Packing of flexible 2D materials in vesicles

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Abstract

To understand the mechanics of cellular packing of two-dimensional (2D) materials, we perform systematic molecular dynamics simulations and theoretical analysis to investigate the packing of a flexible circular sheet in a spherical vesicle and the 2D packing problem of a strip in a cylindrical vesicle. Depending on the system dimensions and the bending rigidity ratio between the confined sheet and the vesicle membrane, a variety of packing morphologies are observed, including a conical shape, a shape of three-fold symmetry, a cylindrically curved shape, an axisymmetrically buckled shape, as well as the initial circular shape. A set of buckling analyses lead to phase diagrams of the packing morphologies of the encapsulated sheets. These results may have important implications on the mechanism of intracellular packing and toxicity of 2D materials.

Keywords: two-dimensional materials, vesicle confinement, buckling analysis, molecular dynamics simulations, vesicle-sheet interaction

Supplementary material for this article is available online
(Some figures may appear in colour only in the online journal)

1. Introduction

More than a decade of research has resulted in an ever increasing variety of engineered two-dimensional (2D) materials, such as graphene, hexagonal boron nitride (h-BN), graphitic carbon nitride, and transition metal dichalcogenides (e.g. MoS\textsubscript{2} and WS\textsubscript{2})\textsuperscript{[1, 2]}. As 2D materials are being actively explored for applications in nanotechnology, their potential toxicity and health risks have become an urgent issue and are calling for more thorough understanding of their interactions with living cells and intracellular organelles\textsuperscript{[3]}. Knowledge about the modes of packing of flexible sheets in vesicles is an important step towards understanding specific biological responses to 2D materials.

Depending on their mechanical, geometrical and surface properties, 2D materials exhibit a variety of modes of interaction with cell membranes\textsuperscript{[3]}. For example, experiments and molecular dynamics (MD) simulations have demonstrated that graphene and graphene oxide (GO) sheets can spontaneously pierce into cell membrane at sharp corner or edge asperities\textsuperscript{[4]}, resulting in destructive lipid extraction and loss of membrane viability\textsuperscript{[5]}. With coating proteins, nanosheets show a variety of size-dependent cell internalization pathways. For example, large (micron-sized lateral dimension) protein-coated GO sheets, following initial attachment to the membrane of PLHC-1 cells, enter cells predominantly through phagocytosis, while small (500 nm) protein-coated GO sheets are internalized through clathrin-mediated endocytosis\textsuperscript{[6]}. Pristine or lightly functionalized graphene nanoflakes can either cut across a bilayer membrane as a transmembrane object or align along the interface between the monolayers when their lateral sizes are comparable to the membrane thickness\textsuperscript{[7–10]}. The extent of hydrophilicity arising from oxidized edges and membrane microstructures play important roles in regulating these different interaction modes.

Following cell uptake, nanomaterials might accumulate in intracellular organelles like endosomes and lysosomes, and as indicated through experimental studies, their behaviors under confinement in intracellular vesicles play critical roles in their cytotoxicity\textsuperscript{[11–13]}. It is found that long and stiff carbon...
nanotubes within lysosomes cause persistent tip contact with the inner lysosomal membrane, leading to lipid extraction, membrane permeabilization, and eventually cell death; while biologically soft carbon nanotubes become curved in lysosomes with low pathogenicity, without activating this nanomechanical toxicity pathway [13]. For 2D materials, new packing morphologies and fundamentally different modes of interaction with vesicles are expected. However, so far only a few experiments and no systematic simulations and theoretical work have been performed on this topic. Experiments have shown that GO sheets of small lateral size (350 nm) remain in their initial flat shapes, while large lateral sized GO sheets (2 µm) form wrinkles and tend to fold into lysosomes [14].

In this work, with an aim to reveal the mechanical behaviors of flexible sheets of 2D materials within (intracellular) vesicles, we consider the packing of a circular sheet in a spherical vesicle and the 2D packing problem of a strip in a cylindrical vesicle through coarse-grained molecular dynamics (CGMD) simulations and theoretical analysis. It is shown that the packing morphologies and modes of interaction of the vesicle-sheet system strongly depend on the bending rigidity ratio between the encapsulated sheet and the vesicle membrane. Phase diagrams of the packing morphologies will be determined based on a buckling analysis, and discussions will be made on differences between the cellular packing of flexible one- and two-dimensional materials. Our results indicate that both the elasticity and geometry of nanomaterials play important roles in regulating their mechanical behaviors under confinement in vesicles. Our findings may help develop a theoretical foundation to understand intracellular toxicity of 2D materials.

