

Effects of Elasticity and Geometry on Cellular Uptake of Nanoparticles

by

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Vitae

Xin Yi was born in 1983, in Changchun, Jilin Province, P. R. China. He attended Peking University in 2001, and received his Bachelor degree in Mechanical Engineering in 2005 and Master degree in solid mechanics in 2008. Then he started his graduate study in the Solid Mechanics group at Brown University in the fall of 2008.

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Abstract of “ Effects of Elasticity and Geometry on Cellular Uptake of Nanoparticles ” by Xin Yi, Ph.D., Brown University, May 2015

A fundamental understanding of cell-nanomaterial interaction is essential for biomedical diagnostics, therapeutics, and nanotoxicity. Here we perform theoretical analysis to investigate the cellular uptake of elastic nanoparticles and one- and two- dimensional rigid nanomaterials. We first show that the extent of adhesive wrapping of an elastic soft nanoparticle by a lipid membrane depends on the particle size, adhesion energy, membrane tension, and bending stiffness ratio between the nanoparticle and membrane. There exist five possible wrapping phases based on the stability of full wrapping, partial wrapping, and no wrapping states. Based on these wrapping phases, we determine the wrapping phase diagrams for cellular uptake of elastic nanoparticles in two and three dimensions. We find that stiffer particles can achieve full wrapping more easily than softer particles. Our results suggest that precise control of the particle elasticity can be a new way to control cellular uptake and drug delivery processes. In another study on the cell uptake of one-dimensional nanomaterials, we show that cell uptake of one-dimensional nanomaterials via receptor-mediated endocytosis is dominated by a single dimensionless parameter that scales with the membrane tension and radius of the nanomaterial and inversely with the membrane bending stiffness. As cell membrane internalizes one-dimensional nanomaterials the uptake follows a near-perpendicular entry mode at small membrane tension but it switches to a near-parallel interaction mode at large membrane tension. This tension-dependent uptake behavior is ubiquitous in the interplay between cell membranes and one-dimensional nanomaterials including nanotubes, nanowires, filamentous bacteria, and certain nanoparticle chains. It also provides a mechanism for the size maintenance of filopodia. Lastly, we investigate the interaction between cell membranes and micro-sized rigid two-dimensional nanomaterials such as graphene-family nanomaterials. We show that a micro-sized two-dimensional trans-membrane nanomaterial always tends to rotate toward an orthogonal configuration with respect to cell membranes. The main driving force of such a rotation comes from both splay and membrane tension energies. In contrast, membrane wrapping of two-dimensional nanomaterials are independent of tension and surface adhering is energetically favorable compared with other inclined configurations.

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CHAPTER ONE

Introduction

1.1 Brief overview of cellular uptake of nanomaterials

A fundamental understanding of cell-nanomaterial interaction is of essential importance to biomedical diagnosis, drug delivery, and other safe applications of nanotechnology. During the last decade, extensive studies have been conducted on the effect of size [1, 2, 3, 4, 5], shape [5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18] and surface structure [19, 20, 21] of nanoparticles on their cellular uptake. For example, receptor-mediated endocytosis of nanoparticles has been shown to depend on the particle size, where particles on the order of several tens of nanometers are most efficiently taken up [1, 2, 3]. Phagocytosis of nanoparticles by macrophages strongly depends on the particle shape [6]. It has been demonstrated that the local curvature of nanoparticle at the point of cell attachment is crucial to the initiation of phagocytosis [6]. Other related studies show that the uptake rate of lower aspect ratio (e.g., 1 : 3) nanorods is greater than that of those with higher aspect ratios (e.g., 1 : 5) [5].

Compared with the rapid progress made in understanding the cell uptake of rigid non-spherical nanoparticles, relatively little is known about the interactions between cell membranes and elastic nanoparticles or one- and two-dimensional nanomaterials. For example, it has been shown that phagocytosis of soft microparticles can be hindered by particle deformation [22]. Nanoparticles decorated with stiffer ligands are more easily engulfed than those coated with softer ligands [20]. Flexible micelles can circulate for prolonged periods in the blood stream due to their flexible structures, allowing them to deliver drugs to target tumor cells more efficiently [23]. These studies suggest that cellular uptake of nanoparticles is strongly influenced by their elastic properties. In addition to the mounting studies focused on the elasticity effect, there has also been increasing interest in the cell interaction with one- and two-dimensional

nanomaterials. For example, it has been demonstrated experimentally and theoretically that cylindrical one-dimensional nanomaterials enter cells through the tip first [9]. Further studies show that one-dimensional nanomaterials without caps on their tips exhibit a different mode of membrane interaction [9]. A recent study has shown that two-dimensional graphene and few-layer graphene microsheets ($0.5\text{-}5\text{ }\mu\text{m}$ lateral dimension) orient their surfaces nearly orthogonal to the membrane surfaces of human lung epithelial cells, keratinocytes and mouse macrophages, after the spontaneous membrane penetration at edge asperities and corner sites [16]. In another related study, human THP-1 macrophages exposed with $25\text{ }\mu\text{m}$ few-layer graphene sheets have been shown to adhere to, then spread on and finally cover the graphene surfaces [24]. These studies indicate that the modes of interaction between cell membranes and one- and two-dimensional nanomaterials are quite different from these for nanoparticles and have unique feature resulting from their intrinsic geometry. The mechanisms behind these interesting phenomena remain to be fully elucidated.

1.2 Thesis organization

In this thesis, we perform theoretical studies on effects of elasticity and geometry on cellular uptake of nanomaterials. We first focus on the elasticity effect. In Chapter 2, we restrict our study in two dimensions and investigate how the membrane wrapping process and morphologies of the nanoparticle and membrane depend on the nanoparticle size and shape, adhesion energy, membrane tension, and bending stiffness ratio between the particle and membrane. In Chapter 3, we perform a three-dimensional study on the adhesive wrapping of an elastic nanoparticle modeled as a fluid vesicle or a solid thin-shelled capsule, for a range of bending stiffness ratio between the particle and membrane. Results from Chapter 2 and Chapter 3 indicate that stiffer particles

can achieve full wrapping more easily than softer particles, while softer particles experience smaller energy changes during wrapping and might be more favorable in case where full wrapping is not necessary. In Chapter 4, we find that there are two basic modes of interaction between cells and one-dimensional nanomaterials: tip recognition followed by near-perpendicular entry versus near-parallel adhering on the membrane. Our analysis indicates that as cell membrane internalizes one-dimensional nanomaterials the uptake follows a near-perpendicular entry mode at small membrane tension but it switches to a near-parallel interaction mode at large membrane tension. Such behavior is ubiquitous in the interplay between cell membranes and one-dimensional nanomaterials including nanotubes, nanowires, filamentous bacteria, and certain nanoparticle chains. In Chapter 5, we investigate two modes of interaction between cell membranes and micro-sized two-dimensional nanomaterials: near-orthogonal membrane translocation after spontaneous membrane penetration at corners of nanomaterials and parallel attachment onto cell membranes. Different from the tension-dependent behavior of one-dimensional nanomaterials uptake discussed in Chapter 4, these two modes in Chapter 5 are independent of membrane tension.

CHAPTER TWO

Cell Uptake of Rod-Shaped Elastic Nanoparticles: A Two-dimensional Study

Understanding the cellular uptake of nanoparticles is essential to a wide range of applications concerning biomedical diagnosis, therapy, and drug delivery. During the last decade, substantial research progresses have been achieved on the effect of size [1, 2, 3, 4, 5], shape [5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18] and surface structure [19, 20, 21] of nanoparticles on their cellular uptake. In addition, there has also been increasing interest in cell interaction with elastic nanoparticles. For example, it has been found that macrophages are unable to phagocytose very soft targets, which has profound implications on the functioning of immune system [25]. Flexible erythrocytes can be strongly distorted during phagocytosis due to strong interactions between cell membrane and soft particles/vesicles [26]. Beningo and Wang [22] have shown that phagocytosis of soft microparticles can be hindered by particle deformation. Nanoparticles decorated with stiffer ligands are more easily engulfed than those coated with softer ligands [20]. Murine leukemia virus (MLV) and human immunodeficiency virus (HIV) particles regulate their mechanical properties at different stages of the life cycle through internal morphological reorganization [27, 28]: Immature HIV viral particles are relatively stiff for budding out of a host while mature HIV particles are substantially softer for entry into a host [27, 28]. Flexible micelles can circulate for prolonged periods in the blood stream due to their flexible structures, allowing them to deliver drugs to target tumor cells more efficiently [23]. In drug delivery, softer, more flexible particles are expected to inhibit phagocytosis, leading to longer lifetime of particles in the circulation. Moreover, elasticity can strongly influence the flow behavior of cells. For example, sickle red blood cells of different morphologies and membrane rigidities exhibit different flow efficiency in shear flow [29, 30, 31]. Although the detailed mechanisms of cellular uptake remain to be fully elucidated and can vary in different cases, a general fact has been established that cellular uptake of nanoparticles is strongly influenced by their elastic properties. This calls for studies aimed at understanding the effect of

elastic deformation of particles on cellular uptake.

Most existing theoretical work on the interaction between nanoparticles and cells has focused on symmetric or axisymmetric geometries [32, 33, 34, 35, 36]. Although some theoretical studies have accounted for asymmetric deformation of a membrane as it wraps around a single rigid rod-shaped nanoparticle or multiple spherical nanoparticles [12, 13, 17, 18, 37, 38, 39, 40], so far no theoretical investigations have been performed for cell membrane wrapping around rod-shaped elastic nanoparticles where both the nanoparticle and membrane can undergo asymmetric deformation.

Here we perform the first theoretical study on the adhesive wrapping of an elastic rod-shaped nanoparticle by a lipid membrane with asymmetric deformation. We will investigate how the wrapping degree and morphologies of the nanoparticle and membrane depend on the nanoparticle size and shape, adhesion energy, membrane tension, and bending stiffness ratio between the particle and membrane. We will determine the wrapping phase diagram for cellular uptake of a class of two-dimensional (2D) elastic nanoparticles that can be modeled as vesicles, probing the transitions between full wrapping, partial wrapping, and no wrapping states, as well as the particle orientation phase diagram for the interaction of a short and stiff rod-shaped nanoparticle with the cell membrane. Although our analysis is limited to two dimensions, some results are generic and can be safely extrapolated to three-dimensional cases. We will also discuss possible implications of our results on relevant biological processes such as viral budding and endocytosis.

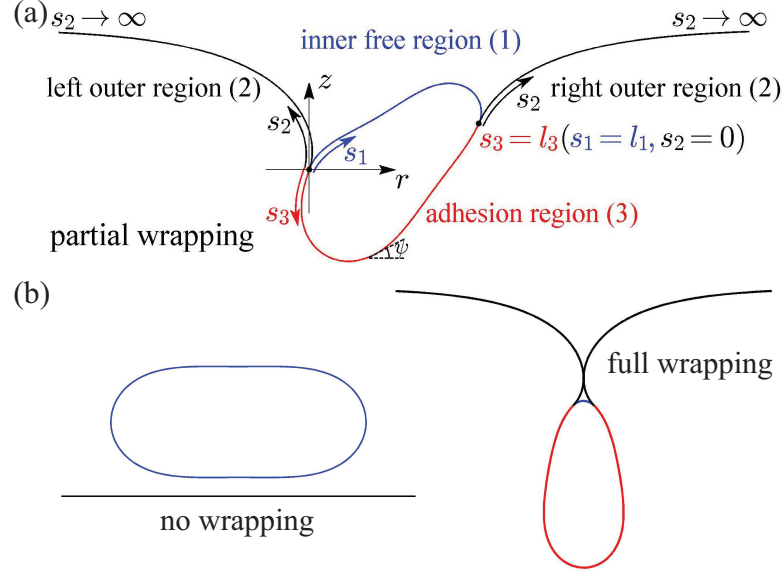


Figure 2.1: Schematic of a soft elastic particle modeled as a vesicle and wrapped by an initially flat membrane in the adopted two-dimensional Cartesian coordinate rz . (a) The geometry of the system with tangent angle ψ . Arclengths s_i ($i = 1, 2, 3$) are defined along the particle and the outer free part of the membrane; s_1 , s_2 of the left outer region, and s_3 are measured from the left contact edge, and s_2 of the right outer region is measured from the right contact edge $s_2 = 0$ ($s_1 = l_1$, $s_3 = l_3$). (b) Schematic for the states of no wrapping with zero contact length and full wrapping in which the left and right sides of the membrane touch each other on the top of the particle. Partial wrapping corresponds to the intermediate scenario with incomplete wrapping as shown in (a).

2.1 Model and methods

While we note that an elastic nanoparticle can be modeled in a number of alternative ways, here we limit our attention to a class of elastic nanoparticles that can be modeled as a 2D vesicle with constant contour length L_t and fixed area A . For such a 2D elastic nanoparticle wrapped by an initially flat cell membrane with elastic deformation in both the particle and membrane, as shown in Fig. 2.1(a), the total energy of the system per unit length in the out-of-plane direction can be described by the Canham-Helfrich functional as [32, 35, 36, 41, 42, 43, 44]

$$E_{tot} = \sum_{i=1,2,3} \int \frac{\kappa_i}{2} \dot{\psi}_i^2 ds_i + PA + \sigma \Delta l - \gamma l_3, \quad (2.1)$$

where P is a Lagrange multiplier enforcing the enclosed area A of the nanoparticle; γ is the specific adhesion energy, and l_3 is the length of the contact region; σ is the membrane tension which is conjugated to the excess length $\Delta l = \sum_{i=2,3} \int (1 - \cos \psi_i) ds_i$ induced by wrapping; ψ_i , s_i , and κ_i ($i = 1, 2, 3$) are the tangent angles, arclength, and bending stiffness of the three regions, respectively [see Fig. 2.1(a)]. The superimposed dot in the first term in Eq. (2.1) denotes derivative with respect to the arclength s , and subscripts 1, 2, and 3 are used to identify quantities associated with the inner free, outer free, and wrapped regions, respectively. Since $\int_0^{l_3} \dot{\psi}_3 ds_3 - \int_0^{l_1} \dot{\psi}_1 ds_1 = 2\pi$ and the circumference L_t of the nanoparticle is fixed, a spontaneous or intrinsic curvature c_0 associated with the elastic nanoparticle would only result in an extra constant energy term in E_{tot} in Eq. (2.1) [43, 44], and therefore needs not be explicitly considered. While recognizing important exceptions such as the possible formation of clathrin or caveolin coats during the wrapping process, for simplicity we will assume $\kappa_3 = \kappa_1 + \kappa_2$ throughout the analysis. All lengths are scaled by the effective radius of the particle $a = L_t/(2\pi)$ and the area of the nanoparticle is reduced as $v = A/(\pi a^2) \leq 1$. As v gradually increases, the shape of such a free-standing particle evolves from a concave dumbbell to a short rod and eventually to a circle at $v = 1$. Hereinafter both the concave dumbbell and short rod shapes are referred to as rod-like shapes. Other dimensionless parameters are

$$\bar{\sigma} \equiv 2\sigma a^2/\kappa_2 \quad \text{and} \quad \bar{\gamma} \equiv 2\gamma a^2/\kappa_2.$$

Since the bending energy of a planar elastica has the same form as the first term in Eq. (2.1), our analysis also applies to the wrapping of two-dimensional inextensible elastic thin-shelled capsules by a lipid membrane.

An isolated particle with a prescribed reduced area $v < 1$ has a rod-like shape. Such a particle with area constraint would exhibit asymmetric deformation during

the wrapping process. Here we employ a numerical optimization technique to determine the minimum energy state at each given wrapping degree $f = l_3/L_t$ defined as the ratio between the length of the contact region and the circumference of the nanoparticle. With geometric relations $\dot{r} = \cos \psi$ and $\dot{z} = \sin \psi$, the elastic deformation energy $E_{el} = E_{tot} + \gamma l_3$ in the region i ($i = 1, 2, 3$) at a given wrapping degree f can be represented as a function of the unknown tangent angle $\psi_i(s_i)$ which is approximated by a Fourier series as [33, 34, 45]

$$\psi_i(s_i) = \psi_0^{(i)} + (\psi_l^{(i)} - \psi_0^{(i)}) \frac{s_i}{l_i} + \sum_{j=1}^N a_j^{(i)} \sin \frac{\pi j s_i}{l_i}. \quad (2.2)$$

Here N is the number of Fourier modes, which is chosen as $N = 80$ in our calculations, $a_j^{(i)}$ are the Fourier amplitudes, s_i is the arclength, and l_i is the length of the considered region i ($i=1,2,3$); $\psi_0^{(i)}$ and $\psi_l^{(i)}$ are the tangent angles at $s_i = 0, l_i$, respectively. For a given wrapping degree f , it can be easily seen that $l_1 = 2(1-f)\pi$ and $l_3 = 2f\pi$. To approximate the condition $s_2 \rightarrow \infty$, the length l_2 of the outer membrane region can be treated as either a certain large number or an unknown parameter determined by a prescribed length of the membrane projection. Our calculations indicate that the choice of these conditions will not affect the final results. For the latter, we set the length of the outer free membrane projection on each side to be $20a$.

The boundary and constraint conditions provide either input parameters or equality constraints during energy minimization. At $s_2 \rightarrow \infty$, the outer free membrane becomes asymptotically flat which requires $\psi_l^{(2)} = 0$. Since we adopt the coordinate with origin at the left contact edge, the continuity of the coordinate (r, z) for the three regions at the origin is satisfied automatically. The continuity of the coordinate (r, z) at the right contact edge, the continuity of the tangent angles ψ at both left and right contact edges, and the prescribed reduced area v are enforced as equality

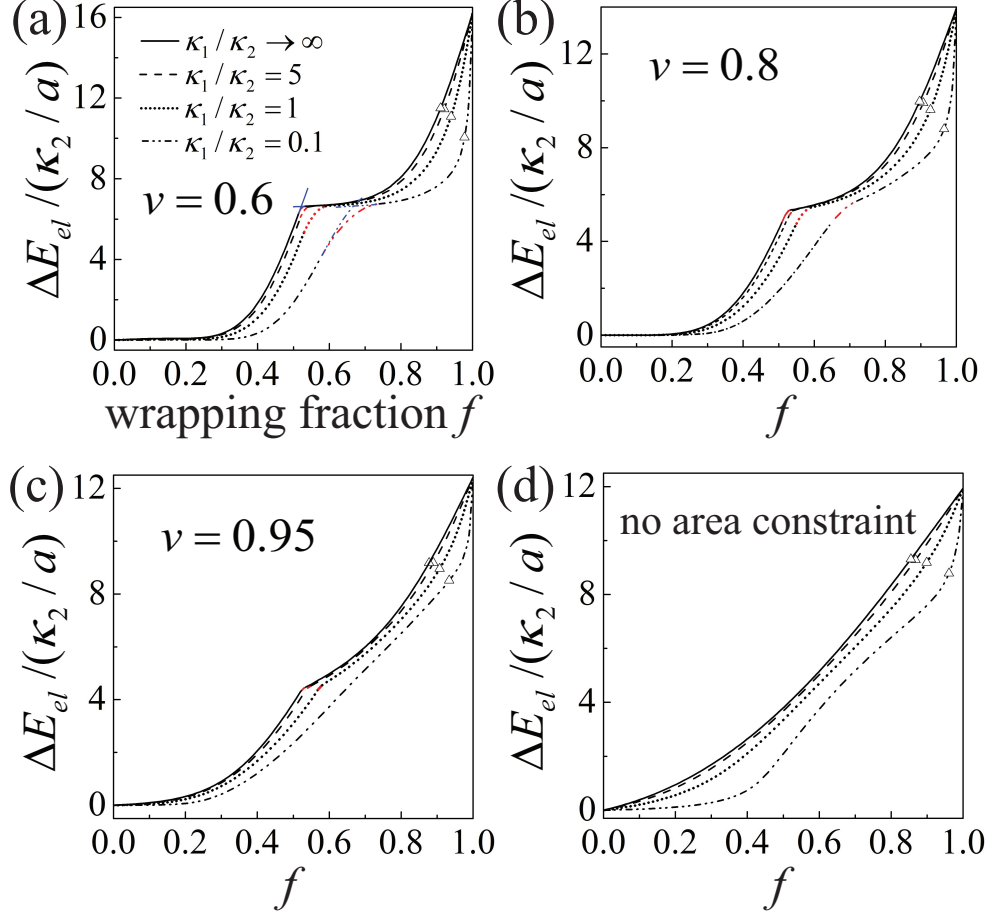


Figure 2.2: (a-d) Elastic energy ΔE_{el} as a membrane wraps around a nanoparticle with wrapping degree f for different reduced areas v and κ_1/κ_2 at $\bar{\sigma} = 1$. The open triangle mark on each curve corresponds to full wrapping. The red regions on some curves indicate asymmetric deformation.

constraints. The elastic energy as a function of $\psi_i(s_i)$ under these constraints at a given f is minimized with respect to the Fourier amplitudes a_j , tangent angles ψ_0 and ψ_l at both contact edges, and l_2 for both outer free regions using sequential quadratic programming [46]. Once the tangent angles ψ given by Eq. (2.2) are known, the elastic energy (Fig. 2.2) and corresponding shapes (of the particle and membrane, see Fig. 2.3) can be determined.

For the nanoparticle without area constraints, both particle and membrane display symmetric morphologies as shown in Fig. 2.3(d). In such case, the corresponding

shape equations of the particle are [44, 47]

$$\kappa_i \ddot{\psi}_i / \kappa_1 = \mu \sin \psi_i \quad (i = 1, 3) \quad \text{and} \quad \dot{\mu} = 0,$$

and the shape equation of the outer free membrane is [48]

$$\ddot{\psi}_2 = \bar{\sigma} \sin \psi_2 / 2,$$

with an analytical solution

$$\psi_2 = 4 \arctan[\tan(\psi_0^{(2)} / 4) \exp(-s_2 \sqrt{\bar{\sigma} / 2})],$$

where s_2 is the rescaled arclength of the outer free part [48]. With the relations $\dot{r} = \cos \psi$ and $\dot{z} = \sin \psi$, the $r(s_2)$ and $z(s_2)$ coordinates of the outer free membrane can be determined as [36, 49]

$$\begin{aligned} r(s_2) &= r_0 + s_2 - \sqrt{\frac{2}{\bar{\sigma}}} \frac{1 - \cos \psi_0^{(2)}}{\coth(\sqrt{\bar{\sigma} / 2} s_2) + \cos(\psi_0^{(2)} / 2)}, \\ z(s_2) &= z_0 + \sqrt{\frac{8}{\bar{\sigma}}} \sin \frac{\psi_0^{(2)}}{2} \left[1 - \frac{\operatorname{csch}(\sqrt{\bar{\sigma} / 2} s_2)}{\coth(\sqrt{\bar{\sigma} / 2} s_2) + \cos(\psi_0^{(2)} / 2)} \right], \end{aligned} \tag{2.3}$$

where r_0 and z_0 are the coordinates of the contact edge and $\psi_0^{(2)}$ is the tangent angle at $r = r_0$.

2.2 Results

Figure 2.2 shows the elastic energy change $\Delta E_{el} = E_{el} - E_{el}^0$ as a function of the wrapping degree f , where E_{el}^0 is the reference energy before the nanoparticle contacts

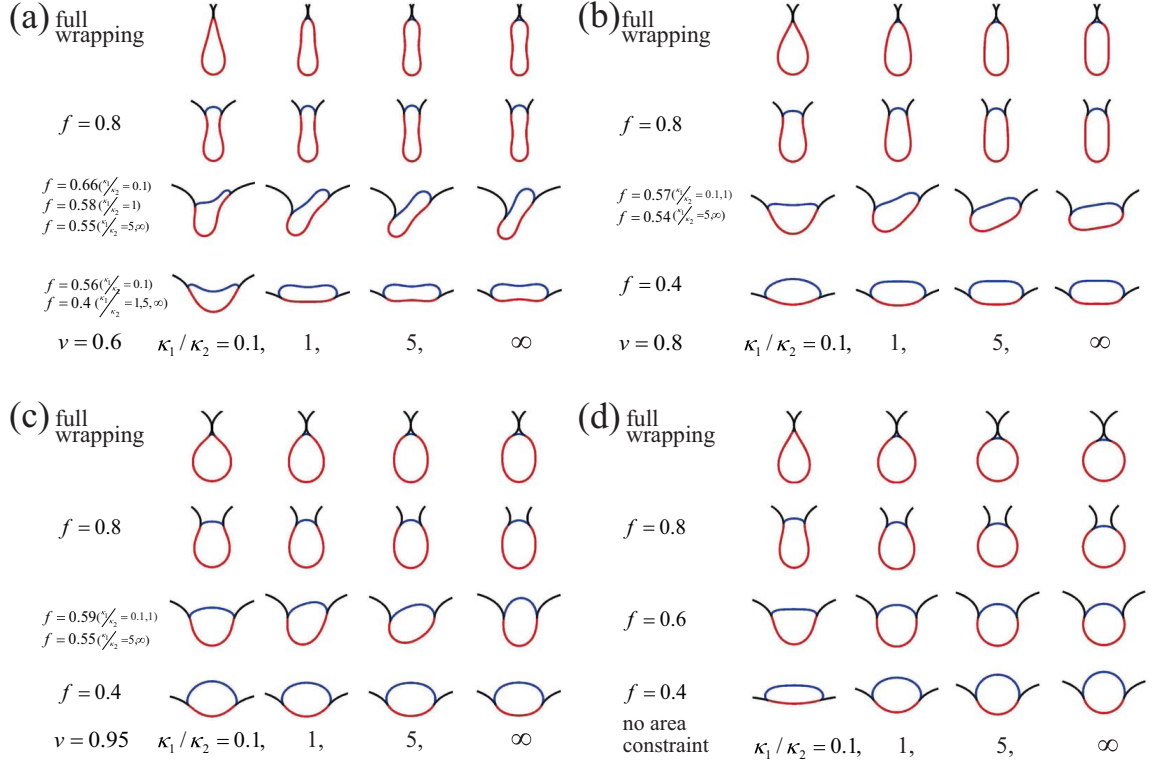


Figure 2.3: Selective wrapping configurations at $\bar{\sigma} = 2$ for different particle-membrane stiffness ratios κ_1/κ_2 and different reduced areas of (a) $v = 0.6$, (b) 0.8 , (c) 0.95 , and (d) no area constraint.

the membrane. The open triangle on each curve corresponds to full wrapping. The red segments on some curves in Fig. 2.2(a-c) indicate the occurrence of asymmetric deformation as the wrapping degree f reaches a critical value f_c slightly larger than 0.5 . At $f < f_c$, a soft particle (e.g., $\kappa_1/\kappa_2 = 0.1$) would contact with the membrane with its flattened side, while a relatively stiff (e.g., $\kappa_1/\kappa_2 = 1$) or rigid ($\kappa_1/\kappa_2 \rightarrow \infty$) rod-like particle would align its long axis parallel to the membrane [see Fig. 2.3(a,b)]. As the wrapping proceeds, the particle and membrane undergo a transition from a symmetric configuration at $f < f_c$ to an asymmetric one, followed by another symmetric configuration in the late stage. Compared to the initial symmetric morphology of particles with long axis parallel to the membrane at $f < f_c$, a soft particle would undergo a dramatic symmetry breaking transition while a stiff particle would reorient itself sharply so that their long axes are realigned perpendicular to the membrane in the second symmetric morphology associated with the

late stage of wrapping [Fig. 2.3(a,b)]. The adoption of an asymmetric morphology (as marked by the red segments) results from a significant decrease in the total elastic energy due to symmetry breaking. As an example, consider the blue lines in Fig. 2.2(a) for the cases of $\kappa_1/\kappa_2 = 0.1$ and $\kappa_1/\kappa_2 \rightarrow \infty$. These lines correspond to the elastic energy change of the system with an unstable symmetric morphology, which is larger than those with the stable asymmetric morphology represented by the red segments. It is found that $f_c - 0.5$ is inversely proportional to the stiffness ratio κ_1/κ_2 . In general, smaller bending stiffness ratio κ_1/κ_2 , which gives rise to more flattened particle with smaller excess length Δl and smaller membrane tension energy $\sigma\Delta l$, and larger membrane tension lead to larger asymmetric regions [see Fig. 2.2(a,b), Fig. A.1(a), and Fig. 2.2(c) and Fig. A.1(b,c) at $\kappa_1/\kappa_2 = 1, 5, \infty$]. A few exceptional examples with no symmetry breaking are observed for very soft particles ($\kappa_1/\kappa_2 = 0.1$) with $v = 0.95$ [Fig. 2.2(c)] and with $v = 0.8$ at $\bar{\sigma} = 2$ in Fig. A.1(b) in the Appendix A, which can be attributed to the fact that there is no longer an energy drop from symmetric to asymmetric morphologies for very soft particles. As demonstrated in Fig. 2.2(a), the energy difference between the unstable symmetric and stable asymmetric morphologies represented by the gap between the blue and red curves is much smaller in the case of $\kappa_1/\kappa_2 = 0.1$ compared to the case of $\kappa_1/\kappa_2 \rightarrow \infty$ for $v = 0.6$. As the gap vanishes, which first occurs in the case of very soft particles, only symmetric morphology would be observed in the whole wrapping process, as demonstrated in the case of $\kappa_1/\kappa_2 = 0.1$ in Fig. 2.3(b,c). It is also observed that the value of f at full wrapping increases as the stiffness ratio κ_1/κ_2 decreases and membrane tension $\bar{\sigma}$ increases.

A free-standing 2D elastic nanoparticle with a small reduced area (e.g., $v = 0.6$) exhibits a concave shape as shown in Fig. 2.3(a) for $\kappa_1/\kappa_2 \rightarrow \infty$. Such a concave shape would lead to a narrow gap between the adhered particle and membrane at

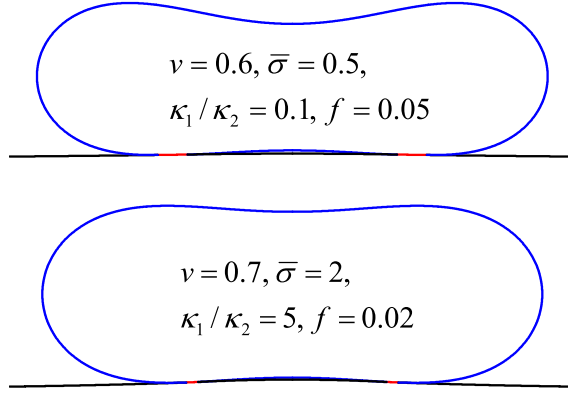


Figure 2.4: A narrow gap exists at the initial contact between a flat membrane and an elastic particle with small reduced area v .

initial contact (Fig. 2.4). The size of the gap decreases as the wrapping degree f increases and a full contact is achieved when f exceeds about 0.1. The existence of a narrow gap was also observed between a round particle adhering to an oblate vesicle [50]. Here the adhesive interaction between the nanoparticle and membrane is characterized by a contact potential with vanishing interaction range as given in Eq. (2.1). Based on a recent study showing that the size of the interaction range plays an important role on the wrapping process [51], the gap size is expected to depend the interaction range.

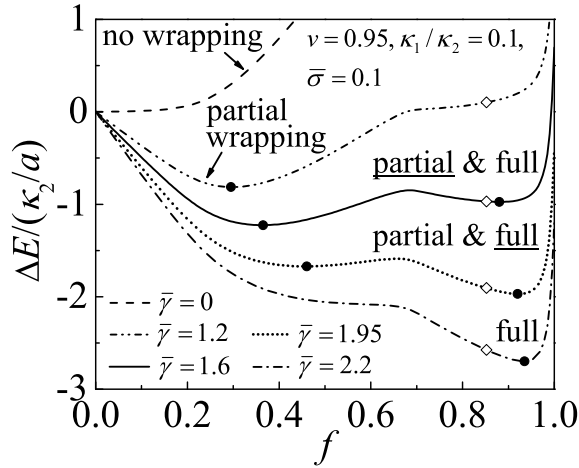


Figure 2.5: Total energy change ΔE of five possible wrapping states as a function of the wrapping degree f for different $\bar{\gamma}$ with $v = 0.95$, $\kappa_1/\kappa_2 = 0.1$, and $\bar{\sigma} = 0.1$. The solid circles on curves correspond to the local minima. The open squares correspond to full wrapping. The underlined wrapping state has energy lower than its coexisting wrapping state.

The total energy difference $\Delta E = E_{tot} - E_{el}^0$ corresponds to the sum of the elastic energy difference ΔE_{el} and adhesion energy $-f\gamma L_t$. Figure 2.5 shows $\Delta E(f)$ as a function of the wrapping degree f for different $\bar{\gamma}$ in the case $v = 0.95$, $\kappa_1/\kappa_2 = 0.1$, and $\bar{\sigma} = 0.1$. The behavior of $\Delta E(f)$ indicates that there exist five possible phases when $\bar{\sigma}$ is very small (Fig. 2.5). For relatively small adhesion energy $\bar{\gamma}$, ΔE increases monotonically with f and no wrapping is possible. As $\bar{\gamma}$ increases, the stable state changes from no wrapping to partial wrapping. Further rise in $\bar{\gamma}$ results in a global minimum at a state of partial wrapping and an energy barrier to reach the (metastable) state of full wrapping. With $\bar{\gamma}$ continuously rising, the stable state of partial wrapping becomes metastable while full wrapping becomes stable. In the regimes of two coexisting states of partial and full wrapping, the one with lower energy is underlined (Fig. 2.5). If $\bar{\gamma}$ is large enough, the energy barrier to full wrapping vanishes and full wrapping becomes the only stable state. For each phase, there exists a stable (global energy minimum) state and possibly a metastable (local energy minimum) state. When $\bar{\sigma}$ is sufficiently large, there are no longer coexisting states of partial and full wrapping, and the stable state simply changes from no wrapping to partial wrapping then to full wrapping as $\bar{\gamma}$ increases. Note that the solutions at solid circles are unphysical in cases when full wrapping (marked by the open squares) arises before f reaches the position marked by the solid circles. In these cases, full wrapping occurs as the membrane comes in contact above the nanoparticle and is not necessarily the state of zero slope along the energy profile.

With the knowledge of energy functions at different values of κ_1/κ_2 , v , $\bar{\sigma}$, and $\bar{\gamma}$, the phase diagrams of wrapping are determined and shown in Figs. 2.6 and 2.7. Note that the solution at $f = 1$ is usually unphysical because in that case the two opposing parts of the membrane will have met and crossed each other above the nanoparticle. Therefore, the state of full wrapping needs to be carefully defined

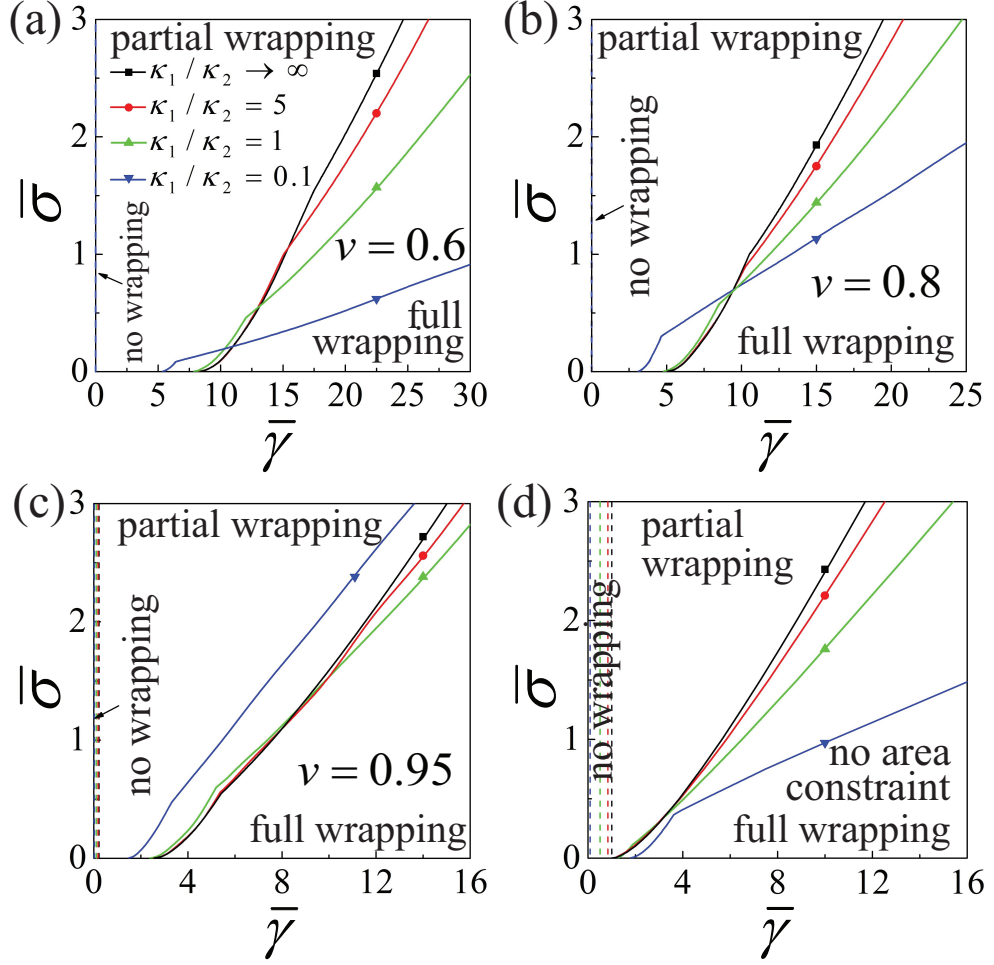


Figure 2.6: Wrapping phase diagrams with respect to normalized adhesion energy $\bar{\gamma}$ and membrane tension $\bar{\sigma}$ at different stiffness ratios κ_1/κ_2 and reduced areas v . (a-c) Cases with reduced areas $v = 0.6, 0.8$, and 0.95 , and (d) the case with no area constraint as a comparison with cases (a-c). Dashed lines indicate boundaries between no wrapping and partial wrapping states. Solid lines indicate boundaries between partial and full wrapping states. In cases (a) and (b), the adhesion energy for partial wrapping is so small that the dashed lines almost overlap with the $\bar{\sigma}$ -axis.

[Fig. 2.1(b)]. At the late stage of wrapping, both particle and membrane display symmetric morphologies, and the shape of the outer free part of the membrane associated with a given wrapping angle $\psi_0^{(2)}$ is given in Eq. (2.3). For a given set of values for r_0 , $\psi_0^{(2)}$, and $\bar{\sigma}$, the minimum of $r(s_2)$ for the right outer free membrane and the maximum of $r(s_2)$ for the left outer free membrane can be determined. The full wrapping condition is then found from the condition that these extreme values are equal, corresponding to the situation when the two opposing parts of the membrane come into contact, as shown in Fig. 2.5. The critical condition for a rigid circular

particle to be fully wrapped by a membrane is then [52]

$$\sqrt{\frac{\bar{\sigma}}{2}} \sin \psi_0^{(2)} = \sqrt{2} - 2 \cos \frac{\psi_0^{(2)}}{2} + \ln \left(\tan \frac{\pi}{8} \cot \frac{\psi_0^{(2)}}{4} \right),$$

with the contact angle $\psi_0^{(2)} = \arccos [1 - (1 - \sqrt{\bar{\gamma}})^2 / \bar{\sigma}]$.

It may be interesting to compare the wrapping phase diagram for particles without area constraints [Fig. 2.6(d)] with those under reduced area constraints [Fig. 2.6(a-c)]. First, for a membrane wrapping around a rigid circular particle, the total energy evolves as

$$\frac{\Delta E}{\kappa_2/a} = 8\sqrt{2\bar{\sigma}} \sin^2 \frac{f\pi}{4} + f\pi + \bar{\sigma}[f\pi - \sin(f\pi)] - f\pi\bar{\gamma}.$$

The condition $\partial\Delta E/\partial f|_{f=0} = 0$ gives the minimum adhesion energy necessary for partial wrapping as $\bar{\gamma}_{\min} = 1$ [52]. For such a particle, $\partial^2\Delta E/\partial f^2 = 0$ gives $f = 1$, which means that the only inflection point of the energy profile is at $f = 1$, and that there is no energy barrier for ΔE for any $\bar{\gamma}$ as f varies and therefore no coexistence of partial and full wrapping states in membrane wrapping of a rigid circular particle regardless of the value of membrane tension $\bar{\sigma}$. For elastic particles without area constraints, $\bar{\gamma}_{\min}$ decreases as κ_1/κ_2 decreases, as the easily flattened softer particles lead to smaller elastic energy; however, $\bar{\gamma}$ needs to increase to attain full wrapping. When $\bar{\sigma} \leq 0.5$, there are small differences between the transition lines from the state of partial wrapping to full wrapping for different κ_1/κ_2 . As $\bar{\sigma}$ increases further, the differences become large and strikingly sensitive to κ_1/κ_2 [see Fig. 2.6(d)]. This phenomenon can also be understood by comparing the maximum slopes of the elastic energy profiles at different κ_1/κ_2 (Figs. 2.2 and A.1), since the condition for full wrapping to be the only stable state requires $\Delta E(f)$ to be a monotonically decreasing function or $\bar{\gamma}$ larger than the maximum slope of the elastic energy profile before f

reaches the critical value for full wrapping indicated by the open symbols in Figs. 2.2 and A.1. Similar effect of elasticity on the wrapping states is also observed for particles with constrained areas, as shown in Fig. 2.6(a-c). For non-circular rod-shaped particles (e.g., those with $v = 0.6, 0.8$, and 0.95), $\bar{\gamma}_{\min}$ is significantly smaller than 1 since the initial wrapping causes less bending deformation. Compared to Fig. 2.6(d), a significant difference in Fig. 2.5(a-c) is that, as $\bar{\sigma}$ is smaller than a certain value, softer particles may require smaller $\bar{\gamma}$ to attain full wrapping. For example, for $\bar{\sigma} = 0.5$ and $v = 0.8$ shown in Fig. 2.6(b), particles with bending stiffness $\kappa_1 = \kappa_2$ require smaller $\bar{\gamma}$ than stiffer ones to attain full wrapping and particles with $\kappa_1 = 0.1\kappa_2$ require even smaller $\bar{\gamma}$ than those with $\kappa_1 = \kappa_2$. This phenomenon is reflected in the elastic energy profile at $\bar{\sigma} = 0.5$ in Fig. A.1(b) where stiffer particles exhibit larger maxima of energy slopes, indicating that higher $\bar{\gamma}$ would be required for full wrapping. As the reduced area v increases, the phase diagram becomes more complex. In the case of $v = 0.95$, particles with $\kappa_1 = 0.1\kappa_2$ require smaller $\bar{\gamma}$ than all the stiffer ones to attain full wrapping for the calculated range of $\bar{\sigma} \leq 3$; When $\bar{\sigma} \leq 1.5$, there are very small differences between the transition lines from the state of partial wrapping to full wrapping for $\kappa_1/\kappa_2 = 1, 5$, and ∞ . The difference becomes larger as $\bar{\sigma}$ increases. The kinks on the phase boundaries in regions of relatively small $\bar{\sigma}$ indicate coexistence of partial and full wrapping states whose detailed phase diagrams are shown in Fig. 2.7. For particles with a prescribed reduced area v , the coexistence region exists only under sufficiently small $\bar{\sigma}$, with size increasing as the stiffness ratio κ_1/κ_2 increases [see Fig. 2.7(a-c)]. In contrast, for particles without area constraints, the coexistence region exists also under sufficiently small $\bar{\sigma}$, but its size decreases as the stiffness ratio κ_1/κ_2 increases, as shown in Fig. 2.7(d).

When $\bar{\sigma}$ is smaller than a certain value, we found a coexistence of the partial and full wrapping states, as indicated in Fig. 2.5. The wrapping phase diagrams

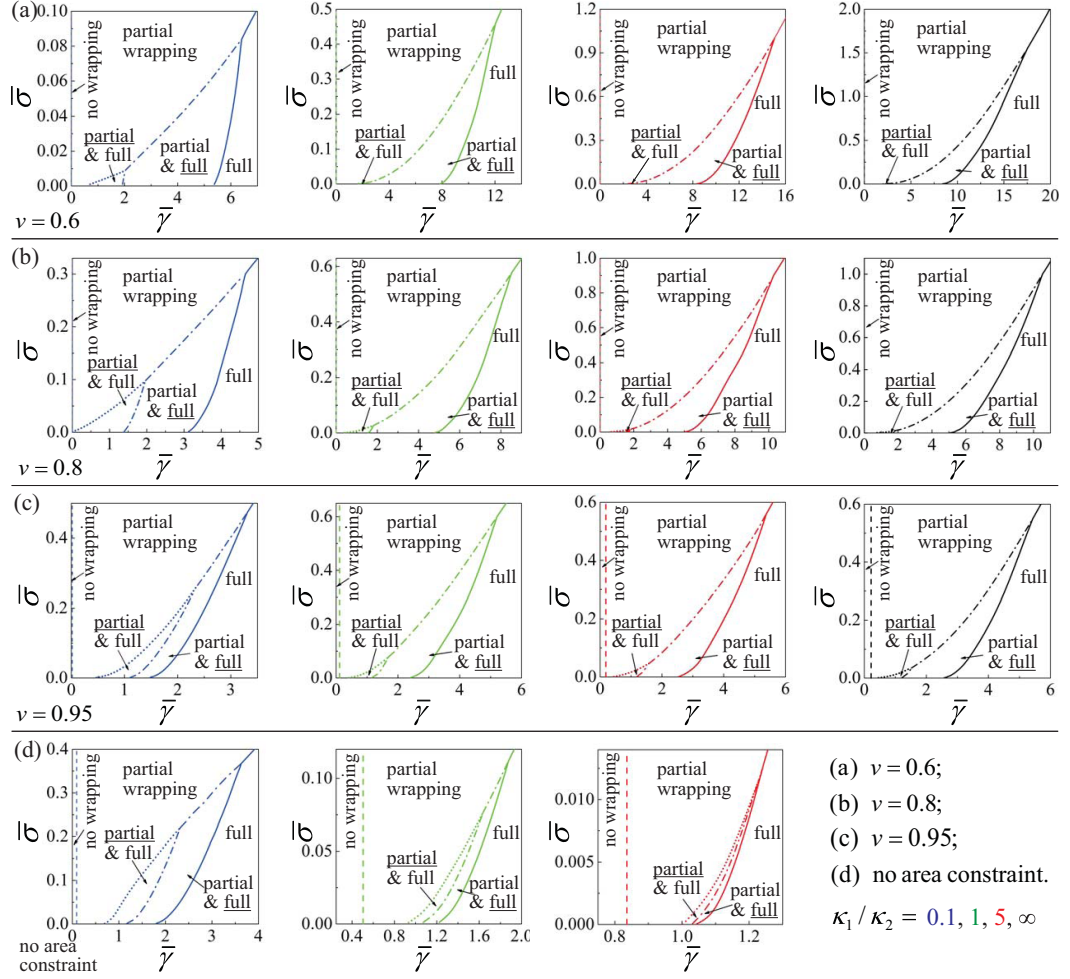


Figure 2.7: Wrapping phase diagrams with respect to normalized adhesion energy $\bar{\gamma}$ and surface tension $\bar{\sigma}$ at different values of the stiffness ratios κ_1/κ_2 and reduced areas v in two dimensions. (a,b,c,d) Zoom-in phase boundaries in the region with small $\bar{\sigma}$ and stiffness ratios $\kappa_1/\kappa_2 = 0.1, 1, 5$, and ∞ (from left to right) in Fig. 2.6(a,b,c,d). In (d) the coexistence of partial and full wrapping states only exists at $\kappa_1/\kappa_2 = 0.1, 1$, and 5 (from left to right). The wrapping state with energy lower than its coexisting state is underlined.

regarding the coexistence regions at different κ_1/κ_2 and v are shown in Fig. 2.7. For particles with a prescribed reduced area v , the size of the coexistence region increases as the stiffness ratio κ_1/κ_2 increases [see Fig. 2.7(a-c)]; while for particles without area constraints, the size of the coexistence region decreases as the stiffness ratio κ_1/κ_2 increases as shown in Fig. 2.7(d).

Figure 2.6 shows that the wrapping phase diagrams strongly depend on the value of the reduced area v , especially when v is close to 1. To find out how the boundary

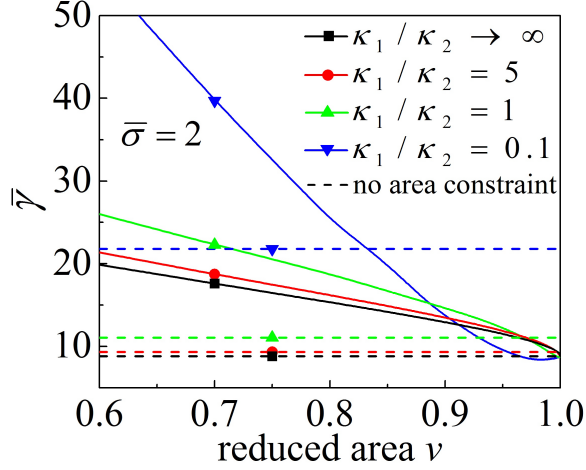


Figure 2.8: Phase diagram of full wrapping as a function of the reduced area v and adhesion energy $\bar{\gamma}$ for different bending stiffness ratios at $\bar{\sigma} = 2$. The dashed lines correspond to values of $\bar{\gamma}$ without any area constraint. Above each curve lies the full wrapping region, and below is the partial wrapping region.

between partial and full wrappings evolves as v varies, we plot the phase boundaries between partial and full wrapping as a function of the reduced area v and adhesion energy $\bar{\gamma}$ for different bending stiffness ratios at a typical value of the normalized membrane tension $\bar{\sigma} = 2$ (see Fig. 2.8). Let us consider the cases with fixed reduced area first. For $\kappa_1/\kappa_2 = 5$ and ∞ , the required $\bar{\gamma}$ for full wrapping is inversely proportional to v . For $\kappa_1/\kappa_2 = 0.1$ and 1 , $\bar{\gamma}$ decreases for most of the interesting and physically important particle shapes but increases slightly after v exceeds 0.985 and 0.9995 , respectively. Another interesting feature is that, at fixed v , the critical $\bar{\gamma}$ for smaller κ_1/κ_2 is not always larger than that for larger κ_1/κ_2 , rather it depends on the value of v . In the case of no area constraints (hence no dependence on v), the critical $\bar{\gamma}$ for full wrapping increases as the bending stiffness ratio decreases. Except for the case of $\kappa_1/\kappa_2 \rightarrow \infty$ where the critical $\bar{\gamma}$ for full wrapping without area constraint is always smaller than that with area constraint, the critical $\bar{\gamma}$ for full wrapping at other κ_1/κ_2 without area constraint can be larger than that with area constraint when v exceeds a certain value.

Rigid particles with different reduced areas v exhibit different shapes. The shape

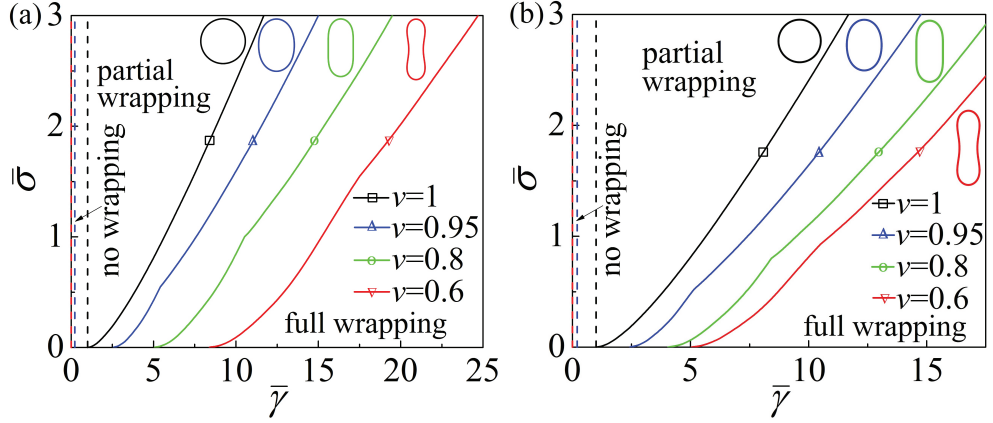


Figure 2.9: Wrapping phase diagram with respect to normalized adhesion energy $\bar{\gamma}$ and membrane tension $\bar{\sigma}$ for rigid nanoparticles with (a) the same circumference but different enclosed areas, and (b) with the same enclosed area but different circumferences. Shapes from left to right correspond to $v = 1, 0.95, 0.8$, and 0.6 , respectively.

effects of rigid 2D particles on cellular uptake can be extracted from Fig. 2.6 for different values of the constrained area v . When this is done, the results are compared together in Fig. 2.9(a). The $\bar{\gamma}$ required for full wrapping decreases as v increases. In other words, thinner and longer 2D rigid particles with larger aspect ratios require larger adhesion energy $\bar{\gamma}$ than thicker and shorter ones with smaller aspect ratios to attain full wrapping or simply, slender particles are more resistant to full wrapping. This behavior is a direct consequence of the energy profile in Fig. 2.2 where the black solid curve corresponding to smaller v is seen to have larger slope. The phase boundary for $\bar{\sigma} = 2$ and different v is indicated by the black curve in Fig. 2.7. Particles with different v (shapes) in Fig. 2.9(a) have the same circumference L_t but different area A . For particles with the same area A but different circumferences L_t [Fig. 2.9(b)], the effective radius is redefined as $a = \sqrt{A/\pi}$ instead of $a = L_t/(2\pi)$. It can be shown that the corresponding phase diagram can be obtained from Fig. 2.9(a) through a linear scaling mapping $(v\bar{\gamma}, v\bar{\sigma}) \rightarrow (\bar{\gamma}, \bar{\sigma})$. For example, the point with coordinate $(15.34, 2)$ on the green curve ($v = 0.8$) in Fig. 2.9(a) is mapped to the point with coordinate $(15.34 \times 0.8, 2 \times 0.8) = (12.272, 1.6)$ on the green curve ($v = 0.8$) in Fig. 2.9(b). A comparison of Fig. 2.9(a) and (b) indicates that the shape effect

is less striking for particles with the same area than with the same circumference. This property should also be valid for soft particles since the linear scaling mapping only depends on v rather than κ_1/κ_2 .

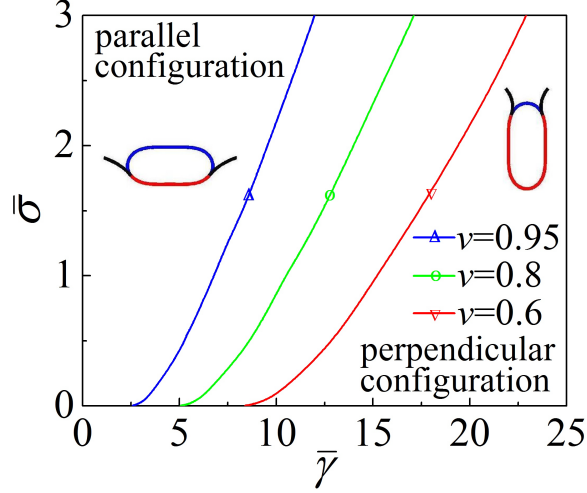


Figure 2.10: Particle orientation phase diagram with respect to normalized adhesion energy $\bar{\gamma}$ and membrane tension $\bar{\sigma}$ for rigid rod-shaped nanoparticles with the same circumference but different enclosed areas. The insets show parallel and perpendicular configurations in the case of $v = 0.8$.

Another interesting phenomenon is the sudden orientational change of rigid or relatively stiff and short rod-shaped nanoparticles around the wrapping degree of $f = 0.5$, as indicated in Fig. 2.2. In the early stage of wrapping, the rod-shaped nanoparticles align their long axes parallel to the membrane and remain in that configuration until the critical point of reorientation is reached, at which the nanoparticles suddenly realign their long axes perpendicular to the membrane and remain in this configuration during the late stage of wrapping. Since the slopes of the elastic energy profile ΔE_{el} have similar values at reorientation and at full wrapping, the orientation phase diagram describing transition boundaries between different nanoparticle orientations (Fig. 2.10) are similar to the wrapping phase diagram describing different wrapping states [Fig. 2.9(a)]. As v decreases, $\bar{\gamma}$ needs to increase in order to maintain the perpendicular configuration. In Fig. 2.10 we have excluded the cases with coexisting parallel and perpendicular configurations, and only consider situations in which the total energy difference $\Delta E(f) = \Delta E_{el}(f) - \gamma f L_t$ is

a monotonically decreasing function when the nanoparticles adopt the perpendicular configuration relative to the membrane. The orientation phase diagram for the situation that particles have the same area A but different circumferences L_t can also be simply obtained from Fig. 2.10 through the same linear scaling mapping $(v\bar{\gamma}, v\bar{\sigma}) \rightarrow (\bar{\gamma}, \bar{\sigma})$ used in the preceding analysis for Fig. 2.8.

2.3 Discussion

Figure 2.2 shows that very soft particles ($\kappa_1/\kappa_2 = 0.1$) with a relatively small reduced area ($v = 0.6$ and 0.8) exhibit a dramatic morphological change from a symmetric parachute-like shape to an asymmetric slipper-like shape in the mid-stage of membrane wrapping as the reduced area v decreases. An interesting phenomenon is that red blood cells moving in a shear flow adopt similar complex morphologies [29, 53]. Numerical studies show that a moving vesicle with the bending stiffness of a healthy red blood cell exhibits symmetric parachute-like shape at the condition of a large reduced area v or a large flow velocity. Once v is below a critical value around 0.7, the symmetric parachute-like shape develops instability and the cell transforms into an asymmetric slipper-like shape no matter how large the flow velocity is [29]. Further numerical analysis shows that membrane bending stiffness also has a dramatic effect on the morphological change of red blood cells with a low reduced area (e.g., $v = 0.6$). Lower stiffness leads to an asymmetric slipper-like shape and larger stiffness results in a pear-like shape with slight asymmetry [29]. These observations are mysteriously similar to those found in the present wrapping problem [see the third row from the top in Fig. 2.3(a)]. Such v - and stiffness-dependent features shared by these two physically different phenomena are suggesting that there may be deep connections between the deformation shape, area constraint, bending stiffness, and

types of loading on the behaviors of elastic particles.

Although the present study is restricted to two-dimensions, some conclusions are generic and can be safely extrapolated to three-dimensions (3D). For example, 2D short rigid anisotropic nanoparticles or elastic nanoparticles with a prescribed reduced area undergo a dramatic morphological change induced by the membrane wrapping, and there exists an asymmetric shape transition in the mid-stage of wrapping. This phenomenon is mainly due to the high bending energy cost at the highly curved regions of rod-shaped nanoparticles and should be generic for both 2D and 3D cases. Theoretical results [12, 13, 17] and molecular dynamics simulations [10, 14, 15] have shown that 3D rigid ellipsoidal, rod-shaped, and even hemisphere-shaped particles experience an orientational change in the mid-stage of wrapping. Another consequence of this high energy cost associated with bending around highly curved tips is that longer and thinner rigid rods with tips of higher curvature require larger adhesive energy than broader, shorter and rounder ones with the same circumference but different enclosed area or the same enclosed area but different circumference to attain full wrapping (see Fig. 2.9). Similar phenomena have been found in numerical simulations and experiments for 3D rod-like nanoparticles under the constraint of the same area but different volume [17] or the same volume but different area [11, 17]. Asymmetric morphology could appear when a 3D elastic nanoparticle with a prescribed reduced volume (defined as the ratio of the enclosed volume over the volume of a sphere having the same surface area) and anisotropic shape is wrapped by a cell membrane.

The orientational change of short rod-shaped nanoparticles indicates that no matter what the initial position or orientation such a particle takes, a complete membrane wrapping or nanoparticle budding always ends up with a configuration with the rod-shaped nanoparticle aligning its long axis perpendicular to the cell membrane. Such

reorientation of short nanoparticles seems ubiquitous in the budding and endocytosis of virus [54, 55, 56], and the orientation combined with membrane deformation provides information on the stage of budding process and endocytosis. For example, short rod-shaped fowlpox and pigeonpox viruses align their long axes parallel to the membrane of infected chick embryo fibroblasts in the initial and mid-stage of budding but eventually bud out of the cells with their long axes perpendicular to the membrane [54]. Similar phenomena are observed during the cell entry of vesicular stomatitis viruses by endocytosis [55]. Recent numerical simulations have shown that two adsorbed nanoparticles form a short linear cluster on the membrane surface of a vesicle at a large reduced volume but induce a short membrane tube on a vesicle at a small reduced volume [40]. This is consistent with our result in Fig. 2.9 in view of the fact that the conjugated membrane tension is greater at a larger reduced volume, though the cooperative wrapping of particles strongly depends on the range of interaction potential between particles and membrane [51]. Based on similar geometries and induced wrapping configurations, the particle-chain induced short linear aggregation of nanoparticles adhering on the surface and short membrane tubulation can be considered as examples analogous to the parallel configuration at large membrane tensions in the early stage of wrapping and perpendicular configuration at small membrane tensions in the late stage of wrapping, respectively.

As Fig. 2.9 indicates, 2D rigid rod-shaped nanoparticles or particles with relatively small reduced areas (e.g., $v = 0.6$) require larger adhesive energy $\bar{\gamma}$ to attain full wrapping than round particles or particles with larger reduced areas. This shape-dependent uptake of short nanoparticles has important implications on the design of efficient endocytosis and drug or gene delivery systems. It has been observed experimentally that ellipsoidal nanoparticles have a lower cellular uptake rate [5, 6, 8, 11]. For example, the rate of cellular uptake of round particles is greater than rod-shaped

particles with aspect ratio of 1 : 3, which in turn is greater than that of nanoparticles with even higher aspect ratio of 1 : 5 [5]. This is consistent with our theoretical results in Figs. 2.6 and 2.9. Taking advantage of the shape effects on cellular uptake, nanoparticles can be designed to be cylindrical or elliptical to extend their circulation time in blood [8, 23], or their labelling and tracking time as biomarkers on cell membranes [13]. Elastic rod-shaped nanoparticles can further amplify the shape effect (see Fig. 2.6). Through precise control of the reduced area of 2D elastic nanoparticles or the reduced volume of 3D elastic nanoparticles, the shape of these soft particles can be adjusted. Such elastic nanoparticles with tunable shapes may be used to design smart drug delivery systems. For example, researchers can design elongated drug nanoparticles (by controlling their enclosed volume in 3D) when the nanoparticles are in the circulation system and expand the particle to a rounder shape (by increasing the enclosed volume) to facilitate membrane wrapping as the particles reach their target cells. For nanoparticles coated with ligands, the adhesion energy $\gamma = \xi_L e_{RL}$ depends on the ligand density ξ_L and the energy of a single receptor–ligand bond e_{RL} . Taking typical parameter values as $\xi_L = 5 \times 10^3/\mu\text{m}^2$ and $e_{RL} = 15 k_B T$ [2], we have $\gamma = 0.075 k_B T/\text{nm}^2$. Considering an area-tunable nanoparticle with an effective radius of $a = 40 \text{ nm}$ interacting with a membrane with bending stiffness $\kappa_2 = 20 k_B T$ and tension $\sigma = 0.03 \text{ mN/m}$ [57], we have $\bar{\sigma} \approx 1.165$ and $\bar{\gamma} = 12$. As suggested in Fig. 2.8, such a nanoparticle with an initial reduced area $v = 0.6$ has a partial wrapping state and remains circulating before it reaches the target cell. As the nanoparticle reaches its target cell, an inflation of its reduced area to a value larger than 0.8 can be imposed to promote full wrapping followed by cell engulfment. This simple idea may be clinically useful as the remotely controlled drug delivery technology is under rapid development [58]. Elastic nanoparticles with or without area constraints can also be used to model or mimic many different types of nanoparticles such as hydrogel nanoparticles with high water content and pleo-

morphic viral particles appearing in different shapes in different situations.

A related but different phenomenon is the cell uptake of long one-dimensional (1D) nanomaterials via receptor-mediated endocytosis (see Chapter 4 for detailed discussion) in which a near-perpendicular entry mode is preferred at a small normalized membrane tension $\bar{\sigma}$ but a near-parallel interaction mode prevails at sufficiently large $\bar{\sigma}$ [18]. In that case, endocytosis can usually be considered as a process limited by the diffusion of receptors in the cell membrane to the contact region [2, 9, 18]. Our study discussed in Chapter 4 indicates that membrane wrapping regulated by two-dimensional diffusion of receptors is much faster than that regulated by one-dimensional receptor diffusion [2, 18]. For 1D nanomaterials, the membrane wrapping of their curved tips should be governed by two-dimensional diffusion of receptors, while the wrapping of their cylindrical walls is regulated by one-dimensional diffusion of receptors. This means that the tip, rather than the lateral wall, of a 1D nanomaterial should be wrapped first. As long as the specific receptor-ligand interaction energy can overcome the deformation energy penalty induced by the tip wrapping, the subsequent interaction is governed by the two interaction modes controlled only by $\bar{\sigma} \equiv 2\sigma a^2/\kappa_2$ with a being the radius of cylindrical nanomaterials [18]. For three-dimensional short nanorods with two curved tips and a much smaller lateral wall, the difference between receptor diffusion near the tip and wall regions are not evident; while for short nanorods wrapped by membrane in two dimensions as we focus in this study, the receptor diffusion is one-dimensional and has no difference between the tip and wall regions. In both cases, the specific receptor-ligand attractive interaction between short nanorods and cell membrane during the dynamic endocytosis can be approximated by adhesive interaction without the consideration of receptor diffusion as we did in the current study. Therefore, the initial wrapping of short nanorods would start at the relatively flat region of nanorods which appears

to be a parallel configuration of the short nanorods with respect to the membrane. Such nanorods then reorient themselves perpendicular to the membrane during the late stage of wrapping regardless of the value of membrane tension as long as the adhesion energy is large enough. The orientational change from a parallel configuration to a perpendicular one relative to the membrane is characteristic of the mode of interaction between short rod-shaped nanoparticles and cell membrane.

2.4 Summary

We have performed a theoretical analysis related to the cellular uptake of elastic rod-shaped nanoparticles with a prescribed reduced area in two dimensions. Using sequential quadratic programming, we have calculated the system energy, determined the corresponding morphologies of nanoparticles and the cell membrane, and obtained the associated wrapping phase diagrams describing transition boundaries between different wrapping phases as well as the nanoparticle orientation phase diagram describing transition boundaries between different nanoparticle orientations. Compared to nanoparticles without any area constraint displaying symmetric morphology during the whole wrapping process, nanoparticles with a prescribed reduced area undergo a dramatic morphological change from a symmetric configuration in the early stage of wrapping to a symmetry breaking transition in the mid-stage, then to a symmetric configuration again in the late stage of wrapping. For nanoparticles without area constraints, stiffer particles always attain full wrapping more easily than softer particles [36]. The geometric constraint of a fixed reduced area complicates the wrapping phase diagram. In the cases of a relatively large normalized membrane tension $\bar{\sigma}$ and relatively small reduced area v , stiffer particles can attain full wrapping more easily than softer particles; In the cases of small $\bar{\sigma}$ and large v ,

the situations become complicated and no general conclusions exist. In the limiting case of rigid and relatively stiff nanoparticles, the particle shape evolves from a circle to a rod as the reduced area v decreases. We find that larger normalized adhesion energy $\bar{\gamma}$ is required to attain full wrapping for slender nanoparticles corresponding to smaller reduced area v . In other words, thinner and longer two-dimensional rigid or stiff nanoparticles require larger adhesion energy $\bar{\gamma}$ than thicker, shorter and rounder ones for full wrapping. This shape-dependent cellular uptake of short nanoparticles has also been observed in experiments [5, 6, 8, 11]. Our results suggest that the particle elasticity and geometric restriction provide new design parameters to control cellular uptake and drug delivery processes. We also found that rigid and stiff rod-shaped nanoparticles align their long axes parallel to the membrane in the early stage of wrapping but suddenly reorient themselves with long axes perpendicular to the membrane and remain in this configuration in the late stage of wrapping. Such nanoparticle reorientation driven by the elastic deformation energy of the membrane is a generic mode of cell uptake of rod-shaped nanoparticles or any other relatively stiff short anisotropic nanoparticles. For soft nanoparticles, there exists a finite asymmetric morphological transition in the mid-stage of wrapping. The distinction in uptake between short rod-shaped nanoparticles and long one-dimensional nanomaterials is also discussed from the view of receptor-mediated endocytosis limited by diffusion of receptors.

The optimization method used here can be employed immediately in the study of interactions between multiple rigid [59, 60, 61] or soft particles in two dimensions, nanoparticle interaction with large cells [35, 50], and elastocapillary interactions between droplets and soft structures such as lipid membranes [62] and elastic thin films [63]. This method can also serve as a foundation for future studies taking into account the deformation of cytoskeleton network [64, 65].

CHAPTER THREE

Cellular Uptake of Round Elastic Nanoparticles

In this Chapter, we present a theoretical model in three dimensions on the adhesive wrapping of an elastic nanoparticle modeled as a fluid vesicle or a solid thin-shelled capsule, for a range of bending stiffness ratio between the particle and membrane. Using theoretical analysis we will show that the physical feature of the membrane wrapping of elastic nanoparticles is independent of the modeling of the elastic particle. We also show how the wrapping degree depends on the particle size, the adhesion energy, the surface tension of the membrane, and the bending stiffness ratio. We will determine the phase diagrams for cellular uptake of three-dimensional (3D) axisymmetric particles, probing the transitions between full wrapping, partial wrapping and no wrapping states. We will also discuss possible implications of our results in Chapter 2 and Chapter 3 on relevant biological processes.

3.1 Model and methods

3.1.1 Elastic nanoparticle modeled as fluid elastic vesicle

Similar to Chapter 2, here we model the engulfed nanoparticle as an elastic vesicle but with a constant total surface area A_t . Consider such an elastic particle wrapped by an initially flat membrane with elastic deformation in both the particle and the membrane, as shown in Fig. 3.1(a). The total energy of the system can be described by the Canham-Helfrich Hamiltonian as [32, 41, 47]

$$E = \sum_{i=1,2,3} \int (2\kappa_i H^2 + \kappa_i^g G) dA_i + \sigma \Delta A - \gamma A_3 + \Gamma A_t, \quad (3.1)$$

where H is the mean curvature, G is the Gaussian curvature, κ_i and κ_i^g are the bending and Gaussian moduli of the three regions, respectively; σ is the surface

tension of the membrane which is conjugated with the excess area ΔA induced by wrapping; γ is the specific adhesion energy and A_3 is the contact area. The last term arises from the constraint that the surface area A_t is fixed, Γ being the Lagrange multiplier. We assume zero pressure difference between the inside and outside of the nanoparticle. Although not considered in our model, a spontaneous curvature can be readily included in the formulation. Hereafter in this chapter we use subscripts 1, 2 and 3 to identify quantities associated with the inner free, outer free and the wrapped regions, respectively. To simplify our description, we assume that the Gaussian modulus of the adhesion region is $\kappa_3^g = \kappa_1^g + \kappa_2^g$, which implies that the Gaussian curvature does not affect the vesicle shape due to the Gauss-Bonnet theorem [66]. We will further assume $\kappa_3 = \kappa_1 + \kappa_2$ throughout the analysis as we have done in Chapter 2. All length scales are scaled by the effective radius of the vesicle $a = \sqrt{A_t/(4\pi)}$. Other dimensionless parameters are $\bar{\gamma} = 2\gamma a^2/\kappa_2$ and $\bar{\sigma} = 2\sigma a^2/\kappa_2$.

The axisymmetric shapes in Fig. 3.1(a) are determined from the tangent angle $\psi(s)$ with geometric relations $\dot{r} = \cos \psi$ and $\dot{z} = \sin \psi$, where dots denote derivatives with respect to the rescaled arclength s of the vesicle or to arclength t of the outer free part of the membrane. For the axisymmetric configuration, $H = \frac{1}{2} \left(\dot{\psi} + \frac{\sin \psi}{r} \right)$, $\Delta A = 2\pi \int r(1 - \cos \psi) ds$, and variation of the energy functional in Eq. (3.1) gives rise to the following governing equation for the vesicle shape

$$\begin{cases} \ddot{\psi} = \frac{1}{r} \left(\frac{\sin \psi}{r} \cos \psi - \dot{\psi} \cos \psi + \frac{\kappa_2}{\kappa_1 + \kappa_2} \frac{\mu}{2} \sin \psi \right), \\ \dot{\mu} = \frac{\kappa_1}{\kappa_2} \left(\dot{\psi} + \frac{\sin \psi}{r} - c_0 \right) \left(\dot{\psi} - \frac{\sin \psi}{r} - c_0 \right) + \left(\dot{\psi}^2 - \frac{\sin^2 \psi}{r^2} \right) + \eta, \\ \dot{r} = \cos \psi \end{cases} \quad (3.2)$$

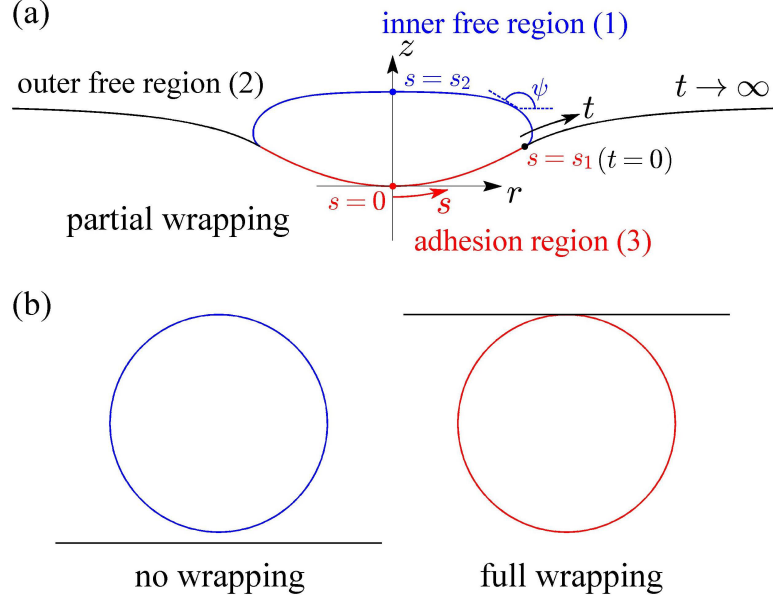


Figure 3.1: Schematic of a soft elastic particle modeled as a vesicle and wrapped by an initially flat membrane. (a) The geometry of the system with wrapping angle α and tangent angle ψ . Arclengths s and t are defined along the vesicle and along the outer free part of the membrane, measured from the bottom pole ($s = 0$) and the adhesion edge $t = 0$ ($s = s_1$), respectively; r_0 is the $r(s)$ coordinate at $s = s_1$. (b) Schematic of the three characteristic wrapping states. No wrapping is the state with zero contact area. Full wrapping is the state in which the left and right sides of the membrane touch each other on the top of the vesicle. Partial wrapping corresponds to the intermediate scenario with incomplete wrapping.

for the wrapped region of the vesicle, and

$$\begin{cases} \ddot{\psi} = \frac{1}{r} \left(\frac{\sin \psi}{r} \cos \psi - \dot{\psi} \cos \psi + \frac{\mu}{2} \sin \psi \right), \\ \dot{\mu} = \left(\dot{\psi} + \frac{\sin \psi}{r} - c_0 \right) \left(\dot{\psi} - \frac{\sin \psi}{r} - c_0 \right) + \eta, \\ \dot{r} = \cos \psi \end{cases} \quad (3.3)$$

for the inner free part of the vesicle. The equations that govern the shape of the outer free part of the membrane are [32]

$$\begin{cases} \ddot{\psi} = \frac{1}{r} \left(\frac{\sin \psi}{r} \cos \psi + \frac{\bar{\sigma} r}{2} \sin \psi - \dot{\psi} \cos \psi + \frac{\mu}{2} \sin \psi \right), \\ \dot{\mu} = \dot{\psi}^2 - \frac{\sin^2 \psi}{r^2} + \bar{\sigma} (1 - \cos \psi), \\ \dot{r} = \cos \psi. \end{cases} \quad (3.4)$$

The boundary conditions are $\psi_3(0) = 0$ and $r_3(0) = 0$ at $s = 0$, and $\psi_1(s_2) = \pi$ and $r_1(s_2) = 0$ at $s = s_2$. The remote boundary conditions are $\lim_{t \rightarrow \infty} \psi_2(t) = 0$ and $\lim_{t \rightarrow \infty} \dot{\psi}_2(t) = 0$ as $t \rightarrow \infty$, which enforce the asymptotic flatness of the membrane at large distances [32]. At the adhesion edge $s = s_1$, the radial coordinate r and tangent angle ψ must be continuous. The equilibrium configurations of the vesicle and membrane can be found numerically by solving Eqs. (3.2-3.4). To calculate the energy at any given value of the wrapping degree $f = A_3/A_t$, we vary the values of $r_0 (\in [0, s_1])$ and α with a step size 0.0005 in r_0 and 0.001 in α for a given f , obtain solutions by the shooting method and then determine the shapes of the vesicle and membrane from solution with the lowest energy. An equilibrium state corresponds to a local extremum of free energy as f increases from 0 to 1. A similar method has been used to study the axisymmetric equilibrium shapes of erythrocytes considering vesicle-vesicle adhesion [67].

3.1.2 Elastic nanoparticle modeled as solid thin-shelled capsule

In the above Subsection 3.1.1, the elastic nanoparticle have been modeled as an elastic vesicle. In two dimensions, the membrane wrapping of an elastic vesicle with a given circumference is mathematically equivalent to the wrapping of a capsule enclosed with an inextensible elastic thin shell as discussed in Chapter 2. In three dimensions, the curved lipid bilayer membrane of a vesicle can be flattened onto a plane without surface stretching and breaking through lipid flow; while the solid elastic thin shell of a capsule can only be flattened a little before breaking caused by the high local stretching. This indicates that the deformation of fluid vesicle and solid thin-shelled capsule is not mathematically equivalent in 3D. To understand how

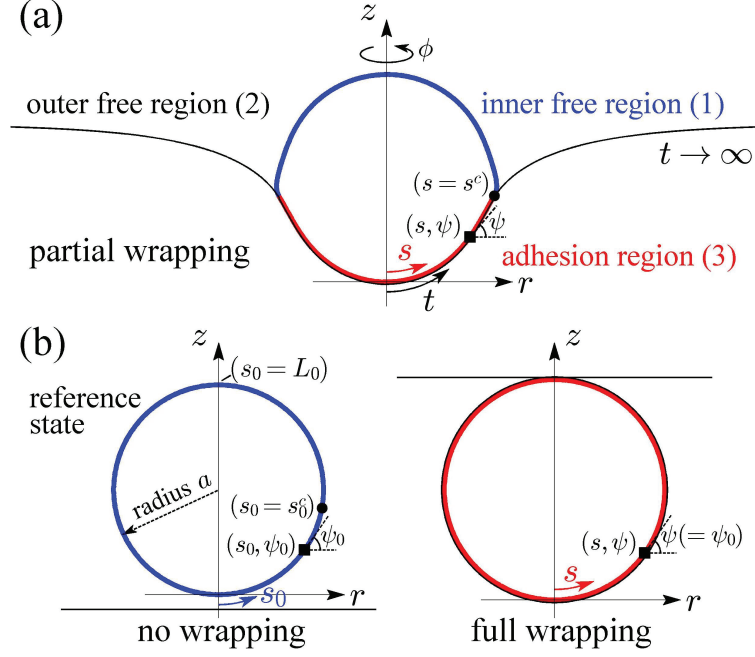


Figure 3.2: Schematic of an elastic thin-shelled spherical capsule (thick line) of an initial radius a wrapped by an initially flat membrane (thin black line). (a) The geometry of the system with tangent angle ψ . Arclengths s and t are defined along the capsule and membrane, measured from the bottom pole ($s = t = 0$), respectively. (b) Schematic for the states of no wrapping (reference state) with zero contact length and full wrapping in which the capsule is fully covered by the membrane. Partial wrapping corresponds to the intermediate scenario with incomplete wrapping as shown in (a).

the membrane wrapping is influenced by a solid thin-shelled capsule, we consider a spherical thin-shelled capsule undergoing an axisymmetric deformation induced by the wrapping of the cell membrane in the adopted cylindrical coordinate (r, ϕ, z) (Fig. 3.2). Since the axisymmetric deformation of the capsule and membrane is independent of the circumferential coordinate ϕ , we restrict our attention to any cross-section of the configuration (e.g., $\phi = 0$). Before the capsule contacts the membrane, the capsule remaining in a stress-free state takes a spherical shape which is taken as its reference configuration [Fig. 3.2(b)]. A material point located at $(r = r_0, z = z_0)$ on that cross-section of the thin-shell in the undeformed reference configuration can be generally parameterized by the curvilinear coordinates $s_0 \in$

$[0, L_0]$ and $\psi_0 \in [0, \pi]$ with the geometrical relations

$$dr_0/ds_0 = \cos \psi_0 \quad \text{and} \quad dz_0/ds_0 = \sin \psi_0.$$

Here s_0 is the arclength measured along the meridian, L_0 denotes the half of the circumference of the cross-section considered, and ψ_0 is the tangent angles. The surface area is $A_0 = 2\pi \int_0^{L_0} r_0 ds_0$. The principal curvatures at the point (s_0, ψ_0) in the meridional (longitudinal) and circumferential (latitudinal) directions are respectively given as

$$c_{s_0} = d\psi_0/ds_0 \quad \text{and} \quad c_{\phi_0} = \sin \psi_0/r_0.$$

For the spherical capsule of a radius a considered here, we have $L_0 = \pi a$, $\psi_0 = s_0/a$, $r_0 = a \sin \psi_0$, $z_0 = a(1 - \cos \psi_0)$, and $c_{s_0} = c_{\phi_0} = 1/a$.

As the membrane wraps around the capsule, the wrapping state evolves from the state of no wrapping (defined as the reference state in Fig. 3.2(b)) to partial wrapping [Fig. 3.2(a)]. In the state of partial wrapping, the material point (s_0, ψ_0) located at (r_0, z_0) is displaced by the membrane to the point (r, z) with the curvilinear coordinate (s, ψ) in the deformed configuration which can be measured by the longitudinal stretch λ_s and the latitudinal stretch λ_ϕ in the meridional and circumferential directions, respectively, as

$$\lambda_s = ds/ds_0 \quad \text{and} \quad \lambda_\phi = r/r_0.$$

For a linearly elastic isotropic shell of a thickness h , the strain energy density W_s is [68]

$$W_s = \frac{Eh}{2(1-\nu^2)}(e_s^2 + 2\nu e_s e_\phi + e_\phi^2) + \frac{D}{2}(C_s^2 + 2\nu C_s C_\phi + C_\phi^2),$$

where E is the Young modulus, $\nu \in [-1, 1]$ is the Poisson ratio of the two-dimensional shell,

$$e_s = \lambda_s - 1 \quad \text{and} \quad e_\phi = \lambda_\phi - 1$$

are the meridional and circumferential strains, respectively. $D = Eh^3/[12(1 - \nu^2)]$ is defined as the bending stiffness of the thin shell, and C_s and C_ϕ are the meridional and circumferential bending strains, respectively, given as

$$C_s = \lambda_s c_s - c_{s_0} \quad \text{and} \quad C_\phi = \lambda_\phi c_\phi - c_{\phi_0},$$

with the meridional curvature $c_s = d\psi/ds$ and circumferential curvature $c_\phi = \sin \psi/r$.

The elastic energy density of the deformed cell membrane is

$$W_m = \frac{\kappa_m}{2} (c_s^{(m)} + c_\phi^{(m)})^2 + \sigma(1 - \cos \psi_m),$$

where κ_m , $c_s^{(m)} = d\psi_m/dt$, $c_\phi^{(m)} = \sin \psi_m/r$, ψ_m , and t are the bending stiffness, meridional curvature, circumferential curvature, tangent angle, and arclength of the cell membrane, respectively. The total energy of the system is

$$E_{tot} = \int_0^{L_0} 2\pi r_0 W_s ds_0 + \int_0^\infty 2\pi r W_m dt - \gamma A_3, \quad (3.5)$$

where γ is the adhesion energy and $A_3 = \int_0^{s^c} 2\pi r ds$ is the contact area with $s = s^c$ as the arclength at the contact edge. The contact edge corresponds to the material point whose arclength is $s_0 = s_0^c$ in the reference configuration. The wrapping degree f for a certain wrapping state is defined as $f = \int_0^{s_0^c} 2\pi r_0 ds_0 / A_0$, that is, the corresponding area of the contact region in the reference configuration divided by the total surface area A_0 of the undeformed capsule.

Here we employ a numerical optimization technique to determine the minimum energy state at each given wrapping degree f . With geometric relations $dr/ds = \cos \psi$ and $dz/ds = \sin \psi$ and the notation of the longitudinal stretch $\lambda_s = ds/ds_0$, the deformation of the capsule as well as the membrane attaching on it is determined by the tangent angle ψ and the longitudinal stretch λ_s , while the shape of the outer free membrane is determined by tangent angle ψ . The functions of tangent angle ψ and the longitudinal stretch λ_s in the adhesion region and inner free region, and the tangent angle function ψ in the outer free region are approximated by cubic B-spline curves as [69]

$$\begin{aligned} \psi_2(a_j^{(2)}, t) &= \sum_{j=0}^n a_j^{(2)} N_j(t), \\ \psi_i(a_j^{(i)}, s_0) &= \sum_{j=0}^n a_j^{(i)} N_j(s_0), \quad \text{and} \quad \lambda_i(b_j^{(i)}, s_0) = \sum_{j=0}^n b_j^{(i)} N_j(s_0) \quad (i = 1, 3). \end{aligned} \quad (3.6)$$

Here control points $a_j^{(i)}$ and $b_j^{(i)}$ are the coefficients of the cubic basis functions N_j with integer indices $0 \leq j \leq n$. The basis functions $N_j(t)$ and $N_j(s_0)$ as presented in Eq. (A.1) can be determined explicitly by specifying sequences of the variable parameters t and s_0 , respectively. A typical choice of the knot vector for a parameter $\xi \in [p, q]$ is taken as $\{\xi_0, \dots, \xi_{n+4}\}$ with $\xi_i = p$ ($i = 0, \dots, 3$) and $\xi_j = q$ ($j = n+1, \dots, n+4$). Here n is chosen as $n = 100$.

The boundary and constraint conditions provide either input parameters or equality constraints during energy minimization. At $t \rightarrow \infty$, the outer free membrane becomes asymptotically flat which requires $\psi_2|_{t \rightarrow \infty} = 0$ or $a_n^{(2)} = 0$. Similarly, $\psi_1|_{s_0=L_0} = 0$ and $\psi_3|_{s_0=0} = 0$ require $a_n^{(1)} = 0$ and $a_0^{(3)} = 0$, respectively. At the contact edge $s_0 = s_0^c$ (or $s = s^c$), the continuity of tangent angle requires $a_0^{(1)} = a_n^{(3)} = a_0^{(2)}$. The continuity of the coordinate (r, z) at the contact edge is enforced as equality constraints. The total energy E_{tot} in Eq. (3.5) as a function of $\psi_i(s_i)$ under these constraints at a given f and adhesion energy γ is minimized

with respect to the coefficients a_j and b_j using the interior point method [46]. Once the tangent angles ψ and the longitudinal stretch λ_s given by Eq. (3.6) are known, the total energy and corresponding shapes (of the capsule and membrane) can be determined.

At the full wrapping state, the spherical capsule with an initial radius a deforms into another sphere with radius ρ . In that state, $\lambda \equiv \lambda_s = \lambda_\phi = \rho/a$, $e_s = e_\phi = \lambda - 1$, and $K_s = K_\phi = 0$, and $W_s = Eh(\lambda - 1)^2/(1 - \nu)$. Since the membrane elastic energy of the outer free region at the full wrapping state is extremely small and negligible compared with the elastic energy of membrane adhering on the capsule, the total energy of the system at full wrapping can be approximated as the sum of the adhesion energy and the elastic energy of the capsule and adhering membrane, that is,

$$E_{tot} = 4\pi a^2 \frac{Eh}{(1 - \nu)} (\lambda - 1)^2 + 2\pi\lambda^2\kappa(\bar{\sigma} - \bar{\gamma}) + 8\pi\kappa_2,$$

with the normalized membrane tension $\bar{\sigma} \equiv 2\sigma a^2/\kappa_2$ and normalized adhesion energy $\bar{\gamma} \equiv 2\gamma a^2/\kappa_2$. $dE/d\lambda = 0$ gives

$$\lambda = \frac{2Ea^2h}{2Ea^2h + \kappa(\bar{\sigma} - \bar{\gamma})(1 - \nu)}.$$

The necessary condition for a stable full wrapping state is $E_{tot} < 0$ (or $\bar{\gamma} > \bar{\sigma}$), which means that $\lambda > 1$ or the spherical capsule is enlarged by the adhesive membrane.

3.2 Results

3.2.1 Results based on fluid vesicle model

We first focus on the fluid vesicle model proposed in Subsection 3.1.1. Figure 3.3 shows the energy change $\Delta E = E - E_0$ as a function of the wrapping degree f , where E_0 is the ground state energy taken as $8\pi\kappa_1$ for a spherical vesicle. The 3D axisymmetric configuration exhibits five possible phases (I-V) as introduced in Ref. [32] [see Fig. 3.3(a)]. In phase I, $\bar{\gamma}$ is low and ΔE increases monotonically with f . As $\bar{\gamma}$ increases, there exists a stable state (no wrapping) and a metastable partial wrapping state and phase II arises. Further increase of $\bar{\gamma}$ results in a global minimum at a partial wrapping state with an energy barrier to reach the metastable full wrapping state (phase III). With increasing $\bar{\gamma}$ the stable partial wrapping state becomes metastable with respect to a stable full wrapping state (phase IV). If $\bar{\gamma}$ is large enough, the energy barrier vanishes and the full wrapping state becomes the only stable state in phase V. For each phase, there is a stable state (global energy minimum) and possibly a metastable state (local energy minimum). For very soft particles (e.g., $\kappa_1/\kappa_2 < 1$), the phase II' no longer has no wrapping as the stable state and partial wrapping as a metastable state; instead the no wrapping state becomes unstable while partial wrapping becomes stable [see Fig. 3.3(b)]. There are three cases of possible energy evolution profiles as a function of the wrapping degree. Case 1 exhibits a global energy minimum at a relatively small f and possibly a metastable state at a relatively large f ; case 2 shows a global minimum at a relatively large f and a possible metastable state at a relatively small f ; case 3 has only one global minimum at large f . Fig. 3.4 shows sequences of vesicle-membrane configurations at $\bar{\sigma} = 2$ for different stiffness ratios κ_1/κ_2 .

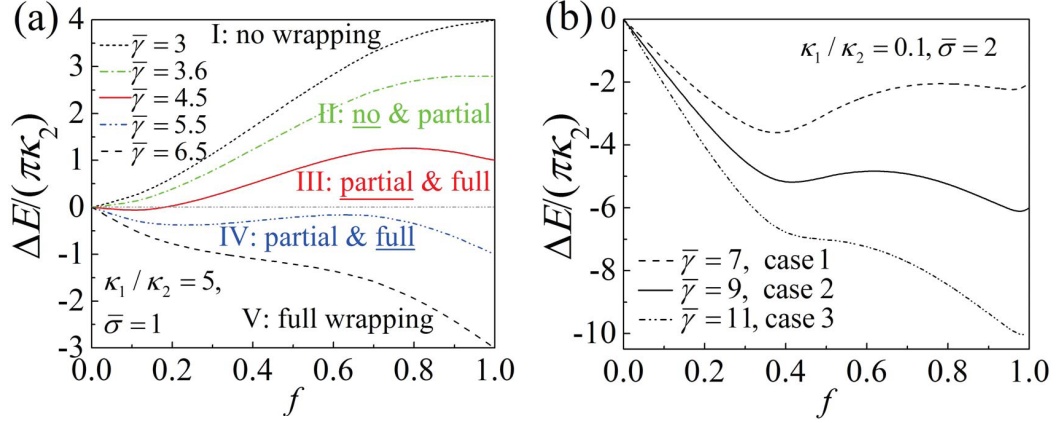


Figure 3.3: Energy change ΔE as a membrane wraps around a particle with wrapping degree f for different $\bar{\gamma}$ at (a) $\kappa_1/\kappa_2 = 5$ and $\bar{\sigma} = 1$, and (b) $\kappa_1/\kappa_2 = 0.1$ and $\bar{\sigma} = 2$. In general, there exist 5 distinct wrapping phases in 3D. Plots shown in (a) show that, under the selected parameters, there are 2 stable (I, V) and 3 metastable phases (II-IV). Among the 3 metastable phases, the underlined wrapping states in (a) are the ones with lower energy. Plots in (b) show typical energy landscapes of the three cases in phase II'.

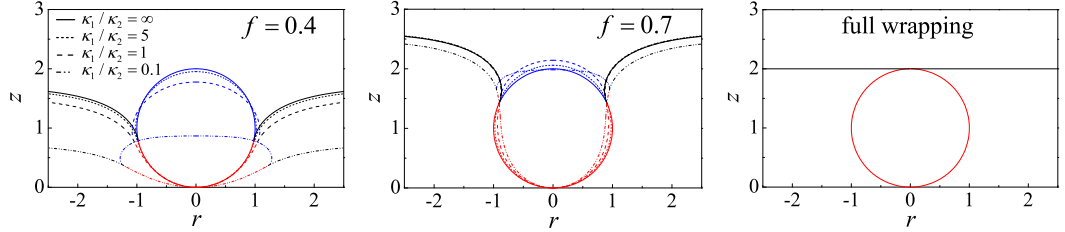


Figure 3.4: Solutions to selective wrapping configurations at $\bar{\sigma} = 2$ for different particle-membrane stiffness ratios κ_1/κ_2 .

With the knowledge of energy functions for κ_1/κ_2 , $\bar{\sigma}$ and $\bar{\gamma}$, the phase diagrams of wrapping have been calculated and shown in Fig. 3.5. Here we take $f = 1$ as the full wrapping condition for simplicity. For a rigid spherical particle, $\bar{\gamma}_{\min} = 4$ [32]. There is no phase II for $\kappa_1/\kappa_2 = 1$ and 0.1 since the adhesion energy can compensate for the bending energy at the initial wrapping process for small κ_1/κ_2 [Fig. 3.5(c) and (d)]. The effects of κ_1/κ_2 on the phase transition is very small when $\kappa_1/\kappa_2 \geq 1$ but large for very soft vesicles ($\kappa_1/\kappa_2 = 0.1$). The thin (dash-dotted and solid) lines in Fig. 3.5(c) and (d) correspond to the boundaries between these three cases in Fig. 3.3(b). In case 3 the energy change between the global minimum and the full wrapping state ($f = 1$) is about $4k_B T$ with $\kappa_2 = 20k_B T$, and might be overcome by thermal fluctuations. This indicates that the thin solid line in Fig. 3.5(d) can

be viewed as the transition line between phase II' and phase V approximately. For readers who are less interested in the precise transitions between these five possible phases but are more willing to get a rough understanding of the transitions, the four subfigures in Fig. 3.5 are merged into a concise phase diagram (Fig. 3.6) where the state of no wrapping represents a combination of phases I and II in Fig. 3.5(a) and (b) or phase I in Fig. 3.5(c) and (d), the partial wrapping state represents the combination of phases III and IV in Fig. 3.5(a) and (b), and phases II', III, and IV in Fig. 3.5(c) and (d), and the full wrapping state represents phase V. A general result from Fig. 3.5 or Fig. 3.6 is that stiffer particles require lower adhesion energy for wrapping. Comparison with Fig. 2.6(d) indicates that $\bar{\gamma}_{\min}$ is larger in 3D than in two-dimensions (2D) for a given κ_1/κ_2 , since the bending energy density is larger in 3D. For a given κ_1/κ_2 , the critical value of $\bar{\gamma}$ for full wrapping is larger in 3D when $\bar{\sigma}$ is small ($\bar{\sigma} < 1$ for $\kappa_1/\kappa_2 \geq 1$, $\bar{\sigma} < 0.5$ for $\kappa_1/\kappa_2 = 0.1$). When $\bar{\sigma}$ exceeds a certain value, the critical value of $\bar{\gamma}$ for full wrapping in 3D becomes smaller than that in 2D, indicating that the uptake of a two-dimensional particle could not be accomplished even if full wrapping becomes possible in three-dimensions. In such cases, spherical particles can be absorbed when cylindrical ones cannot.

3.2.2 Results based on solid thin-shelled capsule model

We now investigate the membrane wrapping of a solid thin-shelled capsule with different bending stiffness D . Although the bending stiffness of a solid shell D is not physically equivalent to the membrane bending stiffness κ_m , the ratio D/κ_m can still be regarded as a measure of the relative difference between thin shell and cell membrane in bending property. Fig. 3.7 compares the elastic deformation energy $E_{el} \equiv E_{tot} + \gamma A_3$ in Eq. (3.5) of the capsule-membrane system with elastic energy

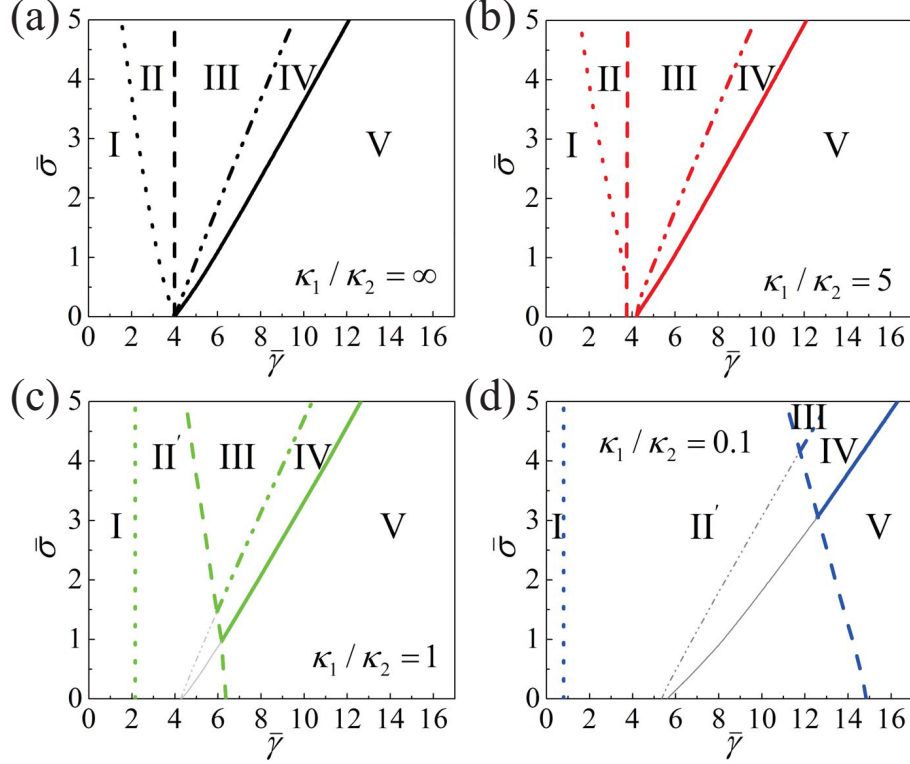


Figure 3.5: Wrapping phase diagrams with respect to normalized adhesion energy $\bar{\gamma}$ and surface tension $\bar{\sigma}$ at different values of the stiffness ratio κ_1/κ_2 . (a-d) Cases for $\kappa_1/\kappa_2 = \infty, 5, 1, 0.1$, dotted lines: boundaries between phases I and II(II'), dashed lines: boundaries between phases II(II') and III (III-V for $\kappa_1/\kappa_2 = 1, 0.1$), dash-dotted lines: boundaries between phases III and IV, solid lines: boundaries between phases IV and V.

$\Delta E = E - E_0$ of the vesicle-membrane system at $\gamma = 0$. It is shown that elastic thin-shelled capsule behaves stiffer than the vesicle particle with the same value of bending stiffness (Fig. 3.7). This results from the high stretching energy involved in the capsule deformation. For stiff capsule and vesicle particles with the same values of bending stiffness (e.g., $D = \kappa_1 = 5\kappa_m$), their elastic energy profiles exhibit slight difference. For soft vesicle particles (e.g., $\kappa_1/\kappa_2 = 0.1$), the elastic energy profile exhibits a significant decrease compared with stiff particles; while for relatively soft thin-shelled capsules with the same value of bending stiffness as soft vesicle particles (e.g., $D/\kappa_m = 0.1$), a relatively slight energy decrease is observed.

For thin-shelled capsules (see Fig. 3.8), there also exhibits five possible wrapping phases as displayed in Fig. 3.3. Comparison with phase diagrams for vesicle particles

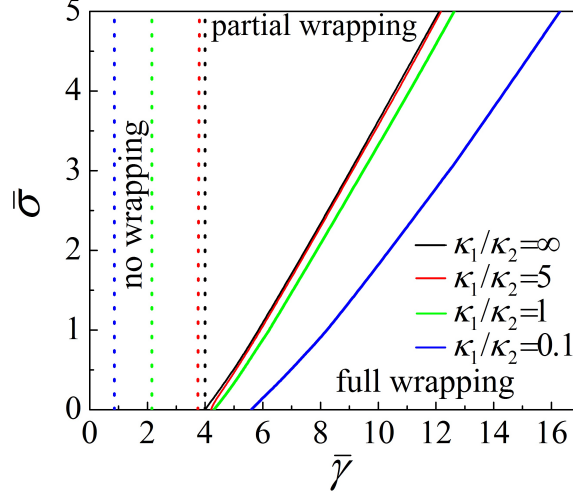


Figure 3.6: Concise wrapping phase diagram with respect to the normalized adhesion energy $\bar{\gamma}$ and surface tension $\bar{\sigma}$ at different values of the stiffness ratio κ_1/κ_2 . Dotted lines: boundaries between no wrapping and partial wrapping states; solid lines: boundaries between partial and full wrapping states.

(Fig. 3.5) indicates that elastic thin-shelled capsule behaves stiffer than the vesicle particle with the same value of bending stiffness, especially in the cases of soft particles (e.g., $D/\kappa_m = 1, 0.1$).

3.3 Discussion

The phase diagrams shown in Fig. 3.5 and Fig. 3.8 may have broad implications for endocytosis/phagocytosis and drug/gene delivery processes. If the adhesive interaction between a particle and membrane is not strong enough, it will be difficult for the cell membrane to engulf a very soft particle. To achieve the full wrapping state and engulf the particle, the bending modulus of the particle needs to increase. Protein coating formation and actin polymerization beneath the membrane are effective methods to increase bending modulus of the particle. For example, binding of clathrin-associated proteins leads to a significant increase in the stiffness of clathrin-coated vesicles which is about 20 times that of the vesicle membrane alone [70]. For

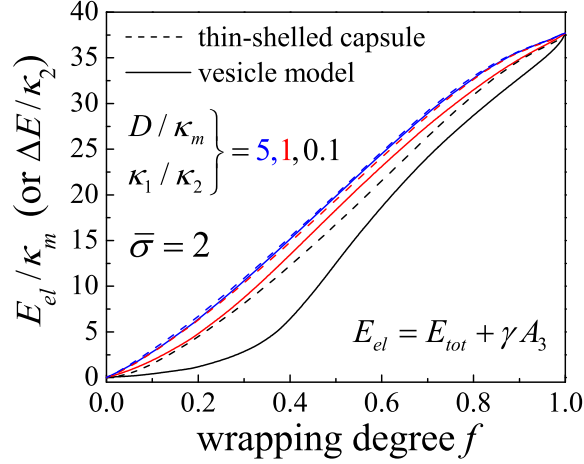


Figure 3.7: Elastic energy change as a membrane wraps around a soft particle modeled as a solid thin-shelled capsule or a fluid vesicle nanoparticle at $\bar{\sigma} = 2$ and $\bar{\gamma} = 0$ for different particle-membrane stiffness ratios D/κ_m (or κ_1/κ_2).

streptavidin- and avidin-coated vesicles $\kappa_1 \approx 320k_B T$ and $\kappa_1 \approx 115k_B T$, respectively, which are much larger than bare SOPC/capBio-DOPE vesicles with $\kappa_1 \approx 10k_B T$ [71]. The actin concentration inside the phagocytic cup has been estimated to increase the local stiffness by a factor of 5 [72]. The phase diagrams in Figs. 3.5 and 3.8 provide a possible alternative view on why clathrin and other protein coatings as well as actin polymerization are involved in many endocytosis/phagocytosis processes.

Macrophages have a preference to engulf rigid targets with more actin filaments concentrated beneath stiffer particles [22]. In some cases, infected macrophages are drug targets; while in other cases, macrophages are not target cells but act as barriers by phagocytosis preventing particles from releasing their therapeutic cargos near or within target cells [73]. Figs. 2.7(d), 3.5 and 3.8 demonstrate that uses of soft particles and cylindrical particles can postpone or prevent uptake. An alternative method is to increase the bending modulus of the cell membrane. For three-dimensional particles, the effects of particle elasticity on cellular uptake is only evident when $\kappa_1 < \kappa_2$. Worm-like viruses and particles with very high aspect ratios [6] may be considered two-dimensional vesicles. For these types of particles, controlling the particle stiff-

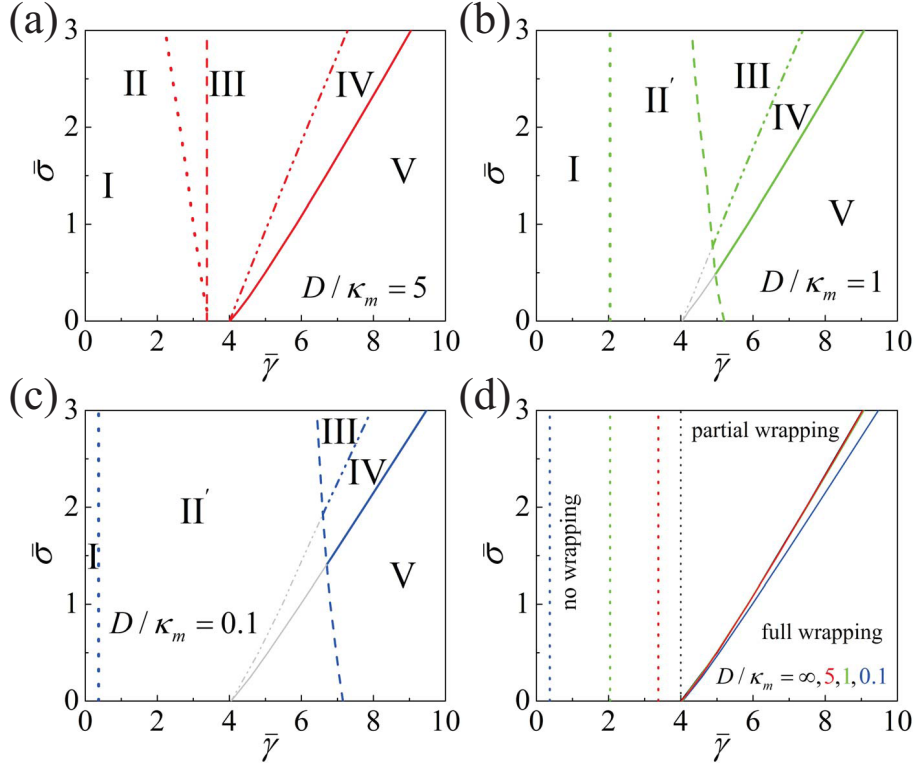


Figure 3.8: Wrapping phase diagrams for solid thin-shelled capsule with respect to normalized adhesion energy $\bar{\gamma}$ and surface tension $\bar{\sigma}$ at different values of the bending stiffness ratio D/κ_m . (a-c) Cases for $D/\kappa_m = 5, 1, 0.1$, dotted lines: boundaries between phases I and II(II'), dashed lines: boundaries between phases II(II') and III (III-V for $D/\kappa_m = 1, 0.1$), dash-dotted lines: boundaries between phases III and IV, solid lines: boundaries between phases IV and V. Case for $D/\kappa_m = \infty$ is the same as Fig. 3.5(a). (d) Concise wrapping phase diagram for solid thin-shelled capsule with respect to normalized adhesion energy $\bar{\gamma}$ and surface tension $\bar{\sigma}$ at different stiffness ratio D/κ_m . Dotted lines: boundaries between no wrapping and partial wrapping states; solid lines: boundaries between partial and full wrapping states.

ness can be essential for controlling cellular uptake. Many particles can be used as soft drug delivery particles such as polymer particles, nanocapsules and nanogel with high water content. The elasticity- and geometry-induced inhibition of cellular uptake will have important applications in the use of those particles as drug delivery carriers.

Experiments show that MLV and HIV particles are stiff during viral budding out of the host and soften during entry activities [27, 28]. For these viral particles, entry into cell often involves endocytosis and membrane fusion, in which case full wrapping is not necessary for the uptake process. In contrast, the budding process

involves no membrane fusion and requires full wrapping, so that the stiffness of the particle can be expected to play a more important role. However, softer particles experience smaller energy changes during the wrapping process (see Chapter 2 and Fig. 2(a) in Ref. [36] for 2D cases) which might be preferred in case full wrapping is not required. These results provide feasible explanations why viral particles harden right before budding and then soften again in the uptake stage [27, 28].

Our analysis suggests that the reason why soft particles are less prone to wrapping than stiff ones could be understood as follows. For a rigid particle, the adhesive interaction between the particle and membrane simply forces the membrane to deform and wrap around the particle. In contrast, Figs. 2.3 and 3.4 show that for a soft particle, the deformation is partitioned between the particle and the membrane at different stages of wrapping. A very soft particle would initially spread along the membrane without significant membrane deformation. Only at a later stage will the membrane be forced to bend around the particle. Thus, wrapping around a rigid particle involves a gentler rise in elastic energy in the membrane as more membrane area deforms around the particle. When wrapping around a soft particle, the membrane does not deform initially but then needs to catch up to almost the same configuration at full wrapping. This means more abrupt rise in elastic energy at the later stage of wrapping and, consequently, larger adhesion energy would be required to balance the more rapid rise in elastic energy. Since it is this partition of deformation at the early stage of wrapping that hinders the full wrapping of a soft particle, alternative particle models, such as a particle with a bulk elastic modulus or a thin-shell with shear rigidity, are not expected to change this basic feature of particle-membrane interaction. Therefore, the conclusions of our study should be generic and not specific to the modeling of the soft particle.

3.4 Summary

We have performed theoretical analysis to study the cellular uptake of elastic nanoparticles in three-dimensional axisymmetric configuration. Using variational methods and free energy functional for cell membrane wrapping around elastic spherical particles, we have calculated the associated phase diagrams describing transition boundaries between different wrapping phases. We find that stiffer particles can achieve full wrapping more easily than softer particles, while softer particles experience smaller energy changes during wrapping and might be more favorable in case full wrapping is not necessary. Our results suggest that precise control of the particle elasticity can be another appealing way to control cellular uptake. The present model can be extended to problems such as phase separations and assembly of programmable soft materials.

CHAPTER FOUR

Cell Uptake of One-Dimensional Nanomaterials

Although extensive studies have been conducted on cellular uptake of (spherical) nanoparticles, relatively little is known about how cells interact with one-dimensional (1D) nanomaterials with radius at the nanoscale but length at a much larger scale, such as nanotubes and filamentous bacteria [9, 74, 75]. Recent experiments showed that carbon nanotubes undergo endocytic entry into liver and mesothelial cells through tip recognition and then rotation to a near-perpendicular entry mode [9]. Similar tip entry phenomena were also observed in phagocytosis of high-aspect ratio filamentous *Escherichia coli* bacteria [75]. In another type of experiment, it was found that nanoparticles adhering to a substrate-supported flat phospholipid vesicle spontaneously form particle-chains encapsulated inside lipid tubes that protrude out of the vesicle at low membrane tension, but when the membrane tension is increased by adding Ca^{2+} to enhance adhesion between the vesicle and substrate, the lipid tubes retract back to the vesicle [76]. Here we show that these seemingly different phenomena can all be understood with a unified theoretical model of an elastic fluid membrane interacting with a 1D nanomaterial.

The above experiments suggest that there exist two basic modes of interaction between cells and 1D nanomaterials: tip recognition followed by near-perpendicular entry versus near-parallel adhering on the membrane. However, the underlying mechanisms of different modes of interaction have not been elucidated. In this Chapter, we present the first theoretical model on cellular uptake of 1D nanomaterials, which accurately accounts for the elastic deformation energy associated with the wrapping of an initially flat membrane around a nanotube oriented at different orientations with respect to the membrane. First, we note that a lipid membrane, subjected to various types of loading, deforms and equilibrates immediately on a time scale of less than one second [77, 78, 79]. By contrast, cell uptake of nanomaterials, with a time scale from hundreds of seconds to tens of minutes [80, 81, 82], can usually

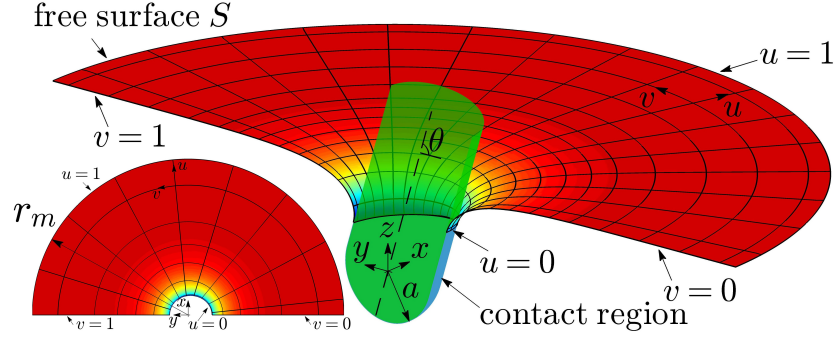


Figure 4.1: Schematic of an initially flat membrane wrapping around a nanotube (green). A nanotube of radius a is seen to enter membrane at an angle θ . Due to limited rate of receptor diffusion, the total area of the contact region (blue) is assumed to be fixed in the analysis while the nanotube can adjust the entry angle θ to reduce the system energy. Curvilinear coordinates $u, v = 0, 1$ define the boundaries of the free part S of the membrane, and colors on S denote the relative height of the membrane. The hemispherical cap of the nanotube is bounded by $x^2 + y^2 + z^2 = a^2$ and $z \sin \theta - y \cos \theta = 0$ in the adopted Cartesian coordinate xyz . (Lower left corner) Top view of the free part S .

be regarded as a process limited by diffusion of receptors in the cell membrane toward the contact region [2, 9]. For simplicity, here we do not consider the dynamic process of receptor diffusion in the membrane, rather we focus on a relatively short time window around a given point of time in which the total area of the contact region remains constant while the nanotube adjusts its entry angle to reduce the energy of the system. It will be shown that membrane tension, membrane bending stiffness, and tube radius collectively determine the uptake mode through a single dimensionless parameter.

4.1 Model and methods

Without loss of generality, we consider an initially flat membrane wrapping around a semi-infinite cylindrical nanotube with a hemispherical end cap (Fig. 4.1). The total energy of the system is expressed in terms of Canham-Helfrich functional as [32, 41]

$$E_{tot}(\theta) = E_b + \sigma \Delta A - \gamma A_c, \quad (4.1)$$

where $E_b = \int 2\kappa H^2 dA$ is the bending energy, κ being the bending rigidity of the membrane, H the local mean curvature, and dA the membrane surface element; σ is the membrane tension and ΔA the excess surface area induced by wrapping; γ is the specific adhesion energy and A_c is the contact area. The bending energy associated with the Gaussian curvature remains constant for the problem and is neglected. Spontaneous curvature is not considered here. All length scales are scaled by the nanotube radius a . Other dimensionless parameters are $\bar{\gamma} \equiv 2\gamma a^2/\kappa$ and $\bar{\sigma} \equiv 2\sigma a^2/\kappa$.

Since the contact area A_c is assumed to be constant, the minimum energy configuration of the nanotube-membrane system should only depend on the single dimensionless parameter $\bar{\sigma}$, which represents the ratio of membrane tension and bending energies. The bending energy plays a dominant role for $\bar{\sigma} \ll 1$, while the tension energy should do so for $\bar{\sigma} \gg 1$. Based on the value of $\bar{\sigma}$, there are two limiting cases: the limit of zero-bending stiffness ($\kappa = 0$), corresponding to extremely large $\bar{\sigma} \rightarrow \infty$, and the limit of zero-tension ($\bar{\sigma} = 0$). A careful analysis of these limiting cases reveals the following two fundamental theorems regarding cell membrane interaction with nanotubes or any other relatively stiff 1D nanomaterials.

Theorem 1. In the limit of vanishing membrane tension ($\bar{\sigma} = 0$), a nanotube would favor the perpendicular entry mode $\theta = 90^\circ$ when interacting with an initially flat membrane at a fixed contact area. In this mode, the configuration of global minimum energy for the membrane is the catenoid

$$x^2 + y^2 = a^2 \cosh^2(h/a), \quad z = z_0 + h, \quad (4.2)$$

where h is the height of the membrane relative to the position of the contact edge at $z = z_0$.

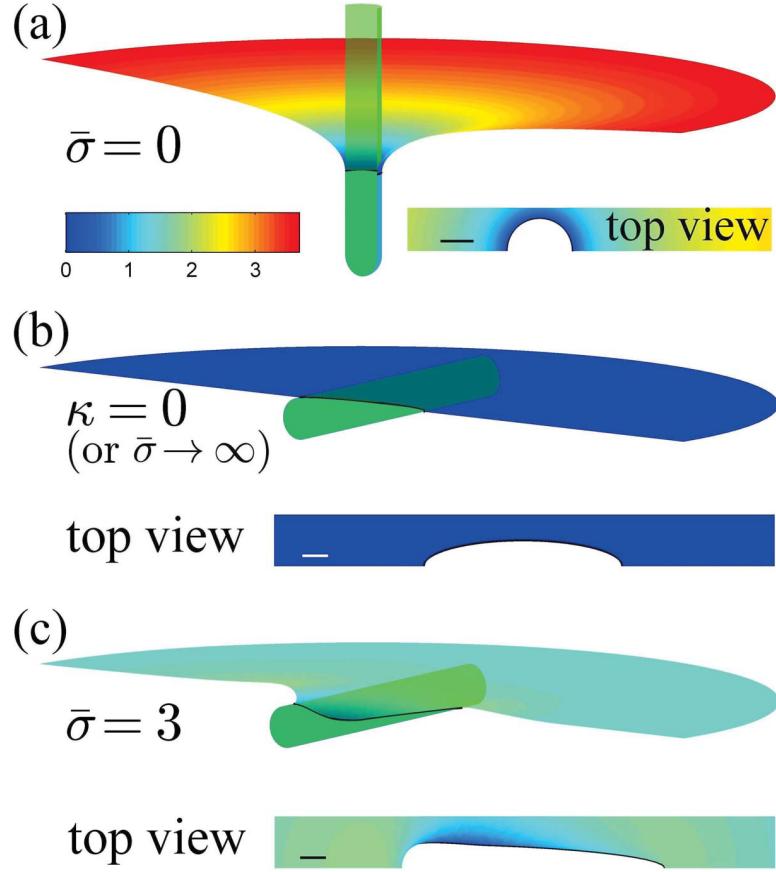


Figure 4.2: Simulated modes of interaction between a patch of cell membrane bounded inside a cylindrical region of radius $r_m = 20a$ and a nanotube of radius a with contact area fixed at $A_c = 10\pi a^2$. (a) In the limiting case of vanishing membrane tension $\bar{\sigma} = 0$, the preferred mode of interaction is the perpendicular entry mode. (b) In the limiting case of zero membrane bending stiffness $\kappa = 0$ ($\bar{\sigma} \rightarrow \infty$), the preferred mode of interaction is a near-parallel surface adhering mode. (c) In the case of normalized membrane tension $\bar{\sigma} = 3$, a nanotube interacts with the membrane at a prescribed entry angle of $\theta = 15^\circ$. The colors represent the height of the membrane relative to the lowest point at the contact edge (black curves). Scale bars are set to be the tube radius a .

Proof. At vanishing membrane tension and fixed contact area, the elastic bending energy stored in the membrane governs the free energy of the system. The catenoid in Eq. (4.2) has unit normal vector $\mathbf{n} = [-ax/(x^2 + y^2), -ay/(x^2 + y^2), \sqrt{1 - a^2/(x^2 + y^2)}]$. At contact edge $h = 0$, $\mathbf{n} = (-x/a, -y/a, 0)$ satisfies the smooth contact boundary condition between the nanotube and membrane at the perpendicular entry angle of $\theta = 90^\circ$, and $\mathbf{n} \rightarrow (0, 0, 1)$ as $x^2 + y^2 \rightarrow \infty$ guarantees the asymptotic flatness of the membrane away from the contact region. The principal curvatures of the catenoid $\pm a^{-1} \text{sech}^2(h/a)$ give rise to vanishing mean curvature

($H = 0$) everywhere in the fluid membrane, leading to zero elastic energy since $2\kappa H^2 = 0$ [32]. Moreover, it has been shown that the catenoid is the only complete, embedded minimal surface in the Euclidean space with zero mean curvature and two ends [83]. Since the bending energy is positive definite, the catenoid in Eq. (4.2), as shown in Fig. 4.2(a), is the only solution of zero deformation energy for the present problem. Due to the smooth contact between the nanotube and membrane and the uniqueness of catenoid [83], any rotation of the nanotube away from the perpendicular entry configuration will cause a deviation in membrane shape from the catenoid, resulting in non-zero mean curvature and positive deformation energy. Therefore, the perpendicular entry mode $\theta = 90^\circ$ corresponds to the state of global minimum energy for a nanotube to interact with an initially flat fluid membrane at zero membrane tension. In this state, the membrane assumes the configuration of a catenoid described in Eq. (4.2).

Theorem 2. In the limit of vanishing membrane bending stiffness ($\kappa = 0$), a nanotube interacting with an initially flat membrane under a fixed contact area would prefer an infinitesimally small entry angle $\theta \rightarrow 0^\circ$.

Proof. At vanishing membrane bending stiffness and fixed contact area, it becomes possible for the membrane to adopt arbitrarily sharp curvatures without any energy penalty, which effectively releases the condition of smooth contact at the contact edge. Since a flat membrane outside the nanotube automatically satisfies the condition of asymptotic flatness away from the contact region and also has smaller excess area compared to any curved surfaces, the minimum energy configuration of the membrane at any entry angle θ is just a flat surface [Fig. 4.2(b)]. Moreover, since the flat surface slices the nanotube along an elliptical contact edge with semimajor axis $a/\sin\theta$ and semiminor axis a , resulting in an enclosed area of $\pi a^2/\sin\theta$, the excess membrane surface area induced by the nanotube is $\Delta A = A_c - \pi a^2/\sin\theta$ and

the membrane tension energy $\sigma\Delta A$ will be minimized when $\theta \rightarrow 0^\circ$. Therefore, the most favorable configuration under vanishing membrane bending stiffness and fixed contact area is for the nanotube to lie nearly parallel to the membrane, i.e. $\theta \rightarrow 0^\circ$. In this state, the membrane wraps around the nanotube but remains a perfectly flat surface outside the contact region.

The above limiting cases define two fundamental modes of interaction between a nanotube (and any other rigid 1D cylindrical nanomaterials) and a fluid membrane. Based on these solutions, we postulate that a nanotube should undergo a transition from the perpendicular entry mode $\theta = 90^\circ$ to the near-parallel mode $\theta \rightarrow 0^\circ$ as the normalized membrane tension $\bar{\sigma}$ increases. In the following, we confirm this transition through a rigorous numerical analysis.

In the numerical analysis, the deformed membrane configuration, as illustrated in Fig. 4.1, is described in the Euclidean space as a tensor product cubic B-spline surface [69]. The entry of the nanotube into cell at a given angle can be regarded as a steady state process as soon as the tip region is covered by the membrane. In such a steady-state entry process limited by receptor diffusion, we can assume that the tip of the nanotube is fully wrapped and focus on energy stored in the free part of the membrane surface S . The position vector along S , $\mathbf{r} = \mathbf{r}(u, v)$, is parameterized by a set of curvilinear coordinates (u, v) on the domain $u, v \in [0, 1]$ as

$$\mathbf{r}(u, v) = \sum_{m=0}^{L_u} \sum_{n=0}^{L_v} \mathbf{c}_{m,n} N_m(u) N_n(v),$$

where $\mathbf{r}(u, v) = [x(u, v), y(u, v), z(u, v)]$ represents the Cartesian coordinates of the membrane and control points $\mathbf{c}_{m,n} = (c_{m,n}^x, c_{m,n}^y, c_{m,n}^z)$ are the coefficients of the basis functions $N_{m(n)}$ with integer indices $0 \leq m \leq L_u$ and $0 \leq n \leq L_v$. The unit normal vector to S is $\mathbf{n} = (\mathbf{r}_u \times \mathbf{r}_v) / |\mathbf{r}_u \times \mathbf{r}_v|$, where $\mathbf{r}_u \equiv \partial \mathbf{r} / \partial u$ and $\mathbf{r}_v \equiv \partial \mathbf{r} / \partial v$ are two

tangent vectors spanning the local tangent plane of S . The basis functions $N_m(u)$ and $N_n(v)$ as presented in Eq. (A.1) can be determined explicitly by specifying two knot vectors $\{u_0, \dots, u_{L_u+4}\}$ and $\{v_0, \dots, v_{L_v+4}\}$, respectively. Here a typical choice is taken as $u_i = v_i = 0$ ($i = 0, \dots, 3$), $u_j = 1$ ($j = L_u + 1, \dots, L_u + 4$) and $v_k = 1$ ($k = L_v + 1, \dots, L_v + 4$). With the knowledge of $\mathbf{r}(u, v)$, geometrical properties such as surface area and local mean curvature can be obtained (see Section A.3 for details) which in turn determine the total elastic energy $E_{el} \equiv E_{tot} + \gamma A_c$ in Eq. (4.1).

The free part of the membrane patch S is bounded inside a cylindrical region of radius r_m , as shown in Fig. 4.1. Since the system energy at entry angles θ and $\pi - \theta$ should be the same, it suffices to consider $\theta \in [0, \pi/2]$. The inner boundary of S at $u = 0$ is constrained on the nanotube wall and described by $x^2 + y_0^2 = a^2$ with $y_0 = y \sin \theta + z \cos \theta$, and a smooth contact between the nanotube and membrane requires $\mathbf{n} \times \mathbf{n}_0 = \mathbf{0}$, where $\mathbf{n}$ is the normal vector to S and $\mathbf{n}_0 = (x/a, y_0 \sin \theta/a, y_0 \cos \theta/a)$ is the normal vector to the nanotube wall. The outer boundary of the patch is at $u = 1$ and satisfies $x^2 + (y + y_c)^2 = r_m^2$, with $y_c = [A_c/(2\pi a) - a] \cos \theta$ to keep the inner boundary located around the center of S . The membrane is assumed to be asymptotically flat with $\mathbf{n} \cdot \mathbf{n}_1 = 0$, where $\mathbf{n}_1 = [x/r_m, (y + y_c)/r_m, 0]$ is the normal vector to the wall of the bounding cylinder [37]. The mirror symmetry of the configuration with respect to the y - z plane at $v = 0, 1$ requires $x(u, 0) = x(u, 1) = 0$, which lead to $c_{m,0}^x = c_{m,L_v}^x = 0$, $c_{L_u,0}^y = -(r_m + y_c)$, and $c_{L_u,L_v}^y = r_m - y_c$; the condition that $\mathbf{r}_v$ stays parallel with, $\mathbf{e}_x$, the base vector in the x -direction leads to $c_{m,0}^{y,z} = c_{m,1}^{y,z}$ and $c_{m,L_v-1}^{y,z} = c_{m,L_v}^{y,z}$ ($m = 0, \dots, L_u$). The combination of conditions $\mathbf{n} \cdot \mathbf{n}_1 = 0$ at $u = 1$ and $\mathbf{r}_v \parallel \mathbf{e}_x$ at $v = 0, 1$ requires $c_{L_u-1,0}^z = c_{L_u,0}^z$ and $c_{L_u-1,L_v}^z = c_{L_u,L_v}^z$. To keep the height of the membrane at the outer boundary on the same level, we take $z(1, 0) = z(1, 1)$ which gives $c_{L_u,0}^z = c_{L_u,L_v}^z$. To avoid penetration between the membrane and nanotube, we require $x^2 + y_0^2 \geq a^2$ for $u > 0$.

At a given value of the normalized membrane tension $\bar{\sigma}$, the minimum energy configuration of the nanotube-membrane system is determined using a Monte Carlo simulated annealing approach. Random adjustments to the values of control points $\mathbf{c}_{m,n}$ are attempted in the Monte Carlo scheme to change the membrane shape. The patch is divided into several regions in which mesh elements have a similar size. The geometrical boundary conditions at $u = 0, 1$ are enforced by the least square method, and the membrane curvature is kept within a range. To minimize E_{tot} , the system temperature is gradually reduced to a value close to zero after initial equilibration. Adjustments to $\mathbf{c}_{m,n}$ are accepted according to the Metropolis algorithm. The adjustment amplitudes are controlled to make sure that about 50% adjustments are accepted on the inner and outer boundaries and in different patch regions. All simulations are performed for about 7×10^6 Monte Carlo moves for each $\mathbf{c}_{m,n}$ with $r_m = 20a$, $L_u = 39$, and $L_v = 38$. Larger membrane patch and denser mesh give similar results.

4.2 Results and discussion

While it is well known that punching a clamped solid membrane would result in wrinkling near the contact edge as a mechanism of releasing energy by relieving the azimuthal compression through bending, there is no wrinkling in the two-dimensional fluid membrane and the energetically favorable configuration is governed by the bending and membrane tension energies [Fig. 4.2(c)]. As θ decreases from 90° , the contact edge buckles from a planar circle to a spoon-like loop [Fig. 4.2(c)]. This edge evolution results from a reduction in membrane tension energy, as the excess membrane area is relieved through out-of-plane displacement.

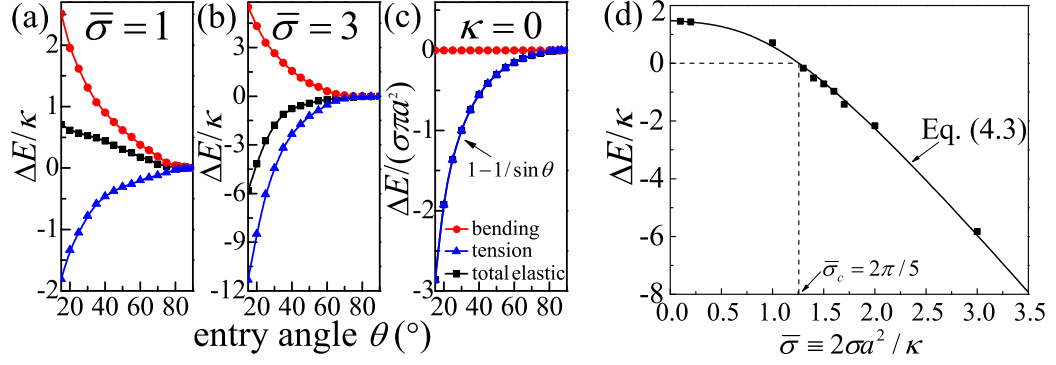


Figure 4.3: Variations of normalized total elastic, bending and tension energies of the membrane-nanotube system as functions of the entry angle θ for membrane with tension (a) $\bar{\sigma} = 1$, (b) $\bar{\sigma} = 3$ and (c) vanishing bending stiffness $\kappa = 0$. (d) Total elastic energy change $\Delta E = E_{el}(15^\circ) - E_{el}(90^\circ)$ as a function of $\bar{\sigma}$. The solid squares in (d) are results from Monte Carlo simulations which are well approximated by Eq. (4.3).

At a fixed contact area A_c , the total elastic energy $E_{el} \equiv E_{tot} + \gamma A_c$ in Eq. (4.1) can be decomposed into a bending energy E_b and a tension part $\sigma \Delta A = \bar{\sigma} \Delta A \kappa / (2a^2)$. Figure 4.3(a-c) plots the variations of the total elastic energy change $\Delta E = E_{el}(\theta) - E_{el}(90^\circ)$ and the associated bending and tension energies as functions of the entry angle θ . It can be seen that the membrane bending energy tends to rotate the nanotube to the perpendicular entry angle while the membrane tension energy prefers interaction at vanishingly small entry angle (i.e., the near-parallel interaction). These behaviors are perfectly consistent with the two limiting solutions discussed earlier in the paper. The larger the membrane tension, the more likely the nanotube would stay parallel to the membrane surface. As shown in Fig. 4.3(d), an empirical relation has been found between the energy difference $\Delta E = E_{el}(15^\circ) - E_{el}(90^\circ)$ and $\bar{\sigma}$ as

$$\bar{\sigma} = \bar{\sigma}_c + c_2 \ln \left(1 - c_1 \frac{\Delta E}{\kappa} \right) - \frac{2 \sin \theta}{\pi(1 - \sin \theta)} \frac{\Delta E}{\kappa}, \quad (4.3)$$

where $c_1 = 5c_2 = 2\pi^2/15$ at $\theta = 15^\circ$ and $\bar{\sigma}_c = 2\pi/5 \approx 1.257$ is the critical point of transition between the two interaction modes. The last linear term in Eq. (4.3) ensures $\Delta E = \sigma \pi a^2 (1 - 1/\sin \theta)$ in the limit of $\bar{\sigma} \rightarrow \infty$ as discussed in theorem 2. In the case of $\bar{\sigma} = \bar{\sigma}_c$ and $\Delta E = 0$, the bending and tension energies play equal role

in the regulation of nanotube rotation, and the nanotube is expected to oscillate due to thermal fluctuation.

Below the critical value $\bar{\sigma}_c$, the bending energy dominates over the tension energy, and the nanotube is driven to a high entry angle (the near-perpendicular entry mode); see Figs. 4.2(a) and 4.3(a). The tension energy becomes dominant at large $\bar{\sigma}$, leading to a low entry angle; see Figs. 4.2(c) and 4.3(b). The tension energy becomes dominant at large $\bar{\sigma}$, leading to a low entry angle; see Figs. 4.2(c) and 4.3(b). A comparison of Fig. 4.3(b) and (c) indicates that the tension energy variation is essentially similar to its limit behavior $-1/\sin\theta$ once $\bar{\sigma}$ rises above its critical transition value. The competition between bending and tension energies thus leads to two different interaction modes, as found in various experiments [9, 74, 75, 76]. The profile of the total elastic energy indicates that the configurational torque $dE_{el}/d\theta$ decreases as θ increases, and its flatness around $\theta = 90^\circ$ suggests that the nanotube would spend much time in thermal oscillation around 90° during near-perpendicular entry. In this sense, the entry angle at low $\bar{\sigma}$ may not be strictly equal to 90° in experiments. This prediction is consistent with recent experimental observation that carbon nanotubes, as well as gold nanowires and asbestos nanofibers, largely adopt a near-perpendicular mode of entry into murine liver cells and human mesothelial cells [9]. Similar tip entry phenomena have also been observed in successful phagocytosis of high aspect ratio filamentous *Escherichia coli* bacteria [75].

Our analysis indicates that the cellular uptake of a 1D nanomaterial can exhibit different rotation behaviors at different normalized membrane tension $\bar{\sigma}$. For $\bar{\sigma} < \bar{\sigma}_c$, the 1D nanomaterial would rotate to a high entry angle during uptake, and the deformed membrane forms a surface of revolution whose axis is the z -axis outside the contact region. For $\bar{\sigma} > \bar{\sigma}_c$, the 1D nanomaterial would rotate to a shallow entry angle and eventually adhere to the membrane surface in a near-parallel con-

figuration as the tip and the wall of the nanomaterial become partially exposed and partially wrapped by the weakly deformed membrane. This $\bar{\sigma}$ -dependent uptake behavior should be ubiquitous in the interactions between 1D nanomaterials and lipid membranes. For example, recent experiments and simulations have demonstrated nanoparticles adhering to a vesicle can spontaneously form particle-chains encapsulated inside lipid tubes or linear aggregates of particles adhering on the membrane surface [38, 39, 40, 76, 84]. In one experiment, transient tubular structures grow vertically on the membrane surface from adsorbed nanoparticles within substrate-supported flat giant unilamellar vesicles in an ambient solution of low Ca^{2+} concentrations (1–4 mM) [76]. These vertically grown tubular structures are in a tensionless state as indicated by thermal fluctuations of the membrane. As the vesicles spread on a substrate, their membrane tension increases and causes the lipid tubes to retract and the nanoparticles to align at the peripheral of the vesicle. At high concentrations of Ca^{2+} (4–10 mM), no membrane tubes form due to the adhesion induced high membrane tension [76]. Also, bacterial toxins (e.g., