# Adsorption-induced vesicle fission

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A theoretical model for vesicle fission induced by particle adsorption and aggregation on a membrane surface is presented. The bulk fluid contains particles that are adsorbed reversibly to the membrane. Adsorbed particles aggregate on the membrane, forming particle-rich domains. Domains at a critical size which contains $n_v$ particles become vesicles and leave the membrane. We find that for parameters that correspond to typical experimental situations, vesicle formation is energetically favored except for a possible energy barrier for domain nucleation at small $n$, where $n$ is the number of particles in a domain. We also find that in typical experimental situations a particle-rich domain grows without being affected strongly by neighboring domains, and the vesicle formation rate is proportional to adsorption rate $j_{\mathrm{on}}$ when $j_{\mathrm{on}}$ is large; when $j_{\mathrm{on}}$ is small the vesicle formation rate scales like $j_{\mathrm{on}}^2$. Because the diffusion flux is small for domains with small in-plane radius $R_n$, in the time-independent state the densities of domains with $n/n_v \ll 1$ and $1-n/n_v \ll 1$ are large.

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## I. INTRODUCTION

An important function of biomembranes is to separate the inner and outer environments of a cell or an organelle. For example, ions and small molecules move through specific channels in a biomembrane [1]. On the other hand, a cell often takes larger particles like proteins and viruses by engulfing these particles with cell membrane, a process termed endocytosis. In many cases, large particles in the bulk fluid outside the cell first adsorb on the membrane, then assemble to form domains, domains with sufficiently large size form buds in the membrane, and eventually become vesicles and leave the membrane with or without help from specific proteins [1]. For example, experiments with the yolk of hen ovum indicated that particles assemble on the cytosolic surface of the membrane and then form vesicles [2]. Experiments on HeLa cells [3] found that transferrin receptors are taken into the cell via coated pits. Recent experiments on retroviruses also indicate that the budding of retroviruses begins with the adsorption and assembly of Gag proteins on the cell membrane [4].

Theoretical studies on endocytosis have focused on how viruses or colloids coated with many specific ligands enter cells. Lipowsky *et al.* [5] studied the free energy of flexible membranes in contact with dispersed nanoparticles or colloids and showed that when membrane-particle interactions are attractive the membrane can wraps itself around the particles. In an analysis based on equilibrium statistical mechanics, Tzlil *et al.* [6] showed that complete viral budding can take place only when the ligand-receptor adhesion energy exceeds a critical bending free energy. Gao *et al.* [7] showed that viruses with sizes in the range of tens to hundreds of nanometers can enter cells via wrapping events in the absence of clathrin coats, and there exists an optimal viral size that has the shortest wrapping time. Effenterre and Roux [8] studied the adhesion of colloidal particles on the surface of a cell and showed distinct behavior of the system at low and high concentrations of colloidal particles. On the other hand, there have been relatively few studies on the formation of fluid-containing vesicles with many particles adhered to their inner surfaces. [9] For example, a recent simulation shows that the attractive interaction induced by membrane curvature elasticity is sufficient to drive the aggregation of particles for vesicle formation [10]. The energetics for human immunodeficiency virus (HIV) self-assembly and budding and the dynamics of the early stage of budding of a partial budded retrovirus capsid were studied by Zhang and Nguyen [11]

This paper discusses several issues that have not been addressed or have only been partially addressed in previous theoretical work on adsorption-induced vesicle formation. The formation of vesicles follows the growth of particle-rich domains which in turn follows the adsorption of particles from bulk fluid. Therefore one should treat the growth of particle-rich domains as a two-dimensional domain growth in the presence of an external particle source (the bulk fluid) and sink (vesicles that leave the membrane). In general, the growth of a particle-rich domain should be affected by other domains in the membrane [12,13]. The following questions are of special interest: Is domain growth and vesicle formation a thermally activated process? How does the number of vesicles produced by the membrane depend on the adsorption rate of particles? What is the density of particle-rich domains with given size? How does the growth of a particle-rich domain depend on its neighboring domains? We shall begin with a discussion on the energetics of domain growth and vesicle formation that follows closely the classic work of Lipowsky [14]. Based on energetic considerations, we construct a minimal model for the kinetics of the vesicle formation process. Our study provides answers for the questions above.

## II. ENERGETIC CONSIDERATION

As shown in Fig. 1, the system under consideration contains a flat membrane that separates the outer and inner fluids. The outer fluid contains particles that can be adsorbed reversibly on the membrane. The adsorption rate is denoted as $j_{\mathrm{on}}$ and the desorption rate is denoted as $j_{\mathrm{off}}$. After being adsorbed on the membrane, these particles aggregate and

 

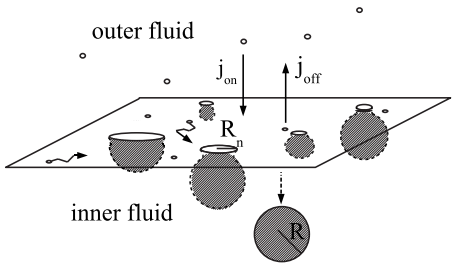


FIG. 1. Schematics of the system under consideration. A flat membrane separates the inner and outer fluids; the outer fluid contains particles that can be adsorbed to the membrane and the inner fluid does not contain any particles. Particles adsorb on the membrane with rate $j_{on}$; after adsorption they can depart from the membrane with rate $j_{off}$. At the same time, particles on the membrane aggregate to form domains that bud toward the inner fluid with radius of curvature $R$. Sufficiently large domains depart from the membrane by vesicle fission.

form particle-rich domains that bud toward the inner fluid with radius of curvature $R$. Typically, $R$ is in the range from tens of nanometers to about 1 $\mu$m, and in general $R$ depends on $n$, the number of particles in the domain. We assume that domains with $n_v$ particles depart from the membrane and enter the inner fluid by vesicle fission. The key physical mechanism for the budding of a particle-rich domain is the tendency for the particles to bend the membrane, although it has been pointed out that line tension of the domain boundary can induce budding even when the spontaneous curvature of a domain is zero [15].

