

## Delamination and restructuring inside multilamellar lipid vesicles by a polycation: formation of multi-compartment vesicles

Rui Cao, Rishabh Jain, Ian K. Torres, Chris Oville, and Anthony D. Dinsmore\*

*Physics Department, University of Massachusetts Amherst.*

\*corresponding author

### Supplemental information

- I. Microscope images of multi-lamellar and multi-vesicular structures without polymer
- II. Mixing vesicles with different fluorescent labels
- III. Additional images from a movie showing formation of a vesicular superparticle
- IV. Additional images from a movie showing breakup of a vesicular superparticle
- V. Details of the proposed model of superparticle formation
- Vi. Description of the supplemental movies

**I. Microscope images of multi-lamellar and multi-vesicular structures without polymer**, showing the distinction from vesicular superparticles.

The as-made vesicles have a very different morphology than vesicular superparticles. We classify the as-made vesicles into three types: **Highly multilamellar vesicles** (HMGVs) appears to be mostly filled with lipid. The high density of the lamellae prevents us from resolving the interior structure. An example is shown in Fig. S1(a). **Slightly multilamellar vesicles** have a large fraction of open interior space. Having fewer lamellae, there are also less bright in fluorescence than the HMGVs (Fig. S1(b,c)). These structures are ideal for superparticles. **Multivesicular**, or **nonconcentric, multilamellar vesicles** have multiple lamellae in the outermost layer, and also internal multilamellar vesicles (Fig. S1(b)). These lamellae are not all concentric. In the literature, these are referred to as ‘multivesicular’.<sup>1</sup>

Crucially, all of the as-made vesicles that we observed lack the characteristic interior structure membrane-bound compartments that nearly or entirely fill up the interior volume.

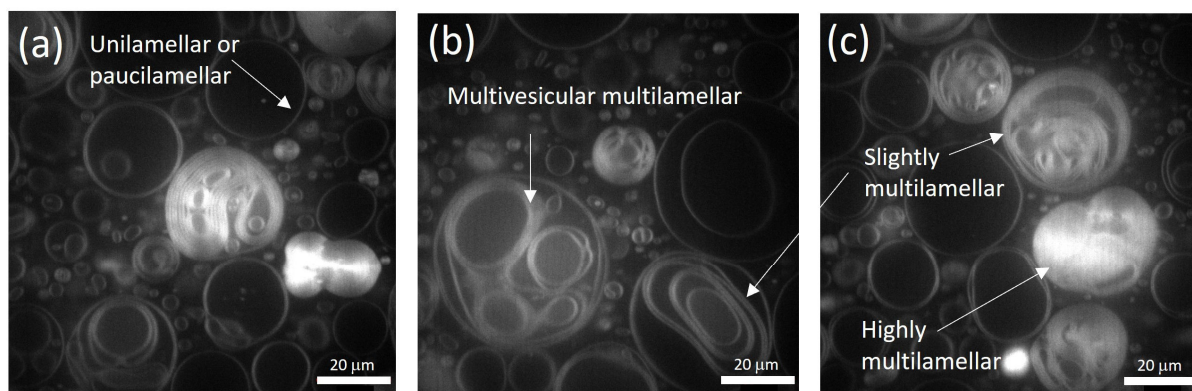
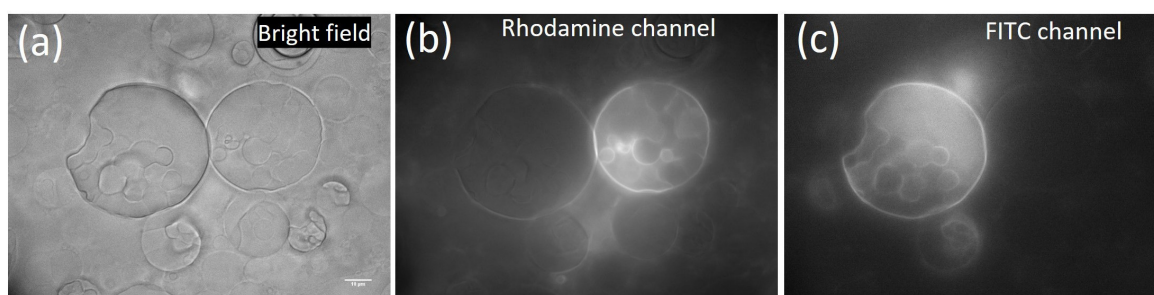


Fig. S1: Fluorescence microscope images of multilamellar vesicles as made, prior to exposure to PDADMAC. The kinds of vesicles are described in the text of this SI document.