2. Modeling and methods

To elucidate the modes of mechanical interaction between a vesicle and an encapsulated flexible sheet of 2D material, we perform systematic CGMD simulations combined with theoretical modeling to demonstrate and analyze how the rigidity and geometrical factors regulate the morphology of the vesicle-sheet system. In our MD simulations, the vesicle membrane is composed of solvent-free lipid molecules, each modeled as three connected beads, one bead representing the hydrophilic head and two the hydrophobic tail [15, 16]. The bending rigidity of the coarse-grained lipid bilayer is \( \kappa \approx 12 k_B T \) [17], where \( k_B T = \frac{4.3 \times 10^{-21}}{J} \) is the thermal energy. The initial radius of simulated spherical and cylindrical vesicles are taken to be \( a = 50 \text{nm} \). A pressure difference across the lipid membrane is maintained by imposing outward forces on the lipid heads along the director of the lipid molecules in the bilayer [13, 18]. The CGMD simulations were performed at a constant pressure difference of \( \Delta p = 40 \text{ kPa} \), corresponding to a normalized value of \( \Delta p a^3/\kappa = 100 \). The encapsulated sheet is modeled as a 2D triangular lattice of CG beads, whose bending rigidity is determined by the stiffnesses of the connected bonds, angles, and dihedrals between the CG beads. Similar schemes have been used to model graphene nanosheets [19]. The simulations were performed at a constant temperature of 310 K under the Nose–Hoover thermostat [20, 21]. More detailed parameterization of the flexible sheet, the simulation methodology, and interaction potentials can be found in the supplementary information (SI) (stacks.iop.org/JPhysD/51/224001/mmedia).

MD simulations could be employed to study mechanical interactions in a general vesicle-sheet system. To validate the simulation results, we focus on a combined MD and theoretical analysis of a couple of benchmark cases with axisymmetry or plane symmetry. The Canham–Helfrich model [22] is employed to characterize the vesicle deformation and the classical buckling analysis is adopted to evaluate the mechanical instability of the confined sheet.

3. Packing of a flexible sheet in a spherical vesicle

We first consider the packing of a rigid circular sheet of diameter \( L_p \) in a spherical vesicle of a fixed surface area \( A_0 = 4\pi a^2 \), \( a \) being the effective vesicle radius (see the inset in figure 1(a)). In the theoretical analysis, we assume that the axisymmetric vesicle adopts a configuration of upside-down symmetry as shown in the inset in figure 1 with the arclength \( s \) starting from the south pole of the vesicle and reaching \( s = l \) at the edge of contact between the vesicle and the encapsulated sheet. The total energy of the system is expressed as [22]
\[ E_{\text{tot}} = 2\pi \kappa \int_0^l r \left( \frac{\dot{\psi}}{r} + \frac{\sin \psi}{r} \right)^2 \, ds - \Delta p (V - V_0) + \sigma (A - A_0) + 2\pi \int_0^l \mu (\dot{r} - \cos \psi) \, ds, \]

where \( \psi, s, \) and \( \kappa \) are the tangent angle, arclength, and bending rigidity of the vesicle membrane, and the dot denotes derivative with respect to the arclength \( s; \) \( \Delta p \) represents the pressure difference between the interior and exterior of the vesicle, \( V = 2\pi \int_0^l r^2 \sin s \, ds \) and \( A = 4\pi \int_0^l r \, ds \) are the volume and surface area of the deformed vesicle, respectively; \( V_0 = 4\pi \alpha^3/3 \) and \( A_0 = 4\pi \alpha^2 \) are the initial volume and surface area of the spherical vesicle. The effective membrane tension \( \sigma \) is introduced as a Lagrange multiplier to enforce the constraint of the vesicle area; and \( \mu (s) \) serves as a Lagrange multiplier function to impose the geometric relations \( \dot{r} = \cos \psi. \)

Introducing

\[ L = \kappa r \left( \frac{\dot{\psi}}{r} + \frac{\sin \psi}{r} \right)^2 - \Delta p r^2 \sin \psi + 2\alpha r + \mu (\dot{r} - \cos \psi) \]

allows us to rewrite \( E_{\text{tot}} = 2\pi \int_0^l L \, ds + \Delta p V_0 - \sigma A_0. \)

Variation of \( E_{\text{tot}} \) leads to a set of Euler–Lagrange equations that govern the vesicle shape

\[
\begin{align*}
2\kappa \dot{\psi} &= -2\kappa \left( \frac{\dot{\psi}}{r} + \frac{\sin \psi}{r} \right) \cos \psi - \Delta p r^2 \cos \psi + \mu \sin \psi, \\
\dot{\mu} &= \kappa \left( \frac{\dot{\psi}}{r} + \frac{\sin \psi}{r} \right) \left( \dot{\psi} - \frac{\sin \psi}{r} \right) - 2\Delta p r \sin \psi + 2\alpha, \\
\dot{r} &= \cos \psi.
\end{align*}
\]

Geometrical boundary conditions for the Euler–Lagrange equation (2) are \( \psi (0) = 0 \) and \( r (0) = 0 \) at the south pole of the vesicle \( (s = 0) \), and \( \dot{\psi} (l) = \pi/2, r (l) = L_0/2 \). The conditions for the area constraint of the vesicle are \( A (0) = 0 \) and \( A (l) = 2\pi \alpha^2 \). To determine the unknown \( l \), we employ the method from [23] and introduce a new independent variable \( r \) such that \( t = 0 \) at the south pole and \( t = 1 \) at \( s = l \). The governing equations are then reparametrized by replacing \( ds \) with \( \lambda dr \). The conservation of the Hamiltonian function \( H = -L + \dot{\psi} \partial L/\partial \dot{\psi} + r \partial L/\partial r \) results in \( \mu (0) = 0 \) as discussed in [23, 24]. With the knowledge of the boundary conditions, the governing equations could be solved numerically for the energy configuration of the system, as well as the membrane tension \( \sigma (= -\partial E_{\text{tot}}/\partial A > 0) \). The contact force per unit length \( f \) upon the closed contact edge is determined as \( f = \partial E_{\text{tot}}/\partial A (L_p/2)^2 \) or \( f = \mu (l)/L_p \). To validate the theoretical model, we perform MD simulations for \( \Delta p a^2/\kappa = 100 \) and different \( L_p/(2a) \).