In many biological examples, the aggregation of adsorbed particles on a membrane is mediated by specific interactions, not weak generic interactions. Therefore we assume that a particle-rich domain with $n$ particles has area $n\pi a^2$; here $a \sim 2-5$ nm is the effective radius of a particle. In this paper, all numerical solutions are performed by taking $a=2$nm, and we have checked that all results are unchanged qualitatively if $a$ is taken to be 5nm. As shown in Fig. 2, we assume that, when a particle-rich domain buds it has the shape of a spherical cap with radius of curvature $R$, the area of the neck of the domain is $\pi R_n^2$, where $R_n$ is the radius of the neck. The membrane outside a particle-rich domain is stretched by the domain because the area of a neck is smaller than that of a flat domain. The free energy of a particle-rich domain includes the energy cost for stretching the membrane, the bending

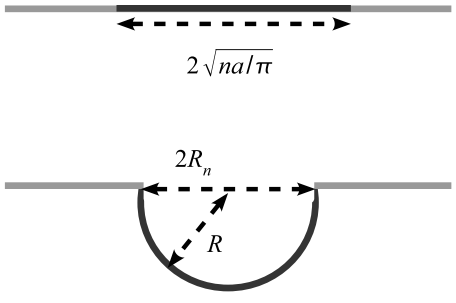


FIG. 2. Schematics of a particle-rich domain. Before budding the domain is a flat disk with radius $\sqrt{na/\pi}$; after budding the domain has a neck with radius $R_n$.

energy of the domain, the line energy of the neck, and the aggregation energy of the particles [14],

$$F = \Gamma(n\pi a^2 - \pi R_n^2) + n\pi a^2 \frac{\kappa}{2}\left(\frac{2}{R} - \frac{2}{R_0}\right)^2 + 2\pi R_n \sigma + E_a n. \tag{1}$$

Here $\Gamma$ is the surface tension of the membrane, $\kappa$ is the bending rigidity of the domain, $R_0$ is the preferred radius of curvature for a particle-rich domain, $\sigma$ is the line tension on the boundary of the domain, and $E_a$ is the chemical potential of a particle in the domain. For living cells $\Gamma \sim 10^{-5}$ J/m$^2$ [16]. The line tension $\sigma$ can be estimated roughly as $\sigma \sim |E_a|/a$. Due to contributions from the adsorbed particles, the bending elastic modulus $\kappa$ of the domain is large compare to that of a pure lipid bilayer, i.e., $\kappa \gg 20k_BT$. For particles that can aggregate on a membrane, $|E_a| \gtrsim k_BT$.

The equilibrium shape of a domain for given $n$ is determined by minimizing the free energy with respect to the radius of curvature, i.e., $(\partial F/\partial R)_n = 0$. The shape of the domain can be described by $\sqrt{na}/R$, and it follows that $R_n = \sqrt{na}\sqrt{1-(\sqrt{na}/2R)^2}$. When the domain is flat, $\sqrt{na}/R = 0$ and $R_n = \sqrt{na}$; as the domain buds $\sqrt{na}/R$ increases until $\sqrt{na}/R \to 2$ and $R_n \to 0$. The equation of state of the domain at given $n$ is

$$8\frac{\kappa/\sigma}{\sqrt{na}}\left(\frac{\sqrt{na}}{R} - \frac{\sqrt{na}}{R_0}\right) = \left(1 - \frac{R_n}{\sigma/\Gamma}\right)\frac{\sqrt{na}}{R}\frac{1}{\sqrt{1-(\sqrt{na}/2R)^2}}. \tag{2}$$

Since $\kappa \gg 20k_BT$, $\sigma \sim |E_a|/a$, $R_n \lesssim O(100\text{ nm})$, and $|E_a| \gtrsim k_BT$, we have $(1-R_n\Gamma/\sigma) \lesssim O(1)$ and $\kappa/\sqrt{na}/\sigma \gg 1$. In this limit the radius of curvature of the domain is expected to be close to $R_0$. Straightforward calculation shows the following.

(a) When $1-\sqrt{na}/2R_0 \sim O(1)$,

$$\frac{1}{R} \approx \frac{1}{R_0}\left[1 + \frac{\sqrt{na}}{8\kappa/\sigma}\left(\frac{1}{\sqrt{1-(\sqrt{na}/2R_0)^2}} - \frac{\sqrt{na}}{\sigma/\Gamma}\right)\right]. \tag{3}$$

(b) When $1-\sqrt{na}/2R_0 = \delta \ll 1$, the curvature of the domain is $1/R = (1/R_0)(1+\epsilon)$, where $\epsilon$ satisfies

$$\delta = \epsilon + \left[4\sqrt{2}\left(\frac{\kappa/\sigma}{R_0}\epsilon + \frac{R_0}{2\sigma/\Gamma}\right)\right]^{-2}. \tag{4}$$

First, Eq. (4) ensures that $\epsilon \ll 1$ in this regime. Therefore the radius of curvature of a domain is always close to $R_0$. Furthermore, Eq. (4) does not have a solution with $\epsilon > 0$ when $\delta < \delta_{\min} \approx \frac{3}{2} \times 4^{-2/3}(\kappa/\sigma\, R_0)^{-2/3} - \frac{1}{2}(R_0\,\Gamma/\sigma)(\kappa/\sigma\, R_0)^{-1}$. This means that, when the domain grows to $\sqrt{na}/2R_0 = 1-\delta_{\min}$, the local free energy minimum disappears, and the bud loses stability and forms a vesicle immediately. This also means that before $\sqrt{na}/2R_0$ reaches $1-\delta_{\min}$ there is a range of $n$ where the incomplete bud is thermodynamically metastable. Indeed, one can look for the value of $\delta$ at which the free energy of the bud with $\epsilon$ given by the solution of Eq. (4) is the same as the free energy of a vesicle with the same number of particles. The result is that this happens when $\delta = \delta^* \approx \frac{3}{4}(\kappa/\sigma\, R_0)^{-2/3} - \frac{1}{2}(R_0\,\Gamma/\sigma)(\kappa/\sigma\, R_0)^{-1}$. Therefore a domain

with $1-\delta_{\min}>\sqrt{n}a/2R_0>1-\delta^*$ may become a vesicle by thermal activation. The energy barrier for a bud with $\sqrt{n}a/2R_0=1-\delta^*$ to change its shape to a complete vesicle is also straightforward to calculate; it is $2\pi\sigma R_0(\alpha^2-3\alpha+2\sqrt{\alpha})(\kappa/\sigma R_0)^{-1/3}$, where $\alpha=(2-\sqrt{3})/2$.