**II. Mixing vesicles with different fluorescent labels** shows that each vesicular superparticle comes from just one parent vesicle.

To identify the origin of the reorganization of the inner membranes, we separately fabricated two vesicle batches, each labeled with a different fluorophore. One had Rhodamine-DOPE (0.1 mol%) and the other had NBD-DOPE (0.1 mol%). We uniformly mixed 10  $\mu\text{L}$  of each labeled vesicle suspension, added 80  $\mu\text{L}$  of non-fluorescent labeled vesicle suspension, then exposed the entire mixture to 20  $\mu\text{L}$  of the 100-200 kDa PDADMAC stock solution. The final solution was 180 mOsm/L and  $c_p=5.8$  mg/mL. Figure S2 shows the superparticles under bright field, rhodamine and fluorescein (FITC) fluorescent channels at the same location after 48 h. We found that superparticles were visible only in one single channel: no superparticles were visible in both fluorescent channels. Hence each fluorescent superparticle was only labeled by a unique fluorescent lipid and there was no mixing of lipids from the starting vesicles. This demonstrates that the interior membranes could only come from the parent vesicle, not by coalescence with a separate vesicle.

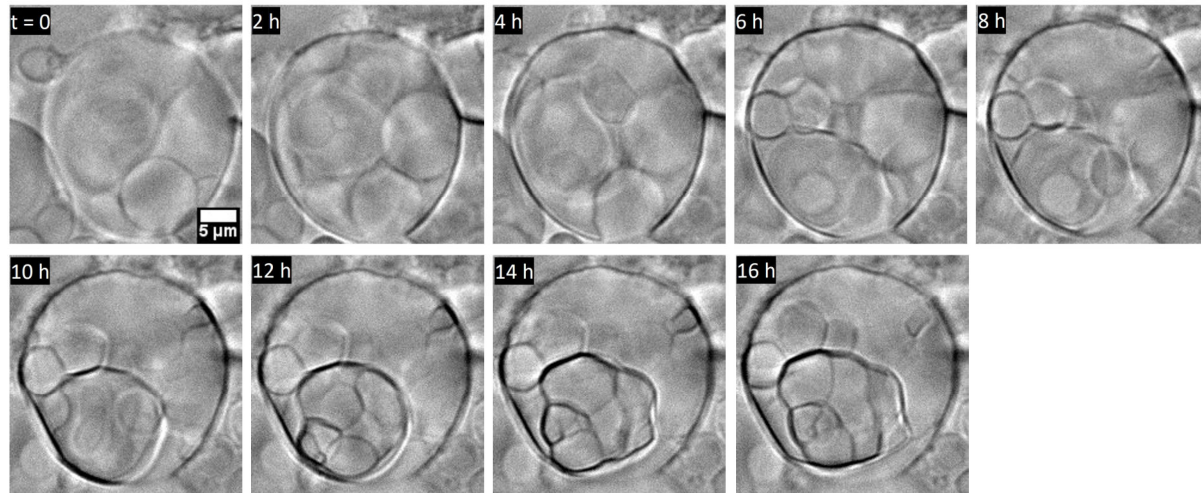


**Fig. S2:** Bright-field and fluorescence images of superparticles in a mixture of vesicles with two separate fluorescence labels. All three images (a,b,c) show the same region in the sample. In (b), one superparticle is visible in the rhodamine channel, with a small amount of scattered light illuminating the nearby ones. (c) An adjacent large superparticle and a few surrounding small ones are visible in the FITC channel. The one from (b) is not visible.

### III. Additional images from a movie showing formation of a vesicular superparticle.

Figure S3 is a set of images showing formation, providing further information for Fig 2 of the main text. 5  $\mu\text{L}$  of concentrated vesicle suspension (180 mOsm/L) was gently mixed with 45  $\mu\text{L}$  glucose solution (180 mOsm/L). We then injected 50  $\mu\text{L}$  of PDADMAC solution (400k – 500k Da, 56 mg/mL, 182 mOsm/L), mixed, and then injected a portion of it into a glass chamber for inspection. The chamber was sealed with vacuum grease to prevent evaporation. The final  $c_p = 28$  mg/mL.