As indicated in figures 1(a) and (b), both the total free energy \( E_{\text{tot}} \) and the contact force \( f \) increase at increasing rates with the sheet diameter \( L_p \) or pressure difference \( \Delta p \). The membrane tension \( \sigma (L_p, \Delta p) \) exhibit similar trends which are not displayed here. Figure 1(c) plots the theoretical prediction of selected vesicle configurations at different \( L_p \) and \( \Delta p \). As \( \Delta p \) increases, the vesicle becomes inflated and the central region of the vesicle bulges. Similar behaviors are observed in MD simulations (insets in figure 1(b)).

Next we consider the packing of a flexible circular nanosheet in a spherical vesicle. As the compressive contact force on the sheet edge is proportional to its size, it is anticipated that an encapsulated sheet of sufficiently large size and small bending rigidity will undergo buckling instability. Figure 2 shows typical time sequenced snapshots of system morphologies at \( L_p/(2a) = 1.24 \) and for different \( \kappa_p/\kappa = 0.1, 1, 5, 26, \) and 43. At \( \kappa_p/\kappa = 0.1 \), a very soft and flexible sheet becomes crumpled into a structure with two developable cone vertices [25] and eventually evolves to a conical structure with no stretching, and the vesicle maintains a nearly spherical shape. In this case, the relatively strong vesicle confinement induces large and complex deformation of the soft nanosheet, with partial edge contact between the crumpled nanosheet and vesicle membrane. This is similar to a flat elastic thin sheet packed in a rigid hollow cylinder exhibiting a conical structure described by the equation of an elastica [26–28]. In the case of \( \kappa_p/\kappa = 1 \), the nanosheet with bending rigidity comparable to that of the vesicle membrane adopts a configuration of mirror symmetry, undergoing significant cylindrical bending (generalized plane strain bending) deformation while the vesicle adopts a dumpling-like shape. As the nanosheet becomes stiffer (e.g. \( \kappa_p/\kappa = 5 \)), it evolves first to a shape of three-fold symmetry with a nearly flat region at the center, then undergoes cylindrical bending deformation with elastic energy released from these three curved regions. In comparison, very stiff nanosheets (e.g. \( \kappa_p/\kappa = 26 \) and 43 here) display no significant deformation in the vesicle. As the side views in the bottom row of figure 2 indicate, the nanosheet of \( \kappa_p/\kappa = 26 \) adopts a cylindrical mode of bending deformation and maintains a configuration of mirror symmetry; while the nanosheet of \( \kappa_p/\kappa = 43 \) undergoes slight axisymmetric bending. This rigidity-dependent shape transformation of the encapsulated nanosheet can be understood as follows. As long as the elastic deformation of the nanosheet is within its linear range, the sheet maintains an essentially axisymmetric shape. When the nanosheet becomes more flexible, its deformation falls into a geometrically nonlinear range, in which maintaining an asymmetrical deformation mode requires both bending and in-plane stretching. In this case, a cylindrical bending mode could occur with minimal in-plane stretching. Therefore, below a critical ratio of bending rigidity, the encapsulated nanosheet adopts a cylindrical mode of bending deformation. A similar deformation mode transition has been predicted theoretically in the bifurcation study of a thin film-substrate system subject to a mismatch strain [29]. In addition to MD simulations which is capable of characterizing the complex packing configurations, significant efforts are required to build a complete theoretical model on packing of a flexible nanosheet in a spherical vesicle. This challenging task calls for fundamental studies aimed at understanding the mechanics of crumpled thin sheets [30] and deserves further dedicated investigations in the future.
Figure 2. Temporal evolution of the packing morphology of an initially spherical vesicle encapsulating a flexible circular sheet of normalized diameter $L_p/(2a) = 1.24$ under normalized pressure $\Delta p a^3/\kappa = 100$. The bottom row displays the side view of the system whose corresponding top view is provided in the fourth row.

Figure 3. Time sequences of MD simulations showing morphological evolutions of the vesicle-sheet system. (a) Buckling of an initially flat nanosheet of bending rigidity $\kappa_p/\kappa = 1$ in a spherical vesicle. (b) Flattening of an initially curved nanosheet of bending rigidity $\kappa_p/\kappa = 80$. The nanosheets in both cases have the normalized diameter of $L_p/(2a) = 1.29$. (c) Variations of distance $d$ between two edges of the nanosheets during the morphological evolutions shown in (a) and (b). Inset in (c) illustrates the definition of the edge distance $d$. 
Figure 4. (a) Phase diagram on the packing instability of the encapsulated flexible circular sheet with respect to the bending rigidity ratio $\kappa_p/\kappa$ and size ratio $L_p/(2a)$ at different pressures. (b) MD simulations showing the buckling instability at $\Delta p a^3/\kappa = 100$. The dashed line corresponds to the red curve in (a). (c) Representative equilibrium morphologies of the vesicle-sheet system at selected $\kappa_p/\kappa$ and $L_p/(2a)$ from MD simulations.

