

Formation of extracellular vesicles depends on mechanical feedback of the cortex and the glycocalyx

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Cell-secreted extracellular vesicles (EVs) play a pivotal role in local and distant cell-to-cell communication by delivering specific cargoes to other cells or to the extracellular space. In many cells, the glycocalyx, a thick sugar-rich layer at the cell surface, and the membrane-cortex attachment are crucially linked to the formation of EVs. However, it remains unclear as to what determines the successful formation of EVs when multiple physical factors are involved. In this work, we developed a model for glycocalyx-membrane-cortex composite to investigate the effects of glycocalyx and membrane-cortex adhesion on the formation of EVs by combining polymer physics-based theory and Helfrich membrane theory. By performing linear stability analysis, we show that modulating the mechanical feedback among the glycocalyx, membrane-cortex attachment, and membrane curvature can give rise to two types of instabilities: a conserved Turing-type instability and a Cahn-Hilliard-type instability. Furthermore, using an equilibrium model, we identified two critical conditions for EV formation: an initial detachment of the membrane from the underlying cortex and then a sufficient driving force to induce membrane deformation for successful EV formation. We further demonstrated that there exists an optimal glycocalyx coating area at which the formation of EVs is most favorable. Finally, we use our model to predict that a heterogeneous size distribution of EVs can be generated through the regulation of glycocalyx properties, shedding insight into how EVs of different radii may be generated.

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I. INTRODUCTION

Extracellular vesicles (EVs) are lipid bilayer-enclosed particles ubiquitously secreted by all cells [1–7] into the extracellular environment under a range of conditions, from normal and stress states to physiological and pathological states [1–3,8,9]. Various types of cellular components and biomolecules, such as signaling proteins, lipids, nucleic acids (mRNAs, miRNAs, and DNA), metabolites, and even pharmacological compounds [1–3,10–12], are shuttled to the extracellular space through many EV subtypes, i.e., exosomes, microvesicles, apoptotic bodies, as well as migrasomes and large oncosomes [1–4]. The ability to transfer biomolecular cargo into target cells enables EVs to play a pivotal role in biological processes such as local and long-distance intercellular communication and the disposal of unwanted materials [1–4]. In addition, a plethora of studies have reported that EVs also contribute to numerous pathological diseases such as cancer, heart disease, infectious diseases, and immunological and neurological diseases (Alzheimer's and Parkinson's) [7,13–17]. For example, many cell stress-inducing conditions, such as inflammation, proapoptotic conditions, and

hyperbaric pressure, can lead to an increase in the formation of membrane vesicles [18]. Many different cell types, including cancer cells, platelets, and endothelial cells, have been known to release EVs and EVs have been implicated in many different physiological and pathological conditions [19–21]. Based on these definitions and classifications, these EVs generated by direct outward budding or blebbing of specific sites of the plasma membrane are generally referred to as ectosomes or microvesicles [MVs; see Fig. 1(a)]. Hence, EVs are fragments of the plasma membrane secreted in response to activating stimuli such as oxidative stress, cytokines, and mechanical forces. In general, EVs are heterogeneous spherical phospholipid bilayer structures with sizes spanning from approximately tens to thousands of nanometers [22,23]. The categorization of heterogeneous EVs population is summarized in Table I. However, the biophysical mechanisms of generating EVs across a spectrum of sizes, along with the probability of their size occurrences, remain poorly understood.

Many experimental studies have characterized different factors that affect the formation of membrane geometries and we briefly summarize them here grouped by protein effects, membrane effects, or cytoskeletal effects [37,38]. One of the predominant views has been that the mechanisms for the generation of curvature are attributed to protein insertion, scaffolding, and crowding [37–44]. However, a majority of the modeling studies are focused on inward membrane bending such as endocytosis [45–48]. Instead, a number of membrane morphologies (EVs, cellular blebs, spherical protrusions, tubular protrusions, microvilli, etc.) are generated by bending

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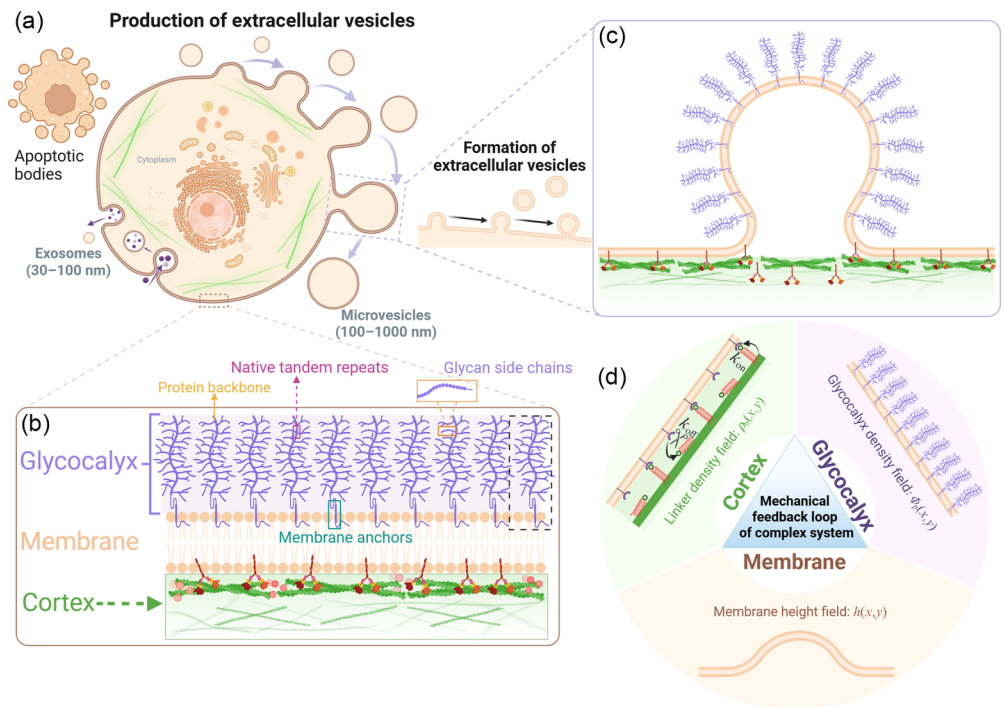


FIG. 1. Schematic representation of the factors involved in the formation of EVs. (a) Production and release of EVs into the extracellular space. (b) Schematic representation of three main factors—glycocalyx, membrane, and cortex—considered in this study for the formation of EVs. (c) An enlarged view of an EV detached from the underlying cortex driven by the glycocalyx. (d) Sketch of the glycocalyx-membrane-cortex composite system. In this work, we explore the feedback between these three elements using biophysical modeling. Panels are prepared using Biorender.com.

the membrane outward, and these structures play a crucial role in a spectrum of biological functions and processes in many cells [49]. Additionally, during membrane shape remodeling, numerous biomacromolecules, membranes, and cytoskeletal structures inside the cell are involved. For example, the glycocalyx [see Fig. 1(b)], a thick layer of heavily glycosylated transmembrane biomacromolecule proteins concentrated on most cell surfaces in a complex brush structure

[50–53], has been reported to regulate the formation of spherical vesicles, pearls, and tubes along the plasma membrane. As a result, another recently acknowledged membrane curvature generating mechanism is that in order to reduce the entropic cost of the system, the glycocalyx on the outer leaflet tends to drive membrane bending so as to provide more space and additional orientational degrees of freedom for glycopolymer chains [38,53–55]. In addition, interactions

TABLE I. Types of extracellular vesicles.

Name	Category	Size range	Origin	Type of cells	References
Exosomes	Small EV	~30–100 nm	Inward budding of endosomal compartments	Normal cells and cancerous cells	[1–3,5,6,24,25]
Small ectosomes	Small EV	~30–150 nm	Direct outward budding of the plasma membrane	Healthy cells, perivascular dendritic cells, T cells, and cancer cells	[1,3,5,6,26,27]
Microvesicles	Small-to-large EV	~100–1000 nm	Direct outward budding of the plasma membrane	Epithelial cells, normal cells, and cancerous cells	[1–3,5,6,28,29]
Apoptotic EVs	Small-to-large EV	~100–5000 nm	Fragments released by dying cells in the late stage of apoptosis	Dying cells	[1–3,5,6,30,31]
Migrasomes	Large EV	~500–3000 nm	Retraction fibers	Migrating cells	[1,3,5,6,32–34]
Oncosomes	Large EV	~1–10 μm	The shedding of nonapoptotic plasma membrane blebbing	Tumor cells	[1–3,5,6,35,36]

between the membrane and the cortex also control membrane shape changes; for instance, the formation of cellular blebs requires the detachment of the membrane from the underlying cortex [56–63] and cell membrane protrusion initiation is triggered by actin-membrane detachment [64–67]. The cortex is attached to the membrane through a large number of linker molecules [49,68,69] such as talin [63,70] and the ezrin, radixin, and moesin (ERM) family of proteins [71–73]. The disruption of links between the cortical cytoskeleton and the plasma membrane is a required condition for successful membrane detachment [see Fig. 1(c)]. A fundamental challenge in predicting EV formation regulated by glycocalyx and membrane-cortex adhesion is to understand the energetic contributions of the glycocalyx, membrane-cortex adhesion, and associated factors, such as glycocalyx area coverage, line tension created by multiple phases on the membrane, spontaneous curvature owing to asymmetries in the bilayer, and membrane tension [45,46,74,75].

Since EVs and highly curved membrane shapes are essential to diverse cellular functions, the formation of curved membrane morphologies has been extensively studied by experiments and theory. Experimentally, it has been shown that polypeptide and a class of large, heavily glycosylated proteins called mucins are frequently accumulated at high densities on protrusive membrane structures such as epithelial microvilli [76–81], cilia [82], and filopodia [83,84]. More recently, Shurer *et al.* [53] reported that bulky brushlike glycopolymers are sufficient to induce a variety of curved membrane features, including spherical-shaped membranes (referred to as blebs), tubes, and unduloids, in a density-dependent manner. Theoretical studies have used mean-field theory, analytical methods [85–88], scaling theory [89], phase field models [90,91], and Monte Carlo simulations [92,93], to show that anchored polymers on membrane give rise to changes in the shape, elastic properties, and spontaneous curvature of the plasma membrane. However, the membrane response to a thick layer of glycocalyx has not been well investigated. Recently, we developed a general energetic framework that couples the mechanics of the glycocalyx with the mechanics of the lipid bilayer to investigate how the glycocalyx can generate spherical vesicles and tubular structures [74,75]. We note that there are plenty of studies examining the role of the cortex in bleb formation by combining experimental observations and continuum mechanics models [57–60,94,95]. Also, a biophysical model was recently developed to account for the interplay between cortical adhesion and membrane bending in the regulation of microparticle formation [96]. Despite the fact that EVs have become the focus of rising interest and it is well known that they are formed by outward membrane bending, the balance of forces required for EV formation is not yet well understood.

To address this gap, in this work, we develop a theoretical model to interpret the formation of EVs based on polymer physics-based theory and Helfrich membrane theory, which captures the combined effects of glycocalyx, membrane-cortex adhesion, glycocalyx coating area, line tension, spontaneous curvature, and membrane tension. Based on this framework, we systematically investigated the influence of different factors on the formation of EVs [Fig. 1(d)]. Specifically, we begin our exploration by providing a linear

stability analysis of the glycocalyx-membrane-cortex complex system (Sec. II A). Next, we propose an equilibrium model to study the role of each factor in the formation of EVs (Sec. II B). Our model predicts that (1) the complex system becomes unstable under the conditions of depletion of the adhesive protein linkers and increasing the density and length of glycopolymers; two types of instabilities can emerge by tuning the control parameters in the unstable regime. (2) A critical condition is required for the onset of membrane detachment from the cortex, and the formation of a complete EV requires a threshold grafting density. (3) There is an optimal glycocalyx coating area for the formation of EVs and a wide range of EV size can be generated by regulating glycocalyx properties. Thus, this work provides a comprehensive model for EV formation by a detailed representation of the plasma membrane going beyond the lipid bilayer alone.

II. MODEL DEVELOPMENT AND METHODS

To investigate the influence of glycocalyx and membrane-cortex attachment on EV formation, we first construct a minimal dynamical model (see Sec. II A) that takes into account both glycopolymer-membrane and membrane-cortex interactions and study the stability of this complex system. Then, we develop a biophysical model (see Sec. II B) to investigate the effects of glycocalyx and membrane-cortex adhesion on membrane shape remodeling (EV formation). We consider a system comprising a cell membrane coated with glycocalyx on the outside of the surface and attached to a thin layer of cortex through protein linkers, as shown in Fig. 1. The total free energy of the system is given by the sum of the following three contributions, i.e.,

$$F_{\text{tot}} = F_{\text{glycocalyx}} + F_{\text{mem}} + F_{\text{ad}}, \quad (1)$$

where the first contribution is the energy associated with the glycocalyx, the second contribution is the Canham-Helfrich free energy due to bending the membrane [97,98], and the last term is the membrane-cortex adhesion energy, which stems from the attachment of the membrane to the cortex.

The first energy term related to the glycocalyx is described separately in Secs. II A and II B. The second energy term on the right-hand side of Eq. (1) can be given by the Canham-Helfrich Hamiltonian [97,98] as

$$\begin{aligned} F_{\text{mem}} &= F_{\text{bending}} + F_{\text{membrane tension}} \\ &= \int_{A_{\text{mem}}} \left[\frac{\kappa}{2} (2H - c_0)^2 + \sigma \right] dA, \end{aligned} \quad (2)$$

where κ is the bending modulus, $H = (c_1 + c_2)/2$ and c_0 are the mean and spontaneous curvatures, c_1 and c_2 are the two principal curvatures, and σ is the membrane surface tension. In Eq. (2), the first term accounts for the bending energy, which arises from the membrane resistance to the out-of-plane deformation, whereas the last term describes the tension energy due to membrane stretching.

Over the years, many theoretical models have been proposed to incorporate cytoskeletal effects into membrane dynamics [56,57,99,100]. For instance, Maitra *et al.* [99] investigated the dynamics of a fluid membrane in an active polar medium, where the membrane is coupled to an actin

cortex composed of orientable, self-driven filaments. In contrast, Gov *et al.* [100] modeled the membrane-cytoskeleton interaction using a harmonic potential, accounting for the inhomogeneous attachment of the cytoskeleton to the plasma membrane. Since membrane-cortex adhesion couples to membrane deformations, we also consider the effect of the tethering of the membrane to the cell cortex. To quantitatively capture this effect on EV formation, we assume that the attachment between the cell membrane and the actin cortex through linker proteins and the linker proteins (i.e., ezrin, radixin, and moesin) behave as springs arranged in parallel. Thus, the membrane-cortex adhesion energy cost due to tethering of the membrane to the cortex can be treated as quadratic with respect to the membrane-cortex linker deformation and is given by a harmonic potential of the form [56,57]

$$F_{\text{ad}} = \int \frac{k}{2} \rho_b z^2 dA, \quad (3)$$

where k is the effective elastic stiffness (spring constant) of each membrane-cortex linker, ρ_b is the local density of bound springlike linkers, and z is the stretching length of linkers.

In the presence of the cortex and glycocalyx, an additional energy contribution, membrane shear energy, may also contribute to the total free energy of the system. In Refs. [101–104], to investigate the formation of echinocyte bump shape observed in red blood cells, it is noted that the membrane shear energy contributed by the skeleton is taken into consideration in the membrane shape remodeling. This shear energy is a measure of the shear deformation of the membrane surface [104]. In the biological context, the cellular actin cortex is a thin layer of active material beneath the cell membrane, where cortical flows are driven by active forces within the cell cortex. These flows generate shear and active stresses. However, since we are not considering the dynamics of EV formation in the present model, we assume that the cortical flow and lateral diffusion of the glycocalyx are negligible. Additionally, we treat the cortex as statically attached to the membrane through linker proteins, and thus, the shear energy is excluded from our current model.

A. Onset of glycopolymer phase separation and initiation of EV formation

To explore whether the glycocalyx undergoes phase separation on the cell membrane and initiates the formation of EV when the membrane is attached to the cortex, we first propose a free energy functional to derive the kinetic equations that govern the dynamics of the complex system, and then perform linear stability analysis to demonstrate the instability of the system under certain conditions. Hence, we employ the Flory-Huggins theory here to describe the energy related to the glycocalyx on the membrane.

1. Free-energy functional

To study the stability of the glycocalyx-membrane-cortex system, we characterize this system by three state variables: $[\phi_p(\mathbf{r}), \rho_b(\mathbf{r}), h(\mathbf{r})]$, where $\phi_p(\mathbf{r})$ represents the area fraction of glycopolymers on the membrane, $\rho_b(\mathbf{r})$ denotes the molecular linker density attached to the membrane, and $h(\mathbf{r})$ is the normal displacement (height) of the membrane above a refer-

ence plane parametrized by $\mathbf{r} = (x, y)$. We partition the total free energy functional of the system into three contributions

$$\mathcal{F}[\phi_p, \rho_b, h] = \int_{\Omega} [f_p(\phi_p) + f_{\text{mem}}(\phi_p, h) + f_{\text{ad}}(\rho_b, h)] dA, \quad (4)$$

where the first term is the functional for the free energy densities of the glycopolymer on the membrane, the second term represents the Canham-Helfrich free energy [97,98], and the last term denotes the membrane-cortex adhesion energy.

For the functional of the free energy density associated with the glycopolymers on the membrane, we adopt the standard Flory-Huggins model [105,106] to account for the entropy of mixing and the interaction of glycopolymers:

$$f_p[\phi_p(\mathbf{r})] = \frac{k_B T}{A_p} [\phi_p \ln \phi_p + (1 - \phi_p) \ln (1 - \phi_p)] - \frac{\omega}{A_p} \phi_p + \frac{\chi(N)}{2A_p} \phi_p^2 + \frac{\gamma}{2A_p} |\nabla \phi_p|^2. \quad (5)$$

where A_p is the surface area occupied by a single grafted polymer on the membrane, ω stands for the chemical potential of the polymer binding, χ is a direct interaction coefficient between glycopolymers, and γ sets the energetic penalty for composition gradients. Note that the length of glycopolymers also affects the inter-polymer interactions here. For simplicity, we assume that χ depends on N through the relation $\chi(N) = (1 + N)\chi_0$, where N is the number of monomers in a polymer brush. In this work, we use the number of monomers, N , to capture the length of the polymer due to that the thickness of polymer brush is related to the total number of monomers [see Eq. (A12)].

Using the Monge representation for membrane deformation under small gradient approximation ($|\nabla h| \ll 1$), the free energy density for the elastic deformation of the membrane is given by

$$f_{\text{mem}}[\phi_p, h] = \frac{\kappa}{2} (\nabla^2 h - C_p \phi_p)^2 + \frac{\sigma}{2} (\nabla h)^2. \quad (6)$$

Here, we assume that a linear dependence of the spontaneous curvature on the glycocalyx density ϕ_p with a proportionality factor C_p encodes the curving strength of glycopolymers [107].

Considering that the local stretching length of linkers is equal to the height of the membrane, i.e., $z = h$, the last energy functional density appearing in Eq. (4) can be expressed as

$$f_{\text{ad}}[\rho_b, h] = \frac{k}{2} \rho_b(\mathbf{r}) h^2(\mathbf{r}). \quad (7)$$

Combining Eqs. (5)–(7), the total free energy functional can finally be written as the sum of these contributions, with

$$\begin{aligned} \mathcal{F}[\phi_p, \rho_b, h] = \int_{\Omega} \left\{ \frac{\kappa}{2} (\nabla^2 h - C_p \phi_p)^2 + \frac{\sigma}{2} (\nabla h)^2 + \frac{k}{2} \rho_b h^2 \right. \\ \left. + \frac{k_B T}{A_p} [\phi_p \ln \phi_p + (1 - \phi_p) \ln (1 - \phi_p)] \right. \\ \left. - \frac{\omega}{A_p} \phi_p + \frac{\chi(N)}{2A_p} \phi_p^2 + \frac{\gamma}{2A_p} |\nabla \phi_p|^2 \right\} dA. \quad (8) \end{aligned}$$

2. Coupled dynamics

Assuming that the membrane deforms to reduce the total free energy, the shape-deformation dynamics can be described by an energy relaxation equation. The time-evolution equation for the shape of the membrane is given by

$$\frac{\partial h}{\partial t} = -M_h \frac{\delta \mathcal{F}}{\delta h}, \quad (9)$$

where M_h is a damping coefficient. Here, we assume that the shape changes of the membrane follow gradient dynamics, with hydrodynamic effects other than friction being neglected [107]. Using Eqs. (8) and (9), we find

$$\frac{\partial h}{\partial t} = M_h (-\kappa \nabla^4 h + \sigma \nabla^2 h + \kappa C_p \nabla^2 \phi_p - k_{\rho_b} h). \quad (10)$$

For the local area fraction of glycopolymers, we assume that the time-evolution equation for ϕ_p obeys the continuity equation $\partial_t \phi_p + \nabla \cdot \mathbf{J} = 0$ with a current density $\mathbf{J} = -M_p \nabla \mu$, where M_p is the mobility coefficient and $\mu = \delta \mathcal{F} / \delta \phi_p$ is the chemical potential. Here, $M_p = D_{\text{diff}} A_p / (k_B T)$ with D_{diff} being the diffusion constant. Performing the functional differentiation, one can obtain a Cahn-Hilliard-type dynamical equation for the glycopolymer density as

$$\begin{aligned} \frac{\partial \phi_p}{\partial t} = M_p \left[-\kappa C_p \nabla^4 h - \frac{\gamma}{A_p} \nabla^4 \phi_p + \left(\frac{\chi(N)}{A_p} + \kappa C_p^2 \right) \nabla^2 \phi_p \right. \\ \left. + \nabla^2 \left(\frac{k_B T}{A_p} \ln \left(\frac{\phi_p}{1 - \phi_p} \right) - \frac{\omega}{A_p} \right) \right]. \end{aligned} \quad (11)$$

We also assume that the linker protein undergoes turnover between a detached state at a force-dependent rate k_{off} and an attached state at a constant rate k_{on} . Then, the kinetics of tethered linker proteins can be simply described by [56,57]

$$\frac{\partial \rho_b}{\partial t} = k_{\text{on}} (\rho_0 - \rho_b) - k_{\text{off}}(h) \rho_b, \quad (12)$$

where ρ_0 is the density of available linkers and $k_{\text{off}}(h) = k_{\text{off}}^0 e^{\beta k h \delta}$ [108] with δ being a bond length in the nanometric scale [109].

3. Linear stability analysis

Next, we begin from a uniform initial state and look for conditions of instability. We perform a linear stability analysis on the system of equations given in Eqs. (10)–(12), by introducing small perturbations around the spatially homogeneous flat membrane state: $h = h_{\text{eq}} + \delta h$, $\phi_p = \phi_{p,\text{eq}} + \delta \phi_p$, and $\rho_b = \rho_{b,\text{eq}} + \delta \rho_b$. Taking the spatial Fourier transform of Eqs. (10)–(12) yields the linearized equations

$$\frac{\partial}{\partial t} \begin{pmatrix} \delta h(\mathbf{q}, t) \\ \delta \phi_p(\mathbf{q}, t) \\ \delta \rho_b(\mathbf{q}, t) \end{pmatrix} = \mathcal{J} \cdot \begin{pmatrix} \delta h(\mathbf{q}, t) \\ \delta \phi_p(\mathbf{q}, t) \\ \delta \rho_b(\mathbf{q}, t) \end{pmatrix} \quad (13)$$

where $\mathcal{J}(q) = [\mathcal{J}_{ij}(q)]_{3 \times 3}$ is the Jacobian matrix that reads

$$\begin{aligned} \mathcal{J}_{hh} &= M_h (-\kappa q^4 - \sigma q^2 - k_{\rho_b, \text{eq}}), \\ \mathcal{J}_{h\phi_p} &= -M_h \kappa C_p q^2, \\ \mathcal{J}_{h\rho_b} &= -M_h k h_{\text{eq}}, \\ \mathcal{J}_{\phi_p h} &= -M_p \kappa C_p q^4, \end{aligned}$$

$$\mathcal{J}_{\phi_p \phi_p} = M_p q^2 \left[-\frac{\gamma q^2}{A_p} - \frac{\chi(N)}{A_p} - \kappa C_p^2 - \frac{k_B T}{A_p \phi_{p,\text{eq}} (1 - \phi_{p,\text{eq}})} \right],$$

$$\mathcal{J}_{\phi_p \rho_b} = \mathcal{J}_{\rho_b \phi_p} = 0,$$

$$\mathcal{J}_{\rho_b h} = -\beta k \delta k_{\text{off}}^0 e^{\beta k h_{\text{eq}} \delta} \rho_{b,\text{eq}},$$

$$\mathcal{J}_{\rho_b \rho_b} = -k_{\text{on}} - k_{\text{off}}^0 e^{\beta k h_{\text{eq}} \delta}, \quad (14)$$

where q is the wave number. The stability of the glycocalyx-coated and cortex-attached membrane is governed by the real or complex eigenvalues Λ_q of the Jacobian associated with the matrix $\mathcal{J}$. The largest eigenvalue of the Jacobian matrix determines the highest growth rate of the perturbations. The dispersion relation $\Lambda(q)$

$$\Lambda(q) = \text{Re}(\Lambda) + i \text{Im}(\Lambda) \quad (15)$$

can be determined from the characteristic cubic equation

$$\Lambda^3 - \text{Tr}(\mathcal{J}) \Lambda^2 + \sum_{i=1}^3 M_{ii}(\mathcal{J}) \Lambda - \text{Det}(\mathcal{J}) = 0, \quad (16)$$

where $\text{Tr}(\mathcal{J})$ stands for the trace of $\mathcal{J}$, $M_{ii}(\mathcal{J})$ for the minors of the diagonal terms of $\mathcal{J}$, and $\text{Det}(\mathcal{J})$ for the determinant of $\mathcal{J}$. This cubic equation can be solved analytically [110,111] but the resulting expressions are rather complex so we present them graphically in the Results section. The real part $\text{Re}(\Lambda)$ is a rate that characterizes the exponential growth ($\text{Re}(\Lambda) > 0$) or decay ($\text{Re}(\Lambda) < 0$) of the corresponding linear mode. The imaginary part $\text{Im}(\Lambda)$ corresponds to a frequency that can be zero (stationary case) or nonzero (oscillatory case) [112].

B. Equilibrium description of EV formation

In this section, we consider the scenario in which the glycocalyx aggregates into a stable and area-fixed domain through phase separation. Thus, the energy contribution associated with the glycocalyx is no longer described by the Flory-Huggins theory here. Instead, we utilize the polymer brush-based theory to characterize this energy contribution because we assume that the glycopolymers on the membrane formed a brushlike structure. We assume that this phase-separated domain is flat because of the small deformation approximation. Our aim here is to investigate the conditions under which this process can initiate membrane bending and facilitate EV formation. In the following, we briefly summarize the theoretical model and include additional details in the Appendixes A and B.

1. Description of the model: geometry of the EV

To investigate the effects of glycocalyx properties and membrane-cortex adhesion on membrane morphologies, i.e., spherical extensions, we consider a glycocalyx-membrane-cortex system, as shown in Fig. 2. The system we considered consists of a membrane patch that is anchored by glycocalyx biopolymers and a thin layer of actin cortex attached beneath the membrane (see Fig. 2). We assume that the patch is much smaller than the average radius of the cell, and this membrane patch with fixed surface area $A_0 = \pi R_0^2$ is deformed into a

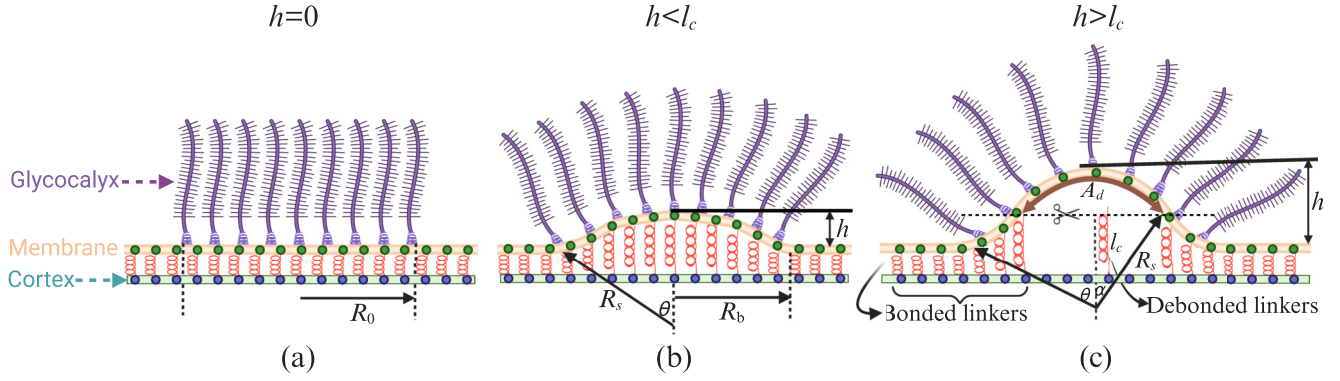


FIG. 2. Schematic of the glycocalyx-membrane-cortex system for EV formation. (a) A patch of glycocalyx with in-plane radius R_0 , represented as a layer of brushlike polymer, is anchored on an initially flat membrane. The cortex beneath the membrane is attached to the membrane through linker proteins, which are represented by springs. (b) The small deformation state where the membrane takes the shape of a spherical cap with its radius of curvature, base radius, opening angle, and height denoted by R_s , R_b , 2θ , and h . The linkers are attached to the membrane when the depth of the spherical cap is smaller than the maximum extension length of the membrane-cortex spring, i.e., $h < l_c$. (c) In the case of $h > l_c$, the spherical cap-shaped membrane is divided into two parts: the membrane-cortex adhesion region below the dashed line with bonded linkers and the membrane-cortex detached region above the dashed line with debonded linkers. We denote the opening angle and area of the membrane-cortex detached region by 2α and A_d . Panels are prepared using Biorender.com.

set of spherical caps with radius R_s due to the presence of glycocalyx, where R_0 is the in-plane radius of the flat membrane (see Fig. 2). The configuration of the membrane system can then be described by a single shape parameter η which characterizes the area ratio between a spherical cap and a sphere. In the one-parameter family of configurations of the spherical cap, $\eta = 0$ corresponds to a completely flat circular bud with radius R_0 , while at $\eta = 1$ the bud is a sphere that implies an EV is formed. The membrane domain takes the shape of spherical caps when η is located in the intermediate value between 0 and 1, i.e., $0 < \eta < 1$. The spherical caps for each η must have equal area as required by the constraint constant area A_0 , implying $\eta = R_0^2/(4R_s^2)$.

Given the attachment between the cell membrane and the actin cortex, membrane bending requires stretching and/or breaking of these adhesive connections. In addition to the glycocalyx and cortex, the line tension caused by the multiple phases on the membrane also affects membrane deformation. Thereby, the total free energy of the system is described as follows.

2. Energetics of the system

After phase separation, we assume that an initially flat circular domain, coated with glycocalyx, is embedded in a membrane matrix, its boundary experiences a line tension λ due to the unfavorable interactions at the interface between the glycocalyx-rich domain and the surrounding bare lipid layer. In this case, the domain edge energy that is proportional to the length of the perimeter and to the line tension is given by

$$F_{\text{line tension}} = \lambda \oint_{\partial l} dl, \quad (17)$$

where λ denotes the strength of line tension along the domain boundary, and the integral is over the periphery line dl of the domain. We assume that the line tension is a constant. Consequently, by adding the line tension energy to Eq. (1), the total free energy of the glycocalyx-membrane-cortex system (F_{tot}) becomes

$$F_{\text{tot}} = F_{\text{glycocalyx}} + F_{\text{mem}} + F_{\text{ad}} + F_{\text{line tension}}. \quad (18)$$

The first energy term on the right-hand side of Eq. (18) includes the elastic stretching of the polymer chains and the interactions between monomers in the polymer brush. Following Refs. [53,113], this energy contribution originating from the glycocalyx polymers is given by

$$\begin{aligned} F_{\text{glycocalyx}} &= F_{\text{glycocalyx}}^{\text{elastic}} + F_{\text{glycocalyx}}^{\text{interaction}} \\ &= \frac{1}{\beta} \int_R^{R+L_{\text{brush}}} \left[\frac{3}{2a^2 c_p(r) s^2(r)} + v c_p^2(r) \right] s(r) dr, \end{aligned} \quad (19)$$

where $\beta = 1/(k_B T)$ and in which $k_B T$ is the thermal energy with k_B being the Boltzmann's constant and T being the absolute temperature, a is the effective monomer size, $c_p(r)$ is the local monomer density profile along the thickness, r is the radial distance defined from the center of the spherical or cylindrical surface, $s(r)$ is the area per chain at distance from the polymer grafting surface, and v is the second virial coefficient. Note that only pairwise monomer-monomer interactions with second virial coefficient v are considered. The detailed derivation of the individual energy component in Eq. (19) is provided in Appendix A. Using these expressions, we can write

$$F_{\text{glycocalyx}} = F_{\text{glycocalyx}}^{\text{elastic}} + F_{\text{glycocalyx}}^{\text{interaction}} = \begin{cases} N_p \frac{9N}{2\beta} \left(\frac{v}{3\xi^2 a} \right)^{\frac{2}{3}}, & \eta = 0, \\ N_p \frac{9R_0}{4\beta\sqrt{\eta}} \left(\frac{3\sqrt{v}}{\xi a^2} \right)^{\frac{2}{3}} \left[\left(1 + \frac{10N\sqrt{\eta}}{3R_0} \left(\frac{va^2}{3\xi^2} \right)^{\frac{1}{3}} \right)^{\frac{1}{5}} - 1 \right], & \eta > 0, \end{cases} \quad (20)$$

where N_p is the number of polymer chains grafted on the membrane and ξ is the grafting distance of polymers on the cell membrane surface.

In this model, the membrane geometry is restricted to spherical bud geometry since we are focused on EV formation. Thus, the two principal curvatures are given by $c_1 = c_2 = 1/R_s$, the excess area is calculated as $\Delta A = A_0 - \pi R_b^2$, where $R_b = R_0 \sqrt{1 - \eta}$ is the in-plane base radius, and the length of the domain boundary can be written as

$\oint_{\partial l} dl = 2\pi R_b$. Therefore, the elastic energy and the line tension of the membrane become

$$F_{\text{mem}} + F_{\text{line tension}} = 8\pi\kappa \left(\sqrt{\eta} - \frac{R_0 c_0}{4} \right)^2 + \pi\sigma\eta R_0^2 + 2\pi\lambda R_0 \sqrt{1 - \eta} \quad (21)$$

and the membrane-cortex adhesion energy appearing in Eq. (18) can be estimated as

$$F_{\text{ad}} = \begin{cases} \frac{\eta}{6}\pi k \rho_b R_0^4, & \eta \leq (l_c/R_0)^2, \\ \frac{1}{2}\pi k \rho_b R_0 l_c^2 (R_0 - \frac{2l_c}{3\sqrt{\eta}}), & \eta > (l_c/R_0)^2, \end{cases} \quad (22)$$

where l_c refers to the maximum length of the membrane-cortex adhesion bond. A complete derivation of this energy contribution can be found in Appendix B.

By substituting Eqs. (20), (21), and (22) into Eq. (18), we can obtain the total free energy of the system

$$\frac{F_{\text{tot}}}{\pi\kappa} = \frac{F_{\text{glycocalyx}} + F_{\text{mem}} + F_{\text{line tension}} + F_{\text{ad}}}{\pi\kappa},$$

$$= \begin{cases} N_p \frac{9N}{2\pi\beta\kappa} \left(\frac{v}{3\xi^2 a} \right)^{\frac{2}{3}} + \frac{(R_0 c_0)^2}{2} + \frac{2\lambda R_0}{\kappa}, & \eta = 0, \\ N_p \frac{9R_0}{4\pi\beta\kappa\sqrt{\eta}} \left(\frac{3\sqrt{v}}{\xi a^2} \right)^{\frac{2}{3}} \left[\left(1 + \frac{10N\sqrt{\eta}}{3R_0} \cdot \left(\frac{va^2}{3\xi^2} \right)^{\frac{1}{3}} \right)^{\frac{1}{5}} - 1 \right] + 8 \left(\sqrt{\eta} - \frac{R_0 c_0}{4} \right)^2 \\ + \frac{\sigma}{\kappa} \eta R_0^2 + 2 \frac{\lambda}{\kappa} R_0 \sqrt{1 - \eta} + \frac{\eta}{6\kappa} k \rho_b R_0^4, & 0 < \eta \leq \left(\frac{l_c}{R_0} \right)^2, \\ N_p \frac{9R_0}{4\pi\beta\kappa\sqrt{\eta}} \left(\frac{3\sqrt{v}}{\xi a^2} \right)^{\frac{2}{3}} \left[\left(1 + \frac{10N\sqrt{\eta}}{3R_0} \cdot \left(\frac{va^2}{3\xi^2} \right)^{\frac{1}{3}} \right)^{\frac{1}{5}} - 1 \right] + 8 \left(\sqrt{\eta} - \frac{R_0 c_0}{4} \right)^2 \\ + \frac{\sigma}{\kappa} \eta R_0^2 + 2 \frac{\lambda}{\kappa} R_0 \sqrt{1 - \eta} + \frac{k \rho_b R_0 l_c^2}{2\kappa} \left(R_0 - \frac{2l_c}{3\sqrt{\eta}} \right), & \eta > \left(\frac{l_c}{R_0} \right)^2. \end{cases} \quad (23)$$

Here, we assume that our system is at equilibrium, implying that the system selects the membrane shape that minimizes the total free energy of the above equation.

3. Numerical implementation

To analyze the stability of the glycocalyx-membrane-cortex system, we numerically solve the eigenvalue problem to obtain the eigenvalues that correspond to the linear growth rates of the perturbations. By displaying the corresponding dispersion relation functions $\Lambda(q)$ at various degrees of control parameters, we can determine the stability behaviors.

