Electronic Supplementary Material (ESI) for Soft Matter.

Supporting information

Asymmetric desorption of lipid oxidation products induces membrane bending

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Derivation of the relation between GUV area change and lipid binding kinetics

We note that all the kinetic equations (Eq. 2, 3 and 4 in the main text) used for fitting experimental data in our study can be obtained from a general equation presented by Zhelev and Needham assuming appropriate initial conditions[1, 2]. In the following, we review the derivation of the kinetic fitting equations for various initial conditions relevant to our experiments.

(1) Lipid adsorption with flows containing LysoPC or PAzePC

We define N_{in} and N_{out} as the numbers of inserted lipids (LysoPC or PAzePC) in the inner leaflet and the outer leaflet, respectively, of a POPC membrane; A_{acc} and a_{POPC} are the accessible membrane area (the spherical portion, which is close to the initial total GUV surface area A_0 since the projection length is short at the very beginning of the adsorption process), and the average area of one POPC headgroup, respectively. We assume constant a_{POPC} and constant A_{acc} during the adsorption process. The total number of POPC lipids on the outer leaflet is A_0/a_{POPC}, which is approximately constant for $N_{out} << A_0/a_{\mathrm{POPC}}$.

We define c_{out} as the (bulk-) concentration of LysoPC or PAzePC outside the GUV and c_{in} as lipid concentration inside the GUV. The binding rate k_{on} is defined as the number of lipids inserted into the membrane, compared to the total lipid number of the membrane, as well as per unit concentration and per unit time. The unbinding rate k_{off} is defined as the number of lipids desorbing from one leaflet of the membrane per unit lipid and per unit time. The rate of transmembrane movement, k_{trans} is the number of lipids moved from one leaflet to the other leaflet per unit number difference comparing the two leaflets per unit time. The total number of inserted lipids in each membrane leaflet can be calculated as:

$$\frac{dN_{out}}{dt} = k_{on}\, c_{out} \frac{A_0}{a_{POPC}} - k_{off} \cdot N_{out} - k_{trans} \cdot \left(N_{out} - N_{in}\right) \tag{S1}$$

$$\frac{dN_{in}}{dt} = k_{on} c_{in} \frac{A_0}{a_{POPC}} - k_{off} \cdot N_{in} + k_{trans} \cdot \left(N_{out} - N_{in}\right) \tag{S2}$$

To note, in our experiment, 3 % to 20 % total area change were observed, which was achieved by an increase of the projection length and the shrinking of the spherical portion of the vesicle to maintain a constant volume. Such total area change corresponded to 1% - 10% decrease of the accessible membrane area.

(I) The flow rate was high enough to effectively remove the effect of a bulk diffusion zone created through the presence of a stagnant solution layer around the GUV surface.[1] We define the concentration of the bulk solution as c,

$$c_{out} = c \tag{S3}$$

(II) We assume that lipid binding and unbinding is always at equilibrium on the inner leaflet:

$$k_{on}\, c_{in} \frac{A_0}{a_{POPC}} = k_{off} \cdot N_{in} \tag{S4}$$

This assumption is proposed considering the limited solution volume inside the GUV. Based on assumption (I) and (II), Eq. S1 and Eq. S2 can be re-written as:

$$\frac{dN_{out}}{dt} = \frac{A_0}{a_{POPC}} k_{on}\, c - \left(k_{off} + k_{trans}\right) \cdot N_{out} + k_{trans} \cdot N_{in} \tag{S5}$$

$$\frac{dN_{in}}{dt} = \frac{A_0}{a_{POPC}} k_{trans} \cdot N_{out} - k_{trans} \cdot N_{in} \tag{S6}$$

With the definition $\epsilon = 2\frac{k_{trans}}{k_{off}}$, with **initial condition N_{out} (t=0) = N_{in} (t=0) = 0**, Eqs. S5 and S6 can be solved to obtain:

$$N_{out} = \frac{A_0}{a_{POPC}} \frac{k_{on}}{k_{off}} c \frac{1}{\sqrt{1+\epsilon^2}} \left\{ \frac{1+\sqrt{1+\epsilon^2}}{\epsilon + 1 + \sqrt{1+\epsilon^2}} \left[1 - e \right. \right. \tag{S7}$$

$$N_{in} = \frac{1}{2} \frac{A_0}{a_{POPC}} \frac{k_{on}}{k_{trans}} c \frac{1}{\sqrt{1+\epsilon^2}} \left\{ - \frac{1}{\epsilon + 1 + \sqrt{1+\epsilon^2}} \right. \tag{S8}$$