(figures 3(a) and (b)). An initially flat nanosheet of relatively small bending rigidity (e.g. $\kappa_p/\kappa = 1$) buckles in a short period of time under the compressive force from the vesicle confinement. After long time simulations, the system reaches an equilibrium state in which the nanosheet is strongly curved in the cylindrical bending mode and the vesicle adopts a dumbbell-like shape with two mirror symmetry planes (figures 3(a) and (c)). The confined nanosheet undergoes shape fluctuations (characterized by the edge distance $d$) due to thermal undulation. For an initially curved nanosheet of large bending rigidity (e.g. $\kappa_p/\kappa = 80$), the sheet returns to the flat configuration with released bending energy compensated by the deformation of the vesicle (figures 3(b) and (c)). At the same time, the vesicle evolves from an initial dumbbell-like shape to a lens-like shape. Note that the nanosheet with large bending rigidity exhibits very small shape fluctuations at equilibrium (figure 3(c)).

As demonstrated in MD simulations, an encapsulated flexible nanosheet of sufficiently large size and small bending rigidity could buckle under the compressive contact force $f$ along the edge. Based on the Euler buckling criterion under the assumption that the maximal deflection of the nanosheet is small compared to its length, the critical buckling force per unit contact length is $f_c = 16.79 \kappa_p/L_p^2$, where the bending rigidity $\kappa_p = E_p h_p^3/12(1-\nu_p^2)$ depends on the Young’s modulus $E_p$, thickness $h_p$, and Poisson ratio $\nu_p$ of the nanosheet [31, 32]. Substituting $f_c$ into $f$, we can determine a buckling phase diagram for the encapsulated nanosheet inside a vesicle with respect to the sheet-vesicle size ratio $L_p/(2a)$ and bending rigidity ratio $\kappa_p/\kappa$ (see figure 4(a)). As shown in figure 4(a), larger and softer encapsulated nanosheets are easier to buckle. The theoretical prediction of buckling at $\Delta p a^3/\kappa = 100$ is confirmed by our MD simulations (figure 4(b)) with selected sheet-vesicle system morphologies demonstrated in figure 4(c). Generally, larger and softer nanosheets are curved more significantly inside their confining vesicles (figure 4(c)).

4. Two-dimensional packing of a flexible strip in a cylindrical vesicle

In a 2D configuration, we limit our attention to the packing of a flexible strip in a cylindrical vesicle with a constant contour length $2\pi a$, $a$ being the effective radius of the vesicle (see the inset in figure 5(a)). The total energy of the system per unit length in the out-of-plane direction is [33, 34]

$$E_{\text{tot}} = \frac{1}{2} \int c^2 ds + \frac{c_p}{2} \int c_p^2 ds_p - \Delta p (A - \pi a^2) + \sigma \left( \int ds - 2\pi a \right) + \int [\mu(\dot{r} - \cos \psi) + \eta(\dot{z} - \sin \psi)] ds,$$

where $c(= \dot{\psi})$, $\psi(s)$, $s$, and $\kappa$ are the curvature, tangent angle, arclength, and bending rigidity of the vesicle membrane, respectively, and the dot denotes derivative with respect to the arclength $s$ starting from the left contact edge. For the encapsulated strip, the corresponding quantities are denoted with subscript ‘p’; the first and second terms correspond to the bending energy of the vesicle and strip, respectively; $\Delta p$ represents the pressure difference between the interior and exterior of the vesicle, and $A$ is the enclosed area of the deformed vesicle in two dimensions. The fourth term is introduced to enforce the constraint that the vesicle circumference $L$ is fixed at $2\pi a$, where the effective membrane tension $\sigma$ serves as the Lagrange multiplier. In the last term, $\mu(s)$ and $\eta(s)$ are the Lagrange multipliers to enforce geometric relations $\dot{r} = \cos \psi$ and $\dot{z} = \sin \psi$.

In the case of encapsulating a rigid flat strip of a uniform width $L_p$, we assume that the vesicle adopts a configuration of upside-down symmetry as shown in the inset in figure 5(a). Variation of $E_{\text{tot}}$ leads to the following Euler–Lagrange equations that govern the vesicle shape

$$\begin{aligned}
\psi &= (\mu \sin \psi - \eta \cos \psi - \Delta p r \cos \psi)/\kappa, \\
\dot{r} &= -\Delta p \sin \psi, \\
\dot{\psi} &= 0, \\
\dot{r} &= \cos \psi, \\
\dot{z} &= \sin \psi.
\end{aligned}$$