The full movie is available as part of this SI as Cao\_SI-Movie\_SP-formation.mp4

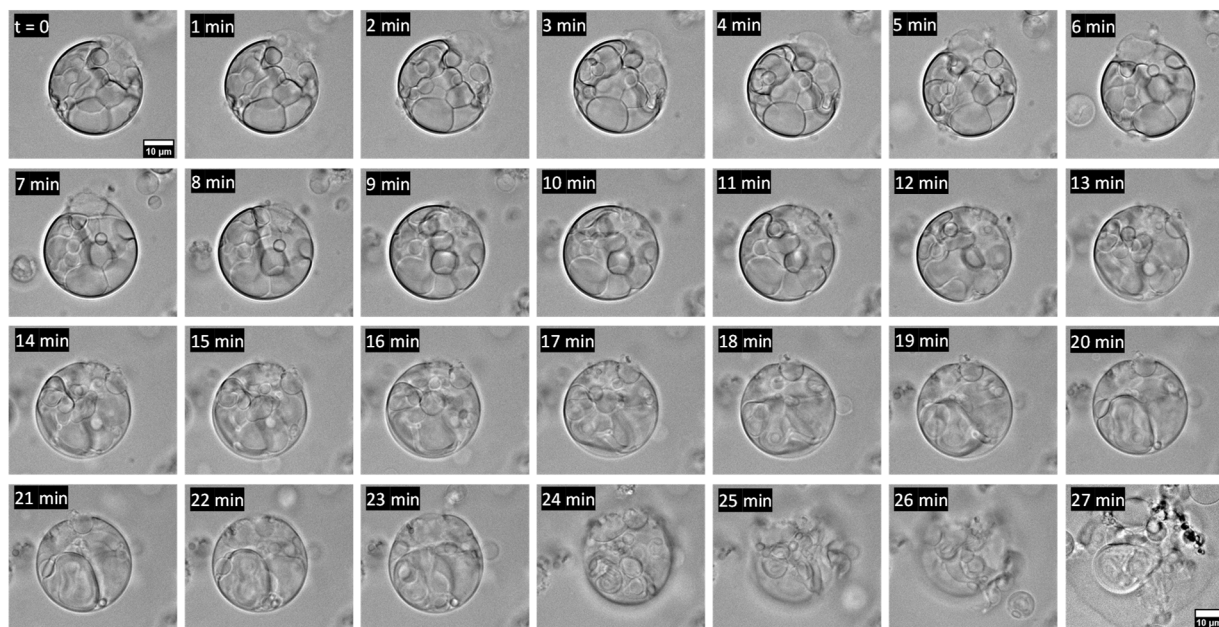


**Fig. S3:** Brightfield images, showing stages of formation of a vesicular superparticle. This is the same example as shown in Fig. 2 of the main text; here we show additional frames.

#### IV. Additional images from a movie showing breakup of a vesicular superparticle.

Figure S4 is a set of images showing breakup after PDADMAC was diluted by a factor of 10 with the osmotic strength kept constant. The superparticle formed with  $c_p = 28$  mg/mL and the images show response after dilution, which took place at approximately  $t = -5$  min. Some of these images appeared in Fig. 3(b).

The movie of this process is included in the SI, Cao\_SI-Movie\_SP-breakup\_B.mp4 (2.4 MB)



**Fig. S4:** Brightfield images, showing stages of disruption of a vesicular superparticle. This is the same example as shown in Fig. 3(b) of the main text; here we show additional frames.

## V. Details of the proposed model of superparticle formation.

Equation (4) of the main text is the double-layer electrostatic repulsion contribution to the final-state free energy:

$$F_{f,rep} = 4\pi R^2 \epsilon \left[ \frac{1}{2} (n+1-m)(n-m) \right] + 4\pi R^2 \epsilon (n+1-m) + \frac{1}{2} \epsilon (4\pi a^2 N - 4\pi R^2) \quad (\text{Eq. 4})$$