(III) When $k_{trans} \ll k_{off}$, we have $\epsilon \ll 1$. Thus Eq. S7 and Eq. S8 can be simplified to yield:

$$N_{out} = \frac{A_0}{a_{POPC}} \frac{k_{on}}{k_{off}} c \left\{ \left(1 - e^{-k_{off} \cdot t}\right) + \frac{k_{trans}}{k_{off}} \left(1 - e^{-k_{trans} \cdot t}\right) \right\} \tag{S9}$$

$$N_{in} = \frac{A_0}{a_{POPC}} \frac{k_{on}}{k_{off}} c \left\{ - \frac{k_{trans}}{k_{off}} \left(1 - e^{-k_{off} \cdot t}\right) + \left(1 - e^{-k_{trans} \cdot t}\right) \right\} \tag{S10}$$

(IV) We assume the change of membrane surface area is proportional to number of inserted lipids. Due to the slightly different headgroup area of LysoPC, PAzePC and POPC in membranes, we introduce a parameter γ, area ratio of PAzePC/LysoPC compared to POPC. MD simulation results have shown that the change of the average lipid area is within 3 % with PAzePC incorporated up to 30 %.[3] Thus we assume $\gamma = 1$ for PAzePC. According to X-ray diffraction studies, the headgroup area of 18:0 LysoPC is 45.5 Å^2 and the area of 18:1-16:0 PC is 64 Å^2 at room temperature.[4, 5] We

assume the area of 18:1 LysoPC to be similar to that of 18:0 LysoPC to obtain $\gamma = 0.7$. Thus the area change **normalized** to the initial area A_0 can be expressed as:

$$\Delta\bar{A} = \frac{\left[\frac{1}{2}(+)\, a_{POPC}\,\gamma\right]}{A_0} \tag{S11}$$

Combining Eq. S11 with Eq. S9 and S10, we obtain

$$\Delta\bar{A} = \frac{k_{on}}{k_{off}}\gamma\, c\left[1 - \frac{1}{2}\left(1 - \frac{k_{trans}}{k_{on}}\right)e^{-k_{off}\cdot t} - \frac{1}{2}\left(1 + \frac{k_{trans}}{k_{on}}\right)e^{-k_{trans}\cdot t}\right] \tag{S12}$$

For fitting purposes, we further simplify this equation with the assumption (III) $k_{trans} \ll k_{off}$

$$\Delta\bar{A} = \frac{k_{on}}{k_{off}}\gamma\, c\left(1 - \frac{1}{2}e^{-k_{off}\cdot t} - \frac{1}{2}e^{-k_{trans}\cdot t}\right) \tag{S13}$$

If the binding starts at $t = t_0$, then we obtain Eq. 2 in the main text.

(2) Lipid desorption under lipid-free flow conditions – after long-time adsorption

We still need to solve Eq. S1 and S2, with the assumptions (I) - (III) and changed conditions: (a) $c_{out} = 0\ \mu M$, (b) Initial condition: we assume (V): $N_{out}(t_0) = N_{in}(t_0) \equiv N_0 \neq 0$. Here, n_0 is the number of inserted lipids on each leaflet after the membrane exchange between two leaflets reaches equilibrium.

$$N_{out} = N_0\left[(1 - \frac{k_{trans}}{k_{off}})e^{-k_{off}\cdot(t-t_0)} + \frac{k_{trans}}{k_{off}}e^{-k_{trans}\cdot(t-t_0)}\right] \tag{S14}$$

$$N_{in} = N_0\left[-\frac{k_{trans}}{k_{off}}e^{-k_{off}\cdot(t-t_0)} + \left(1 + \frac{k_{trans}}{k_{off}}\right)e^{-k_{trans}\cdot(t-t_0)}\right] \tag{S15}$$

With assumption (IV), the normalized area change

$$\frac{\Delta\bar{A}(t)}{\Delta\bar{A}(t_0)} = \frac{\frac{1}{2}(N_{in} + N_{out})}{N_0} \tag{S16}$$

Further simplification can be obtained with assumption (III) $k_{trans} \ll k_{off}$:

$$\Delta\bar{A} = \frac{1}{2}\Delta\bar{A}(t_0)\left[e^{-k_{off}(t\,-\,t_0)} + e^{-k_{trans}(t\,-\,t_0)}\right] \tag{S17}$$

(3) Lipid desorption in lipid-free flows – after short-time adsorption

When the adsorption time does not last enough to meet the equilibrium of the lipid exchange between two leaflets, we have $N_{out} > N_{in}$.