Six boundary conditions corresponding to equation (4) are $\psi(0) = -\pi/2$, $r(0) = -L_p/2$, and $z(0) = 0$ at $s = 0$ (left contact edge), and $\psi(\pi a) = \pi/2$, $r(\pi a) = L_p/2$, and $z(\pi a) = 0$ at $s = \pi a$ (right contact edge). By using the shooting method, the governing equations could be solved numerically for the elastic energy and configuration of the system. The edge contact force $f$ per unit length of the encapsulated strip is determined as [35]

$$f = \partial E_{\text{tot}}/\partial L_p$$

or $f = 2\mu(0)$

and the effective membrane tension $\sigma$ is [34, 36, 37]
\[ \sigma = -\partial E_{\text{tot}}/\partial L \quad \text{or} \quad \sigma = (\bar{c} + \bar{c}/2 + \Delta \rho)/c. \]

Figures 5(a) and (b) show the total energy \( E_{\text{tot}} \) and edge contact force \( f \) at different \( \Delta \rho \) in the 2D packing of a rigid strip in a cylindrical vesicle. Similar to the packing of a rigid circular sheet in a spherical vesicle, both \( E_{\text{tot}} \) and \( f \) increase at increasing rates with the strip size \( L_p \). A higher \( \Delta \rho \) induces higher \( E_{\text{tot}} \) and \( f \). Theoretical prediction of selected vesicle configurations at different \( L_p \) and \( \Delta \rho \) are shown in figure 5(c). In the presence of a pressure difference, the vesicle is inflated and its central region bulges. The contact force and equilibrium vesicle morphologies at \( \Delta \rho^2/\kappa = 100 \) have also been obtained from MD simulations (see the red dots and insets in figure 5(b)) and are consistent with the theoretical predictions.

An encapsulated flexible strip of sufficiently large size and small bending rigidity will buckle into a curved shape due to the compressive edge contact force by the vesicle confinement. Based on the Euler buckling criterion, the critical buckling force per unit contact length is \( f_c = \pi^2 \kappa_p / L_p^2 \) [38]. Comparing the edge contact force \( f \) to the critical buckling force \( f_c \), we can theoretically determine a buckling phase diagram for the encapsulated strip (figure 6(a)) with respect to the bending rigidity ratio and size ratio. As \( L_p \) and \( \Delta \rho \) increase, so does the critical bending rigidity of the encapsulated strip. The theoretical prediction at \( \Delta \rho^2/\kappa = 100 \) is confirmed by our MD simulations (figure 6(b)).

Considering large deflections of a bent strip, the deformed shape subject to a pair of edge contact forces \( f \) is locally governed by \( \kappa_p^2 \psi_p/dx_p^2 + f \sin \psi_p = 0 \), which can be integrated as [39]

\[ \left( \frac{d\psi_p}{dx_p} \right)^2 = \frac{2f}{\kappa_p} (\cos \psi_p - \cos \psi_0), \]

where \( \psi_0 = \psi_p(0) \) is the tangential angle of the strip at the left contact edge. Here we have treated the encapsulated strip as an inextensible planar elastica. Assuming that the strip buckles downwards (\( \psi_0 \leq 0 \)), the contact force \( f \) at a certain \( \psi_0 \) can be expressed as an elliptic integral,

\[ L_p = \sqrt{\frac{\kappa_p}{f}} \int_{\psi_0}^{\psi} \frac{d\psi'}{\sqrt{2(\cos \psi' - \cos \psi_0)}}, \tag{5} \]

and the bending energy of the strip can be determined as [39]
Figure 7. (a) Bending energy of an inextensible strip with finite deflection. (b) The strip-vesicle configurations for selected bending rigidity ratios at \( \Delta \rho \alpha^3 / \kappa = 400 \) and \( L_p/(2a) = 1.4 \) from theoretical modeling. (c) Representative equilibrium system morphologies of selected size ratio and bending rigidity ratio at \( \Delta \rho \alpha^3 / \kappa = 100 \) from MD simulations. Inset in (a) plots the strip configuration at \( d/L_p = 0.8 \) with \( d \) as the edge distance.

\[
E_p = \frac{1}{2} \kappa_p \int \left( \frac{d\psi}{dx} \right)^2 dx = \int \left( \cos \psi - \cos \psi_0 \right) dx_p
\]

With geometric relations \( d\psi/dx_p = \cos \psi_p \) and \( dz_p/dx_p = \sin \psi_p \), the shape of the deformed strip is [39]

\[
r = -\sqrt{\frac{\kappa_p}{f}} \int_{\psi_0}^{\psi} \frac{\cos \psi'(d\psi')}{\sqrt{2(\cos \psi' - \cos \psi_0)}}
\]

and

\[
z = -\sqrt{\frac{\kappa_p}{f}} \int_{\psi_0}^{\psi} \frac{\cos \psi'(d\psi')}{\sqrt{2(\cos \psi' - \cos \psi_0)}}
\]

According to equations (5)–(7), we can obtain the bending energy and the corresponding configuration of the strip at different bending levels. For example, figure 7(a) plots the strip bending energy \( E_p \) as a function of the edge distance \( d \), and \( E_p(d) \) exhibits an approximately linear relation with \( d \). In the 2D problem of encapsulating a flexible strip inside a cylindrical vesicle, substantial mechanical deformation can take place. The summation of \( E_p \) in figure 7(a) and \( E_{tot} \) in figure 5(a) enables us to determine the energetically favorable system configurations as illustrated in figure 7(b). As \( \kappa_p / \kappa \) decreases, the encapsulated strip is more significantly curved, which is also seen in MD simulations (see figure 7(c)). The temporal evolutions of the encapsulated strips of \( \kappa_p / \kappa = 11 \) and 85 at \( L_p/(2a) = 1.24 \) are analyzed in figure S2, which suggest that the equilibrium state of the vesicle-strip system is insensitive to the initial system configuration, similar to the packing of a flexible circular sheet in a vesicle (figure 3). In this section, we limit our studies to the cases in which only the edge contact is fully formed. For more flexible strips or those of much larger widths, the surface contact between the vesicle and strip could be modeled by, for example, the Lennard-Jones interaction potential [40].

5. Discussions

Packing of flexible sheets in vesicles serves as a model to understand cellular interaction with 2D materials. Through endocytosis, nanosheets engulfed by cells might undergo intracellular transport into and become encapsulated by subcellular vesicles such as endosomes and lysosomes. Though the bending rigidity of lipid bilayers could vary in a wide range from \( 20 \ k_B T \) to \( 150 \ k_B T \), depending on the membrane composition [41], a value on the order of \( 20 \ k_B T \) can be taken as a typical bending rigidity for the lipid membrane of a subcellular vesicle [13, 42], in which the most abundant phospholipids are phosphatidylcholine, phosphatidylethanolamine and sphingomyelin [43]. Depending on the number of atomic layers and chemical composition, the bending rigidity \( \kappa_p \) of engulfed 2D nanosheets could be comparable to, or significantly larger than, that of the biomembrane. For example, MD simulations indicate that \( \kappa_p \) is about \( 56 \ k_B T \) for single-layered graphene [44], \( 700 \ k_B T \) for MoS 2 bilayer [44], and \( 375 \ k_B T \) for MoS 2 monolayer [45]. In experiments, the bending rigidity of a monolayered GO nanosheet with a carbon-to-oxygen ratio (4:1) is measured around \( 180 \ k_B T \) [46]. A typical radius of lysosome is about \( a = 0.5 \mu m \), and the pressure difference due to osmolarity change is about \( 300 \ Pa \) [13]. According to the buckling analysis in figure 4(a), the critical bending rigidity of an encapsulated sheet at bucking is on the order of few hundred \( k_B T \). Therefore, for packing in intracellular vesicles, the MoS 2 bilayer can be regarded as rigid, while single-layered graphene, GO, and MoS 2 monolayer might be considered flexible.

Experiments and MD simulations indicate that the packing of one-dimensional (1D) nanofibers in vesicles strongly depends on the nanofiber-membrane bending stiffness ratio and size ratio [18, 47, 48]. For example, an encapsulated long and stiff nanofiber could give rise to a cherry-shaped vesicle.
with a single tubular protrusion or a φ-shaped vesicle with a pair of tubular protrusions [18, 47]; while an encapsulated flexible nanofiber could exhibit, depending on the nanofiber length, an axisymmetric lemon-like vesicle shape with a pair of protruding tips or a non-axisymmetric dumpling-like shape with the nanofiber curved against the vesicle membrane [18, 48]. In comparison with the packing morphology of nanofibers in vesicles, our present study shows that the packing of 2D sheets without edge asperities typically involves milder deformation and no (planar) protrusions. The packing morphology of a 1D nanofiber in a vesicle typically depends on the initial configuration of the vesicle-nanofiber system [18]. For the packing of an initially curved long nanofiber inside a spherical vesicle, the vesicle could exhibit, depending on the nanofiber stiffness, a non-axisymmetric dumpling-like shape without tubular protrusions or a cherry-like shape with a single tubular protrusion, and the boundary between these two packing morphological phases is well characterized by the classical Euler buckling theory [18]. If the nanofiber is initially straight in the vesicle, the same morphological phases exist but the phase boundary does not obey the Euler buckling criterion [18]. In contrast, the packing of 2D sheets in vesicles under consideration appears to be insensitive to the initial vesicle-sheet configurations, as shown in figures 3 and S2. The reason for this is that the packing process of a nanofiber involves a significant barrier associated with vesicle tubulation [18, 49], while that of a 2D sheet involves no such barrier. An interesting open question is under what conditions an energy barrier would emerge as the encapsulated nanomaterial varies from a 1D fiber to a 2D sheet.

In this work, we have assumed that there is no (evident) adhesive interaction between the encapsulated sheet and vesicle. It is not clear whether such adhesion could lead to 2D membrane protrusions. Experimental and theoretical studies reveal that a linear aggregation of adhesive nanoparticles within a vesicle could give rise to vesicle tubulation [50, 51], and binding between microtubule and vesicle membrane through certain proteins could stabilize a φ-shaped vesicle [52]. However, relatively little is known about the packing of adhesive sheets in a vesicle.

The encapsulated sheets considered here are assumed to have smooth edges without edge asperities or sharp corners. Previous theoretical modeling and MD simulations have shown that edge asperities could significantly influence the manner in which membrane interacts with nanosheets [4, 5, 8–10, 53]. For example, edge asperities of graphene nanosheets can reduce the energy barrier of membrane penetration to only a few $k_B T$, enabling spontaneous penetration [4]. Theoretical analysis accounting for the bilayer microstructure of the lipid membrane indicates that transmembrane nanosheets exhibit a near-perpendicular configuration with respect to the membrane surface, driven by membrane splay and tension energies [10]. Once membrane penetration by graphene takes place, lipid molecules could be extracted out of the membrane due to strong interactions between the hydrophobic lipid hydrocarbon chains and graphene surface [5, 13], and lipid extraction could result in membrane permeabilization, membrane damage, and cell death [5, 13]. These phenomena suggest that nanosheets such as graphene with edge asperities or sharp corners might pierce through a confining vesicle or even escape from it. In these cases, the buckling of a nanosheet under strong confinement might not be applicable. For sheets free of edge asperities or those polar 2D materials like highly oxidized graphene oxide, h-BN, and MoS$_2$, direct piercing of membrane requires overcoming a large energy barrier or is energetically unfavorable. Barring direct penetration, the mechanical interaction modes discussed in our study should be applicable. Recent experimental studies of intracellular trafficking of nanoparticles report that nanoparticles with low sharpness could stably reside in the intracellular vesicles such as endosomes or lysosomes, while those sharp-shaped nanoparticles could escape from the vesicles and accumulate in the cytosol [12]. Further simulations and theoretical studies will be required to fully understand the coupling between elastic deformation and geometrical effect in the packing of 2D materials in cells or vesicles.

6. Conclusions

Molecular dynamics simulations and theoretical analysis have been performed to explore the packing of flexible sheets in vesicles. In particular, we have studied the packing of a flexible circular sheet in a spherical vesicle and 2D packing of a strip in a cylindrical vesicle. It is shown that the sheet-vesicle size and rigidity ratios play important roles in regulating the packing morphologies of the vesicle-sheet system. As the sheet rigidity increases, it undergoes complex shape transformations to a rich variety of morphologies including a conical shape, a shape of three-fold symmetry with a nearly flat central region and three curved marginal regions, a generalized cylindrically curved shape, an axisymmetrically buckled shape, and the initial circular shape. Phase diagrams of the packing morphology have been established based on the critical conditions for buckling instability. In comparison to cellular packing of long and stiff 1D materials with tubular membrane protrusions, no membrane protrusion has been observed for a vesicle encapsulating a 2D sheet. This study shows how size and elasticity of 2D materials could regulate their packing morphology in vesicles. Our findings may have important biological implications on the cytotoxicity of 2D materials and may help provide a theoretical foundation to understand biological and environmental interactions of 2D materials.

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1. Interaction potentials in CGMD simulations

The bead-bead interaction potentials in our simulations are defined as follows.

\[ U_{\text{WCA}}(r) = 4\varepsilon \left( (a\sigma / r)^6 - (a\sigma / r)^{12} + 1/4 \right) \quad (0 < r < r_{\text{cut}}), \]

\[ U_{\text{COS}}(r) = \begin{cases} 
-\varepsilon + U_{\text{WCA}}(r) & (0 < r < r_{\text{cut}}), \\
-\varepsilon \cos^2 \left[ \pi (r - r_{\text{cut}})/(2w) \right] & (r_{\text{cut}} < r < r_{\text{cut}} + w), 
\end{cases} \]

\[ U_{\text{FENE}}(r) = \frac{1}{2} k_{\text{FENE}} r_{\infty}^2 \ln \left( 1 - \frac{r^2}{r_{\infty}^2} \right) \quad (0 < r < r_{\infty}), \]

\[ U_{\text{harmonic}}(r) = \frac{1}{2} k_{\text{harmonic}} (r - r_0)^2 \quad (0 < r < r_{\infty}), \]

where \( \varepsilon \) and \( \sigma \) represent the energy well depth and bead diameter, respectively, and \( r_{\text{cut}} = 2^{1/6} a\sigma \). Here \( \sigma \) is set at 1 nm to construct a lipid bilayer with an appropriate membrane thickness and areal density. To ensure the mechanical properties of the lipid membrane falling in a range measured experimentally, we chose \( \varepsilon = 0.56 \text{ kcal mol}^{-1} \), and the thermal energy is \( k_B T = 1.1 \varepsilon \) (\( T = 310 \text{ K} \)). Each lipid molecule is approximated by three connected beads with one hydrophilic head bead and two hydrophobic tail beads. The nearest neighbor beads in each lipid molecule are connected by FENE bonds with \( k_{\text{FENE}} = 30\varepsilon \) and \( r_{\infty} = 1.5 \text{ nm} \). The head bead is connected to the second tail bead by a harmonic bond with a rest length \( r_0 = 4 \text{ nm} \) and force constant \( k_{\text{harmonic}} = 10\varepsilon \). Parameterization of nanosheets can be found in the following section in SI. The non-bonded interaction parameters between two beads of the nanosheet, lipid heads and tails are detailed in Table S1. The MD simulations were performed under a constant ambient temperature 310 K with a time step fixed at 100 fs. In the study of rigid nanosheets, the encapsulated nanosheet was fixed while the vesicle membrane could move freely during the simulations. After equilibrium, another 100 ns simulation was performed for each case to get the average compression force due to the contact between the vesicle membrane and nanosheet. In the cases of flexible nanosheets, both the nanosheets and vesicle were allowed to relax to obtain the equilibrium morphologies.

<table>
<thead>
<tr>
<th>bead type</th>
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<th>interaction</th>
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<tbody>
<tr>
<td>lipid head</td>
<td>lipid head</td>
<td>WCA</td>
<td>( \alpha = 0.95 )</td>
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<tr>
<td>lipid head</td>
<td>lipid tail</td>
<td>WCA</td>
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<td>COS</td>
<td>( \alpha = 0.95, w = 1.6\sigma )</td>
</tr>
<tr>
<td>nanosheet</td>
<td>lipid head/tail</td>
<td>WCA</td>
<td>( \alpha = 0.95 )</td>
</tr>
<tr>
<td>nanosheet</td>
<td>nanosheet</td>
<td>WCA</td>
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2. Modeling of a flexible nanosheet

The flexible nanosheet is constructed as a two-dimensional triangular lattice of CG beads with a nearest neighbor distance of 1.3 nm and internal angle $\theta$ of $\pi/3$. The nearest beads are connected by harmonic bonds with stiffness $k_{\text{harmonic}}=200.0$ kcal mol$^{-1}$ rad$^{-2}$, and the rest length between two nearest beads is $r_1=1.3$ nm. The internal angle $\theta$ of each triangle is maintained by the harmonic potential $U_{\text{angle}}(\theta)=k_{\text{angle}}(\theta-\pi/3)^2$ with spring constant $k_{\text{angle}}=1.0$ kcal mol$^{-1}$ rad$^{-2}$, the planar dihedral angle $\phi$ of every two connected triangles is maintained by potential $U_{\text{dihedral}}(\phi)=k_{\text{dihedral}}(1+\cos\phi)^2$, where $k_{\text{dihedral}}$ is used to tune the bending rigidity of the nanosheet.

To measure the rigidity of the nanosheet, a triangular lattice of area $A$ is folded into a cylinder of radius $R$ as shown in figure S1a. The elastic energy of a curved nanosheet is $\Delta E=\kappa_p AR^2/2$ with $\kappa_p$ as the bending rigidity of the nanosheet. Cylinders of different radius are constructed at each $k_{\text{dihedral}}$, and $\kappa_p$ is calculated by the linear fitting between $2\Delta E/A$ and $R^{-2}$ as shown in figure S1b. Figure S1c shows that the nanosheet bending rigidity $\kappa_p$ linearly depends on $k_{\text{dihedral}}$ with a relation $\kappa_p=0.8k_{\text{dihedral}}$.

**Figure S1.** Modeling of a flexible nanosheet in CGMD simulations. (a) A two-dimensional triangular lattice structure is folded to a cylinder of radius $R$. (b) The relationship between $2\Delta E/A$ and $R^{-2}$ at $k_{\text{dihedral}}=10$ kcal/mol, 20 kcal/mol, and 40 kcal/mol. The corresponding fitted bending rigidity of the nanosheets are 8.1 kcal/mol, 16.0 kcal/mol, and 31.9 kcal/mol, respectively. (c) The relationship between the nanosheet bending rigidity and dihedral stiffness is fitted as $\kappa_p=0.8k_{\text{dihedral}}$. 


3. CGMD simulations on two-dimensional packing of flexible strip in a cylindrical vesicle

Figure 7c in the main text plots the vesicle morphologies at different $\kappa_p/\kappa$ and $L_p/(2a)$. Here we provide the temporal evolution of the vesicle-sheet system from a configuration far from the equilibrium state. As demonstrated in the MD simulations (figure S2a), a relatively flexible flat strip of a width larger than the vesicle diameter ($\kappa_p/\kappa=11$ and $L_p/(2a)=1.24$) buckles upon the compressive force on the contact edge; while for a relatively stiff strip ($\kappa_p/\kappa=85$) of the same $L_p$ in an initial curved configuration, it gradually becomes flattened as shown in figure S2b. Further analysis on the temporal evolution of the edge distance $d$ of the strip indicates that the strip undergoes shape fluctuations with a reducing amplitude as the system evolves to an equilibrium state upon thermal undulation (figure S2c). These results suggest that the equilibrium state of the vesicle-strip system is insensitive to the initial system configuration. A similar conclusion is obtained in the packing of circular sheets in spherical vesicles as shown in figure 3 in the main text.

![Figure S2](image)

**Figure S2.** Time sequences of MD simulations showing morphological evolution of the vesicle-strip system in two dimensions. (a) Buckling of an initially flat strip of bending rigidity $\kappa_p/\kappa=11$. (b) Flattening of an initially curved strip of bending rigidity $\kappa_p/\kappa=85$. (c) Time evolution of the distance $d$ between the two contact edges. Inset in (c) illustrates the definition of the edge distance $d$. 