Incorporation of Soft Particles into Lipid Vesicles: Effects of Particle Size and Elasticity

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ABSTRACT: The interaction between particles and lipid biomembranes plays an essential role in many fields such as endocytosis, drug delivery, and intracellular traffic. Here we conduct a theoretical study on the incorporation of elastic particles of different sizes and rigidities into a lipid vesicle through adhesive wrapping. It is shown that while the incorporation of relatively small particles involves smooth shape evolution, the vesicle wrapping of large particles exhibits a discontinuous shape transition, followed by a protrusion of the vesicle membrane at infinitesimal cost of elastic deformation energy. Moreover, softer particles require stronger adhesion energy to achieve successful internalization and delay the onset of discontinuous shape transition to a higher wrapping degree. Depending on the adhesion energy, particle-vesicle size, and rigidity ratios, and the spontaneous curvature of the vesicle, a rich variety of wrapping phase diagrams consisting of stable and metastable states of no-wrapping, partial-wrapping, and full-wrapping are established. The underlying mechanism of the discontinuous shape transformation of the vesicle and the relation between the uptake proneness and uptake efficiency are discussed. These results shed further light on the elasticity effects in cellular uptake of elastic particles and may provide rational design guidelines for controlled endocytosis and diagnostics delivery.

INTRODUCTION

Lipid vesicles, in the fields of cell mechanics and endocytosis, serve as a minimal biomimetic model of cells with reduced structural complexity. Understanding the interaction between particles and lipid vesicles is of fundamental importance not only to the unravelling of endocytic pathways and mechanisms,1,2 but also to a broad range of applications concerning drug delivery,2,3 biomedical diagnosis,4 intracellular distribution,5,6 virology, cell property measurement,7 and liposome stabilization.8 Over the past decade, it has become well established through both theoretical and experimental investigations that the vesicle–particle interaction depends strongly on particle concentration,9–15 size,14–22 shape,23 and surface physiochemical properties.15,16 For example, multiple nanoparticles adsorbed on vesicles can form linear aggregation and induce large vesicle deformation.10–12 Smaller nanoparticles require higher adhesion energy to attach onto vesicles.17–19 An ellipsoidal nanoparticle during uptake undergoes reorientation from an initial configuration with its long axis parallel to the vesicle membrane to a late stage configuration with long axis perpendicular to the membrane.23 Ultrafine nanoparticles functionalized with hydrophobic coatings form a stable structure embedded within the lipid bilayers.15 Similar phenomena on particle geometry and surface properties have also been observed for particle interaction with a lipid membrane patch.24–35,38,39 However, compared to extensive experimental and theoretical studies focusing on the interaction between vesicles and relatively small rigid particles, there have been no systematic studies on the size and elasticity effects in the vesicle incorporation of soft particles except for a recent study focusing on the wrapping and budding of a deformable particle of few specific small size ratios with respect to the vesicle34 and case studies on adhesive contact between vesicles.35–37

Typical elastic particles used in the fields of drug delivery and particle incorporation into vesicles include capsules, polymerosomes, polymer-coated nanoparticles,33,38,39 and vesicular particles such as conventional liposomes, niosomes, and ethosomes.40–48 While there have been a number of theoretical studies on the wrapping of elastic particles modeled as fluid vesicular particles or solid thin-shelled capsules by a patch of lipid membrane,49–51 they become increasingly inaccurate as the particle size increases and the global deformation of the vesicles is no longer negligible. To elucidate the collective roles of particle size and elasticity in vesicle–particle interactions, here we perform the first and comprehensive theoretical study on the adhesive interaction between lipid vesicles and soft spherical elastic particles of a wide size and rigidity range. We investigate how the particle incorporation process and vesicle morphology depend on the particle size, adhesion energy, and bending rigidity ratio between the particle and vesicle membrane. On the basis of the energy evolution profiles during the wrapping process, phase diagrams are established to demarcate different stable and metastable states of no-
wrapping, partial-wrapping, and full-wrapping. The mechanism underlying a discontinuous shape transformation of vesicle wrapping around a large particle is investigated. Moreover, the effect of possible spontaneous curvature of a vesicle on particle incorporation is analyzed. The relationship between uptake proneness and uptake efficiency is also discussed. Our results provide insights into the role of elasticity in vesicle wrapping of large particles and may provide rational design guidelines for controlled endocytosis and diagnostics delivery.

■ THEORETICAL MODELING

Previous numerical analysis indicates that elastic vesicular and solid thin-shelled particles exhibit similar stiffness-dependent behaviors when wrapped by a patch of lipid membrane. Here we model elastic particles also as vesicular particles (e.g., polymesomes), and consider the adhesive wrapping of an initially spherical elastic particle of radius \( a \) by a unilamellar vesicle of radius \( R \). Both particle and vesicle are of fixed surface areas with vanishing osmotic pressure, and subject to axisymmetric elastic deformation as illustrated in Figure 1a. In our model, quantities pertaining to the vesicular elastic particle and the wrapping lipid vesicle are identified by subscripts \( i \) and \( f \), respectively. The total system energy is assumed to obey the Canham-Helfrich model as

\[
E_{\text{tot}} = E_{\text{el}} - \gamma A_c.
\]

where

\[
E_{\text{el}} = 2\kappa_1 \int M^2_i \, dA_i + \frac{\kappa_2}{2} \int (2M_2 - C_0) \, dA_2
\]

is the system elastic energy with \( M_i, \kappa_1 \), and \( dA_i (i = 1, 2) \) representing the mean curvature, bending rigidity, and surface element of the particle and the vesicle, respectively. \( C_0 \) is the spontaneous curvature of the vesicle membrane, \( \gamma > 0 \) is the adhesion energy and \( A_i \) the surface area of the contact region. The initial radii of the elastic particle and vesicle are \( a = \sqrt{A_i/(4\pi)} \) and \( R = \sqrt{A_f/(4\pi)} \), respectively. At a certain \( A_c \), or wrapping degree \( f = A_c/A_f \), the bending energy of the system is \( E_{\text{el}} \equiv E_{\text{el}0} + \gamma A_c = E_{\text{el}0} + \delta A \). Introducing a reduced spontaneous curvature \( c_0 = c_0 R \), we can obtain \( E_{\text{el}}/\kappa \) as a function of \( a/R, k_i/k_f \), and \( c_0 \). We assume that the particle and the vesicle form perfect contact and they can slide against each other freely, so that the bending rigidity of the contact region can be approximated as \( k_i/k_f \).