For the extreme situation that $N_{in} \approx 0$ and $N_{out} \approx 2\,\frac{\Delta\bar{A}}{a_{POPC}\,\alpha}$,

we can use a simplified version of Eq. S1,

$$\frac{dN_{out}}{dt} = -\,k_{off} \cdot N_{out} \tag{S18}$$

The solution is:

$$N_{out}(t) = 2\,\Delta\bar{A}(t_0)\frac{A_0}{a_{POPC}}\,e^{-k_{off}(t-t_0)} \tag{S19}$$

With $N_{in} \approx 0$ and Eq. S11,

$$\Delta\bar{A} = \Delta\bar{A}(t_0)\,e^{-k_{off}(t-t_0)} \tag{S20}$$

(4) Experimental initial condition for lipid desorption studies

With known kinetic parameters from table 1, we estimate the difference between N_{out} and N_{in} after a 300 s adsorption process. We obtain $\frac{N_{out}}{N_{in}} \approx 3$ for 18:1 LysoPC, and $\frac{N_{out}}{N_{in}} \approx 2.2$ for PAzePC.

It follows that the experimental situations considered in this work did not completely satisfy the assumed initial condition for either of the desorption scenarios presented in (2) and (3). The equations derived above for the desorption process (Eq. S17 and Eq. S20, which correspond to Eq. 3 and Eq. 4 in the main content) allow for an approximate determination of the kinetic parameters only. This is particularly the case for k_{trans}, whose influence on the distribution among leaflets of inserted lipids depends on the number difference between the two membrane leaflets (see the term $k_{trans} \cdot (N_{out} - N_{in})$ in Eq. S1 and S2).

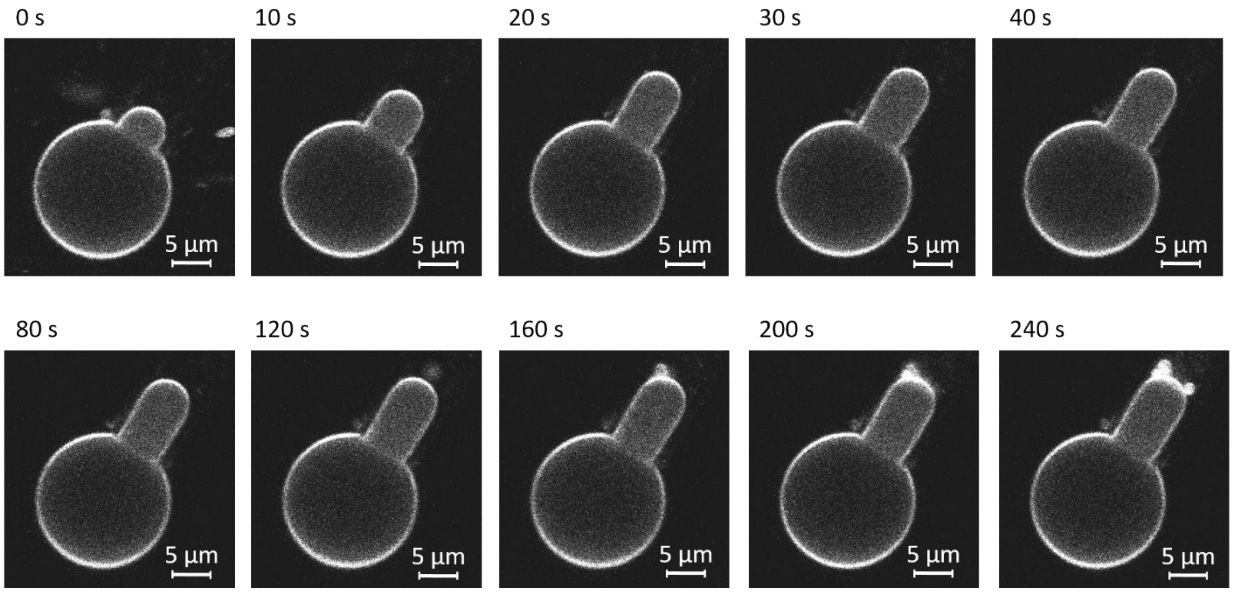
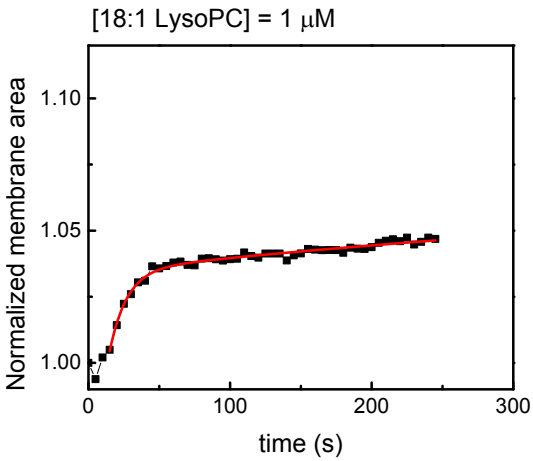
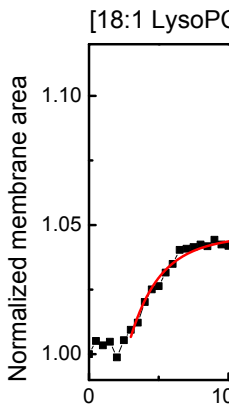