The above discussion indicates that, although thermally activated and spontaneous vesicle formation is possible, the radius of curvature is $R\approx R_0$ for all particle-rich domains in the membrane, and vesicle formation occurs when $n\pi a^2 \approx 4\pi R_0^2$. Therefore in the rest of this paper we shall assume $R=R_0$ for all domains, and domains with $n_v=4\pi R_0^2/\pi a^2$ particles become vesicles. By taking $R=R_0$ the free energy of a domain becomes

$$F = E_a n + \Gamma\frac{\pi a^2}{4\pi R_0^2}\pi a^2 n^2 + 2\pi\sigma\sqrt{n}a\sqrt{1-\frac{n\pi a^2}{4\pi R_0^2}}$$
$$= E_a n_v\left[\frac{n}{n_v} - \tilde{\Gamma}\left(\frac{n}{n_v}\right)^2 - \frac{\tilde{\sigma}}{\sqrt{n_v}}\sqrt{\frac{n}{n_v}\left(1-\frac{n}{n_v}\right)}\right], \quad (5)$$

where $\tilde{\Gamma}=-\pi a^2\Gamma/E_a$ is the dimensionless surface tension of the membrane, and $\tilde{\sigma}=-2\pi a\sigma/E_a$ is the dimensionless line tension of the domain boundary. Substituting typical values of $\Gamma$, $a$, and $\sigma$, we find that $\tilde{\Gamma}\ll O(1)$ and $\tilde{\sigma}\sim O(1)$.

Equation (5) tells us that particle-rich domains can exist when $E_a<0$. As a domain grows, it has to pull nearby lipids in order to form a neck; this process costs a surface energy that scales as $(n/n_v)^2$. The line energy of the neck also contributes to the free energy of a domain. For small $n/n_v$ the line energy increases as $n^{1/2}$; as $n/n_v$ approaches 1 the line energy approaches zero as $(1-n/n_v)^{1/2}$. This indicates that there are two possible energy barriers for vesicle formation. (i) When the line tension of the domain is sufficiently large, there is an energy barrier at $n/n_v\ll 1$ for domain nucleation. (ii) When the surface tension of the membrane exceeds some critical value, a local free energy minimum appears at $n/n_v \approx 1$. Figure 3 shows $F(n)$ in situations where there is a barrier due to line tension, a minimum due to surface tension, and no energy barrier for vesicle formation. The sign of $(\partial F/\partial n)_{n=1}$ can be used to check whether there is an energy barrier at small $n$. From

$$\left(\frac{\partial F}{\partial n}\right)_{n=1} \approx E_a n_v\left(1-\frac{2\tilde{\Gamma}}{n_v}-\frac{\tilde{\sigma}}{2}\right) \approx E_a n_v\left(1-\frac{\tilde{\sigma}}{2}\right) \quad \text{at } n_v \gg 1,$$

as long as $\tilde{\sigma}<2$ the free energy of the domain decreases with increasing $n$ at small $n$, and there is no line-tension-induced energy barrier. When surface tension is sufficiently large, an energy minimum appears at $n/n_v=1-\varepsilon$ where $\varepsilon\ll 1$. Therefore when this energy minimum just appears both $\partial F/\partial n$ and $\partial^2 F/\partial n^2$ vanish at $n/n_v=1-\varepsilon$. These two conditions give us

$$2\tilde{\Gamma}\varepsilon + \frac{\tilde{\sigma}}{2\sqrt{n_v\varepsilon}} + (1-2\tilde{\Gamma}) = 0 \quad (6)$$

and

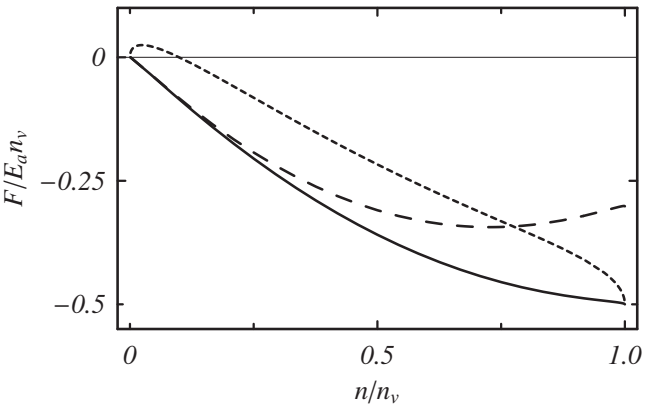


FIG. 3. Free energy of a domain with $n$ particles for $n_v=1000$. Solid line, $\tilde{\Gamma}=0.5$, $\tilde{\sigma}=1$; short-dashed line, $\tilde{\Gamma}=0.5$, $\tilde{\sigma}=10$; long-dashed line, $\tilde{\Gamma}=0.5[1+5(\tilde{\sigma}^2/2n_v)^{1/3}]>\tilde{\Gamma}_c$, $\tilde{\sigma}=1$. The dimensionless surface tension $\tilde{\Gamma}=-\pi a^2\Gamma/E_a$, and the dimensionless line tension of the domain, $\tilde{\sigma}=-2\pi a\sigma/E_a$. Solid line does not have local minimum, short-dashed line has a barrier at $n/n_v\ll 1$ due to the line energy of the domain, and long-dashed line has an energy minimum at large $n/n_v$ due to surface tension of lipids outside the domain.

$$2\tilde{\Gamma} - \frac{\tilde{\sigma}}{4\sqrt{n_v[\varepsilon(1-\varepsilon)]^3}} = 0. \quad (7)$$

It follows that $\varepsilon=(\tilde{\sigma}/8\tilde{\Gamma}\sqrt{n_v})^{2/3}$ and $\tilde{\Gamma}=\tilde{\Gamma}_c=\frac{1}{2}[1+\frac{3}{2}(\tilde{\sigma}^2/2n_v)^{1/3}]\sim O(1)$. Since $\tilde{\Gamma}\ll O(1)$ for typical values of $\Gamma$, $a$, and $\sigma$, we find that $F$ is a monotonically decreasing function of the number of particles $n$ in the domain except for a possible energy barrier at small $n$ due to the line tension of the domain boundary.