For the equilibrium description model of the formation of EVs, we investigate the equilibrium geometry of the glycocalyx-membrane-cortex system under the constraint of fixed membrane patch area $A_0 = \pi R_0^2$ and the restriction of a spherical cap of membrane shape. We minimize the total free energy with respect to the shape parameter η to determine the stable and therefore the energetically most favorable membrane shapes η_{min} . Note that an analytic solution for η_{min} is not feasible due to the fact that the total free energy is nonlinear in the shape parameter η . Thus, we numerically determine η_{min} throughout this work by minimizing F_{tot} . The values of parameters used in the model are summarized in Table II unless otherwise specified.

III. RESULTS

A. Different stability behaviors

In this section, we focus on stability analysis. In order to classify the types of instability exhibited by the system, we

begin by examining the dispersion relations $\Lambda(q)$, i.e., growth rates as a function of the wave number at different values of control parameters. We plot the dispersion relations $\Lambda(q)$ at different values of ρ_b in Fig. 3(a). For low linker density ($\rho_b = 0.01$), there exists an instability band that starts at q_- and lasts until q_+ , with the most unstable mode at $q_- < q_{\text{max}} < q_+$. As we decrease the linker density, the instability band narrows to only the most unstable mode $q_c = q_{\text{max}}$ when the linker density reaches a threshold value $\rho_b^c = 0.126$. Here, q_c corresponds to the critical wave number where at the onset of instability a maximum of $\Lambda(q)$ touches zero, $q_{\pm}$ represents the marginal wave numbers where $\text{Re}(\Lambda_{\text{max}})$ crosses zero above the onset, and q_{max} denotes the fastest growing wave number at where $\text{Re}(\Lambda_{\text{max}})$ has a maximum above the onset. A density beyond ρ_b^c , i.e., $\rho_b > \rho_b^c$, leads to the disappearance of the instability band, which indicates that the system is stable in any mode and the initial perturbations decay exponentially. This type of dynamic behavior corresponds to the conserved Turing-type (cT-type) instability [107,112] which occurs for some range of wave numbers [$\text{Re}(\Lambda_i) > 0$], $q_- < q < q_+$, and where there are nonoscillatory unstable modes [$\text{Im}(\Lambda_i) = 0$], while the zero wave number remains stable. In this region, the amplitudes of these modes grow exponentially from small initial perturbations but do not oscillate or propagate on the membrane surface. The physical mechanism behind this type of instability is that, when the linker density is low, the perturbed homogeneous glycopolymer starts to aggregate and undergoes phase separation, and protrusions start to appear on the initially flat membrane. This indicates the relevant biological prediction that decreases in membrane-cortex

TABLE II. Values of the different parameters used in the model.

Parameter	Description	Estimated range	References	Default value
k	Spring constant	$\sim 10^{-4}$ N/m	[56,57]	10^{-4} N/m
ρ_0	Density of available linkers	$\sim 10^{14}-10^{16}$ m $^{-2}$	[56,57]	10^{14} m $^{-2}$
k_{on}	Linker attachment rate	$\sim 10^3-10^4$ s $^{-1}$	[56,57]	10^4 s $^{-1}$
k_{off}^0	Free linker detachment rate	$\sim 0.1-10$ s $^{-1}$	[56,57]	10 s $^{-1}$
δ	Linker bond length	$\sim 10^{-9}$ m	[56,57]	10^{-9} m
l_c	Critical length of membrane-cortex spring	$\sim 2-10$ nm	[56,57]	5 nm
A_p	Polymer area	~ 100 nm ^{2}	[114]	100 nm ^{2}
D_{diff}	Polymer diffusion constant	$0.1-1$ $\mu\text{m}^2/\text{s}$	[111]	1 $\mu\text{m}^2/\text{s}$
M_h	Damping coefficient	$\sim 10^8$ nm $^4/(k_B T \text{ s})$	[107]	10^8 nm $^4/(k_B T \text{ s})$
M_p	Mobility coefficient	$\sim 10^8$ nm $^4/(k_B T \text{ s})$	[107]	10^8 nm $^4/(k_B T \text{ s})$
$\phi_{p,\text{eq}}$	Polymer area fraction	$\sim 0-1$	[57]	0.3
ω	Polymer-membrane interaction	$\sim -10-10$ $k_B T$	[74,115]	$3 k_B T$
χ_0	Polymer-polymer interaction	$\sim -10-10$ $k_B T$	[107]	$-6 k_B T$
γ	Interfacial energy	$\sim 10^2$ $k_B T$ nm ^{2}	[107,115]	$200 k_B T$ nm ^{2}
κ	Bending rigidity	$10-400$ $k_B T$	[116-119]	$10 k_B T$
σ	Membrane tension	$10^{-7}-10^{-3}$ N/m	[117,120,121]	4.8×10^{-5} N/m
λ	Line tension	$0-100$ pN	[117,122,123]	2 pN
R_0	Patch radius	$20-100$ nm	[93,117]	100 nm
ξ	Grafting distance	$10-100$ nm	[53,124]	15 nm
a	Effective monomer size in the glycocalyx	$10-20$ nm	[50,53,93,125-127]	10 nm
N	Number of effective monomers in a brush	$10-50$	[50,53,93,127]	20
c_0	Spontaneous curvature	$0-0.1$ nm $^{-1}$	[53]	0.02 nm $^{-1}$

detachment can give rise to an unstable state and initiate cell protrusions. Furthermore, we present the band of unstable wave numbers (q_- , q_+) as a function of the linker density

ρ_b , as shown in Fig. 3(b). For $\rho_b < \rho_b^c$, the dispersion relation exhibits a cT-type instability, which exists in some ranges of modes, $q_- < q < q_+$. Above a threshold value ρ_b^c , the band

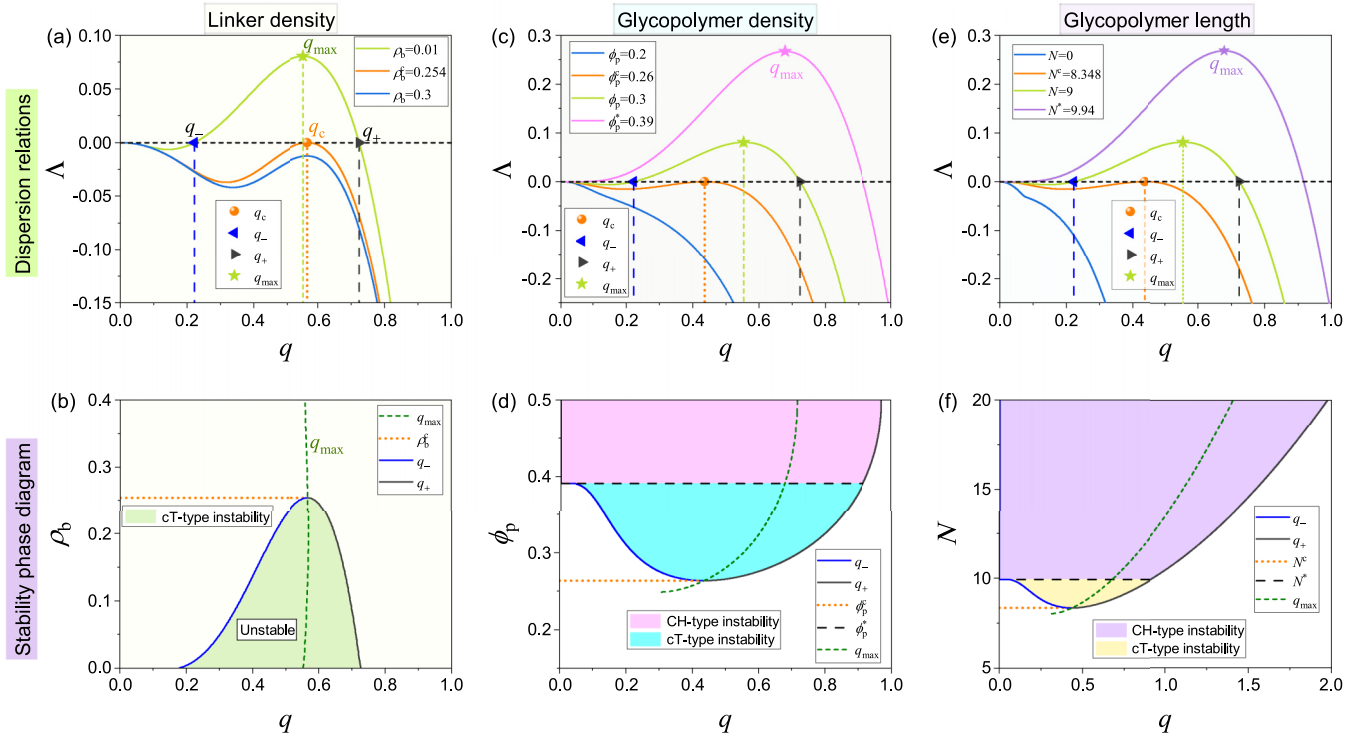


FIG. 3. Linear stability analysis of the system. (a) Dispersion relation functions Δ_q at different values of ρ_b . (b) Band of unstable modes (q_- , q_+) in the plane spanned by wave number q and control parameter linker density, ρ_b . (c) Dispersion relations for different values of ϕ_p . (d) Band of unstable modes (q_- , q_+) in the q - ϕ_p plane. (e) Growth rate for different values of the control parameter N . (f) Stability diagram of the q - N plane illustrating the band of unstable wave numbers in its dependence on the length of glycopolymer N .

of unstable modes disappears and the system remains steady state.

In addition to the effect of membrane-cortex attachment, the properties of the glycocalyx also affect the stability behaviors of the system. Figure 3(c) shows the growth rate as a function of wave number at different values of ϕ_p . At low glycocalyx density (see blue curve), the largest growth rates $\Lambda(q)$ for all modes are negative except the neutral mode at zero wave number, i.e., $\Lambda = 0$ at $q = 0$. Upon increasing the glycocalyx density to a critical value ϕ_p^c (see orange curve), we observe that a nonzero marginal wave number appears where the maximum of $\Lambda(q)$ touches zero, which implies the onset of instability. Then, above this critical value ϕ_p^c results in an instability band that starts at q_- and lasts until q_+ (see light green curve). This instability pattern corresponds to the cT-type instability. While as the glycocalyx density above another threshold value ϕ_p^* , the dispersion curve exhibits an instability band starting at $q = 0$ and ending at some $q = q_+$, with the most unstable mode at $0 < q_{\max} < q_+$ (see magenta curve). This type of instability is referred to as Cahn-Hilliard-type (CH-type) instability [107,128], which is a long-wavelength instability with the band extending to the zero wave vector ($q_- = 0$). Finally, we present the stability diagram of the q - ϕ_p plane to demonstrate the band of unstable wave numbers (q_-, q_+) as a function of the glycocalyx density ϕ_p , as shown in Fig. 3(d). For $\phi_p^c < \phi_p < \phi_p^*$, the system displays a cT-type instability, while when $\phi_p > \phi_p^*$, the dispersion relation shifts from a cT-type instability to a CH-type long-wavelength instability. This means that when the glycocalyx density is high enough, the glycocalyx can undergo phase separation on the membrane surface and initiate EV formation.

In addition to grafting density, the length of the glycopolymers is another important feature of the glycocalyx layer on the cell membrane surface. To probe the influence of glycopolymer length on the stability behaviors of the system, the analysis of dispersion relations for various numbers of monomers N is presented in Fig. 3(e). For short glycopolymer lengths (i.e., $N < N^c$), the system does not demonstrate an instability. Similarly, depending on the length of glycopolymer, we observe two types of instabilities. For glycopolymer lengths in the regime $N^c < N < N^*$, the instability behavior shows a cT-type instability, which exists for some range of modes, $q_- < q < q_+$. However, further increasing the length of glycopolymer to a threshold value N^* leads to an extending of the instability band (q_-, q_+) which corresponds to a reduction of the left marginal wave number q_- to zero. As a result, above N^* , a change in the type of instability from cT to CH type occurs, where the dispersion relation exhibits an instability band starting at $q = 0$ and ending at some $q = q_+$, with the most unstable mode at $0 < q_{\max} < q_+$. Therefore, varying the length of glycopolymer provides a practical strategy to tune the aggregation and phase separation on the membrane and to regulate membrane curvature generation. Similarly, we constructed a two-dimensional phase diagram on the q - N plane to characterize the band of unstable modes in its dependence on the length of glycopolymer N , as demonstrated in Fig. 3(f). Our results show that the dispersion relation shifts from a cT-type instability to a CH-type long-wavelength instability as N reaches a critical value N^* . This suggests that long glycopolymers have the tendency to destabilize the system

and trigger membrane-cortex release and facilitate the onset of EV formation.

The stability analysis reveals that the regimes in the control parameter space where the system is stable and where it is unstable. The unstable regimes exhibit two types of instabilities: cT-type instability and CH-type instability, and there the glycopolymer undergoes aggregation and the membrane protrusions appear. Analogous to Fig. 3, the influence of spontaneous curvature and membrane properties (i.e., membrane tension) on the stability of the system is presented in Fig. 10 (see Appendix C). Next, we will employ the equilibrium description model to probe the conditions under which the EV can be successfully formed.

B. Effects of glycocalyx and membrane-cortex adhesion on EV formation

In this section, we numerically calculate the total energy for different shape parameters η ranging from 0 to 1 based on the equilibrium description model of the formation of EVs. The global energy minimum corresponds to the optimal $\eta_{\min}$ that gives the equilibrium geometry of the cell membrane patch. Here, we first investigate the influence of the level of membrane-cortex protein linkers on energy as well as membrane shape. To quantify the effect of membrane-to-cortex attachment on the initiation of EV formation, we focus on the total energy profile as a function of the shape parameter η for a series of fixed linker density ρ_b , as shown in Fig. 4(a). Immediately, we observe that there exists a global energy minimum (solid dot) which corresponds to the formation of a spherical EV ($0 < \eta < 1$ or a partial blebbing state). As ρ_b increases (from $\rho_b = 10^{-4} \text{ nm}^{-2}$ to $\rho_b = 3 \times 10^{-2} \text{ nm}^{-2}$), the global stable state slightly shifts to a shape corresponding to a smaller η . Interestingly, we observe that the stable partial blebbing state shifts to a stable no blebbing state with increasing ρ_b to a high level (see the curve with $\rho_b = 4 \times 10^{-4} \text{ nm}^{-2}$), indicating that the membrane remains attached to the cortex and undeformed when the density of cortex-membrane protein linkers is higher enough. Note that the initial flat membrane, in order to grow up to a spherical EV, needs to overcome an energy barrier ΔF .

Apart from membrane-cortex adhesion, the properties of glycocalyx such as grafting density and length also have a big impact on EV formation. In Fig. 4(b), we present the total energy profile as a function of the shape parameter η for a sequence of fixed glycopolymer grafting density ρ_p . Similar to Fig. 4(a), a global energy minimum also exists (see the solid dot), and the stable state still corresponds to the partial blebbing state ($\eta < 1$) at low polymer grafting density (i.e., the curve with $\rho_p = 0.002 \text{ nm}^{-2}$). As the grafting density increases, the stable partial blebbing state shifts to a stable state with a full EV formed (refer to the curve with $\rho_p = 0.0045 \text{ nm}^{-2}$). This implies that a higher grafting density of glycopolymer on the cell membrane is necessary to achieve a full EV.

Next, to intuitively visualize the effect of membrane-cortex attachment, we focus on the stable state corresponding to the optimal shape parameter $\eta_{\min}$. Figure 4(c) shows the dependence of $\eta_{\min}$ on linker density at different linker stiffness, polymer length, and grafting density. We note three

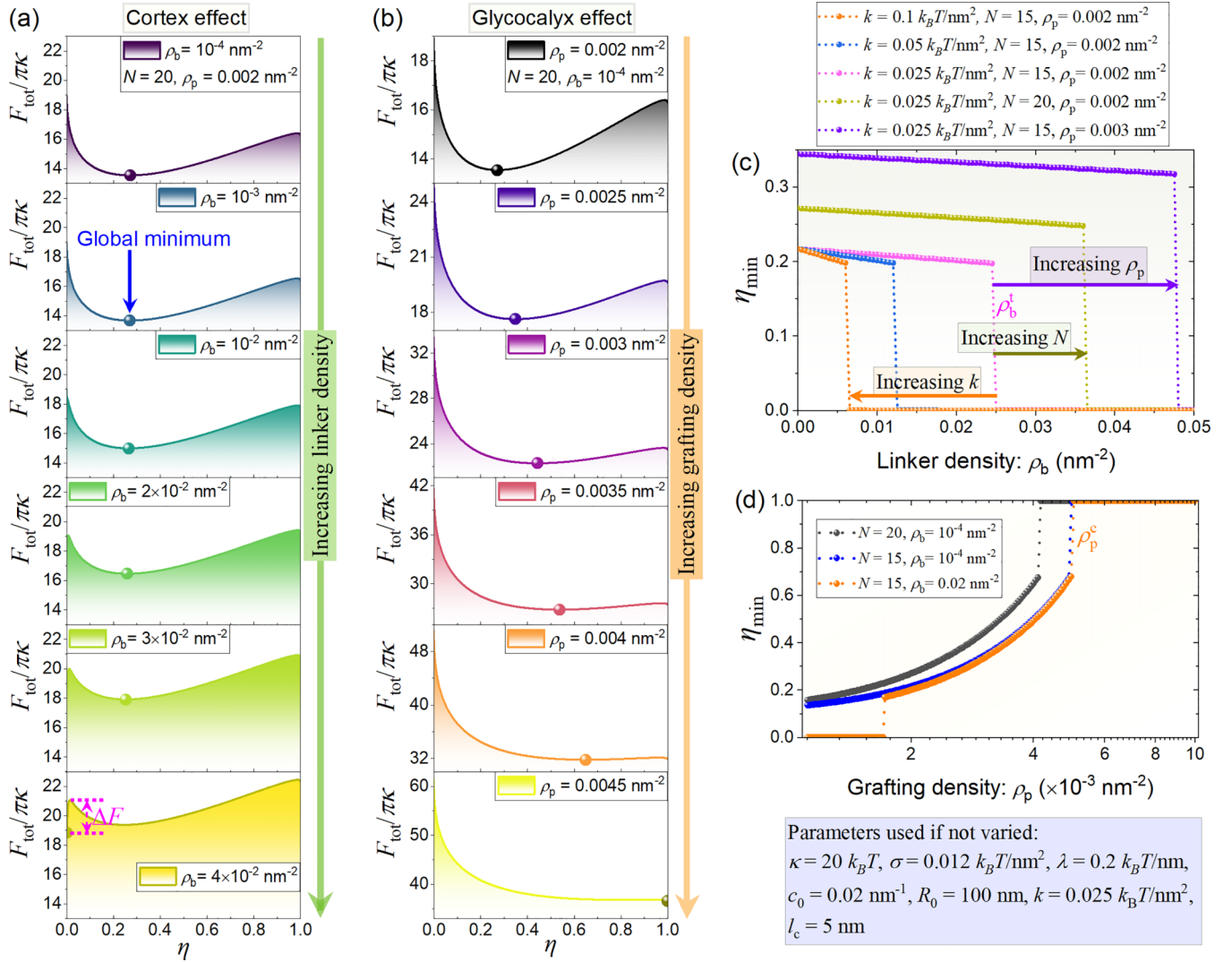


FIG. 4. Total energy profile as a function of shape parameter η for different values of (a) linker density ρ_b and (b) glycopolymer density ρ_p . The dependence of minimum shape parameter η_{min} on (c) linker density ρ_b and (d) glycopolymer grafting density ρ_p .