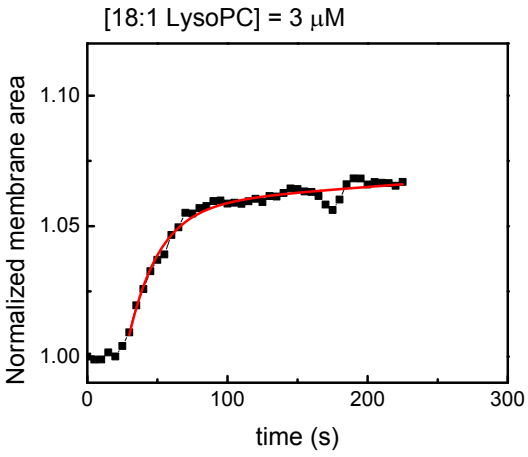
Figure S1. Time series of GUVs imaged during the adsorption process. Additional frames obtained between t = 0 s and t = 350 s are presented for the same GUV shown in Fig. 3. The top row shows images with 10 s intervals while the bottom row contains images with 40 s intervals. The projection length inside the aspiration pipette increased quickly in the first 30 s. Meanwhile, the spherical portion of this aspirated GUV shrank slightly to maintain constant total volume of the vesicle. After t = 30 s, the projection length increase was less significant, suggesting the binding and unbinding process almost reached equilibrium and more lipids were adsorbed slowly due to the transmembrane movement of lysolipids from the outer layer to the inner leaflet.



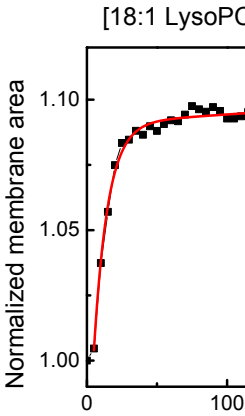
k_{off} (s^{-1})	0.082 ± 0.006
k_{on} $(s^{-1}\mu M^{-1})$	0.0029 ± 0.0002
k_{trans} (s^{-1})	0.0017 ± 0.0002



k_{off} (s^{-1})	0.048 ± 0.005
k_{on} $(s^{-1}\mu M^{-1})$	0.0010 ± 0.0001
k_{trans} (s^{-1})	0.0008 ± 0.0003

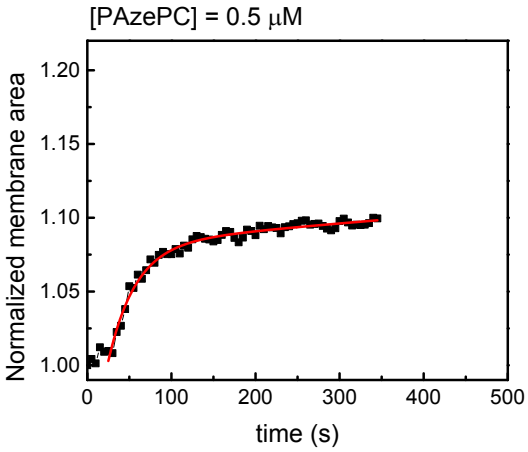


k_{off} (s^{-1})	0.046 ± 0.005
k_{on} $(s^{-1}\mu M^{-1})$	0.0009 ± 0.0001
k_{trans} (s^{-1})	0.0008 ± 0.0004

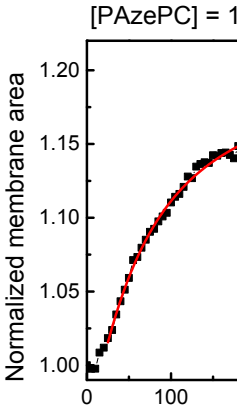


k_{off} (s^{-1})	0.101 ± 0.005
k_{on} $(s^{-1}\mu M^{-1})$	0.0023 ± 0.0001
k_{trans} (s^{-1})	0.0004 ± 0.0001

Figure S2. Examples of 18:1 LysoPC binding experiments with different LysoPC concentration in the flow. The red curve is the fitting curve based on Eq. 2 and fitting results of each example are shown below the figure. The errors are the standard error from the fitting.



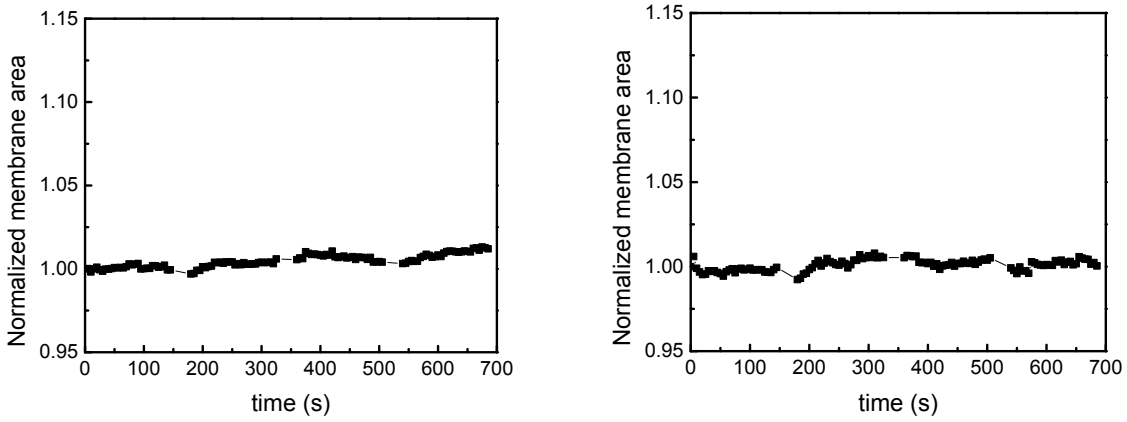
k_{off} (s^{-1})	0.036 ± 0.003
k_{on} ($s^{-1}\mu M^{-1}$)	0.0057 ± 0.0004
k_{trans} (s^{-1})	0.0009 ± 0.0002

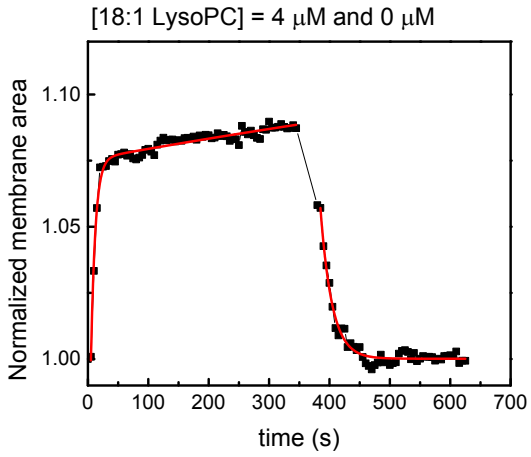


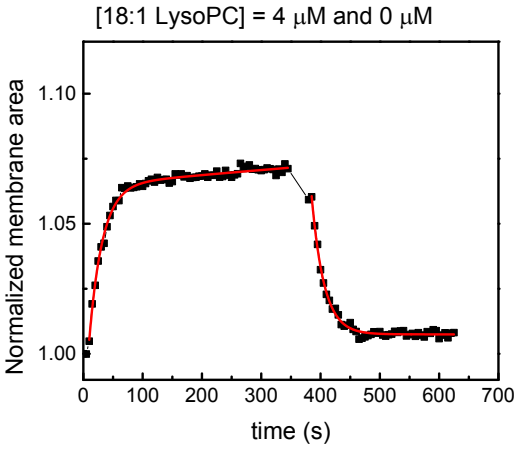
k_{off} (s^{-1})	0.017 ± 0.002
k_{on} ($s^{-1}\mu M^{-1}$)	0.0021 ± 0.0004
k_{trans} (s^{-1})	0.0019 ± 0.0010

Figure S3. Examples of PAzePC binding experiments with different PAzePC concentration in the flow. The red curve is the fitting curve based on Eq. 2. The table below each figure shows kinetic parameters in the form of fitting result ± standard error from the fitting.

Figure S4. GUV area barely changes in control experiments with lipid-free flows. Lipid-free flows injected from inlet 1 for 150 s and then from inlet 2 for 150 s. Two examples are presented with two cycles of lipid-free flows. Area fluctuations within 2% are observed which can be related to potential small position changes of the aspirated GUV in the solution flow which may induce less accurate measurement of the geometric parameters of the GUV.





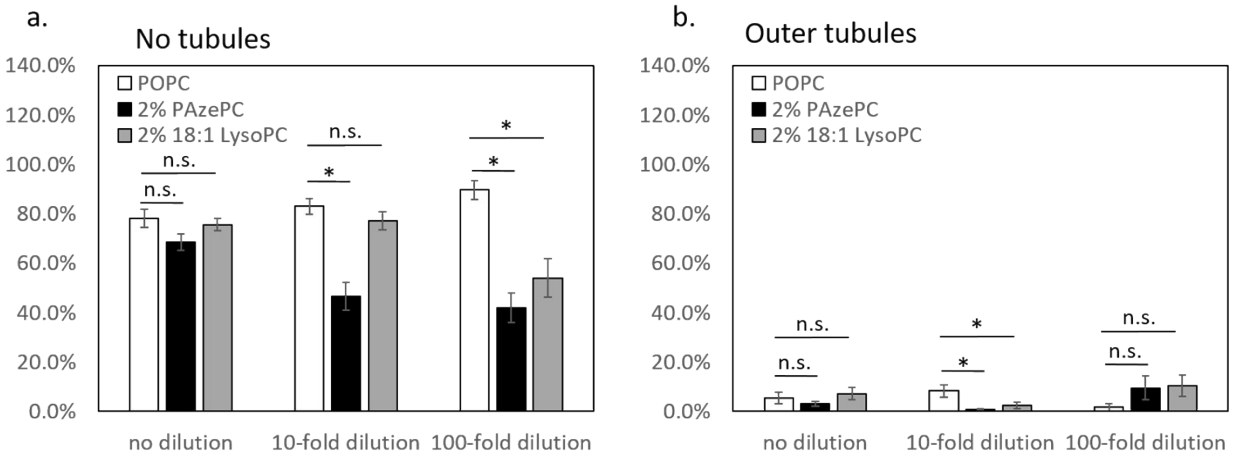


	k_{off} (s^{-1})
Adsorption	0.138 ± 0.007
desorption	0.052 ± 0.003

	k_{off} (s^{-1})
Adsorption	0.045 ± 0.002
desorption	0.048 ± 0.002

Figure S5. Examples of 18:1 LysoPC binding and unbinding cycles. Two examples of different GUVs with 4 μM LysoPC and lipid-free solution flows. The left panel is the same as Fig. 3b. The k_{off} fitted by Eq. 2 for adsorption and Eq. 4 for desorption are represented in bottom tables. The values obtained from adsorption and desorption are of similar magnitude and around the average value of k_{off} (0.08 ± 0.01, mean ± SEM) obtained from multiple adsorption trials. All of them are larger than the average k_{off} for PAzePC (0.02 ± 0.01, mean ± SEM). Note, less desorption trials were carried out compared to adsorption trials due to (1) the fact that GUVs tended to break with long projection length after the adsorption step; (2) the fitting equations (Eq. 3 and 4) for the desorption process suffer larger errors compared to Eq. 2 for the adsorption step.

Figure S6. Percentages of outer tubulation and no tubulation of different lipid composition in the dilution experiments. (a) Comparison of GUV percentages showing no tubules suggests that the number of no-tubule GUVs decreased with 10-fold dilution for 2 % PAzePC composition and with 100-fold dilution for 2 % 18:1 LysoPC composition. The decrease in numbers of no-tubule GUVs corresponds to the observed increase in numbers of inner-tubule GUVs. (b) GUVs of all the conditions show < 10 % outer tubulation. No significant differences were observed for different lipid composition comparing no dilution and 100-fold dilution. For 10-fold dilution, there was a small decrease (< 10 %) in outer tubulation for both 2 % PAzePC GUVs and 2 % LysoPC GUVs. This can be explained by the increased spontaneous curvature which may prohibit the membrane remodeling in the opposite direction.



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