## III. MODELING THE KINETICS

Based on the study of the free energy of a particle-rich domain, in the analysis of the kinetics of vesicle fission we assume that (i) the particles adjust their positions quickly once they are attached to the domains, and as a result a domain always has a circular boundary, (ii) due to strong binding energy between the particles, particles in a domain with $n>1$ do not leave the membrane, (iii) domain growth is a diffusion-limited process—once a particle reaches the boundary of another domain it joins that domain immediately, and (iv) there is a critical number of particles in a domain, $n_v=4\pi R_0^2/\pi a^2$, such that domains containing $n<n_v$ particles grow, and once $n$ reaches $n_v$ a domain becomes a vesicle immediately. Since $n_v\gg 1$, in the following we shall take $n_v\pm 1\approx n_v$. Assumption (ii) should hold for $\tilde{\sigma}<2$ and we expect this approximation will not affect our analysis seriously because $\tilde{\sigma}/2\sim O(1)$, so the critical size for domain nucleation should be small anyway.

Let $\rho(n,t)$ be the number of $n$-particle domains per unit area at time $t$; the areal fraction of particle-rich domains on the membrane is $\Sigma_{n=1}^{n_v-1} n\pi a^2\rho(n,t)$. The time evolution of the total areal fraction of particle-rich domains on the membrane is described by

$$\frac{d}{dt}\left(\sum_{n=1}^{n_v-1} n\pi a^2\rho(n,t)\right) = j_{\text{on}}\left(1-\sum_{n=1}^{n_v-1} n\pi a^2\rho(n,t)\right) - j_{\text{off}}\pi a^2\rho(1,t) - n_v\pi a^2 k(n_v-1)\times\rho(n_v-1,t), \tag{8}$$

where $k(n)$ is the growth rate of domains containing $n$ particles. The first two terms on the right-hand side are the number of particles adsorbed to or desorbed from the membrane per unit area per unit time. The last term comes from particles leaving the membrane by vesicle fission. Typically $j_{\text{off}}^{-1} \approx \tau_0 \exp(\epsilon/k_BT) \sim 10^{-6}-10^2$ s, where $\tau_0 = 6\pi\eta a^3/k_BT \sim 10^{-7}$ s [17] is the characteristic microscopic time for a particle to escape from the membrane; because usually several hydrogen bonds are involved in binding between a particle and the membrane, the typical binding energy of a particle is $\epsilon \sim (2\text{–}20)k_BT$. $j_{\text{on}}^{-1}$ gives the time scale for particles to fill the membrane surface for irreversible adsorption. $j_{\text{on}}/j_{\text{off}}$ gives the characteristic areal fraction of particles for adsorption and desorption; in most experiments $j_{\text{on}} \ll j_{\text{off}}$.

Since domain growth is assumed to be irreversible, $\rho(n,t)$ obeys

$$\frac{\partial\rho(n,t)}{\partial t} = -k(n)\rho(n,t) + k(n-1)\rho(n-1,t), \tag{9}$$

for $n_v - 1 \geqslant n > 1$. In Eq. (9) we have neglected the possibility that particles in the outer fluid adsorb directly to the periphery of a domain. Combining Eqs. (8) and (9), one finds that

$$\frac{\partial\rho(1,t)}{\partial t} = -k(1)\rho(1,t) - \sum_{n=1}^{n_v-1} k(n)\rho(n,t) + \frac{j_{\text{on}}}{\pi a^2}\left(1-\sum_{n=1}^{n_v-1}\pi a^2 n\rho(n,t)\right) - j_{\text{off}}\rho(1,t) = \frac{j_{\text{on}}}{\pi a^2} - j_{\text{off}}\rho(1,t) - \left(k(1)\rho(1,t) + \sum_{n=1}^{n_v-1} k(n)\rho(n,t)\right). \tag{10}$$

The first two terms of the last expression come from particles adsorbed to and desorbed from the membrane; the remaining terms come from particle-particle collisions and particles joining other domains. Because we are interested in the small areal fraction limit $\pi a^2 \Sigma_{n=1}^{n_v-1} n\rho(n,t) \ll 1$, thus $j_{\text{on}}[1-\Sigma_{n=1}^{n^*-1}\pi a^2 n\rho(n,t)] \approx j_{\text{on}}$.

When the distribution of domain size reaches a time-independent state, $\rho(n,t) \to \rho_s(n)$, and Eq. (9) gives

$$k(n)\rho_s(n) = k(1)\rho_s(1), \tag{11}$$

for $1 < n \leqslant n_v - 1$. In this state the number of vesicles produced per unit area per unit time is $\rho_s(n_v-1)k(n_v-1) = \rho_s(1)k(1)$, and the number of single-particle domains in the membrane can be found from Eq. (10),

$$\rho_s(1) = \frac{j_{\text{on}}}{[n_v k(1) + j_{\text{off}}]\pi a^2}. \tag{12}$$

This expression simply states that in the time-independent state the number of particles adsorbed by the membrane per unit time is the sum of the number of particles joining other domains [the first term in the denominator of Eq. (12)] and the number of particles leaving the membrane without joining other domains [$j_{\text{off}}$ term in Eq. (12)].