The variation of \( E_{\text{el}} \) then gives rise to the governing equations for the system morphology, \( E_{\text{el}} \). For example, the shape of the contact region of a fixed \( A_c \) is given by the governing equations

\[
\begin{align*}
\frac{\partial r}{\partial s} &= \frac{\kappa_1}{\kappa_2} \left( 1 + \frac{\kappa_1}{\kappa_2} \right) \sin \psi, \\
\frac{\partial \psi}{\partial s} &= \frac{\cos \psi}{r} + \frac{\kappa_1}{\kappa_2} \frac{\kappa_1}{\kappa_2} \sin \psi.
\end{align*}
\]

where dots denote derivatives with respect to the rescaled arclength \( s \) of the contact region measuring from the intersection between the \( z \)-axis and the contact region to the contact edge, \( \psi \) is the tangent angle, and \( \mu \) and \( \Sigma \) are introduced to enforce the relation \( \tau = \cos \psi \) and the constraint of a fixed \( A_c \), respectively. In our calculations, all length scales are scaled by the radius \( R \) of the vesicle. Derivations of the governing equations in the contact region, inner free region, and free region of the vesicle are listed in the Supporting Information. At a given \( f \), the values of \( \psi \) and \( r \) at the contact edge in certain ranges and pinpoint the state of the minimum system energy in \( \psi, r \) space by solving the equations numerically together with \( \psi = 0 \) at the pole of each region, as well as the continuity of \( \psi \) and \( r \) crossing the contact edge. The numerical procedure for the governing equations is described in detail in refs 49, 53, and 54, and the system morphology and associated \( E_{\text{el}} \) are obtained. The system energy is then determined as \( E_{\text{el}} = E_{\text{el}0} - \delta A_c \) or \( E_{\text{el}0}/k_f = E_{\text{el}0}/k_f - 2\delta A \), where \( \gamma \equiv 2\mu r^3k_f/k_c \). Depending on the wrapping degree \( f \), the system can exhibit three characteristic wrapping states: no-wrapping \((f = 0)\), partial-wrapping \((0 < f < 1)\), and full-wrapping \((f = 1)\).

■ RESULTS

We now investigate the effects of stiffness and size on the incorporation of an elastic particle into a lipid vesicle. Figure 2a-c plots the elastic energy change \( \Delta E_{\text{el}} = E_{\text{el}} - E_{\text{el}0} \) as a function of the wrapping degree \( f \) in the case of zero spontaneous curvature, where \( E_{\text{el}} \equiv E_{\text{el}0} + \gamma A_c \) (see Methods for the expression of \( E_{\text{el}0} \)) denotes the system elastic energy and \( E_{\text{el}0} = 8\tau (k_i + k_f) \) is the reference energy before the particle contacts the vesicle. For a vesicle with a small reduced spontaneous curvature \( c_0 \), \( E_{\text{el}} \) remains spherical when free. For a relative small particle, the energy curve \( \Delta E_{\text{el}}(f) \) is smooth (Figure 2a,b and Figures S1a and S2a in the Supporting Information), indicating that the wrapping configuration evolves continuously, as illustrated in Figure 2d,e.

For the incorporation of rigid particles into a vesicle of \( c_0 = 0 \), the energy profiles \( \Delta E_{\text{el}} \) for \( a/R < 0.35 \) shown in Figure S1a are similar to those reported in refs 16, 22. As the particle stiffness
decreases, the slope $d(\Delta E_{el})/df$ decreases in the early stage and increases in the late stage of incorporation (see Figure 2a-c), which indicates that, compared to rigid particles, the incorporation of softer particles requires smaller adhesion energy in the early stage of wrapping but larger adhesion energy toward the late stage of wrapping. This is also reflected in the morphologies of the particle and the vesicle. As $\kappa_1/\kappa_2$ decreases, the particle deforms more while the vesicle deforms less in the early stage of wrapping, and the reverse is true in the late stage of wrapping (Figure 2d,e). Similar phenomena were previously observed in the wrapping of elastic vesicular particles and solid capsules by a membrane patch, 49,50 and the underlying mechanism is attributed to a partition of elastic deformation energy between the particle and membrane patch (vesicle in the current study).

As the size ratio $a/R$ reaches a critical value $\rho_c$, the energy curve $\Delta E_{el}(f)$ exhibits a kink at a critical wrapping degree $f_c$, followed by an energy plateau. The kink in $\Delta E_{el}(f)$ corresponds to a discontinuous shape transformation of the vesicle from a uniconcave shallow bowl-shaped stomatocyte into an almost closed cup (Figure 2c,f and Figures S2b and S3b), and the plateau characterizes wrapping through propagation of a membrane protrusion during which the particle (regardless of bending rigidity) remains nearly spherical in shape. As the high curvature region of the narrow membrane neck contributes negligible membrane bending energy,55 the protrusion part of the vesicle propagates at almost no change in bending energy (see Figure 2f and Figures S1a and S3). In the case of a rigid particle and $c_0 = 0$, no kink is observed until $a/R$ exceeds $\rho_c \approx 0.36$ (Figure S1a), and as $c_0$ increases, both $\rho_c$ and $f_c$ increase (Figure S1b). Further numerical analysis indicates that softer particles correlate with larger $\rho_c$ and $f_c$ (Figure 2c and Figure S2b). In other words, the stiffer and larger the particle is, the longer is the plateau. Though $\Delta E_{el}(f)$ is higher at a larger $a/R$, it is insensitive to $\kappa_1/\kappa_2$, which is consistent with the fact that similar vesicle–particle morphologies are observed at different values of $\kappa_1/\kappa_2$ (Figures S2b and S3b).

Compared with energetically favorable solid lines in Figure 2c, dotted segments at $f < f_c$ and $f > f_c$ correspond to metastable wrapping states in which the vesicle exhibits an almost closed cup and a uniconcave stomatocyte, respectively. Note that in the case of $\kappa_1/\kappa_2 = 0.1$ the dotted segment corresponding to the vesicle configuration of an almost closed cup are very small and almost invisible. An interesting question is whether there exists a transient state or an evolution trajectory between these two metastable configurations enforcing a continuing evolution of the vesicle volume and the incorporation depth of the particle. To explore the possibility of that transient state, we employ numerical optimization30,56 and perform additional calculations on the vesicle wrapping of a rigid spherical particle of size $a/R > \rho_c$ at different incorporation depths (see Figure S4). As shown in Figure S4, only the almost closed cup and the uniconcave stomatocyte configurations of the wrapping vesicle are observed during the particle incorporation. The theoretical determination of the most possible transient scenario between the metastable wrapping states is challenging and requires further investigation.

**Figure 2.** Evolution profiles of elastic deformation energy and particle–vesicle interaction configurations for $c_0 = 0$. (a–c) Elastic energy $\Delta E_{el}$ as a vesicle wraps around a particle with increasing wrapping degree $f$ for different size ratios $a/R$ and bending rigidity ratios $\kappa_1/\kappa_2$. (d–f) Selected wrapping configurations at $a/R = 0.1$, 0.3, and 0.4; $\kappa_1/\kappa_2 = \infty$, 1, and 0.1; and various wrapping degrees indicated in the figure.
Besides the energy profiles, the particle incorporation process could also be characterized by the evolution of vesicle volume $V$, as shown in Figure 3. As $f$ increases, the vesicle volume decreases first and then increases as $f$ exceeds a certain value until the particle becomes fully internalized. The larger the $a/R$, the smaller the minimum value of $V/(4\pi R^3/3)$. For small particles with $a/R < \rho_c$ (e.g., $a/R = 0.1$ in Figure S5, and $a/R = 0.3$ in Figure 3a), $V$ evolves continuously and smoothly, but it exhibits a discontinuous jump at $f = f_c$ for large particles with $a/R \geq \rho_c$ (Figure 3b). Compared to the wrapping of rigid particles, a vesicle internalizing a softer particle has a larger volume $V$ in the early and middle stages of wrapping, but a smaller $V$ in the late stage of wrapping. At full wrapping ($f \to 1$), the vesicle adopts the shape of an almost closed inverted spherical cup and $V/(4\pi R^3/3) \to 1 - (a/R)^3$, independent of the particle stiffness, as shown in Figures 2 and 3.

Figure 4 shows typical profiles of the total system energy $\Delta E_{\text{tot}} = \Delta E_{\text{id}} - \gamma R$, as a function of the wrapping degree $f$ at $c_0 = 0$ and nine other selected system parameters ($\kappa_1/\kappa_2, a/R, \gamma$). The behavior of $\Delta E_{\text{tot}}(f)$ indicates that there exist five possible wrapping phases (1 to V), depending on the stability of no-wrapping, partial-wrapping, and full-wrapping states. In phase I, which is usually correlated with small adhesion energy $\gamma$, $\Delta E_{\text{tot}}$ increases monotonically with $f$ and the no-wrapping state prevails. As $\gamma$ increases, phase II arises in which a stable state of no-wrapping and a metastable state of partial- or full-wrapping coexist. For soft particles (e.g., $\kappa_1/\kappa_2 = 1$ and 0.1), increase in $\gamma$ could also lead to a global minimum at a state of partial-wrapping and a possible energy barrier to reach a metastable state of full-wrapping (phase III). In phase IV there is a global minimum at a state of full-wrapping as well as a metastable state of no- or partial-wrapping. If $\gamma$ is large enough, a stable full-wrapping state arises with a single energy minimum at $f = 1$ (phase V). For particles of size $a/R > \rho_c$, the wrapping vesicle exhibiting an almost closed cup $f < f_c$ and a uniconcave shallow bowl-shaped stomatocyte at $f > f_c$ is in an energetically unfavorable state, as analyzed in Figure 2c. As the slopes $d(\Delta E_{\text{total}})/df$ in the metastable states of $f < f_c$ are smaller than the maximum of $d(\Delta E_{\text{total}})/df$ in the energetically favorable solid lines (Figure 2c), we do not consider the effects of these metastable wrapping states on the wrapping phase diagrams here.

With the knowledge of energy profiles $\Delta E_{\text{tot}}(f)$ for $c_0 = 0$, the wrapping phase diagrams at different $\kappa_1/\kappa_2$ are determined in the parameter space of $a/R$ and $\gamma$ (Figure 5). For simplicity, we focus on the stable wrapping states and do not distinguish between subcategories in phases II, III, and IV. For very small rigid particles, the minimum adhesion energy necessary for partial wrapping is $\gamma_{\text{min}} = 4$. The same value has been obtained in the case of particle interaction with a membrane patch,57,58 which is expected as $\gamma_{\text{min}}$ only depends on the particle size and local deformation of the (vesicle) membrane in the vicinity of the contact region. Note that there is no phase III in the wrapping phase diagram of a rigid particle. As $\kappa_1/\kappa_2$ decreases, $\gamma_{\text{min}}$ decreases because the deformation of the vesicle is reduced by the flattening of the particle during adhesion, and phase III emerges and expands in the phase diagram. Note that stiffer particles require less adhesion energy $\gamma$ to attain full wrapping (phase V). This phenomenon is reflected in the elastic energy profiles in Figure 2a–c and Figures S1a and S2, where softer particles exhibit larger maximum slope in the energy profiles, which has also been previously observed in the wrapping of vesicular elastic nanoparticles80 or solid thin-walled nanocapsules81 by a membrane patch. Compared to the wrapping phases in the case of interaction between a particle and a membrane patch, new coexistences of no- and full-wrapping states (II-2 and IV-1) are observed in the current case of wrapping by vesicles. This is attributed to the higher energy cost of vesicle deformation during particle incorporation. Note that $\gamma(\equiv 2\gamma a^2/\kappa_2)$ in Figure 5 is proportional to the square of the particle radius $a$. If $\gamma$ itself is employed, instead of $\gamma$, we would find that larger particle requires less adhesion energy $\gamma$ to achieve full wrapping. On the basis of the stability of the wrapping states, larger $a$, $\kappa_2$, and lower $R$ and $\kappa_2$ would facilitate the full internalization of the particle. Since $\Delta E_{\text{tot}}(f)$ for $c_0 = \pm 0.2$ exhibit similar trends to those for $c_0 = 0$ (Figure S6), the wrapping phase diagrams at relative small spontaneous curvatures $c_0 = \pm 0.2$ in Figure S7 exhibit similar structures to Figure 5. As polymersomes exhibit similar features as vesicular particles in their mechanical behaviors and water permeation to some extent, much of our analysis above should be applicable to polymersomes.

**DISCUSSION**

Several methods have been proposed and developed to measure the bending rigidity $\kappa$ of lipid membranes, including indentation by atomic force microscopy, optical stretching, micropipette aspiration, electrodeformation, fluctuation analysis, and membrane relaxation.57,58 As demonstrated in Figure 2d–f, the system configuration at a given wrapping degree depends on the size ratio and bending rigidity ratio between the particle and the vesicle. With the knowledge of the size ratio and the bending rigidity of either the vesicular particle or the...
vesicle, the \( \kappa \) of the other can be determined by comparing the theoretical and experimental results. This new deformation-based approach can be a valuable complement to the existing measurement techniques mentioned above, especially in the case of soft and large particles with weak to intermediate adhesion energy, where the morphological deformation of both particle and vesicle is striking. For example, a comparison between Figure 2e in the present study and Figure 10 in ref 59 suggests that the bending rigidities of the lipid membrane composed of sphingomyelin/cholesterol (SPM/CHOL) in ratio 2:1 and dioleoylphosphatidylcholine (DOPC) membrane are on the same order, with a ratio moderately larger than 1, which is consistent with the experimental measurement.\(^{57}\) Note that volumes of the vesicles in Figure 10 in ref 59 are controlled by the gain or loss of osmotically active solutes,\(^{60}\) while our results are restricted to the case of free volume variation. Therefore, a more precise prediction of \( \kappa \) is expected when this deformation-based approach is employed with a theoretical input of the vesicle volume suggested by experimental observation.

As demonstrated in Figure 2d-f and Figure S3, a vesicle wrapping around a particle of a relatively large \( a/R \) would transform from a uniconcave shallow bowl-shaped stomatocyte to the shape of an almost closed cup and remain in that

![Figure 4](image)

**Figure 4.** Typical profiles of the total energy change \( \Delta E_{\text{tot}} \) as a function of \( f \) at nine selected sets of \((\kappa_1/\kappa_2, a/R, \bar{\gamma})\) and \( c_0 = 0 \). Depending on the stability of no-wrapping, partial-wrapping and full-wrapping states, the behavior of \( \Delta E_{\text{tot}}(f) \) can be categorized into five wrapping phases, I to V. Phase I, a stable no-wrapping state with a single energy minimum at \( f = 0 \); phase II, coexistence of a stable no-wrapping state and a metastable partial- or full-wrapping state; phase III, a stable partial-wrapping state or coexistence of a stable partial- and a metastable full-wrapping states; phase IV, coexistence of a metastable state of no- or partial-wrapping and a stable full-wrapping state; phase V, a stable full-wrapping state with a single energy minimum at \( f = 1 \). Depending on the values of the set parameters, the global energy minimum in phase III-1 with two local minima could be located at a relatively either low or high \( f \). Here in the central subfigure, only the former case is presented as an example. In each profile, the underlined wrapping state indicates the phase of lowest system energy. Magnitudes of \( \Delta E_{\text{tot}} \) are rescaled to a unified value. As indicated in **Figure 5**, some wrapping phases might not be observable at a certain \( \kappa_1/\kappa_2 \).

![Figure 5](image)

**Figure 5.** Wrapping phase diagrams with respect to the normalized adhesion energy \( \bar{\gamma}(=2\gamma a^2/\kappa_2) \) and particle-vesicle size ratio \( a/R \) at different bending rigidity ratios \( \kappa_1/\kappa_2 = \infty, 5, 1, \) and 0.1 in the case of \( c_0 = 0 \). Typical system energy profiles of phases I to V are exemplified in **Figure 4**.
configuration until full internalization (Figure 2f). Such configurational transformation of the vesicle into a closed cup must occur beyond a certain \( a/R \) and can be understood as follows. Theoretical analysis shows that a free-standing vesicle of relatively small \( c_0 \) as considered in the present study tends to adopt a cup-shape at reduced volumes smaller than \( V/(4\pi R^3/3) \approx 0.6 \) (see Figure 10 in ref 53). It can be confirmed that the observed values of \( V/(4\pi R^3/3) \) in the wrapping of a rigid particle of large \( a/R \) are indeed around that range and the sizes of the adhesive particle and cup-shaped vesicle match each other, we can expect that the almost closed cup-like vesicle shape as predicted in our results are energetically favorable. As the particle stiffness decreases, the elastic energy change becomes smaller. Therefore, the vesicle wrapping around a soft particle of large \( a/R \) would adopt a cup-shape at higher reduced volumes as indicated in Figure 3b. In the cases of large \( c_0 \), theoretical analysis indicates that a free-standing vesicle would adopt various shapes including prolate, pear-shapes, and spherical shape with a necklace-like protrusion\(^{61} \) as well as cup-like shapes at small \( V/(4\pi R^3/3) \).\(^{53} \) Therefore, a vesicle of large \( c_0 \) wrapping around the particle of large \( a/R \) might undergo shape transformation into prolate and pear-shapes or cup-like shapes, depending on the sign and magnitude of \( c_0 \). This deserves further detailed investigations in the future.

The present and our previous studies based on the free energy show that softer particles require stronger adhesion energy to achieve successful internalization.\(^{30, 49, 50} \) In other words, softer particles are energetically less prone to full wrapping than stiffer ones. This stiffness-enhanced uptake proneness has recently been observed in the interaction between lipid-covered polymeric nanoparticles of radius 40 nm and HeLa as well as endothelial cells.\(^{46} \) While softer liposomes merely attach onto a cell membrane after 5-h incubation, stiffer lipid-covered nanoparticles undergo successful internalization.\(^{46} \) As the cell uptake of a single nanoparticle occurs usually on a time scale in the range of tens of seconds to tens of minutes,\(^{62} \) these experimental results clearly demonstrate the stiffness-enhanced uptake proneness. Besides studies on stiffness-dependent uptake proneness, there have been investigations on the stiffness effect on the cell uptake rate. Cell uptake typically involves hydrodynamic effects of cell membrane, ligand diffusion, reaction of ligand−receptor binding, and protein coat assembly, each having its own time scale. Therefore, it is not surprising that softer particles in experiments exhibit higher or lower uptake rates, depending on the particle sizes, material compositions, and cell types.\(^{41, 43−47} \) These two distinct aspects, that is, the uptake proneness and uptake rate, may seem at first glance to be somewhat confusing, as the free energy of wrapping might be involved in the determination of both uptake proneness and uptake rate. We emphasize that the uptake proneness characterizes the tendency of an elastic nanoparticle to be fully internalized.\(^{30, 49, 50} \) In this sense, the uptake proneness is governed by the free energy in a conclusive and time-independent way and cannot be employed alone and straightforwardly to describe time related quantities such as the wrapping time and uptake rate.

In our previous work\(^{30, 49} \) and the present theoretical study, an internalized elastic nanoparticle is modeled as a homogeneous vesicular nanoparticle to facilitate a minimal physical model of particle deformation in the wrapping process. This approximation is not unreasonable in the case of a liposome composed of a single species of phospholipid. For general vesicular nanoparticles composed of a mixture of different species of lipids, proteins, and/or small molecules, the composition of the vesicle plays an important role in regulating the mechanical behavior of nanoparticles during the wrapping process. For example, recent molecular dynamics simulations demonstrate that at a low surface density the ligand molecules would undergo diffusional aggregation, leading to the formation of a ligand-free domain and incomplete cell uptake.\(^{66} \) Addition of cholesterol to POPC liposomes can stabilize the liposome membrane and increase its stiffness, which in turn increases the uptake proneness. For liposomes with a membrane consisting of two chemically different monolayers, a nonzero spontaneous curvature might need to be introduced in the theoretical modeling to capture the asymmetry in the bilayer.\(^{19} \) Similar theoretical approaches can be employed to model liposomes subject to insertion or adsorption of small molecules into or onto the external liposome monolayer. Polymersomes as a relatively new class of artificial polymer vesicles exhibit a similar bending property as lipid vesicles but are of orders of magnitude higher lysis tension and larger surface viscosity.\(^{53} \) Therefore, polymersomes are generally more mechanically stable in comparison with liposomes, and can serve as another kind of ideal drug-delivery agents. Moreover, the membrane thickness of polymersomes can be controlled by tuning the molecular weight of building blocks. The higher the molecular weight of hydrophobic blocks, the thicker and tougher the polymersome membrane. More specific and comprehensive discussion on the mechanical properties of polymersomes can be found in ref 63. The thickness dependence of particle stiffness is also a characteristic feature for the thin-shelled solid capsules.\(^{50} \) For polymer-coated nanoparticles, the mechanical properties as well as the cell−nanoparticle interaction modes strongly depend on the length, grafting density, and hydrophilicity of polymer chains.

The water permeability of vesicles depends on the packing of the composing chains and temperature, and can also be enhanced by the incorporation of water channel proteins and peptides.\(^{64−66} \) Here we have focused on the case of zero pressure difference, which is valid for high permeable vesicles or particle incorporation at a long time scale. For vesicles and polymersomes of low permeability or in the case of fast particle incorporation, the vesicle volume can be approximated as constant as water permeation could not occur instantaneously.\(^{10} \) Depending on the value of the reduced volume, a free-standing vesicle subject to volume constraint can exhibit axisymmetric shapes such as prolate, oblates, and stomocytes,\(^{53} \) as well as asymmetric shapes such as a starfish.\(^{57} \) Particle incorporation into these vesicles might induce dramatic shape transformations of the vesicle and even break the vesicle symmetry to achieve a state of lower energy. Theoretical studies based on the continuum Helfrich membrane theory have been carried out to investigate the adsorption of a rigid spherical particle of a specific size onto a vesicle of different reduced volumes under the restriction of an axisymmetric configuration.\(^{56} \) Further studies have been conducted on the size effect of particle adsorption onto an oblate-shaped vesicle, in which an axisymmetric vesicle deformation is presumably energetically favorable.\(^{20} \) Recently, theoretical analysis considering the wrapping of multiple nanoparticles at nonaxisymmetric positions of prolate, oblate, and stomocyte vesicles was performed.\(^{68} \) Numerical studies have also been performed on a rigid particle interacting with a vesicle of large reduced volume\(^{57} \) or zero osmotic pressure\(^{55} \) and on multiple particle
adsorption on a vesicle of zero osmotic pressure difference,\textsuperscript{12} where asymmetric vesicle deformation is captured by a triangulated vesicle model. Comprehensive wrapping and morphological phase diagrams taking into account more general vesicle deformation induced by adhesive particles remain to be fully elucidated.

\section{CONCLUSIONS}

We have conducted a theoretical analysis to investigate the incorporation of an elastic particle of different size and rigidity into a lipid vesicle of fixed surface area and vanishing osmotic pressure. It is shown that the vesicle volume decreases first and then increases as the wrapping proceeds beyond a certain degree until full internalization. When incorporating a small particle, the vesicle volume evolves continuous and the vesicle undergoes smooth shape transformation. In contrast, a vesicle wrapping a large particle exhibits a discontinuous shape transition from a uniconcave shallow bowl-shaped stomatocyte into an almost closed cup. The discontinuous shape transition occurs at a larger wrapping degree and a larger particle-vesicle size ratio for a softer particle. Due to the elastic energy partition between the elastic particle and the wrapping vesicle, a softer particle exhibits the smaller slope in its free energy profile as a function of the wrapping degree in the early wrapping stage and larger slope in the late wrapping stage. Therefore, softer particles are more prone to partial wrapping but require stronger adhesion energy to achieve successful internalization. Depending on the adhesion energy, size, and rigidity ratios between the particle and vesicle, as well as the spontaneous curvature of the vesicle, a variety of wrapping phase diagrams consisting of the stable and metastable states of no-wrapping, partial-wrapping, and full-wrapping have been determined. Moreover, the relation between the uptake proneness and uptake efficiency has been discussed. Since polymersomes exhibit similar mechanical features as lipid vesicles, similar elasticity effects on particle incorporation into polymersomes are expected. Our results shed light on the size and elasticity effects in cell uptake of nanoparticles and may provide rational design guidelines for controlled endocytosis and diagnostics delivery.

\section{ASSOCIATED CONTENT}

\textbullet{} Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.6b03184.

Supplemental figures are presented on additional morphologies of particle-vesicle system in the case of zero membrane spontaneous curvature and the energy profiles, wrapping phase diagrams at finite spontaneous curvatures (PDF).

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Notes

During the review of our manuscript, we became aware of the theoretical study of Tang et al.\textsuperscript{13} on a similar topic. Their work focused on the elasticity effects on both adhesive wrapping and budding of particles of few specific small size ratios with respect to a vesicle of vanishing spontaneous curvature. In our work, we investigated the effects of particle size, stiffness and spontaneous curvature of the vesicle on the adhesive wrapping, with results showing that the vesicle wrapping of large particles exhibits a discontinuous shape transition.

The authors declare no competing financial interest.

\section{ACKNOWLEDGMENTS}

This work was supported by the National Science Foundation (Grants CBET-1344097 and CMMI-1562904).

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Supporting Information for "Incorporation of Soft Particles into Lipid Vesicles: Effects of Particle Size and Elasticity"

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1 Governing Equations for Axisymmetric System Configurations

1.1 Free Vesicle Region

On the basis of the Canham-Helfrich membrane theory, the bending energy \( E_{cl}^f \) of the free region of the vesicle at a given wrapping degree \( f \) is

\[
E_{cl}^f = \frac{\kappa_2}{2} \int (2M_2 - C_0)^2 dA_2 = \pi \kappa_2 \int_0^{l_f} r \left( \psi + \frac{\sin \psi}{r} - c_0 \right)^2 ds,
\]

where \( M_2, \kappa_2, \) and \( dA_2 \) represent the mean curvature, bending rigidity, and surface element of the free region of the wrapping vesicle, respectively. \( C_0 \) is the spontaneous curvature of the vesicle and \( c_0 = C_0 R \) is introduced. \( \psi \) is the tangent angle and \( \dot{\psi} \equiv \frac{\partial \psi}{\partial s} \). \( s, r, \) and \( l_f \) are the rescaled arclength, radical coordinate, and undetermined total arclength of the free vesicle region. The arclength in this region is measured from the bottom pole of the vesicle to the contact edge. In the derivation of the governing shape equations, all length scales are scaled by the vesicle radius \( R \).

The shape of the free vesicle region can be determined by minimizing \( E_{cl}^f \) subject to a given surface area \( A_2 \) (\( \int_0^{l_f} 2\pi r ds = \int dA_2 / R^2 = 4\pi - f \times 4\pi a^2 / R^2 \)) and boundary conditions at the contact edge and bottom pole of the vesicle. Thus, \( E_{cl}^f \) could be determined by minimizing the following functional

\[
E_{cl}^f = \frac{\kappa_2}{2} \int \left[ \frac{\mu}{r} (\dot{r} - \cos \psi) + \Sigma \right] dA_2
\]

\[
= \pi \kappa_2 \int_0^{l_f} \left[ r \left( \psi + \frac{\sin \psi}{r} - c_0 \right)^2 + \mu (\dot{r} - \cos \psi) + r \Sigma \right] ds.
\]

Here, \( \mu \) and \( \Sigma \) are introduced to enforce the relation \( \dot{r} = \cos \psi \) and the constraint of a fixed surface area \( A_2 \).

Introducing

\[
L_f = r \left( \psi + \frac{\sin \psi}{r} - c_0 \right)^2 + \mu (\dot{r} - \cos \psi) + r \Sigma,
\]

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we have \( E_{\text{el}}^{\text{fr}}/(\pi \kappa_2) = \int_0^l f_l ds \). The variation of \( E_{\text{el}}^{\text{fr}} \) or \( E_{\text{el}}^{\text{fr}}/(\pi \kappa_2) \) then gives rise to the governing equations in the free vesicle region as follow.

\[
\left\{ \begin{array}{l}
\frac{\partial L_f}{\partial \psi} - \frac{d}{ds} \frac{\partial L_f}{\partial \dot{\psi}} = 0, \\
\frac{\partial L_f}{\partial r} - \frac{d}{ds} \frac{\partial L_f}{\partial \dot{r}} = 0, \\
\frac{\partial L_f}{\partial \mu} = 0.
\end{array} \right.
\]

or

\[
\left\{ \begin{array}{l}
2r \dot{\psi} = -2 \left( \psi - \frac{\sin \psi}{r} \right) \cos \psi + \mu \sin \psi, \\
\dot{\mu} = \left( \psi + \frac{\sin \psi}{r} \right) \left( \psi - \frac{\sin \psi}{r} \right) - 2c_0 \psi + c_0^2 + \Sigma, \\
\dot{r} = \cos \psi.
\end{array} \right.
\]

Another geometric relation \( \dot{\psi} = \sin \psi \) could also be used to plot the configuration of the free vesicle region, though it is not required in the derivation of the above governing equations.

### 1.2 Inner Free Region of the Elastic Particle

The bending energy \( E_{\text{el}}^p \) of the inner free region of the particle at a given wrapping degree \( f \) is

\[
E_{\text{el}}^p = \kappa_1 \int (2M_1^2) dA_1 = \pi \kappa_1 \int_0^{l_p} r \left( \psi + \frac{\sin \psi}{r} \right)^2 ds,
\]

where \( M_1, \kappa_1, \) and \( dA_1 \) represent the mean curvature, bending rigidity, and surface element of the inner free region of the particle, respectively; \( l_p \) is the rescaled undetermined total arclength of the inner free particle region. The arclength in this region is measured from the top pole of the particle to the contact edge. Following a procedure similar to that in the above subsection, we have

\[
E_{\text{el}}^{p}\pi \kappa_1 = \int_0^{l_p} L_p ds
\]

with

\[
L_p = r \left( \psi + \frac{\sin \psi}{r} \right)^2 + \mu (\dot{r} - \cos \psi) + r \Sigma.
\]

The fixed surface area is given as \( \int_0^{l_p} 2\pi r ds = \int dA_1 / R^2 = (1 - f) \times 4\pi a^2 / R^2 \).

The variation of \( E_{\text{el}}^p \) or \( E_{\text{el}}^{p}/(\pi \kappa_1) \) then gives rise to the following governing equations for the inner free region of the particle as

\[
\left\{ \begin{array}{l}
\frac{\partial L_p}{\partial \psi} - \frac{d}{ds} \frac{\partial L_p}{\partial \dot{\psi}} = 0, \\
\frac{\partial L_p}{\partial r} - \frac{d}{ds} \frac{\partial L_p}{\partial \dot{r}} = 0, \\
\frac{\partial L_p}{\partial \mu} = 0.
\end{array} \right.
\]

or

\[
\left\{ \begin{array}{l}
2r \dot{\psi} = -2 \left( \psi - \frac{\sin \psi}{r} \right) \cos \psi + \mu \sin \psi, \\
\dot{\mu} = \left( \psi + \frac{\sin \psi}{r} \right) \left( \psi - \frac{\sin \psi}{r} \right) + \Sigma, \\
\dot{r} = \cos \psi.
\end{array} \right.
\]
1.3 Contact Region

The bending energy $E_{cl}^c$ of the contact region at a given wrapping degree $f$ is

$$E_{cl}^c = \kappa_1 \int (2M_1^2) \, dA_c + \frac{\kappa_2}{2} \int (2M_2 - C_0)^2 \, dA_c$$

$$= \pi \int_0^{l_c} \left[ \kappa_1 r \left( \psi + \frac{\sin \psi}{r} \right)^2 + \kappa_2 r \left( \psi + \frac{\sin \psi}{r} + c_0 \right)^2 \right] \, ds,$$

where $dA_c$ represents the surface element of the contact region. $l_c$ is the rescaled undetermined total arclength of the inner free particle region. The arclength in this region is measured from the bottom pole of the particle to the contact edge. Similarly, we have $E_{cl}^c/(\pi \kappa_2) = \int_0^{l_c} L_c \, ds$ with

$$L_c = \frac{\kappa_1}{\kappa_2} r \left( \psi + \frac{\sin \psi}{r} \right)^2 + r \left( \psi + \frac{\sin \psi}{r} + c_0 \right)^2 + \mu (\dot{r} - \cos \psi) + r \Sigma.$$

The given surface area is given as $\int_0^{l_c} 2\pi r \, ds = \int dA_c / R^2 = f \times 4\pi a^2 / R^2$.

The variation of $E_{cl}^c$ or $E_{cl}^c/(\pi \kappa_2)$ gives rise to the following governing equations for the contact region as

$$\begin{cases} 
\frac{\partial L_c}{\partial \psi} - \frac{d}{ds} \frac{\partial L_c}{\partial \psi} = 0, \\
\frac{\partial L_c}{\partial r} - \frac{d}{ds} \frac{\partial L_c}{\partial r} = 0, \\
\frac{\partial L_c}{\partial \mu} = 0.
\end{cases}$$

1.4 Boundary Conditions for Governing Equations

The above governing equations can be numerically solved with specific boundary conditions. At the poles $(r = 0)$, we have $\psi = 0$ for all three regions. At the contact edge, the values of $\psi$ and $r$ corresponding to the system configuration of the lowest elastic energy are generally unknown. To obtain the information at a given wrapping degree $f$, we vary $\psi$ and $r$ at the contact edge in certain wide ranges and pinpoint the state of the minimum system energy in $(\psi, r)$ space by solving the set of equations numerically together with $\psi = 0$ at the pole of each region, as well as the continuity of $\psi$ and $r$ crossing the contact edge. At a given $f$, the surface area constraints for these three regions are $\int_0^{l_f} 2\pi rds = 4\pi - f \times 4\pi a^2 / R^2$, $\int_0^{l_p} 2\pi rds = (1 - f) \times 4\pi a^2 / R^2$, and $\int_0^{l_c} 2\pi rds = f \times 4\pi a^2 / R^2$. To determine the unknown $l_f$, $l_p$, and $l_c$, we employed the method from ref 54 with a new independent variable $t$ in each region such that $t = 0$ at the poles and $t = 1$ at the contact edge. For example, in the contact region, $s = l_c t$. The governing equations are then reparametrized by replacing $ds$ with $ldt$, where $l$ represents $l_f$, $l_p$, or $l_c$ depending on the
region of concern. The conservation of the Hamiltonian function $H \equiv -L + \dot{\psi} \partial L / \partial \dot{\psi} + i \partial L / \partial \dot{i}$ with $H(t = 0) = H(t = 1) = 0$ results in $\mu(0) = 0$ in each region as discussed in refs 53,54. With the knowledge of these boundary conditions, the governing equations could be solved numerically, and then the energy and configuration of the system determined.

2 Supplementary Numerical Results

Figure S1a shows the elastic energy change $\Delta E_{el}$ as the vesicle of $c_0 = 0$ wraps around a spherical rigid particle. As the size ratio $a/R$ reaches a critical value $\rho_c$, $\Delta E_{el}(f)$ exhibits a kink at a critical wrapping degree $f_c$ which represents a discontinuous shape transformation of the vesicle from a relative weakly deformed stomatocytelike shape into an almost closed cup-shape. As $c_0$ increases, both $\rho_c$ and $f_c$ increase (Figure S1b). For example, $\rho_c \approx 0.33, 0.36$ and 0.39 at $c_0 = -0.2, 0$ and 0.2, respectively.

![Figure S1](image.png)

Figure S1: Incorporation of a spherical rigid particle into a vesicle. (a) Elastic deformation energy $\Delta E_{el}$ as a function of the wrapping degree $f$ for different particle-vesicle size ratios $a/R$ in the case $c_0 = 0$. Inset, configurations of the particle-vesicle system at $f = 0.42$ and 0.45 with $a/R = 0.5$. (b) Critical wrapping degree $f_c$ where the kink in the energy curve located as a function of $a/R$ at different vesicle spontaneous curvatures $c_0 = -0.2, 0$ and 0.2.

Figure S2 plots the elastic energy curves $\Delta E_{el}$ at different size ratios $a/R$ and bending rigidity ratios $\kappa_1/\kappa_2$ in the case $c_0 = 0$. At $a/R = 0.01$, all of energy curves are smooth as a function of $f$, which indicates that the shape transformation of the vesicle during the wrapping process is continuous. Kinks are observed in all energy curves in the case $a/R = 0.5$ (see Figure S2b).

The system configurations at $\kappa_1/\kappa_2 = \infty, 1$ and 0.1 are shown in Figure 2 in the main text. Here we plot the configurations at $\kappa_1/\kappa_2 = 5$ in Figure S3a. Figure S3b and the fourth column in Figure S3a shows that vesicles wrapping around particles at $a/R = 0.5$ and $\kappa_1/\kappa_2 = \infty, 5, 1$ and 0.1 undergo discontinuous shape transformation, which is consistent with Figure S2b.
Figure S2: Elastic energy $\Delta E_{\text{el}}$ as a vesicle of $c_0 = 0$ wraps around a particle at different size ratios $a/R$ and bending rigidity ratios $\kappa_1/\kappa_2$.

Figure S3: Selected wrapping configurations as the vesicle of $c_0 = 0$ wraps a particle (a) at $\kappa_1/\kappa_2 = 5$ and different $a/R$, (b) at $a/R = 0.5$ and different $\kappa_1/\kappa_2$.

Figure S4 plots the elastic energy curves $\Delta E_{\text{el}}$ as a vesicle of $c_0 = 0$ wraps around a rigid spherical particle of size ratio $a/R = 0.4$ at different wrapping degrees $f$. In the main text, it has been shown that the wrapping vesicle could adopt two kinds of configurations: the almost closed cup and the uniconcave shallow bowl-shaped stomatocyte. Figure S4 further demonstrates that only these two kinds of shapes are observed during the particle incorporation in the numerical calculations.

The volume evolution of wrapping vesicle at $a/R = 0.1$ and $c_0 = 0$ is plotted in Figure S5. Similar to the case $a/R = 0.3$ in Figure 3, the vesicle volume undergoes continuous evolution, decreases first and then increases as $f$ becomes larger than a certain value till the full internalization of the particle.
Figure S4: Elastic energy $\Delta E_{el}$ as a function of the distance $h$ between the bottom pole of the rigid spherical particle of size ratio $a/R = 0.4$ and the bottom pole of the wrapping vesicle of $c_0 = 0$ at $f = 0.2$ (a), 0.4 (b) and 0.6 (c).

Figure S5: Normalized volume $V$ of the wrapping vesicle as a function of wrapping degree $f$ at $a/R = 0.1$ and $c_0 = 0$.

As indicated in Figure S6, the system associated with a smaller $c_0$ has a higher $\Delta E_{el}$, and the effects of $c_0$ on $\Delta E_{el}$ is negligible in the early wrapping stage and becomes striking in the late wrapping stage. Since $\Delta E_{el}(f)$ at different spontaneous curvatures ($c_0 = -0.2$, 0 or 0.2) exhibit similar trends, the wrapping phase diagrams at these spontaneous curvatures exhibit similar structures as demonstrated in Figures 5 and S7.
Figure S6: Elastic energy $\Delta E_{el}$ as a vesicle of different $c_0 = -0.2, 0$ or 0.2 wraps around a particle at $a/R = 0.4$ for $\kappa_1/\kappa_2 = \infty$ (a) and 0.1 (b).

Figure S7: Wrapping phase diagrams for different bending rigidity ratios $\kappa_1/\kappa_2$ at $c_0 = \pm 0.2$. 