main effects of varying linker density. First, all curves in Fig. 4(c) illustrate that the optimal shape parameter η_{min} shows a sharp jump [$0 \rightarrow \eta_{\text{min}} \in (0, 1)$] at a critical onset value, ρ_b^t , a characteristic of discontinuous transition. Such a transition indicates that reducing the linker density to a specific threshold is necessary to trigger the detachment of the membrane from the cortex. In other words, the membrane remains flat ($\eta = 0$) and the shape transformation is unsuccessful when the linker density is above a certain value ρ_b^t , which reflects that increasing the level of membrane-cortex protein linkers and enhancing the membrane-cortex attachment reduces the formation of EV. This implies that local depletion or disruption of membrane-cortex protein linkers is needed for membrane deformation initiation to occur. This prediction is consistent with experimental evidence from previous studies [64], where Welf *et al.* [64] have shown that plasma membrane detachment from actin is needed to initiate cell protrusion, and increased actin-membrane attachment reduces protrusion. Second, this critical onset value depends on the linker stiffness (see the orange, blue, and magenta

curves), glycopolymer grafting density (comparing the dark yellow curve with the magenta curve), and polymer length (see the dark yellow curve and the violet curve). The curves in Fig. 4(c) show that the decreasing of linker stiffness or increasing glycopolymer grafting density and length results in a shift of the threshold ρ_b^t , which triggers the discontinuous transition from $\eta = 0$ to $\eta < 1$. Third, at low stiffness, the optimal shape parameter η_{min} exhibits a gradual reduction as we increase the linker density ρ_b . In contrast, the variation of the optimal shape parameter η_{min} becomes more pronounced and is accompanied by a steeper slope with the increasing of linker stiffness k . This effect can be intuitively understood by considering the role of the membrane-cortex adhesion energy in total energy. Since this adhesion energy opposes membrane bending, a higher energy penalty is incurred when membrane deformation occurs. As a result, it becomes more difficult to detach the cell membrane if the cortex contains a higher linker density with stronger stiffness. We also observe that increasing the glycopolymer grafting density or length raises the optimal shape parameter, shifting

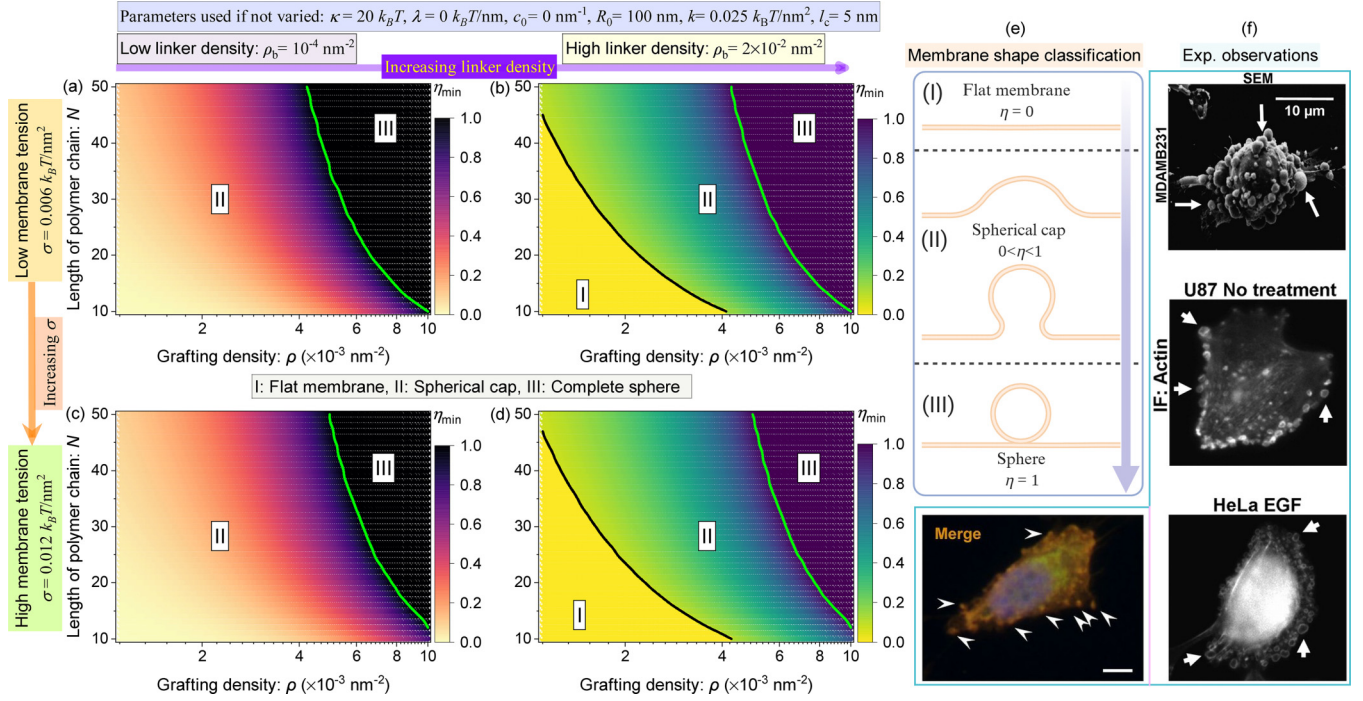


FIG. 5. Heatmaps of optimal shape parameter $\eta_{\min}$ as a function of glycopolymer grafting density ρ_p and polymer chain length N are presented for various scenarios. Panels (a) and (b) correspond to low membrane tension, while panels (c) and (d) correspond to high membrane tension. Specifically, panels (a) and (c) represent low linker density, and panels (b) and (d) represent high linker density. (e) Sketch of various membrane morphologies corresponds to flat, spherical cap, and sphere shapes. (f) Observed MVs, a subtype of EVs, releasing from different types of cancer cells [129,130]. (f) Reprinted from Refs. [129,130] with permission.

curves $\eta_{\min}$ upward with increasing ρ_p or N (comparing the magenta curve with the dark yellow curve or the violet curve). This rise in the optimal shape parameter indicates that the glycocalyx serves as a driving mechanism for the formation of EVs.

Finally, we plot the optimal shape parameter $\eta_{\min}$ against the glycopolymer grafting density ρ_p at different lengths of the polymer chain (see the black and blue dotted curve) and linker density (refer to blue and orange dotted curve), as shown in Fig. 4(d). We find that the optimal shape parameter $\eta_{\min}$ exhibits a snap-through jump at a threshold ρ_p^c at high grafting density, a characteristic of a first-order transition. This suggests that the membrane geometry transits from a partially spherical EV to a fully spherical EV. Comparison of the black and blue curves reveals that shortening the polymer chain length lowers the threshold grafting density required to trigger the formation of a fully spherical membrane geometry. Moreover, comparing the blue curve with the orange curve, we observe that, at low surface coverage of glycopolymers, (1) the undeformed state occurs when the linker density is higher enough; (2) the optimal shape parameter $\eta_{\min}$ undergoes a sharp jump (also indicates a first-order transition) as the grafting density reaches a critical value. In other words, a critical value of grafting density, ρ_p^t is needed to initiate membrane detachment from an initially flat membrane. However, the blue and orange curves in Fig. 4(d) almost overlap, indicating that increased linker density has minimal effect on $\eta_{\min}$ and ρ_p^c once membrane detachment is onset.

Therefore, we find that there exist two threshold grafting densities, one is the critical value required to initiate membrane detachment from a flat membrane, and the other one corresponds to the critical value that triggers a transition from a spherical EV to a full sphere EV.

C. Phase diagrams for glycocalyx-driven EV formation

To characterize the interrelated effects of glycopolymer grafting density and length on the formation of EV, we constructed two-dimensional phase diagrams on the (ρ_p-N) plane in four different cases, as shown in Fig. 5. For both low and high membrane tension, each situation corresponds to two distinct cases: (1) low linker density [see Figs. 5(a) and 5(c)] and (2) high linker density [see Figs. 5(b) and 5(d)]. Figure 5 illustrates three regimes corresponding to an undeformed membrane (regime I), a spherical cap (regime II), and full sphere (regime III), with the color representing the value of the optimal shape parameter $\eta_{\min}$. The two-dimensional phase diagrams in the (ρ_p-N) plane show a common feature that as the glycocalyx density or polymer chain length increases, the shape of the cell membrane undergoes a transition from a spherical cap to a full sphere when the values of ρ_p and N exceed certain thresholds. A comparison between Figs. 5(a) and 5(c) reveals that the glycocalyx can drive the membrane to deform into a full EV (or a complete sphere morphology) under the condition of crossing the threshold of the grafting density and polymer length. However, high membrane tension leads to a narrow regime III and shifts the boundary curve

to the right (see the green curve), which indicates that a higher threshold of the grafting density and polymer length is required to regulate the formation of full sphere EV. When comparing Fig. 5(a) [or Fig. 5(c)] with Fig. 5(b) [or Fig. 5(d)], we observe that an increase in the level of linker density will result in the emergence of the nondeformed regime (flat membrane regime). This validates the prediction presented in Fig. 4, namely, that membrane detachment cannot occur if the glycocalyx grafting density is below a certain threshold. Furthermore, the comparison of regime I between Figs. 5(b) and 5(d) illustrates that the nondeformed regime becomes broader, and the boundary curve (black curve) separating regime I from regime II shifts to the right in the case of high membrane tension. Figure 5(e) shows several typical cell membrane morphologies identified in the phase diagrams. We compare the computed EV geometries with those observed in Refs. [129,130] for distinct types of cancer cells [Fig. 5(f)]. The right column in Fig. 5(f) shows that aggressive tumor cells secrete spherical MVs (are indicated by arrows) at their plasma membrane for long-range delivery of cargoes [129]. The left column in Fig. 5(f) demonstrates that, to mediate intercellular communication, the production of MVs (are denoted by arrowheads) is enhanced when breast cancer cells are exposed to decreased oxygen availability (hypoxia) [130]. The EV shapes observed in the experiments bear resemblance to spherical geometry, which supports the applicability of our model in predicting the formation of EVs.

In summary, membrane-cortex adhesion and glycocalyx properties, such as its density and length, play a crucial role in determining the equilibrium geometry of the cell membrane. The boundary curves that separate the distinct states shift when linker density is altered. Also, to explore the influence of spontaneous curvature and line tension on EV formation, a two-dimensional phase diagram on the $(\lambda - c_0)$ plane is constructed to characterize the interrelated effects of line tension and spontaneous curvature on the shape of the membrane, as shown in Fig. 11 (see Appendix D). Our results demonstrate that EV formation is more easily achieved in the presence of line tension and spontaneous curvature.

D. Influence of membrane-cortex adhesion and glycocalyx coverage on EV formation

It has been reported that EVs exhibit a broad size distribution, typically ranging from 30 to 1000 nm [22,23]. Therefore, it is necessary to understand the role of the glycocalyx coating area in determining EV formation. We first perform the analysis of total free energy for different values of patch radius R_0 . In Fig. 6(a), we plot the total free energy landscape for different R_0 . A direct comparison of the global energy minimums for $R_0 = 50$ nm, $R_0 = 100$ nm, and $R_0 = 300$ nm reveals that a larger glycocalyx grafting area tends to induce the formation of EV. However, further increase of R_0 leads to the formation of EV more unfavorable (see the curves for $R_0 = 500$ nm, $R_0 = 700$ nm, and $R_0 = 900$ nm, respectively). This implies that the effects of the glycocalyx grafting area on EV formation show a nonmonotonic behavior. Figures 6(b)–6(d) show the dependence of $\eta_{\min}$ on R_0 for a series of fixed linker density, glycocalyx grafting density, and glycopolymer length. At low linker density, the blue curve in Fig. 6(b)

shows that $\eta_{\min}$ depends on R_0 in a nonmonotonic manner, which increases first and is followed by a monotonic drop-off. However, the minimum shape parameter $\eta_{\min}$ undergoes a discontinuous reduction to zero at a critical R_0 (see open star) under the circumstance of high linker density. Here, we define the optimal radius R_0^{opt} (solid star) at which $\eta_{\min}$ reaches maximum.

By inspecting the dependence of the optimal shape parameter $\eta_{\min}$ on R_0 for a series of fixed glycocalyx grafting density ρ_p [see Fig. 6(c)] and glycopolymer length N [see Fig. 6(d)], we observe an analogous variation trend, which is $\eta_{\min}$ showing a monotonous rise as $R_0 < R_0^{\text{opt}}$ while a gradual reduction for $R_0 > R_0^{\text{opt}}$. Notably, when the glycocalyx grafting density or glycopolymer length reaches a critical value, the variation of the optimal shape parameter $\eta_{\min}$ is accompanied by a plateau in some range of radii [see the band between the open stars in Figs. 6(c) and 6(d)], indicating the successful formation of a sphere EV.

Furthermore, we scan the phase spaces of $R_0 - \rho_b$, $R_0 - \rho_p$, and $R_0 - N$ in numerical calculations to determine their influence on the optimal shape parameter $\eta_{\min}$, as shown in Figs. 6(e)–6(g), where the magnitude of $\eta_{\min}$ is shown as a color map. Note that all features of Figs. 6(b), 6(c), and 6(d) can be captured by Figs. 6(e), 6(f), and 6(g), respectively. Additionally, Fig. 6(e) shows that two regimes corresponding to nondeformed (regime I: $\eta = 0$) and inflated (regime II: $0 < \eta < 1$) states are identified. While Figs. 6(f) and 6(g) include regime II and regime III ($\eta = 1$) due to that the glycocalyx serves as a driving mechanism for EV formation. The open star dotted line that separates regime I from regime II represents the critical values. Data below this critical line imply that membrane detachment is initiated and an inverse dependence relationship is displayed between the critical R_0 and ρ_b . Intriguingly, the optimal radius slightly depends on linker density (see the solid star dotted line). Remarkably, in contrast to Fig. 6(e), the dependence of R_0^{opt} on glycocalyx grafting density and glycopolymer length in Figs. 6(f) and 6(g) shows an almost linear proportional relationship (see the solid star dotted line). However, the critical curves that separate regime II from regime III exhibit a nonmonotonic behavior. Two more phase diagrams on the $(R_0 - \rho_b)$ plane at different glycocalyx grafting density and glycopolymer length are presented in Appendix E (see Fig. 12) to show that increasing the grafting density and polymer length can narrow regime I as compared to Fig. 6(e).

In summary, our results demonstrate that membrane-cortex adhesion inhibits membrane detachment, while glycocalyx plays a positive role in assisting EV formation. Inspecting the role of the glycocalyx grafting area, we can infer that the formation of EVs becomes more favorable as R_0 reaches the optimal value R_0^{opt} . Here, we predict that the glycocalyx plays an essential role in the formation of EVs. Including model parameters such as glycocalyx grafting density and length is important, as various cell surface morphologies are closely linked to the glycocalyx [7,53,76–84,129,131]. For instance, aggressive tumor cells often produce an excess of mucins and hyaluronan on their surface, facilitating the formation of spherical MVs at the plasma membrane for long-distance intercellular communication and the elimination of

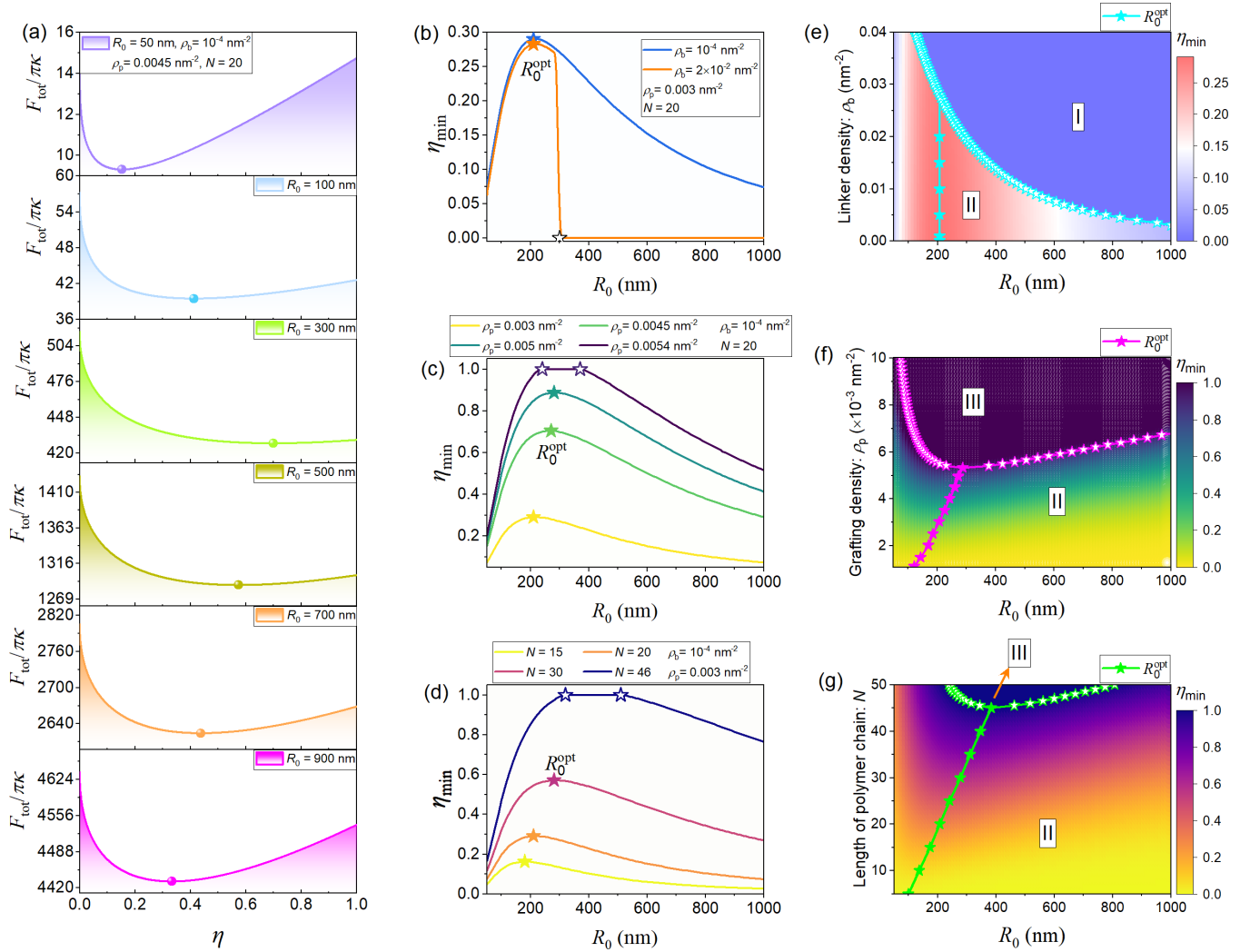


FIG. 6. (a) Total free energy profile as a function of shape parameter η for different values of R_0 . The dependence of the optimal shape parameter η_{min} on R_0 at different (b) linker density ρ_b , (c) glycolyx grafting density ρ_p , and (d) glycopolymer length N . Phase diagrams present the optimal shape parameter η_{min} as a function of R_0 and (e) linker density ρ_b , (f) glycolyx grafting density ρ_p , and (g) glycopolymer length N .

unwanted materials [1–4,7,53,129,131]. On the contrary, the removal of the glycolyx significantly reduces vesicle release [53], suggesting a close correlation between glycolyx presence and cell membrane geometry. Furthermore, investigating the impact of polymer length is essential, as this can be experimentally tested, especially considering the feasibility of modulating the number of tandem repeats in Muc1 [53,55,132].