In our model a domain is a perfect sink that grows by recruiting other adsorbed particles within the "basin of attraction" around it; the domain growth rate $k(n)$ can be found from the particle diffusion flux on the domain boundary. Introducing number density of one-particle domains $\rho_1(\mathbf{r},t)$, whose spatial average is $\rho(1,t)$, Equation (10) provides us the following picture for the time evolution of $\rho_1(\mathbf{r},t)$. Depending on the characteristic size of the basins of attraction and the average interdomain distance, there are two limiting situations. In the first case the basins of attraction of neighboring domains do not overlap and therefore the growth of a domain is not affected by other domains. This case is called the "independent growth regime." In the second case the typical size of the basins of attraction becomes large compared to the average interdomain distance; therefore a domain grows by sharing the same "particle source" with many neighboring domains. This situation is called the "strongly coupled regime." This leads to the following equation for the local density $\rho_1(\mathbf{r},t)$ in the rest frame of an $n$-particle domain with $n \gg 1$ located at $\mathbf{r}=\mathbf{0}$:

$$\frac{\partial\rho_1(\mathbf{r},t)}{\partial t} = D\nabla^2\rho_1 + \frac{j_{\text{on}}}{\pi a^2} - j_{\text{off}}\rho_1 - D\xi^{-2}\rho_1. \tag{13}$$

$D$ is the diffusion coefficient of an adsorbed particle in the rest frame of the domain, $D \sim 1\ \mu\text{m}^2/\text{s}$ [17]. The last term on the right-hand side represents other sinks (domains) located at $\mathbf{r}$. In the independent growth regime $D\xi^{-2}=0$; in the strongly coupled regime $D\xi^{-2}$ can be determined by the following method [18]. Because $k(1)\rho(1,t)+\Sigma_{n=1}^{n_v-1}k(n)\rho(n,t) = n_v k(1)\rho_s(1)$ when $\rho(n) \to \rho_s(n)$, it follows by comparing to Eq. (10) that $(D\xi^{-2})_{\rho(n)\to\rho_s(n)} = n_v k(1)$ in the strongly coupled regime. In short, when $\rho(n) \to \rho_s(n)$,

$$D\xi^{-2} = \begin{cases} 0 & \text{in independent growth regime,} \\ n_v k(1) & \text{in the strongly coupled regime,} \end{cases} \tag{14}$$

and

$$\rho_1(r=\infty) = \frac{j_{\text{on}}}{\pi a^2(j_{\text{on}} + j_{\text{off}} + D\xi^{-2})} \equiv \frac{j_{\text{on}}}{\pi a^2 D m^2}. \tag{15}$$

Here the inverse characteristic length $m = \sqrt{(j_{\text{on}}+j_{\text{off}}+D\xi^{-2})/D} \approx \sqrt{(j_{\text{off}}+D\xi^{-2})/D}$.

As in the phase-ordering kinetics of binary fluids [19], the characteristic time scale for domain growth is much longer than that of particle diffusion in the membrane. Therefore $\rho_1(\mathbf{r})$ satisfies the steady state solution of Eq. (13) with $\rho_1(R_n)=0$ and $\rho_1(\infty) = j_{\text{on}}/(\pi a^2 D m^2)$. The domain growth rate $k(n)$ is related to $\rho_1(\infty)$ by

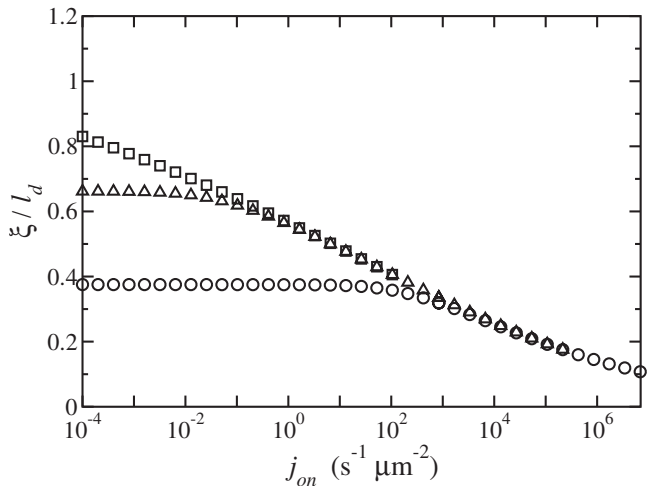


FIG. 4. Ratio between $\xi$, the characteristic length associated with sinks in the strongly coupled regime, and $l_d$, the average interdomain distance for $n_v=1000$. $j_{\rm off}=10^3$ (circles), 10 (triangles), and $10^{-2}$ s$^{-1}$ (squares). The result indicates that on average the number of particle-rich domains in a region of size $\sim\xi$ is small [i.e., $\leqslant O(1)$]. This result holds for $100\leqslant n^*\leqslant 10\,000$ (data not shown).

$$k(n)=[2\pi rD(\partial\rho_1/\partial r)]_{r=R_n}=2\pi R_n Dm\frac{K_1(mR_n)}{K_0(mR_n)}\rho_1(\infty). \tag{16}$$

Here $K_m(x)$ is the modified Bessel function of order $m$ [20]. Equation (16) describes the growth rate of domains with $n>1$, but $k(1)$ has to be treated differently because the growth of a domain from $n=1$ to 2 is caused by binary collision, and in the rest frame of a one-particle domain that is located at the origin, the first term of Eq. (13) should be replaced by $2D\nabla^2\rho_1$. Furthermore, the capture radius for a binary collision is approximately $2a$ [21]. Straightforward algebra similar to the derivation of $k(n>1)$ gives

$$k(1)=4\sqrt{2}\pi Dma\frac{K_1(\sqrt{2}ma)}{K_0(\sqrt{2}ma)}\rho_1(\infty). \tag{17}$$

Equatios (11), (12), and (15)–(17) form a closed set of equations for the system in the limit $\rho(n,t)\to\rho_s(n)$.

## IV. KINETICS OF DOMAIN GROWTH

### A. Typical domain growth is in the independent growth regime

Let us first check whether domain growth is strongly coupled or not. Equation (13) indicates that when domain growth is strongly coupled, the average interdomain distance $l_d\equiv 1/\sqrt{\sum_{n=1}^{n_v-1}\rho_s(n)}$ should be small compared to $\xi=\sqrt{D/n_vk(1)}$, the characteristic length in the strongly coupled regime.

