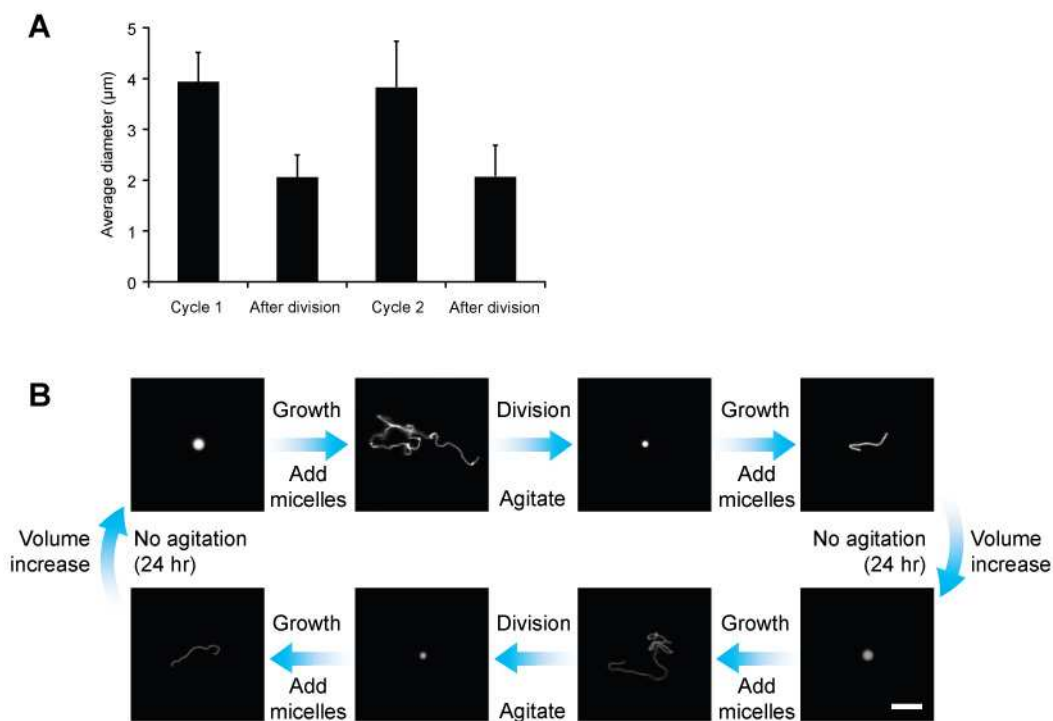
Figure S3. Vesicle Shapes During Cycles of Growth and Division in a Model Prebiotic Buffer

(A) Average vesicle diameters before and after division in 2 cycles, n = 10. (B) Vesicle shapes in cycles of growth and division (in 0.2 M Na-glycine, pH 8.5, ~1 mM initial oleic acid, vesicles contain 10 mM HPTS for fluorescence imaging). Dimmer images in the second row reflect the dilution of the encapsulated fluorescent dye during vesicle volume increase. (Same images with enhanced contrast are shown in Figure 2B.) Scale bar, 20 μm.
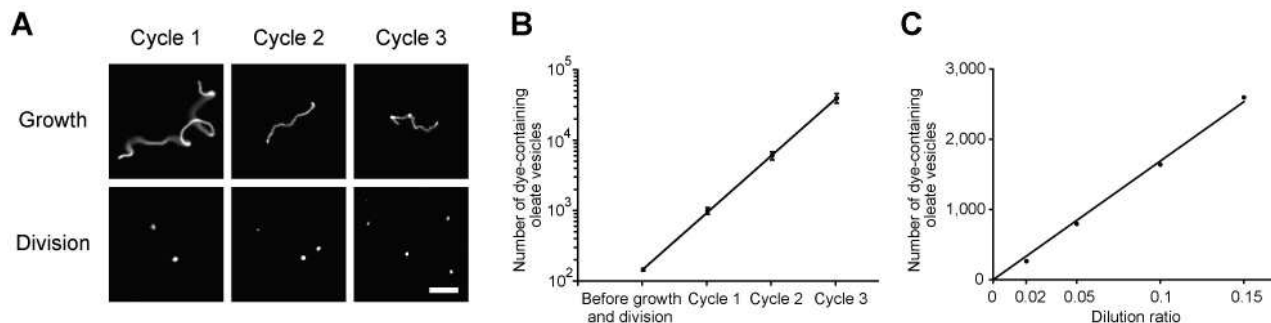
Figure S4. Three Sequential Cycles of Vesicle Growth and Division