E. Size-dependent probability of EV formation

Furthermore, to explore the possibility of generating a spherical EV, indicating that the EV is in a state with $\eta = 1$, we assume that the morphology of the EV is governed by a Boltzmann distribution. As a result, the probability of forming a closed EV can be obtained from statistical mechanics, which is given by

$$P(\eta = 1) = \frac{e^{-\beta F_{\text{tot}}(\eta=1)}}{\sum_{\eta=0}^{\eta=1} e^{-\beta F_{\text{tot}}(\eta)}}, \quad (24)$$

where F_{tot} is given by Eq. (18). We numerically evaluate $P(\eta = 1)$ in Eq. (24) to predict the probability of generating a spherical EV. Figures 7(a) and 7(b) show the $P(\eta = 1)$ as a function of R_0 for different values of the glycolyx grafting density and polymer length. We observe that, for glycolyx with a sparse grafting density and a short length [i.e., $\rho_p = 0.005$ nm $^{-2}$ in Fig. 7(a) and $N = 15$ nm $^{-2}$ in Fig. 7(b)], the possibility of an EV in the closed state is nearly zero, which implies that generating a closed EV is almost impossible. Compared to the flat curve, the rest of the curves in Figs. 7(a) and 7(b) demonstrate that the larger the values of grafting density and polymer length, the greater the likelihood of generating a closed EV. In addition, we also note that the width of the spectrum of R_0 ($R_0^{\text{left}} < R_0 < R_0^{\text{right}}$) spanned by the nonzero probability distribution profile becomes wider with increasing glycolyx grafting density or polymer length, indicating that a wide range of EV sizes can be produced. Based on the probability distribution profile, we also defined the most possible radius R_0^{Pmax} (solid dot) at which the odds

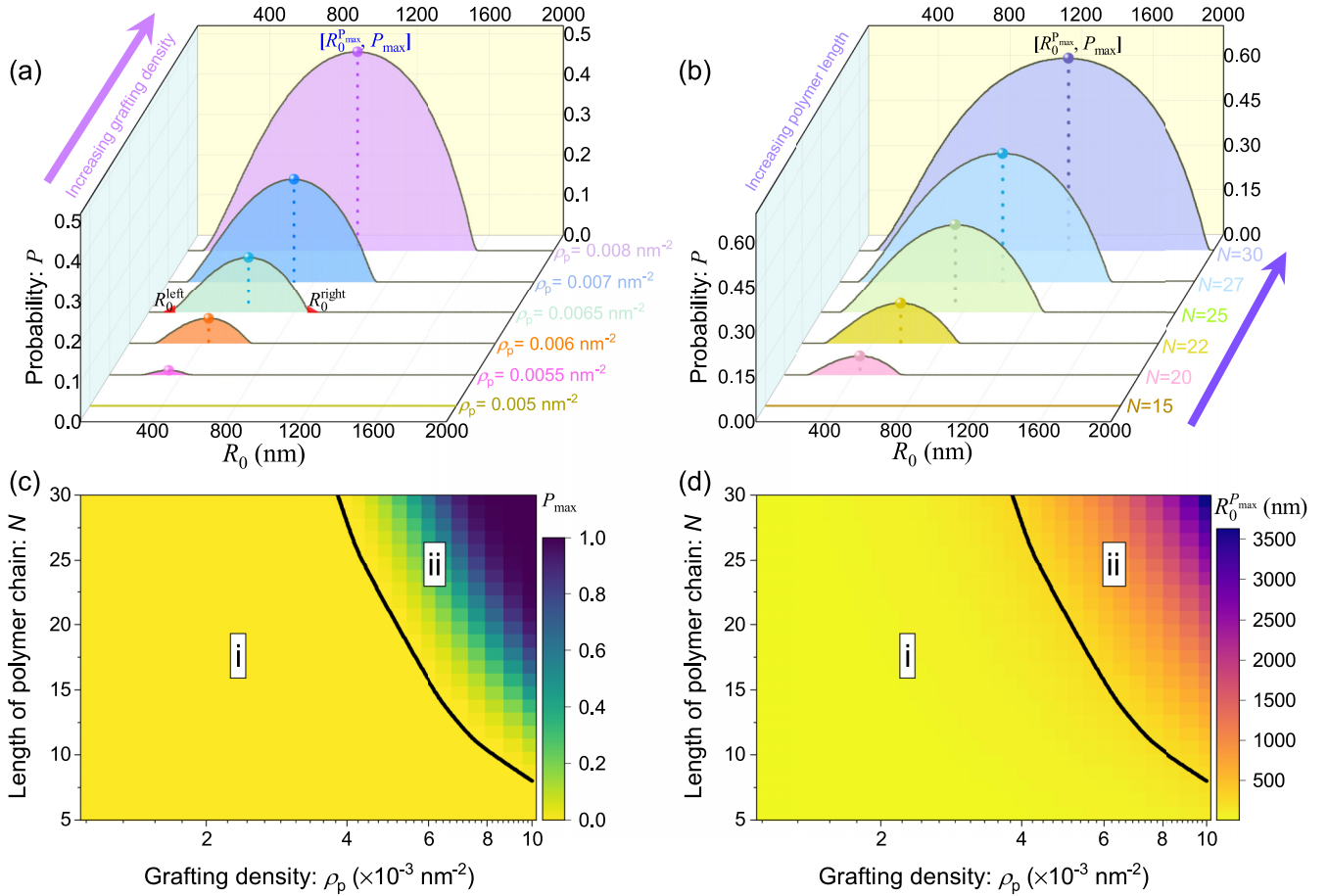


FIG. 7. Probability of generating a sphere EV as a function of R_0 for a set of fixed values of (a) glycocalyx grafting density ρ_p , and (b) glycopolymer length N . Heatmap of (c) maximum probability of generating a closed EV, P_{max} and (d) corresponding $R_0^{\text{P}_{\text{max}}}$ as a function of glycocalyx grafting density ρ_p and glycopolymer length N .

of generating a closed EV have the highest value P_{max} . The interrelated effects of glycocalyx grafting density and polymer length on $R_0^{\text{P}_{\text{max}}}$ and P_{max} are summarized in Figs. 7(c) and 7(d). Specifically, depending on the properties of the glycocalyx, two regimes corresponding to $P_{\text{max}} = 0$ and $P_{\text{max}} \neq 0$ cases are identified in Fig. 7(c), in which the color indicates the maximum probability of forming a closed EV. In regime i, it suggests that forming a closed EV is nearly impossible. In contrast, in regime (ii), the feasibility of producing a closed EV is more higher at denser and longer glycocalyx. The value of $R_0^{\text{P}_{\text{max}}}$ corresponding to regime ii is presented in Fig. 7(d). Similarly, Fig. 7(d) illustrates that larger EV can be produced at higher glycocalyx grafting density and longer polymer length. Therefore, our results predict that within the range of ρ_p and N relevant for EV, different combination of ρ_p and N tend to yield distinct EV sizes. This suggests that cells may self-regulate glycocalyx properties to produce a heterogeneous collection of EV size. Our prediction can be supported by previous experimental observations in Ref. [53] (refer to Fig. 6) where Shurer *et al.* observed that massive concentrations of particles ranging in size from approximately 100 to 400 nm can be secreted from Muc1-42TR-expressing cells. Importantly, they verified that the released particles are membrane vesicles with glycocalyx grafted on their surface

by using cryo-transmission electron microscopy (cryo-TEM) analysis, and the removal of the glycocalyx significantly reduces the production of vesicles. Our results in Figs. 6 and 7 also imply that reducing glycocalyx grafting density will result in unsuccessful EV formation.

E. Critical values required for initiating membrane detachment and EV formation

In Figs. 4–6, we have identified two distinct critical conditions: one for the onset of glycocalyx-driven membrane detachment and another for the regulation of the formation of full EV states. Based on our theoretical model, we expect that several parameters can affect these two critical conditions. To gain more insight into the effects of linker density on the critical condition that initiates membrane detachment, we plot the dependence of the critical value of different parameters to initiate membrane detachment on linker density in Fig. 8(a). Intriguingly, we find that ρ_p^t and ρ_b exhibit a logarithmic linear dependence, and N_t , $R_{0,t}$, λ_t , $c_{0,t}$, and σ_t are almost proportional to ρ_b linearly. Then, focusing on the dependence of the critical grafting density on various parameters in Fig. 8(b), we also find that ρ_p^c depends on λ , c_0 , and σ in a nearly linear manner, while ρ_p^c and N display a logarithmic linear

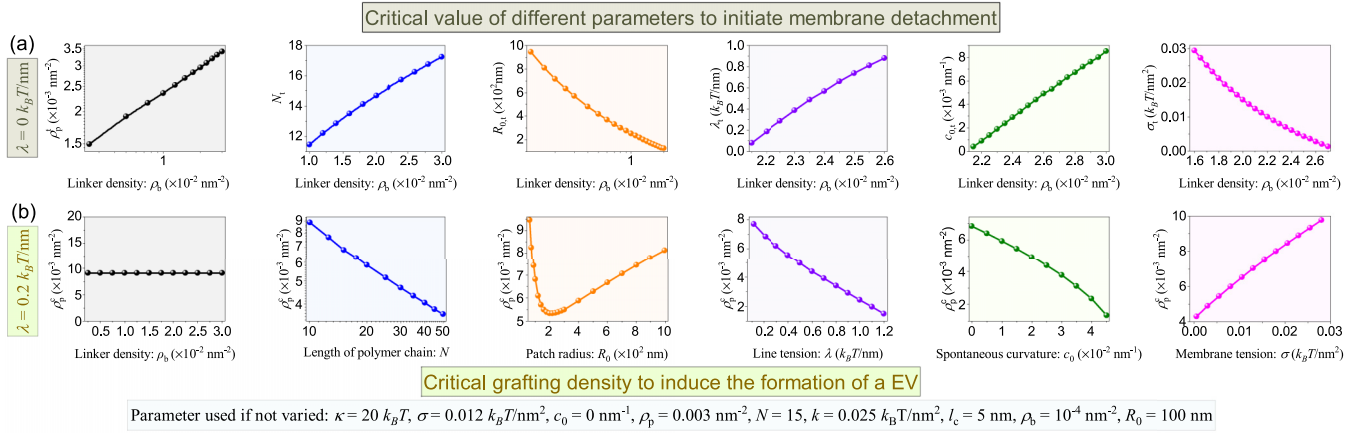


FIG. 8. The dependence of (a) critical value of different parameters to initiate membrane detachment on linker density and (b) critical grafting density on different parameters, including linker density, length of polymer chain, patch radius, line tension, spontaneous curvature, and membrane tension.

dependence. Notably, the variation of the linker density rarely affects ρ_p^c and the dependent relation between ρ_p^c and R_0 exhibits a nonmonotonic behavior. In general, these observations predict that an increase in the level of linker proteins requires a higher grafting density (or a longer polymer length, a larger line tension, spontaneous curvature, a smaller grafting area, and a lower membrane tension) to initiate membrane delamination from the cortex. Longer polymer chains and looser membranes, along with the presence of line tension and spontaneous curvature, reduce the critical grafting density of the polymer required to trigger the formation of a spherical membrane structure.

IV. DISCUSSION

Despite the fact that many experimental studies have shown that glycocalyx can regulate membrane morphologies [53] and that local depletion of actin-membrane bonds is needed for the initiation of membrane protrusions [64], we know surprisingly little about the critical conditions for the onset of membrane detachment and the formation of EVs. From a modeling perspective, we developed a comprehensive biophysical model that incorporates various factors to provide deeper insight into how the interplay between membrane-cortex adhesion and glycocalyx regulates EV formation. Based on our theoretical results, we make the following experimentally relevant predictions. The reduction of membrane-cortex linkers is necessary for the onset of membrane bending, which is qualitatively consistent with the experimental results observed by Welf *et al.* [64]. This could be applied to understand the correlation between the level of membrane-cortex linker molecules and the potential for EV or bleb formation. For example, reduced blebbing has been experimentally observed in zebrafish germ cells [133], A375 melanoma cells [134], and mast cells [135] as the level or activity of ERMs increases. Bleb formation at the leading edge of the cell is facilitated due to the reduction of membrane-to-cortex attachment at the cell front, while the level of the actin-membrane linker ezrin is elevated at the back of the cell [136,137]. These observations suggest that membrane-cortex attachment could play a role in membrane

deformations. Meanwhile, we quantitatively determined the critical conditions for this onset event and found a linear dependence between these thresholds and linker density. In addition to the glycocalyx and cortex adhesion, several other factors may play a role in the formation of EVs. For instance, Iglič *et al.* [138] proposed an alternative mechanism for microexovesicle formation, wherein an elevation in the area difference between the outer and inner membrane lipid layers of a skeleton-free membrane segment leads to the formation of spherical microexovesicles. By using the calculus of variations, a limiting sphere shape can be obtained in the case of the maximum possible value of extreme relative area difference. The mechanism for the microexovesicle formation in Ref. [138] is attributed to the difference between the two membrane layer areas of the skeleton-free membrane segment. In contrast, we are considering a membrane system where the outer membrane is covered by a glycocalyx layer, and the inner membrane is attached to a cortex, rather than being a skeleton-free membrane segment. This distinction implies that the factors contributing to EV formation are more complex and may involve additional elements that need to be further confirmed. The second prediction of our model is that the glycocalyx alone can drive the production of extracellular spherical microvesicles under the condition that a threshold grafting density is reached. This prediction is supported by experimental evidence showing that the removal of the glycocalyx leads to a significant reduction in the release of vesicles [53]. This might also partially have implications for understanding microvesicle generation in many cancer-cell types. For instance, tumor cells with high mucin expression have a propensity to generate a high number of microvesicles [131]. Moreover, the predicted dependence of critical grafting density on various system parameters for the successful generation of extracellular spherical microvesicles may provide guidance on how to adjust control parameters to fine-tune membrane geometries in experiments. In the end, our model also predicts that a heterogeneous collection of EV population can be produced through the regulation of glycocalyx properties. This prediction is supported by the experimental observations made by Shurer *et al.* [53], and the underlying mechanisms may help explain why EVs are heterogeneous

structures spanning a wide range of sizes. We expect that the findings in this work can serve as a better understanding of the role of membrane-cortex attachment and individual glycocalyx properties in regulating membrane shapes and provide deeper insights into how extracellular vesiculation occurs under various biophysical conditions.

To investigate the effects of membrane-cortex adhesion and the glycocalyx on EV formation and to incorporate these factors into our theoretical model, we made several assumptions and simplifications in the present work. First, we approximated the shape of the membrane as a family of spherical caps, which is a reasonable simplification since Shurer *et al.* [53] reported that bulky brushlike glycocalyx polymers are sufficient to induce spherical-shaped membranes (referred to as blebs). Second, the model does not include the electrostatic effects because we treated the glycocalyx as an uncharged polymer brush network. Note that the glycocalyx is typically negatively charged [38,53]. For example, mucins generally carry about five electron charges per 20 amino acids, and the glycocalyx of an erythrocyte contains approximately $1.8 \sim 2 \times 10^7$ negative elementary charges [139,140]. Consequently, when considering the glycocalyx as a charged polymer brush network, it is crucial to account for the electrostatic effects. As such, the total free energy of the system must incorporate the electrostatic energy contribution. Many mathematical models [140,141] have been developed to predict the electric potential profile at the membrane of erythrocytes generated by these charge distributions. Also, numerous efforts of theoretical modeling [142–146] have been devoted to evaluate the electrostatic energy contribution to the total energy of glycocalyx-membrane composite systems. However, in this study, we treat the glycocalyx as electrically neutral and exclude the electrostatic effects. For negatively charged glycocalyx [38,53], we speculate that the electrostatic repulsive forces generated by the negative charges in the glycocalyx simply serve to enhance the steric effects. To investigate the entropic interactions alone, we assume the polymers to be uncharged, thereby neglecting the electrostatic interactions in our analysis. Moreover, the charge gradient generated by ion channels may also be linked to the charge effects that contribute to membrane deformation. Third, we neglected the spatial heterogeneity of the distribution of the glycocalyx polymers on the membrane. Fourth, beyond the local breakage of bonds between the cortex and the membrane, diffusion and binding and unbinding of the protein linker may also affect glycocalyx-mediated membrane architectures within the cell [57,64]. Here, we neglected the diffusion and unbinding kinetics of the protein linker in the cortex. In addition, the energy associated with membrane-cortex adhesion was calculated using the spring constant of the linker and its displacement. Future modeling and experimental studies could incorporate the diffusion of glycocalyx polymers, changes in the bulk physical properties of the glycocalyx, charged polymers, as well as the effects of linker binding and unbinding in the actin cortex to better capture the biological complexity of cell membranes.

Combining polymer physics-based theory and Helfrich membrane theory, we proposed a theoretical model to investigate the mechanisms by which glycocalyx polymers and membrane-cortex adhesion influence membrane shape

remodeling. Specifically, a minimal model allows us to quantify the roles of glycocalyx, membrane-cortex adhesion, grafting area, line tension, and spontaneous curvature in the formation of EVs. The physics behind the transition between different deformation states comes from the competition among the energy associated with the glycocalyx, the elastic energy (consisting of bending energy and tension energy), and membrane-cortex adhesion energy. The energy associated with the glycocalyx drives membrane deformation at the cost of the bending energy due to local membrane curvature formation, of stretching energy due to lateral membrane tension, and of adhesion energy due to the attachment between the membrane and the cortex. Line tension and spontaneous curvature are also driving factors assisting membrane deformation.

In conclusion, based on the developed theoretical framework, we study the EV formation regulated by glycocalyx and membrane-cortex adhesion and find that the presence of glycocalyx is able to initiate membrane deformation and regulate EV size distribution. Such membrane shape remodeling behaviors induced by glycocalyx and membrane-cortex adhesion provide a deeper understanding of EV secretion. EV formation is tunable by glycocalyx, cortex-membrane adhesion, grafting area, line tension, and spontaneous curvature, which can be characterized by a phase diagram in the two-parameter space among these factors. We identify that the degree of membrane deformation can be increased upon regulating many parameters, such as increasing glycocalyx grafting density and length, reducing cortex-membrane adhesion strength by tuning the linker density and stiffness, and increasing line tension and spontaneous curvature.

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DATA AVAILABILITY

The code that support the findings of this article are openly available [147].