Figure 4 shows $\xi/l_d$ as a function of $j_{\rm on}$ for $n_v=1000$ and several typical values of $j_{\rm off}$. We find that $\xi/l_d\lesssim 1$ for all $j_{\rm on}$ and $j_{\rm off}$ shown in the figure. In the Appendix we show analytically that in general $\xi/l_d\lesssim 1$. Thus, for typical experiments, domain growth is not in the strongly coupled regime. In the rest of this paper we shall focus on the independent growth regime. In this regime, $D\xi^{-2}=0$ and $\rho_1(\infty)\approx j_{\rm on}/j_{\rm off}$.

### B. Vesicle production rate shows two regimes with distinct dependence on particle adsorption rate

The main physical quantity of interest is, of course, the number of vesicles leaving the membrane per unit time per unit area. When $\rho(n)\to\rho_s(n)$ this quantity is simply $\rho_s(n_v)k(n_v)=\rho_s(1)k(1)$. From Eq. (12) we find two limiting cases.

(i) When $n_vk(1)\gg j_{\rm off}$, most particles adsorbed by the membrane join other domains without leaving the membrane. By substituting the asymptotic forms of the modified Bessel functions and keeping leading terms, this limit can be expressed as

$$D\frac{n_v}{a^2}\frac{j_{\rm on}}{j_{\rm off}^2}\gg O(1), \tag{18}$$

i.e., this limit occurs at sufficiently large $j_{\rm on}$. In this limit the density of single-particle domains is $\rho_s(1)=j_{\rm on}/[n_vk(1)\pi a^2]$, and the number of vesicles leaving the membrane per unit area per unit time is

$$\rho_s(n_v)k(n_v)=\rho_s(1)k(1)=\frac{j_{\rm on}}{n_v\pi a^2}. \tag{19}$$

That is, when particles seldom leave the membrane, the number of particles adsorbed by the membrane per unit time divided by the number of particles in a vesicle is the number of vesicles leaving the membrane per unit time.

(ii) When $n_vk(1)\ll j_{\rm off}$ [i.e., when $Dn_vj_{\rm on}/a^2j_{\rm off}^2\ll O(1)$], most of the adsorbed particles leave the membrane without joining other domains. In this limit Eq. (12) becomes $\rho_s(1)=j_{\rm on}/(j_{\rm off}\pi a^2)$. By combining Eqs. (11) and (17) one finds that

$$\begin{aligned}\rho_s(n_v)k(n_v)&=\rho_s(1)k(1)\\&\approx\frac{4D}{\pi a^4}\frac{(j_{\rm on}/j_{\rm off})^2}{-\ln(\sqrt{2}ma)-\gamma+\ln 2}\\&\sim\frac{D}{a^4}\left(\frac{j_{\rm on}}{j_{\rm off}}\right)^2\sim\frac{D}{a^4}[\rho_1(\infty)]^2.\end{aligned} \tag{20}$$

Here $\gamma=0.5772...$ is the Euler-Mascheroni constant [20]. In this limit the number of vesicles produced per unit time is proportional to the probability of particle-domain collision, which is proportional to $[\rho_1(\infty)]^2$.

Figure 5 shows the number of vesicles produced per unit time by a membrane with area 100 $\mu$m$^2$ as a function of $j_{\rm on}$. This is obtained by substituting $j_{\rm off}=10^2$ s$^{-1}$ and $n_v=100$, 1000, and 10000, respectively, into Eqs. (11), (12), (15), and (17). Indeed $\rho(n_v)k(n_v)\sim j_{\rm on}$ at large $j_{\rm on}$ and $\rho(n_v)k(n_v)\sim j_{\rm on}^2$ at small $j_{\rm on}$. There are two additional interesting features in Fig. 5. The first is that, for large $n_v$ (e.g., $n_v\gtrsim 10\,000$), $\rho(n_v)k(n_v)\sim j_{\rm on}^2$ occurs only at extremely small $j_{\rm on}$. This suggests that for particles that form large vesicles single-particle domains seldom leave the membrane because the domain density is high and most single-particle domains join other domains within the time scale $j_{\rm off}^{-1}$. The second is

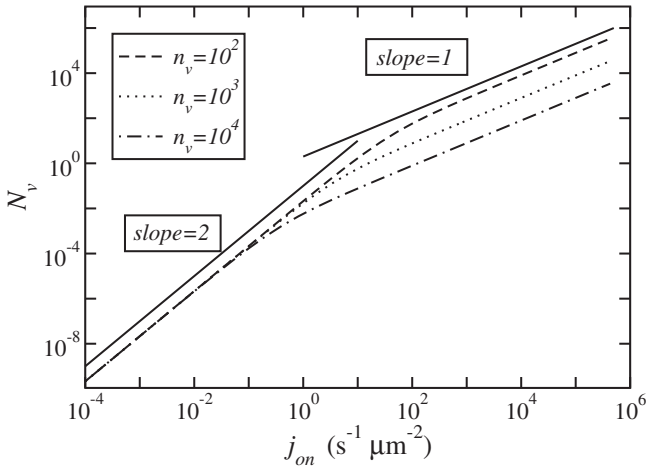


FIG. 5. $N_v$, number of vesicles produced in a 100 $\mu m^2$ area membrane per second, versus $j_{on}$. $j_{off}=10^2$ $s^{-1}$ and $n_v=10^2$ (dashed line), $10^3$ (dotted line), and $10^4$ (dot-dashed line). The rate of vesicle production scales as $\sim j_{on}^2$ at small $j_{on}$, and $\sim j_{on}$ at large $j_{on}$.