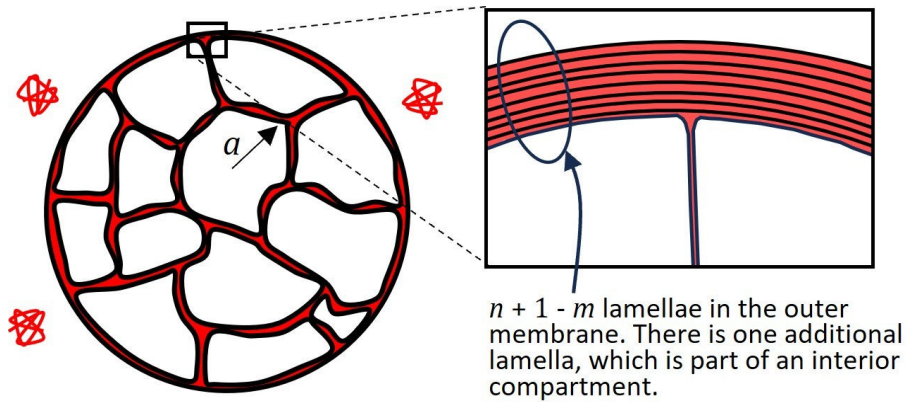
The first term of Eq. 4 accounts for the pairwise repulsion among the  $(n+1-m)$  lamellae in the outer membrane. As shown in Fig. S4, there is one more inner lamella that is part of the interior compartments. This lamella contributes  $4\pi R^2 \epsilon$  for each of the  $(n+m-1)$  nearby lamellae. The 3<sup>rd</sup> term accounts for the pair-wise interactions among the interior membranes. We must subtract the area  $4\pi R^2$  because the outermost layer was already accounted for in the 2<sup>nd</sup> term of Eq. (4). The prefactor of  $\frac{1}{2}$  accounts for the fact that pair-wise interactions involves two sections of membrane. This term accounts for the assumption that surface of each compartment *only* repels the membrane of the adjacent compartment because  $a \gg l_D$ .

$$\text{In the main text, we showed } \frac{\Delta F}{4\pi(2\kappa+\kappa_G)} = (m-1)[m^2 + m + \chi(m-2n-1)] \quad (\text{Eq. 5})$$

From this, we can find the equilibrium or free-energy-minimizing value of  $m$ . We assume is  $m \gg 1$  and therefore treat it as a continuous variable. The condition  $\left. \frac{d\Delta F}{dm} \right|_{m=m^*} = 0$  leads to:

$$m^* = n \text{ or } m^* = \left( \frac{1}{3} \right) \left( -\chi + \sqrt{\chi^2 + 6(n+1)\chi + 3} \right), \quad (\text{Eq. S1})$$

whichever is smaller.



**Fig. S5.** Illustration that shows the counting of lamellae for the repulsive free energy, Eq. (4) of the main text. The outermost membrane has  $n+1$  lamellae but there is one additional lamella from interior compartments.

## V. Description of the supplemental movies.

**Cao\_SI-Movie\_SP1.avi** (avi format, 2.0 MB)

**Cao\_SI-Movie\_SP2.avi** (avi format, 1.3 MB)

These two movies show z-scans through the interior of several vesicular superparticles, 400 kDa PDADMAC,  $c_P = 28$  mg/mL. The conditions are like those of Fig. 1 but the vesicles are different.

**Cao\_SI-Movie\_SP-formation.mp4** (16 MB)

This file is a time-lapse microscope movie of superparticle formation after giant multilamellar vesicles are exposed to 400 kDa PDADMAC with  $c_P = 28$  mg/mL. The movie is sped up by a factor of 1,000. The movie shows a cluster of vesicles, each of which is changing. Approximately 20% of the way into this movie, the focal plane shifts to the vesicle that is shown in Fig. 2 and Fig. S3.

**Cao\_SI-Movie\_SP-breakup\_A.mp4** (3.3 MB)

**Cao\_SI-Movie\_SP-breakup\_B.mp4** (2.4 MB)

These two files are time-lapse microscope movies of superparticle disruption. Vesicular superparticle was formed in conditions like those Fig. 1 and of Cao\_SI-Movie\_SP-formation.mp4. Here, the PDADMAC concentration was then diluted ten-fold (to  $c_P = 2.8$  mg/mL) while maintaining osmotic strength with glucose. Internal rearrangement is seen until, near the end, each vesicle ruptures and adheres to the glass coverslip. Each movie is sped up by a factor of 200. The same vesicles are shown in Fig. 3(a,b), respectively. Additional images from Cao\_SI-Movie\_SP-breakup\_B.mp4 are shown in Fig. S4.

### Reference cited in the SI:

1. Talsma, H.; Jousma, H.; Nicolay, K.; Crommelin, D. J. A., Multilamellar or Multivesicular Vesicles. *Int. J. Pharm.* **1987**, *37*, 171-173.