APPENDIX A: DERIVATION OF THE ENERGY CONTRIBUTION ASSOCIATED WITH THE GLYCOCALYX

Here, we derive Eq. (20) of the main text. Notably, the crowding of large glycosylated proteins appears to regulate the shape of the underlying bilayer plasma membrane [53]. According to polymer physics, two regimes can be defined depending on the grafting density of glycocalyx polymers on the cell membrane, which are the mushroomlike regime and the brushlike regime. Shurer *et al.* [53] have reported that the mucins are in the coiled mushroom state in the case of low-density glycocalyx polymers. We focus on densely grafted

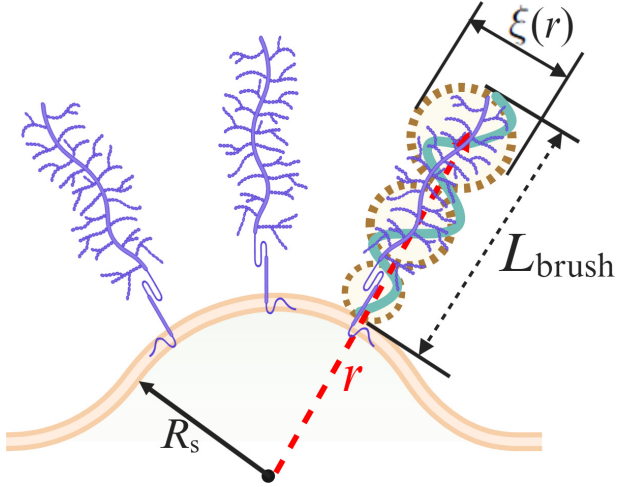


FIG. 9. Schematic of a layer of brushlike polymers attached on a spherical cap membrane surface with radius R_s . At a given position r , the coarse grained blob (dashed black circle) size in a polymer brush is $\xi(r)$ and the thickness of the brush is L_{brush} .

regions of the membrane and therefore model the glycocalyx in the brush regime.

Here, we consider a layer of glycocalyx grafted on a membrane of radius R_s in a brushlike structure with thickness L_{brush} , as shown in Fig. 9. From the viewpoint of coarse graining, the polymer brush is envisioned as an array of blobs. The size of each blob, ξ , at a given position r equals the square root of the local area per chain $s(r)$, where $r = x + R_s$ is the radial distance that is defined from the center of the spherical surface, and in which x is the distance from the membrane surface. Thus, the blob size $\xi = \sqrt{s(r)}$ grows as a function of r , and the grafting density of glycocalyx polymer brush on the membrane surface can be obtained as $\rho = 1/\xi^2$. Assuming that the layer of glycopolymers are extended nonuniformly but equally in the height, L_{brush} , the area per chain at distance x from the membrane surface is given by [113]

$$s(r) = s(x + R_s) = s(R_s) \left(\frac{r}{R_s} \right)^{i-1} \quad (i = 1, 2, 3). \quad (\text{A1})$$

The index $i = 1, 2, 3$ indicates planar, cylindrical, and spherical-shaped membranes, respectively. When the membrane is bent, the changes of polymer configuration give rise to the local extension of the polymer chain. Here, the local chain extension at a height r is characterized by dr/dn , where the variable n denotes the current monomer. This local extension is related to local density profile of monomers $c_p(r)$ as [113]

$$c_p(r) = \frac{dn}{s(r)dr}. \quad (\text{A2})$$

Then, the thickness of the brush L_{brush} is found from the conservation condition (the constraint of conservation of the total number of monomers N) [113],

$$\int_{R_s}^{R_s + L_{\text{brush}}} c_p(r) s(r) dr = N. \quad (\text{A3})$$

As a result, in the brush regime, since electrostatic effects are excluded, the energy contribution originating from glycocalyx polymers includes two terms [53,113]: the elastic energy of the polymer chain ($F_{\text{glycocalyx}}^{\text{elastic}}$) and the free energy caused by the excluded volume interactions of polymer monomers ($F_{\text{glycocalyx}}^{\text{interaction}}$). Based on the hypotheses in the main text, according to Ref. [113], the elastic energy per chain in the brush can be represented as

$$\begin{aligned} F_{\text{glycocalyx}}^{\text{elastic}} &= \int_{R_s}^{R_s + L_{\text{brush}}} f_{\text{glycocalyx}}^{\text{elastic}} s(r) dr \\ &= \int_{R_s}^{R_s + L_{\text{brush}}} \frac{3k_B T}{2a^2 c_p(r) s^2(r)} s(r) dr, \end{aligned} \quad (\text{A4})$$

where a is the monomer length and $f_{\text{glycocalyx}}^{\text{elastic}}$ is the elastic energy density of the polymer chain. Within the mean-field approximation, the energy density of the excluded volume interactions (van der Waals interactions) between monomers can be modeled in terms of the virial expansion

$$f_{\text{glycocalyx}}^{\text{interaction}} = k_B T [v c_p^2(r) + w c_p^3(r) + \dots], \quad (\text{A5})$$

where v and w are the second and third virial coefficient, respectively. As a result, the excluded volume interactions between monomers per chain in the brush are given by

$$F_{\text{glycocalyx}}^{\text{interaction}} = \frac{1}{\beta} \int_{R_s}^{R_s + L_{\text{brush}}} [v c_p^2(r) + w c_p^3(r) + \dots] s(r) dr. \quad (\text{A6})$$

Moving forward, the cubic and higher terms are neglected. Therefore, the sum of Eqs. (A4) and (A6) yields the energy contribution of glycocalyx polymers

$$\begin{aligned} F_{\text{glycocalyx}} &= N_p (F_{\text{glycocalyx}}^{\text{elastic}} + F_{\text{glycocalyx}}^{\text{interaction}}) \\ &= \frac{N_p}{\beta} \int_{R_s}^{R_s + L_{\text{brush}}} \left[\frac{3}{2a^2 c_p(r) s^2(r)} + v c_p^2(r) \right] s(r) dr, \end{aligned} \quad (\text{A7})$$

where $N_p = \rho A_0$ is the number of polymer chain grafted on the membrane. Here, A_0 stands for the membrane patch area covered by glycocalyx polymers and ρ is the density.

In order to calculate the free energy, we need to further determine the local concentration of monomers $c_p(r)$ and the brush thickness L_{brush} . On a planar membrane surface, the planar brush area per chain, $s(r)$, is constant, i.e., $s(r) = s$. Minimizing the free energy of a planar brush $F_{\text{glycocalyx}}$ with respect to c_p by using $c_p(r) \frac{\delta f[c_p(r), r]}{\delta c_p(r)} - f[c_p(r), r] = 0$ leads to the equilibrium polymer concentration c_p^{flat} (see Ref. [113] for detailed steps)

$$c_p^{\text{flat}} = \left(\frac{3}{v s^2 a^2} \right)^{\frac{1}{3}}. \quad (\text{A8})$$

Here, $f[c_p(r), r] = f_{\text{glycocalyx}}^{\text{elastic}} + f_{\text{glycocalyx}}^{\text{interaction}}$. Using the conservation condition defined by Eq. (A3) yields the brush thickness $L_{\text{brush}}^{\text{flat}}$ (see Ref. [113] for detailed steps)

$$L_{\text{brush}}^{\text{flat}} = N \left(\frac{v a^2}{3 s} \right)^{\frac{1}{3}}, \quad (\text{A9})$$

where we can see that the thickness of the polymer brush is proportional to the total number of effective monomers N . Hereafter, in our model, we use the number of effective monomers, N , to capture the length of the polymer. Note that the relationship for c_p^{flat} and $L_{\text{brush}}^{\text{flat}}$ are special cases of electrically neutral brushes as described in Ref. [113]. Substituting Eqs. (A8) and (A9) into Eq. (A7) yields the energy contribution of glycocalyx polymers on a planar membrane surface

$$\frac{F_{\text{flat}}^{\text{glycocalyx}}}{\pi\kappa} = N_p \frac{9N}{2\pi\beta\kappa} \left(\frac{v}{3sa} \right)^{\frac{2}{3}}, \quad (\text{A10})$$

which corresponds to the case $\eta = 0$ in Eq. (20). Thus, even for a flat membrane, the energy contribution by the glycocalyx is directly proportional to the extent of grafting N_p and the length of the polymer brush N .

On a spherical membrane surface, the corresponding monomer density profile $c_p(r)$ and brush thickness L_{brush} are, respectively, expressed as (see Ref. [113] for a detailed calculation)

$$c_p(r) = \left[\frac{3}{vs^2(r)a^2} \right]^{\frac{1}{3}} = c_p^{\text{flat}} \left(\frac{R_s}{r} \right)^{\frac{4}{3}} \quad (\text{A11})$$

and

$$L_{\text{brush}} = R_s \left(1 + \frac{5}{3} \cdot \frac{L_{\text{brush}}^{\text{flat}}}{R_s} \right)^{\frac{3}{2}} - R_s. \quad (\text{A12})$$

Similarly, substituting Eqs. (A11) and (A12) into Eq. (A7) leads to the energy contribution of glycocalyx polymers on a spherical membrane surface as

$$\begin{aligned} \frac{F_{\text{glycocalyx}}}{\pi\kappa} &= \frac{N_p (F_{\text{glycocalyx}}^{\text{elastic}} + F_{\text{glycocalyx}}^{\text{interaction}})}{\pi\kappa} \\ &= \frac{9N_p R_0}{4\pi\beta\kappa\sqrt{\eta}} \left(\frac{3\sqrt{v}}{\xi a^2} \right)^{\frac{2}{3}} \\ &\quad \times \left[\left(1 + \frac{10N\sqrt{\eta}}{3R_0} \left(\frac{va^2}{3\xi^2} \right)^{\frac{1}{3}} \right)^{\frac{1}{5}} - 1 \right], \quad (\text{A13}) \end{aligned}$$

where we use $R_s = R_0/(2\sqrt{\eta})$.

APPENDIX B: DERIVATION OF THE ENERGY ASSOCIATED WITH MEMBRANE-CORTX ADHESION

Here, we derive Eq. (22). To estimate the energy associated with membrane-cortex adhesion, we assume that the membrane-cortex adhesion energy is quadratic with respect to the membrane-cortex linker deformation and is thus given by

$$F_{\text{ad}} = \int \frac{k}{2} \rho_b z^2 dA, \quad (\text{B1})$$

where k is the elastic stiffness (spring constant) of each membrane-cortex linker, ρ_b is the local density of bound springlike linkers, and z is the stretching length of linkers. For

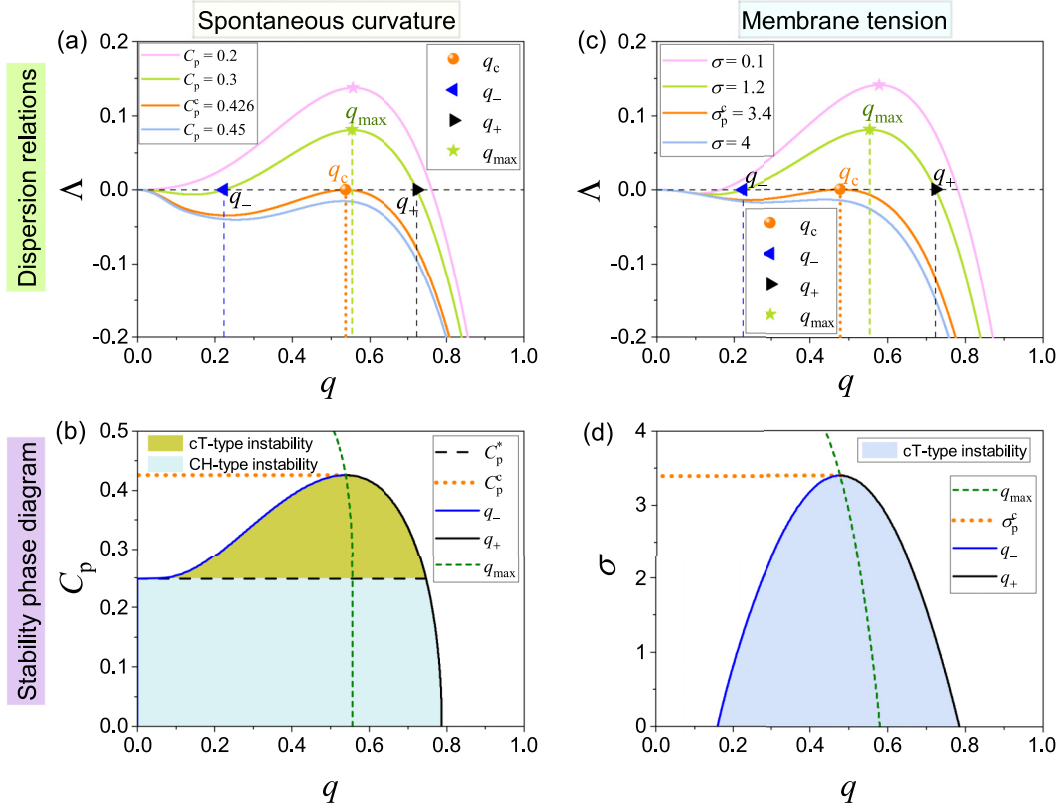


FIG. 10. Linear stability analysis of the composite system. Plot of the dispersion relation $\Delta(q)$ for different values of (a) spontaneous curvature and (c) membrane tension. The dependence of the unstable wave numbers on the (b) spontaneous curvature and (d) membrane tension.

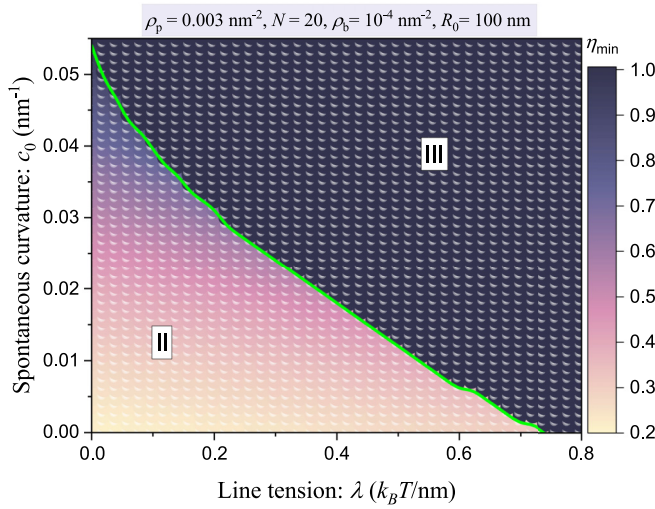


FIG. 11. Contour plot of optimal shape parameter $\eta_{\min}$ as a function of line tension λ and spontaneous curvature c_0 , where the color bar represents the magnitude of the optimal shape parameter.

simplicity, we further assume that (1) the density of bound springlike linkers equals the density of available linkers ρ_0 , i.e., $\rho_b = \rho_0$; (2) the adhesion bond breaks as the membrane-cortex adhesion bond reaches its maximum length l_c .

When the membrane deformation is small, the maximum height of the deformed membrane is smaller than the maximum extension length of linkers [$R_0\sqrt{\eta} = h \leq l_c$, which corresponds to $\eta \leq (l_c/R_0)^2$; see Fig. 2(b)], the membrane-cortex adhesion energy can be calculated as

$$\begin{aligned} F_{\text{ad}} &= \int \frac{k}{2} \rho_b z^2 dA \\ &= \int_0^\theta \frac{k}{2} \rho_b R_s^2 (\cos \Theta - \cos \theta)^2 2\pi R_s^2 \sin \Theta d\Theta \\ &= \frac{\eta}{6} \pi k \rho_b R_0^4, \end{aligned} \quad (\text{B2})$$

where $\cos \theta = 1 - 2\eta$ with θ being the opening angle of the spherical cap and Θ is the integral variable.

If the membrane is deformed into a spherical cap with its maximum height larger than the maximum extension length of the linkers, that is, $h > l_c$ [see Fig. 2(c)]. Then, it means that part of the linkers are broken [see the regime above the dashed line in Fig. 2(c)] and some of the linkers are still attached to the membrane but with some degree of extension [see the regime below the dashed line in Fig. 2(c)] for the deformed membrane. Thereby, we can treat the membrane as two parts, one with the bond linkers are broken, and the other one with the linkers binding on it. As a result, in the case of $h > l_c$, the membrane-cortex adhesion energy consists of two parts, the first one is the part with linker broken, and the second one is the detached part with the linkers still attached but extended.

When the membrane is detached from the cortex, the total number of broken adhesion bonds can be approximated by $N_b = \rho_b A_d(\eta)$, where $A_d(\eta)$ is the area of the detached membrane, which represents the area where the adhesion between the membrane and the cortex has been disrupted, as shown in Fig. 2(c). Specifically, if we assume the detachment between

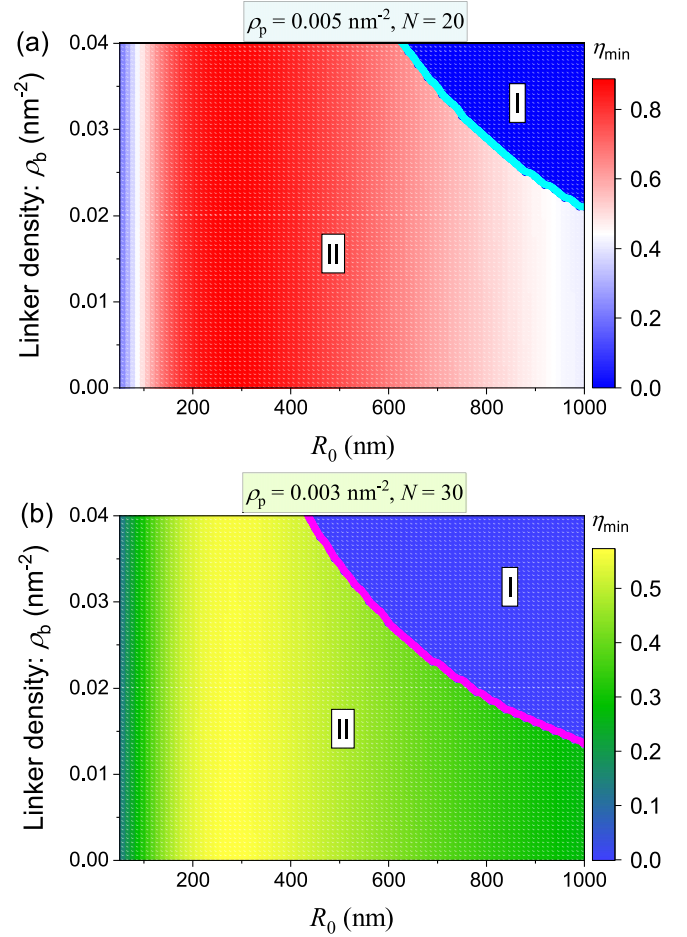


FIG. 12. Contour plot of optimal shape parameter $\eta_{\min}$ as a function of patch radius R_0 and linker density ρ_b for the grafting density and length of the glycocalyx are fixed as (a) $\rho_p = 0.005 \text{ nm}^{-2}$ and $N = 20$, and (b) $\rho_p = 0.003 \text{ nm}^{-2}$ and $N = 30$, where the color bar represents the magnitude of the optimal shape parameter.

the membrane and the cortex occurs once their separation reaches l_c , then $A_d(\eta)$ can be shown to take the form

$$A_d(\eta) = 2\pi R_s^2 \left(2\eta - \frac{l_c}{R_s} \right) = \pi R_0^2 \left(1 - \frac{l_c}{R_0\sqrt{\eta}} \right). \quad (\text{B3})$$

Therefore, the energy contribution from the first part can be obtained as

$$\begin{aligned} F_{\text{ad}}^{\text{broken}} &= N_b \frac{1}{2} k l_c^2 = \frac{k}{2} \rho_b l_c^2 A_d(\eta) \\ &= \frac{1}{2} \pi k \rho_b l_c^2 R_0^2 \left(1 - \frac{l_c}{R_0\sqrt{\eta}} \right). \end{aligned} \quad (\text{B4})$$

The energy contribution from the second part can be obtained as

$$\begin{aligned} F_{\text{ad}}^{\text{extended}} &= \int \frac{k}{2} \rho_b z^2 dA \\ &= \int_0^\theta \frac{k}{2} \rho_b R_s^2 (\cos \Theta - \cos \theta)^2 2\pi R_s^2 \sin \Theta d\Theta \\ &= \frac{1}{6\sqrt{\eta}} \pi k \rho_b R_0 l_c^3. \end{aligned} \quad (\text{B5})$$

Consequently, the membrane-cortex adhesion energy can be expressed as

$$F_{\text{ad}} = \begin{cases} \frac{\eta}{6}\pi k \rho_b R_0^4, & \eta \leq (l_c/R_0)^2, \\ \frac{1}{2}\pi k \rho_b R_0 l_c^2 (R_0 - \frac{2l_c}{3\sqrt{\eta}}), & \eta > (l_c/R_0)^2, \end{cases} \quad (\text{B6})$$

which coincides with Eq. (22).