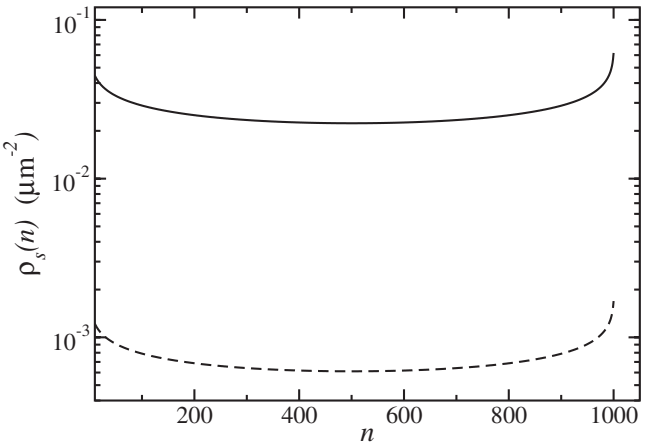


FIG. 6. Size distribution of particle-rich domains for $j_{on}=10^{-1}$ (dotted line) and $10^2$ $s^{-1}$ $\mu m^{-2}$ (solid line) at $j_{off}=10^2$ $s^{-1}$ and $n_v=1000$ from $n=10$ to $n_v$.

that when $\rho(n_v)k(n_v)\sim j_{on}^2$ the vesicle production rate is independent of $n_v$, as predicted by Eq. (20).

### C. Domain size distribution is strongly affected by the size of domain necks

Another physical quantity of interest is the domain size distribution in the time-independent state. From Eqs. (11) and (12),

$$\rho_s(n) = \frac{j_{on}}{\pi a^2}\frac{k(1)}{k(n)}\frac{1}{n_v k(1)+j_{off}} = \begin{cases} \dfrac{j_{off}}{2\pi n_v D}[-\ln(mR_n)-\gamma+\ln 2] & \text{when } n_v k(1) \gg j_{off}, \\ \dfrac{2j_{on}}{\pi a^2 j_{off}}\dfrac{-\ln(mR_n)-\gamma+\ln 2}{-\ln(\sqrt{2}ma)-\gamma+\ln 2} & \text{when } n_v k(1) \ll j_{off}. \end{cases} \tag{21}$$

In the final expression only $R_n$ depends on $n$; therefore $\rho_s(n)$ depends weakly on $n$; except at $n/n_v \ll 1$ and $1-n/n_v \ll 1$. Figure 6 shows the density of $n$-particle domains for $j_{off}=10^2$ $s^{-1}$, $n_v=1000$, $j_{on}=10^2$ $s^{-1}$ $\mu m^{-2}$ [when $n_v k(1) \gg j_{off}$], and $j_{on}=10^{-1}$ $s^{-1}\mu m^{-2}$ [when $n_v k(1) \ll j_{off}$] from $n=10$ to $n=n_v$. Indeed, except for the magnitude of $\rho_s(n)$, both curves look similar to each other: $\rho_s(n)$ is large at $n/n_v \ll 1$ and $n/n_v \approx 1$, and $\rho_s(n)$ is small when $n\sim n_v/2$. This suggests that the periphery of a domain is the main factor that determines the domain size distribution and the domain growth rate. Domains with $n<n_v/2$ grow faster as $n$ increases; domains with $n>n_v/2$ grow more slowly as $n$ increases. As a result there are many large domains with $n/n_v \approx 1$ and many small domains with $n/n_v \ll 1$, but there are few domains with $n\sim n_v/2$.

### D. Collisions between domains are negligible when $j_{off}$ is small and $j_{on}$ is large

Previous studies on vesicle formation from a bulk solution of amphiphilic molecules via the formation and aggregation of disklike micelles have shown that collisions between micelles (clusters) is very important for vesicle formation [22]. However, in our system the vesicles are formed by aggregation of particles on a bilayer membrane surface, and so far we have neglected the collisions between domains with $n>1$ in our model. To check if collisions between domains with $n>1$ are important, we define $T=\Sigma_{n=1}^{n_v-1} 1/k(n)$, the time for $n$ to grow from 1 to $n_v$. Then we compare the diffusion length of a domain during time $T$ with $1/\sqrt{\Sigma_{n=2}^{n_v}\rho_s(n)}$, the average distance between neighboring $n>1$ domains. When the average domain diffusion constant during time $T$ is large compared to $[\Sigma_{n=2}^{n_v}\rho_s(n)T]^{-1}$, collisions between $n>1$ domains are important. The diffusion constant of a rigid domain in a lipid membrane is a long-standing problem [23]; as a simple estimate we use the diffusion constant of a typical membrane protein ($\sim 1$ $\mu$ $m^2/s$) as the upper limit for the average diffusion constant of a domain during time $T$. Thus collisions between $n>1$ domains become important when $[\Sigma_{n=2}^{n_v}\rho_s(n)T]^{-1} \ll 1$ $\mu$ $m^2/s$.

As shown in Fig. 7, $[\Sigma_{n=2}^{n_v}\rho_s(n)T]^{-1}$ increases as $j_{on}$ increases or $j_{off}$ decreases. For particles with $j_{off}=10^{-2}$ $s^{-1}$, collisions between domains with $n>1$ are expected to be important only when $j_{on}$ is extremely small; but for particles with $j_{off} \gtrsim 10^3$ $s^{-1}$, collisions between domains with $n>1$ should be important for typical $j_{on}$ in experiments. Therefore we conclude that collisions between domains with $n>1$ can be neglected for particles that are adsorbed strongly on the membrane; for particles that are adsorbed weakly on the membrane, collisions between domains with $n>1$ should be important, unless those domains are fixed by linking to the cytoskeleton under the membrane. The effect of inter-domain collision on the kinetics of domain growth is beyond the scope of our model; it will be discussed in a future work [24].