(A) Vesicle shapes in cycles of growth and division (containing 10 mM HPTS, in 0.2 M Na-bicine, pH 8.5, ~1 mM initial oleic acid). Scale bar, 10 μm. (B) Total number of dye-labeled oleate vesicles (conditions as above) during 3 cycles of growth and division. The total vesicle count increased exponentially ($r^2 > 0.99$), n = 3. (C) Validating the vesicle counting assay. The counted number of dye-labeled oleate vesicles is proportional to the total number of dye-labeled vesicles in the vesicle suspension ($r^2 > 0.99$).
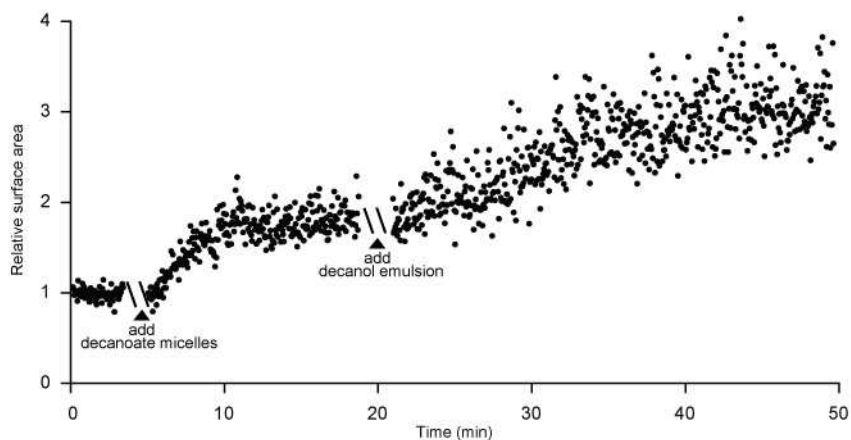
Figure S5. Growth of Decanoate:decanol (2:1) Vesicles

Relative surface area (solid circles), measured using a FRET assay after the addition of 2 equivalents of decanoate micelles and 1 equivalent of decanol emulsion. Both decanoate micelles and decanol emulsion can be incorporated into the decanoate:decanol (2:1) vesicles (in 0.2 M Na-bicine, pH 8.5, at room temperature, ~20 mM initial amphiphile concentration).
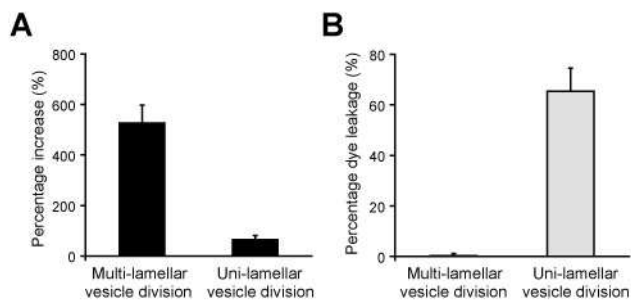
Figure S6. Division of Multilamellar versus Unilamellar Vesicles

(A) Percentage increase in the number of dye-labeled oleate vesicles from baseline count, after growth and division, comparing multilamellar with unilamellar vesicles (both containing 10 mM HPTS, in 0.2 M Na-bicine, pH 8.5, ~1 mM initial oleic acid, briefly agitated at 30 min after the addition of 5 equivalents of oleate micelles), n = 3. (B) Percentage dye leakage from vesicles after division, comparing multilamellar with unilamellar vesicles, n = 3.

Movies S1 to S3

Movie S1. Vesicle Growth and Division

An oleate vesicle (containing 2 mM HPTS, in 0.2 M Na-bicine, pH 8.5, ~1 mM initial oleic acid) grows over a period of ~25 min after the addition of 5 equivalents of oleate micelles, and divides under the influence of mild fluid agitation (QuickTime; 3 MB).

Movie S2. Vesicle Growth and Division

An oleate vesicle (conditions as in Movie S1) grows over a period of ~25 min after the addition of 5 equivalents of oleate micelles, and divides under the influence of mild fluid agitation (QuickTime; 3 MB).

Movie S3. Vesicle Division After the Addition of 1 Equivalent of Micelles

An oleate vesicle (containing 2 mM HPTS, in 0.2 M Na-bicine, pH 8.5, ~1 mM initial oleic acid) at ~10 min after the addition of 1 equivalent of oleate micelles, divides under the influence of mild fluid agitation (QuickTime; 2.5 MB).