APPENDIX C: THE EFFECTS OF SPONTANEOUS CURVATURE AND MEMBRANE TENSION ON SYSTEM STABILITY

To investigate the effects of spontaneous curvature and membrane tension on system stability, the dispersion relations for different values of spontaneous curvature and membrane tension are plotted, as shown in Fig. 10. Figure 10(a) shows that the instability appears when the spontaneous curvature reaches C_p^c , and the eigenvalue becomes unstable in a range of wave vectors $q_- < q < q_+$ when C_p is smaller than C_p^c . Moreover, q_- can vanish if C_p is small enough (pink curve). In Fig. 10(b), we plot the dependence of the unstable wave numbers on the spontaneous curvature, where we also observe two types of instabilities similar to those of Fig. 3(d). In Fig. 10(c), we plot the dispersion relations for a set of fixed membrane tensions, where the curves show a trend similar to that in Fig. 3(a). In the end, the stability phase diagram of the $q-\sigma$ plane demonstrates a cT-type instability [Fig. 10(d)].

APPENDIX D: EFFECTS OF SPONTANEOUS CURVATURE AND LINE TENSION ON EV FORMATION

To intuitively observe the influence of spontaneous curvature and line tension on EV formation, a phase diagram on the $(\lambda-c_0)$ plane is constructed, as shown in Fig. 11. For zero line tension and spontaneous curvature, the cell membrane deformation is in a shallow spherical cap state. By increasing line tension and spontaneous curvature to certain values, the transition from an inflated state to a full EV state can be observed, indicating a first-order transition. This suggests that line tension λ and spontaneous curvature c_0 play a positive role in the formation of EVs.

APPENDIX E: PHASE DIAGRAMS ON THE $(R_0-\rho_b)$ PLANE

To gain more insight into the effects of glycocalyx properties on EV formation, two phase diagrams are constructed on the $R_0-\rho_b$ plane for different values of glycocalyx grafting density and glycopolymer length, as shown in Fig. 12. When comparing Fig. 12(a) with Fig. 6(e), we observe that an elevate of glycocalyx grafting density leads to a narrow regime I and shifts the boundary curve to the right (see the cyan curve). Furthermore, the comparison of regime I between Figs. 12(b) and 6(e) illustrates that the flat membrane regime also becomes narrower, and the boundary curve (magenta curve) that separates regime I from regime II shifts to the right in the case of longer glycopolymer length. These indicate that a higher threshold of the linker density and glycocalyx grafting area is required to prohibit the formation of EVs in the presence of a dense and long glycocalyx cell membrane. This also further validates the prediction that the glycocalyx promotes the formation of EVs.

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- [1] D. K. Jeppesen, Q. Zhang, J. L. Franklin, and R. J. Coffey, Extracellular vesicles and nanoparticles: Emerging complexities, *Trends Cell Biol.* **33**, 667 (2023).
 - [2] D. K. Jeppesen, A. M. Fenix, J. L. Franklin, J. N. Higginbotham, Q. Zhang, L. J. Zimmerman, D. C. Liebler, J. Ping, Q. Liu, R. Evans, W. H. Fissell, J. G. Patton, L. H. Rome, D. T. Burnette, and R. J. Coffey, Reassessment of exosome composition, *Cell* **177**, 428 (2019).
 - [3] M. A. Kumar, S. K. Baba, H. Q. Sadida, S. A. Marzooqi, J. Jerobin, F. H. Altemani, N. Algehainy, M. A. Alanazi, A.-B. Abou-Samra, R. Kumar, A. S. Al-Shabeeb Akil, M. A. Macha, R. Mir, and A. A. Bhat, Extracellular vesicles as tools and targets in therapy for diseases, *Sig. Transduct. Target. Ther.* **9**, 27 (2024).
 - [4] J. A. Welsh, *et al.*, Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches, *J. Extracell. Vesicles* **13**, e12404 (2024).
 - [5] M. Sheta, E. A. Taha, Y. Lu, and T. Eguchi, Extracellular vesicles: New classification and tumor immunosuppression, *Biology* **12**, 110 (2023).
 - [6] T. Ahmadzade, S. Kao, G. Reid, S. Clarke, G. E. Grau, and E. Hosseini-Beheshti, Extracellular vesicles as biomarkers in malignant pleural mesothelioma: A review, *Crit. Rev. Oncol. Hematol.* **150**, 102949 (2020).
 - [7] A. Becker, B. K. Thakur, J. M. Weiss, H. S. Kim, H. Peinado, and D. Lyden, Extracellular vesicles in cancer: Cell-to-cell mediators of metastasis, *Cancer Cell* **30**, 836 (2016).
 - [8] L. A. Hargett and N. N. Bauer, On the origin of microparticles: From “platelet dust” to mediators of intercellular communication, *Pulm. Circ.* **3**, 329 (2013).
 - [9] K. Segawa, J. Suzuki, and S. Nagata, Constitutive exposure of phosphatidylserine on viable cells, *Proc. Natl. Acad. Sci. USA* **108**, 19246 (2011).
 - [10] J. D. Arroyo, J. R. Chevillet, E. M. Kroh, I. K. Ruf, C. C. Pritchard, D. F. Gibson, P. S. Mitchell, C. F. Bennett, E. L. Pogosova-Agadjanyan, D. L. Stirewalt, J. F. Tait, and M. Tewari, Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma, *Proc. Natl. Acad. Sci. USA* **108**, 5003 (2011).
 - [11] K. C. Vickers, B. T. Palmisano, B. M. Shoucri, R. D. Shamburek, and A. T. Remaley, MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins, *Nat. Cell Biol.* **13**, 423 (2011).
 - [12] H. Zhao, A. Achreja, E. Iessi, M. Logozzi, D. Mizzoni, R. Di Raimo, D. Nagrath, and S. Fais, The key role of extracellular vesicles in the metastatic process, *Biochim. Biophys. Acta Rev. Cancer* **1869**, 64 (2018).

- [13] D. E. Reynolds, P. Vallapureddy, R.-T. T. Morales, D. Oh, M. Pan, U. Chintapula, R. L. Linardi, A. M. Gaesser, K. Ortved, and J. Ko, Equine mesenchymal stem cell derived extracellular vesicle immunopathology biomarker discovery, *J. Ex. Bio.* **2**, e89 (2023).
- [14] S. Muraoka, A. M. DeLeo, M. K. Sethi, K. Yukawa-Takamatsu, Z. Yang, J. Ko, J. D. Hogan, Z. Ruan, Y. You, Y. Wang, M. Medalla, S. Ikezu, M. Chen, W. Xia, S. Gorantla, H. E. Gendelman, D. Issadore, J. Zaia, and T. Ikezu, Proteomic and biological profiling of extracellular vesicles from Alzheimer's disease human brain tissues, *Alzheimer's Dement.* **16**, 896 (2020).
- [15] C. Jiang, F. Hopfner, D. Berg, M. T. Hu, A. Pilotto, B. Borroni, J. J. Davis, and G. K. Tofaris, Validation of α -synuclein in L1CAM-immunocaptured exosomes as a biomarker for the stratification of Parkinsonian syndromes, *Mov. Disord.* **36**, 2663 (2021).
- [16] M. O. Gonçalves, J. F. Di Iorio, G. V. Marin, P. Meneghetti, N. G. S. Negreiros, and A. C. Torrecilhas, Extracellular vesicles, in *Extracellular Vesicles from Basic Research to Clinical Applications*, Current Topics in Membranes, edited by A. C. Torrecilhas (Academic Press, Cambridge, 2024), Vol. 94, pp. 1–31.
- [17] K. Abhange, A. Makler, Y. Wen, N. Ramnauth, W. Mao, W. Asghar, and Y. Wan, Small extracellular vesicles in cancer, *Bioact. Mater.* **6**, 3705 (2021).
- [18] K. D. Brett, N. Z. Nugent, N. K. Fraser, V. M. Bhopale, M. Yang, and S. R. Thom, Microparticle and interleukin-1 β production with human simulated compressed air diving, *Sci. Rep.* **9**, 13320 (2019).
- [19] J. H. W. Distler, D. S. Pisetsky, L. C. Huber, J. R. Kalden, S. Gay, and O. Distler, Microparticles as regulators of inflammation: Novel players of cellular crosstalk in the rheumatic diseases, *Arthritis Rheum.* **52**, 3337 (2005).
- [20] D. Ruhela, V. M. Bhopale, M. Yang, K. Yu, E. Weintraub, A. Greenblatt, and S. R. Thom, Blood-borne and brain-derived microparticles in morphine-induced anti-nociceptive tolerance, *Brain Behav. Immun.* **87**, 465 (2020).
- [21] D. Ruhela, V. M. Bhopale, S. Kalakonda, and S. R. Thom, Astrocyte-derived microparticles initiate a neuroinflammatory cycle due to carbon monoxide poisoning, *Brain Behav. Immun. Health* **18**, 100398 (2021).
- [22] C. Ciardiello, R. Migliorino, A. Leone, and A. Budillon, Large extracellular vesicles: Size matters in tumor progression, *Cytokine Growth Factor Rev.* **51**, 69 (2020).
- [23] Z. Varga, B. Fehér, D. Kitka, A. Wacha, A. Bóta, S. Berényi, V. Pipich, and J.-L. Fraikin, Size measurement of extracellular vesicles and synthetic liposomes: The impact of the hydration shell and the protein corona, *Colloids Surf. B* **192**, 111053 (2020).
- [24] M. F. Baietti, Z. Zhang, E. Mortier, A. Melchior, G. Degeest, A. Geeraerts, Y. Ivarsson, F. Depoortere, C. Coomans, E. Vermeiren, P. Zimmermann, and G. David, Syndecan-syntenin-1 regulates the biogenesis of exosomes, *Nat. Cell Biol.* **14**, 677 (2012).
- [25] M. Ostrowski, N. B. Carmo, S. Krumeich, I. Fanget, G. Raposo, A. Savina, C. F. Moita, K. Schauer, A. N. Hume, R. P. Freitas, B. Goud, P. Benaroch, N. Hacohen, M. Fukuda, C. Desnos, M. C. Seabra, F. Darchen, S. Amigorena, L. F. Moita, and C. Thery, Rab27a and Rab27b control different steps of the exosome secretion pathway, *Nat. Cell Biol.* **12**, 19 (2010).
- [26] F. K. Fordjour, C. Guo, Y. Ai, G. G. Daaboul, and S. J. Gould, A shared, stochastic pathway mediates exosome protein budding along plasma and endosome membranes, *J. Biol. Chem.* **298**, 102394 (2022).
- [27] M. Mathieu, N. Nevo, M. Jouve, J. I. Valenzuela, M. Maurin, F. J. Verweij, R. Palmulli, D. Lankar, F. Dingli, D. Loew, E. Rubinstein, G. Boncompain, F. Perez, and C. Thery, Specificities of exosome versus small ectosome secretion revealed by live intracellular tracking of CD63 and CD9, *Nat. Commun.* **12**, 4389 (2021).
- [28] T. Matsui, F. Osaki, S. Hiragi, Y. Sakamaki, and M. Fukuda, Alix and ceramide differentially control polarized small extracellular vesicle release from epithelial cells, *EMBO Rep.* **22**, e51475 (2021).
- [29] J. Kowal, G. Arras, M. Colombo, M. Jouve, J. P. Morath, B. Primdal-Bengtson, F. Dingli, D. Loew, M. Tkach, and C. Thery, Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes, *Proc. Natl. Acad. Sci. USA* **113**, E968 (2016).
- [30] M. Dieudé, *et al.*, The 20s proteasome core, active within apoptotic exosome-like vesicles, induces autoantibody production and accelerates rejection, *Sci. Transl. Med.* **7**, 318ra200 (2015).
- [31] M. Hristov, W. Erl, S. Linder, and P. C. Weber, Apoptotic bodies from endothelial cells enhance the number and initiate the differentiation of human endothelial progenitor cells *in vitro*, *Blood* **104**, 2761 (2004).
- [32] L. Ma, Y. Li, J. Peng, D. Wu, X. Zhao, Y. Cui, L. Chen, X. Yan, Y. Du, and L. Yu, Discovery of the migrasome, an organelle mediating release of cytoplasmic contents during cell migration, *Cell Res.* **25**, 24 (2015).
- [33] X. Zhao, Y. Lei, J. Zheng, J. Peng, Y. Li, L. Yu, and Y. Chen, Identification of markers for migrasome detection, *Cell Discov.* **5**, 27 (2019).
- [34] Y. Huang, B. Zucker, S. Zhang, S. Elias, Y. Zhu, H. Chen, T. Ding, Y. Li, Y. Sun, J. Lou, M. M. Kozlov, and L. Yu, Migrasome formation is mediated by assembly of micron-scale tetraspanin macrodomains, *Nat. Cell Biol.* **21**, 991 (2019).
- [35] V. R. Minciacci, C. Spinelli, M. Reis-Sobreiro, L. Cavallini, S. You, M. Zandian, X. Li, R. Mishra, P. Chiarugi, R. M. Adam, E. M. Posadas, G. Viglietto, M. R. Freeman, E. Cocucci, N. A. Bhowmick, and D. Di Vizio, MYC mediates large oncosome-induced fibroblast reprogramming in prostate cancer, *Cancer Res.* **77**, 2306 (2017).
- [36] D. Di Vizio, M. Morello, A. C. Dudley, P. W. Schow, R. M. Adam, S. Morley, D. Mulholland, M. Rotinen, M. H. Hager, L. Insabato, M. A. Moses, F. Demichelis, M. P. Lisanti, H. Wu, M. Klagsbrun, N. A. Bhowmick, M. A. Rubin, C. D'Souza-Schorey, and M. R. Freeman, Large oncosomes in human prostate cancer tissues and in the circulation of mice with metastatic disease, *Am. J. Pathol.* **181**, 1573 (2012).
- [37] A. Callan-Jones and P. Bassereau, Curvature-driven membrane lipid and protein distribution, *Curr. Opin. Solid State Mater. Sci.* **17**, 143 (2013).
- [38] M. M. Kozlov and J. W. Taraska, Generation of nanoscopic membrane curvature for membrane trafficking, *Nat. Rev. Mol. Cell Biol.* **24**, 63 (2023).