## V. CONCLUSION

Our study shows that in typical experiments the radius of a particle-rich domain is close to the preferred value $R_0$ for

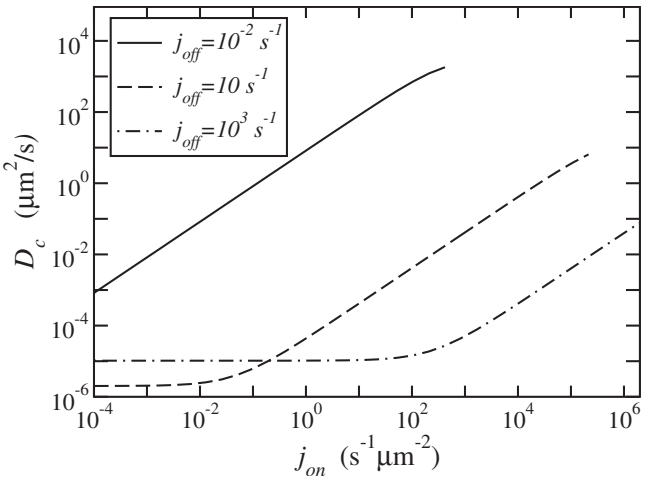


FIG. 7. $[\Sigma_{n=2}^{n_v}\rho_s(n)T]^{-1}$ for $j_{\rm off}=10^{-2}$ (solid curve), 10 (dashed curve), and $10^3$ $s^{-1}$ (dash-dotted curve) as a function of $j_{\rm on}$. Here $D_c \equiv [\Sigma_{n=2}^{n_v}\rho_s(n)T]^{-1}$. Collisions between $n>1$ domains become important when $[\Sigma_{n=2}^{n_v}\rho_s(n)T]^{-1} \ll 1$ $\mu m^2 s$.

the adsorbed particles, and vesicle formation is energetically favored except for a possible energy barrier for domain nucleation at small $n$. On the study of the kinetics of vesicle formation, assuming that domains grow independently without being affected by other domains is a reasonable zeroth-order approximation. Thus the multiple vesicle formation problem of endocytosis can be reduced to a much simpler problem. We find that when $Dn_v j_{\rm on}/a^2 j_{\rm off}^2 \ll O(1)$ only a small fraction of single-particle domains join other domains within time $j_{\rm off}^{-1}$ and the number of vesicles produced per unit time is proportional to $(j_{\rm on}/j_{\rm off})^2$. When $Dn_v j_{\rm on}/a^2 j_{\rm off}^2 \gg O(1)$ most single-particle domains join other domains without leaving the membrane, and the number of vesicles produced per unit time is proportional to $j_{\rm on}$. The growth rate of a domain is largely determined by its in-plane radius $R_n$; as a result in the time-independent state the densities of domains with $n/n_v \ll 1$ and $1-n/n_v \ll 1$ are large because of small $k(n)$ at small $R_n$.

In our model it is assumed that the membrane tension does not change with time. This is a good approximation when the membrane is a self-assembled bilayer in coexistence with a lipid reservoir. In a cell, the mirror-image process of endocytosis, exocytosis, also occurs all the time, and caveolae can also serve as a lipid reservoir [25]; thus surface tension can be treated as a constant during endocytosis. The parameters $j_{\rm on}$ and $j_{\rm off}$ are related to interaction energy between particles and the membrane; in particular, $j_{\rm on}$ also depends on the geometry of the system. It is straightforward to estimate the values of $j_{\rm on}$ and $j_{\rm off}$ for *in vivo* and *in vitro* experiments; thus our model will help the analysis of future experiments on this important nonequilibrium process.

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## APPENDIX

In this appendix we show analytically that domain growth in typical experiments is not in the strongly coupled regime. First, straightforward algebra gives us

$$\sum_{n=1}^{n_v-1}\rho_s(n) = k(1)\rho_s(1)\sum_{n=1}^{n_v-1}\frac{1}{k(n)} = \frac{mk(1)}{2\pi[n_v k(1)+j_{\rm off}]}\sum_{n=1}^{n_v-1}\frac{K_0(mR_n)}{R_n K_1(mR_n)}. \quad \text{(A1)}$$

Assuming that domain growth is strongly coupled, one finds the expression

$$\frac{\xi}{l_d} = \frac{\sqrt{D/n_v k(1)}}{l_d} = \sqrt{\frac{Dm}{2\pi n_v[n_v k(1)+j_{\rm off}]}\sum_{n=1}^{n_v-1}\frac{K_0(mR_n)}{R_n K_1(mR_n)}} = \begin{cases} \sqrt{\dfrac{Dm}{2\pi n_v j_{\rm off}}\displaystyle\sum_{n=1}^{n_v-1}\frac{K_0(mR_n)}{R_n K_1(mR_n)}} & \text{when } n_v k(1) \ll j_{\rm off}, \\ \sqrt{\dfrac{Dm}{2\pi (n_v)^2 k(1)}\displaystyle\sum_{n=1}^{n_v-1}\frac{K_0(mR_n)}{R_n K_1(mR_n)}} & \text{when } n_v k(1) \gg j_{\rm off}. \end{cases} \quad \text{(A2)}$$

Figure 4 shows that the magnitude $\xi/l_d$ decreases monotonically as $j_{\rm on}$ increases; therefore it is sufficient to show that $\xi/l_d \lesssim 1$ in the small-$j_{\rm on}$ limit. As Fig. 5 indicates, at small $j_{\rm on}$ the vesicle formation rate scales as $j_{\rm on}^2$ because most single-particle domains do not encounter another domain within the time $j_{\rm off}^{-1}$. Thus $n_v k(1) \ll j_{\rm off}$ and $m \approx \sqrt{j_{\rm off}/D}$. Using the asymptotic forms $K_0(x) \approx -\ln(x) - \gamma + \ln 2 + \cdots$ (here $\gamma = 0.577\,215\,66\ldots$ is the Euler-Mascheroni constant), $K_1(x) \approx x^{-1} + \cdots$ [20], and $R_n = \sqrt{n}a\sqrt{1-(\sqrt{n}a/2R_0)^2} = \sqrt{n}a\sqrt{1-n/n_v}$, one finds that for typical values of $D$, $n_v$, and $j_{\rm off}$,

$$\frac{\xi}{l_d} = \sqrt{\frac{1}{4\pi}\left[\ln\left(\frac{4D}{j_{\rm off}a^2 n_v}\right) + 2(1-\gamma)\right]} \lesssim O(1) \quad \text{when } n_v k(1) \ll j_{\rm off}. \quad \text{(A3)}$$

Thus domain growth in typical experiments is not strongly coupled.

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