- [39] J. Derganc and A. Čopič, Membrane bending by protein crowding is affected by protein lateral confinement, *Biochim. Biophys. Acta* **1858**, 1152 (2016).
- [40] J. C. Stachowiak, E. M. Schmid, C. J. Ryan, H. S. Ann, D. Y. Sasaki, M. B. Sherman, P. L. Geissler, D. A. Fletcher, and C. C. Hayden, Membrane bending by protein-protein crowding, *Nat. Cell Biol.* **14**, 944 (2012).
- [41] D. J. Busch, J. R. Houser, C. C. Hayden, M. B. Sherman, E. M. Lafer, and J. C. Stachowiak, Intrinsically disordered proteins drive membrane curvature, *Nat. Commun.* **6**, 7875 (2015).
- [42] S. Liese and A. Carlson, Membrane shape remodeling by protein crowding, *Biophys. J.* **120**, 2482 (2021).
- [43] V. T. Ruhoff, G. Moreno-Pescador, W. Pezeshkian, and P. M. Bendix, Strength in numbers: Effect of protein crowding on the shape of cell membranes, *Biochem. Soc. Trans.* **50**, 1257 (2022).
- [44] H. Alimohamadi and P. Rangamani, Modeling membrane curvature generation due to membrane-protein interactions, *Biomolecules* **8**, 120 (2018).
- [45] N. Walani, J. Torres, and A. Agrawal, Endocytic proteins drive vesicle growth via instability in high membrane tension environment, *Proc. Natl. Acad. Sci. USA* **112**, E1423 (2015).
- [46] J. E. Hassinger, G. Oster, D. G. Drubin, and P. Rangamani, Design principles for robust vesiculation in clathrin-mediated endocytosis, *Proc. Natl. Acad. Sci. USA* **114**, E1118 (2017).
- [47] R. Ma and J. Berro, Endocytosis against high turgor pressure is made easier by partial coating and freely rotating base, *Biophys. J.* **120**, 1625 (2021).
- [48] K. Xiao, C.-X. Wu, and R. Ma, Vesiculation mechanisms mediated by anisotropic proteins, *Phys. Rev. Res.* **5**, 023176 (2023).
- [49] B. Alberts, A. Johnson, J. Lewis, D. Morgan, M. Raff, K. Roberts, and P. Walter, *Molecular Biology of the Cell* (Garland Science, New York, 2015).
- [50] J. C.-H. Kuo, J. G. Gandhi, R. N. Zia, and M. J. Paszek, Physical biology of the cancer cell glycocalyx, *Nat. Phys.* **14**, 658 (2018).
- [51] S. Weinbaum, J. M. Tarbell, and E. R. Damiano, The structure and function of the endothelial glycocalyx layer, *Annu. Rev. Biomed. Eng.* **9**, 121 (2007).
- [52] L. Möckl, The emerging role of the mammalian glycocalyx in functional membrane organization and immune system regulation, *Front. Cell Dev. Biol.* **8**, 253 (2020).
- [53] C. R. Shurer, J. C.-H. Kuo, L. M. Roberts, J. G. Gandhi, M. J. Colville, T. A. Enoki, H. Pan, J. Su, J. M. Noble, M. J. Hollander, J. P. O'Donnell, R. Yin, K. Pedram, L. Möckl, L. F. Kourkoutis, W. Moerner, C. R. Bertozzi, G. W. Feigenson, H. L. Reesink, and M. J. Paszek, Physical principles of membrane shape regulation by the glycocalyx, *Cell* **177**, 1757 (2019).
- [54] J. C.-H. Kuo and M. J. Paszek, Glycocalyx curving the membrane: Forces emerging from the cell exterior, *Annu. Rev. Cell Dev. Biol.* **37**, 257 (2021).
- [55] S. Gollapudi, S. Jamal, A. Kamatar, F. Yuan, L. Wang, E. M. Lafer, B. Belardi, and J. C. Stachowiak, Steric pressure between glycosylated transmembrane proteins inhibits internalization by endocytosis, *Proc. Natl. Acad. Sci. USA* **120**, e2215815120 (2023).
- [56] R. Alert and J. Casademunt, Bleb nucleation through membrane peeling, *Phys. Rev. Lett.* **116**, 068101 (2016).
- [57] R. Alert, J. Casademunt, J. Brugués, and P. Sens, Model for probing membrane-cortex adhesion by micropipette aspiration and fluctuation spectroscopy, *Biophys. J.* **108**, 1878 (2015).
- [58] F. Y. Lim, K.-H. Chiam, and L. Mahadevan, The size, shape, and dynamics of cellular blebs, *Europhys. Lett.* **100**, 28004 (2012).
- [59] W. Strychalski and R. D. Guy, A computational model of bleb formation, *Math. Med. Biol.* **30**, 115 (2013).
- [60] W. Strychalski and R. D. Guy, Intracellular pressure dynamics in blebbing cells, *Biophys. J.* **110**, 1168 (2016).
- [61] E. K. Paluch and E. Raz, The role and regulation of blebs in cell migration, *Curr. Opin. Cell Biol.* **25**, 582 (2013).
- [62] G. Charras and E. Paluch, Blebs lead the way: How to migrate without lamellipodia, *Nat. Rev. Mol. Cell Biol.* **9**, 730 (2008).
- [63] R. Merkel, R. Simson, D. A. Simson, M. Hohenadl, A. Boulbitch, E. Wallraff, and E. Sackmann, A micromechanic study of cell polarity and plasma membrane cell body coupling in dictyostelium, *Biophys. J.* **79**, 707 (2000).
- [64] E. S. Welf, C. E. Miles, J. Huh, E. Sapoznik, J. Chi, M. K. Driscoll, T. Isogai, J. Noh, A. D. Weems, T. Pohlkamp, K. Dean, R. Fiolka, A. Mogilner, and G. Danuser, Actin-membrane release initiates cell protrusions, *Dev. Cell* **55**, 723 (2020).
- [65] J. Dai and M. P. Sheetz, Membrane tether formation from blebbing cells, *Biophys. J.* **77**, 3363 (1999).
- [66] A. Diz-Muñoz, M. Krieg, M. Bergert, I. Ibarlucea-Benitez, D. J. Muller, E. Paluch, and C.-P. Heisenberg, Control of directed cell migration *in vivo* by membrane-to-cortex attachment, *PLoS Biol.* **8**, e1000544 (2010).
- [67] A. Paraschiv, T. J. Lagny, C. V. Campos, E. Coudrier, P. Bassereau, and A. Šarić, Influence of membrane-cortex linkers on the extrusion of membrane tubes, *Biophys. J.* **120**, 598 (2021).
- [68] M. Sheetz, Cell control by membrane-cytoskeleton adhesion, *Nat. Rev. Mol. Cell Biol.* **2**, 392 (2001).
- [69] F.-C. Tsai, G. Guérin, J. Pernier, and P. Bassereau, Actin-membrane linkers: Insights from synthetic reconstituted systems, *Eur. J. Cell Biol.* **103**, 151402 (2024).
- [70] M. Tsujioka, S. Yumura, K. Inouye, H. Patel, M. Ueda, and S. Yonemura, Talin couples the actomyosin cortex to the plasma membrane during rear retraction and cytokinesis, *Proc. Natl. Acad. Sci. USA* **109**, 12992 (2012).
- [71] S. Tsukita and S. Yonemura, Cortical actin organization: Lessons from ERM (ezrin/radixin/moesin) proteins*, *J. Biol. Chem.* **274**, 34507 (1999).
- [72] R. G. Fehon, A. I. McClatchey, and A. Bretscher, Organizing the cell cortex: The role of ERM proteins, *Nat. Rev. Mol. Cell Biol.* **11**, 276 (2010).
- [73] E. Korkmazhan and A. R. Dunn, The membrane-actin linker ezrin acts as a sliding anchor, *Sci. Adv.* **8**, eabo2779 (2022).
- [74] K. Xiao, S. Park, J. C. Stachowiak, and P. Rangamani, Biophysical modeling of membrane curvature generation and curvature sensing by the glycocalyx, *Proc. Natl. Acad. Sci. USA* **122**, e2418357122 (2025).
- [75] K. Xiao and P. Rangamani, Glycocalyx-induced formation of membrane tubes, *Biophys. J.* **124**, 1631 (2025).
- [76] C. L. Hattrup and S. J. Gendler, Structure and function of the cell surface (tethered) mucins, *Annu. Rev. Physiol.* **70**, 431 (2008).

- [77] Y. Jung, I. Riven, S. W. Feigelson, E. Kartvelishvili, K. Tohya, M. Miyasaka, R. Alon, and G. Haran, Three-dimensional localization of T-cell receptors in relation to microvilli using a combination of superresolution microscopies, *Proc. Natl. Acad. Sci. USA* **113**, 5916 (2016).
- [78] G. Kesavan, F. W. Sand, T. U. Greiner, J. K. Johansson, S. Kobberup, X. Wu, C. Brakebusch, and H. Semb, Cdc42-mediated tubulogenesis controls cell specification, *Cell* **139**, 791 (2009).
- [79] M. Kesimer, C. Ehre, K. A. Burns, C. W. Davis, J. K. Sheehan, and R. J. Pickles, Molecular organization of the mucins and glycocalyx underlying mucus transport over mucosal surfaces of the airways, *Mucosal Immunol.* **6**, 379 (2013).
- [80] S. Makabe, T. Naguro, and T. Stallone, Oocyte–follicle cell interactions during ovarian follicle development, as seen by high resolution scanning and transmission electron microscopy in humans, *Microsc. Res. Tech.* **69**, 436 (2006).
- [81] S. P. Evanko, M. I. Tammi, R. H. Tammi, and T. N. Wight, Hyaluronan-dependent pericellular matrix, *Adv. Drug Delivery Rev.* **59**, 1351 (2007).
- [82] B. Button, L.-H. Cai, C. Ehre, M. Kesimer, D. B. Hill, J. K. Sheehan, R. C. Boucher, and M. Rubinstein, A periciliary brush promotes the lung health by separating the mucus layer from airway epithelia, *Science* **337**, 937 (2012).
- [83] J. Richard Bennett, Jr., T. Järvelä, P. Engelhardt, L. Kostamovaara, P. Sparks, O. Carpen, O. Turunen, and A. Vaheri, Mucin MUC1 is seen in cell surface protrusions together with ezrin in immunoelectron tomography and is concentrated at tips of filopodial protrusions in MCF-7 breast carcinoma cells, *J. Histochem. Cytochem.* **49**, 67 (2001).
- [84] V. Koistinen, R. Kärnä, A. Koistinen, A. Arjonen, M. Tammi, and K. Rilla, Cell protrusions induced by Hyaluronan Synthase 3 (HAS3) resemble mesothelial microvilli and share cytoskeletal features of filopodia, *Exp. Cell Res.* **337**, 179 (2015).
- [85] C. Hiergeist and R. Lipowsky, Elastic properties of polymer-decorated membranes, *J. Phys. II France* **6**, 1465 (1996).
- [86] R. Lipowsky, Bending of membranes by anchored polymers, *Europhys. Lett.* **30**, 197 (1995).
- [87] M. Breidenich, R. R. Netz, and R. Lipowsky, The shape of polymer-decorated membranes, *Europhys. Lett.* **49**, 431 (2000).
- [88] T. Bickel, C. Jeppesen, and C. Marques, Local entropic effects of polymers grafted to soft interfaces, *Eur. Phys. J. E* **4**, 33 (2001).
- [89] Y. W. Kim and W. Sung, Membrane curvature induced by polymer adsorption, *Phys. Rev. E* **63**, 041910 (2001).
- [90] F. Campelo and A. Hernández-Machado, Model for curvature-driven pearling instability in membranes, *Phys. Rev. Lett.* **99**, 088101 (2007).
- [91] F. Campelo and A. Hernández-Machado, Polymer-induced tubulation in lipid vesicles, *Phys. Rev. Lett.* **100**, 158103 (2008).
- [92] M. Werner and J. U. Sommer, Polymer-decorated tethered membranes under good- and poor-solvent conditions, *Eur. Phys. J. E* **31**, 383 (2010).
- [93] S. Kutti Kandy and R. Radhakrishnan, Crowding-induced membrane remodeling: Interplay of membrane tension, polymer density, architecture, *Biophys. J.* **121**, 3674 (2022).
- [94] G. Charras, A short history of blebbing, *J. Microsc.* **231**, 466 (2008).
- [95] J.-Y. Tinevez, U. Schulze, G. Salbreux, J. Roensch, J.-F. Joanny, and E. Paluch, Role of cortical tension in bleb growth, *Proc. Natl. Acad. Sci. USA* **106**, 18581 (2009).
- [96] A. Mahapatra, S. A. Malingen, and P. Rangamani, Interplay between cortical adhesion and membrane bending regulates microparticle formation, *bioRxiv* 2024.02.07.579325 (2024).
- [97] P. Canham, The minimum energy of bending as a possible explanation of the biconcave shape of the human red blood cell, *J. Theor. Biol.* **26**, 61 (1970).
- [98] W. Helfrich, Elastic properties of lipid bilayers—Theory and possible experiments, *Z. Naturforsch. C* **28**, 693 (1973).
- [99] A. Maitra, P. Srivastava, M. Rao, and S. Ramaswamy, Activating membranes, *Phys. Rev. Lett.* **112**, 258101 (2014).
- [100] N. Gov, A. G. Zilman, and S. Safran, Cytoskeleton confinement and tension of red blood cell membranes, *Phys. Rev. Lett.* **90**, 228101 (2003).
- [101] E. A. Evans and R. Skalak, *Mechanics and Thermodynamics of Biomembranes* (CRC Press, Boca Raton, 1980).
- [102] H. Hägerstrand, V. Kralj-Iglič, M. Bobrowska-Hägerstrand, and A. Iglič, Membrane skeleton detachment in spherical and cylindrical microexovesicles, *Bull. Math. Biol.* **61**, 1019 (1999).
- [103] A. Iglič, A possible mechanism determining the stability of spiculated red blood cells, *J. Biomech.* **30**, 35 (1997).
- [104] R. Waugh, Elastic energy of curvature-driven bump formation on red blood cell membrane, *Biophys. J.* **70**, 1027 (1996).
- [105] P. J. Flory, Thermodynamics of high polymer solutions, *J. Chem. Phys.* **9**, 660 (1941).
- [106] M. L. Huggins, Solutions of long chain compounds, *J. Chem. Phys.* **9**, 440 (1941).
- [107] A. Winter, Y. Liu, A. Ziepe, G. Dadunashvili, and E. Frey, Phase separation on deformable membranes: Interplay of mechanical coupling and dynamic surface geometry, *Phys. Rev. E* **111**, 044405 (2025).
- [108] H. Kramers, Brownian motion in a field of force and the diffusion model of chemical reactions, *Physica* **7**, 284 (1940).
- [109] E. Evans, Probing the relation between force-lifetime-and chemistry in single molecular bonds, *Annu. Rev. Biophys.* **30**, 105 (2001).
- [110] A. Veksler and N. S. Gov, Calcium-actin waves and oscillations of cellular membranes, *Biophys. J.* **97**, 1558 (2009).
- [111] R. Shlomovitz and N. S. Gov, Membrane waves driven by actin and myosin, *Phys. Rev. Lett.* **98**, 168103 (2007).
- [112] T. Frohoff-Hülsmann and U. Thiele, Nonreciprocal Cahn-Hilliard model emerges as a universal amplitude equation, *Phys. Rev. Lett.* **131**, 107201 (2023).
- [113] E. B. Zhulina, T. M. Birshtein, and O. V. Borisov, Curved polymer and polyelectrolyte brushes beyond the Daoud-Cotton model, *Eur. Phys. J. E* **20**, 243 (2006).
- [114] A. Veksler and N. S. Gov, Phase transitions of the coupled membrane-cytoskeleton modify cellular shape, *Biophys. J.* **93**, 3798 (2007).
- [115] C. Tozzi, N. Walani, and M. Arroyo, Out-of-equilibrium mechanochemistry and self-organization of fluid membranes interacting with curved proteins, *New J. Phys.* **21**, 093004 (2019).
- [116] R. Lipowsky, Domain-induced budding of fluid membranes, *Biophys. J.* **64**, 1133 (1993).

- [117] J. Liu, M. Kaksonen, D. G. Drubin, and G. Oster, Endocytic vesicle scission by lipid phase boundary forces, *Proc. Natl. Acad. Sci. USA* **103**, 10277 (2006).
- [118] J.-M. Allain, C. Storm, A. Roux, M. Ben Amar, and J.-F. Joanny, Fission of a multiphase membrane tube, *Phys. Rev. Lett.* **93**, 158104 (2004).
- [119] L. Foret, Shape and energy of a membrane bud induced by protein coats or viral protein assembly, *Eur. Phys. J. E* **37**, 42 (2014).
- [120] Z. Shi and T. Baumgart, Membrane tension and peripheral protein density mediate membrane shape transitions, *Nat. Commun.* **6**, 5974 (2015).
- [121] R. Phillips, T. Ursell, P. Wiggins, and P. Sens, Emerging roles for lipids in shaping membrane-protein function, *Nature (London)* **459**, 379 (2009).
- [122] T. Baumgart, S. Hess, and W. Webb, Imaging coexisting fluid domains in biomembrane models coupling curvature and line tension, *Nature (London)* **425**, 821 (2003).
- [123] R. Lipowsky, Budding of membranes induced by intramembrane domains, *J. Phys. II France* **2**, 1825 (1992).
- [124] D. Bracha, E. Karzbrun, G. Shemer, P. A. Pincus, and R. H. Bar-Ziv, Entropy-driven collective interactions in DNA brushes on a biochip, *Proc. Natl. Acad. Sci. USA* **110**, 4534 (2013).
- [125] L. Foret and P. Sens, Kinetic regulation of coated vesicle secretion, *Proc. Natl. Acad. Sci. USA* **105**, 14763 (2008).
- [126] J. Paturej, S. S. Sheiko, S. Panyukov, and M. Rubinstein, Molecular structure of bottlebrush polymers in melts, *Sci. Adv.* **2**, e1601478 (2016).
- [127] J. G. Gandhi, D. L. Koch, and M. J. Paszek, Equilibrium modeling of the mechanics and structure of the cancer glycocalyx, *Biophys. J.* **116**, 694 (2019).
- [128] M. C. Cross and P. C. Hohenberg, Pattern formation outside of equilibrium, *Rev. Mod. Phys.* **65**, 851 (1993).
- [129] M. A. Antonyak, B. Li, L. K. Boroughs, J. L. Johnson, J. E. Druso, K. L. Bryant, D. A. Holowka, and R. A. Cerione, Cancer cell-derived microvesicles induce transformation by transferring tissue transglutaminase and fibronectin to recipient cells, *Proc. Natl. Acad. Sci. USA* **108**, 4852 (2011).
- [130] T. Wang, D. M. Gilkes, N. Takano, L. Xiang, W. Luo, C. J. Bishop, P. Chaturvedi, J. J. Green, and G. L. Semenza, Hypoxia-inducible factors and RAB22A mediate formation of microvesicles that stimulate breast cancer invasion and metastasis, *Proc. Natl. Acad. Sci. USA* **111**, E3234 (2014).
- [131] V. Muralidharan-Chari, J. W. Clancy, A. Sedgwick, and C. D'Souza-Schorey, Microvesicles: Mediators of extracellular communication during cancer progression, *J. Cell Sci.* **123**, 1603 (2010).
- [132] C.-H. Lu, K. Pedram, C.-T. Tsai, T. Jones, X. Li, M. L. Nakamoto, C. R. Bertozzi, and B. Cui, Membrane curvature regulates the spatial distribution of bulky glycoproteins, *Nat. Commun.* **13**, 3093 (2022).
- [133] M. Goudarzi, T. Banisch, M. Mobin, N. Maghelli, K. Tarbashevich, I. Strate, J. van den Berg, H. Blaser, S. Bandemer, E. Paluch, J. Bakkers, I. Tolić-Nørrelykke, and E. Raz, Identification and regulation of a molecular module for bleb-based cell motility, *Dev. Cell* **23**, 210 (2012).
- [134] A. Lorentzen, J. Bamber, A. Sadok, I. Elson-Schwab, and C. J. Marshall, An ezrin-rich, rigid uropod-like structure directs movement of amoeboid blebbing cells, *J. Cell Sci.* **124**, 1256 (2011).
- [135] Y. Yanase, I. Hide, S. Mihara, Y. Shirai, N. Saito, Y. Nakata, M. Hide, and N. Sakai, A critical role of conventional protein kinase C in morphological changes of rodent mast cells, *Immunol. Cell Biol.* **89**, 149 (2011).
- [136] V. Niggli and J. Rossy, Ezrin/radixin/moesin: Versatile controllers of signaling molecules and of the cortical cytoskeleton, *Int. J. Biochem. Cell Biol.* **40**, 344 (2008).
- [137] S. Martinelli, E. J. Chen, F. Clarke, R. Lyck, S. Affentranger, J. K. Burkhardt, and V. Niggli, Ezrin/radixin/moesin proteins and flotillins cooperate to promote uropod formation in T cells, *Front. Immunol.* **4**, 84 (2013).
- [138] A. Iglič and H. Hägerstrand, Amphiphile-induced spherical microexovesicle corresponds to an extreme local area difference between two monolayers of the membrane bilayer, *Med. Biol. Eng. Comput.* **37**, 125 (1999).
- [139] E. Donath and D. Lerche, Electrostatic and structural properties of the surface of human erythrocytes. I-cell-electrophoretic studies following neuraminidase treatment, *J. Electroanal. Chem. Interfacial Electrochem.* **116**, 41 (1980).
- [140] R. Heinrich, M. Gaestel, and R. Glaser, The electric potential profile across the erythrocyte membrane, *J. Theor. Biol.* **96**, 211 (1982).
- [141] A. Iglič, M. Brumen, and S. Svetina, Determination of the inner surface potential of the erythrocyte membrane, *Bioelectrochem. Bioenerg.* **43**, 97 (1997).
- [142] D. Lerche, M. Kozlov, and V. Markin, Electrostatic free energy and spontaneous curvature of spherical charged layered membrane, *Biorheology* **24**, 23 (1987).
- [143] S. McLaughlin, The electrostatic properties of membranes, *Annu. Rev. Biophys.* **18**, 113 (1989).
- [144] M. M. Kozlov, M. Winterhalter, and D. Lerche, Elastic properties of strongly curved monolayers. Effect of electric surface charges, *J. Phys. II France* **2**, 175 (1992).
- [145] V. Kralj-Iglič and A. Iglič, A simple statistical mechanical approach to the free energy of the electric double layer including the excluded volume effect, *J. Phys. II France* **6**, 477 (1996).
- [146] E. Gongadze, A. Velikonja, Š. Perutkova, P. Kramar, A. Maček-Lebar, V. Kralj-Iglič, and A. Iglič, Ions and water molecules in an electrolyte solution in contact with charged and dipolar surfaces, *Electrochim. Acta* **126**, 42 (2014).
- [147] https://github.com/RangamaniLabUCSD/Glycocalyx_Membrane_Cortex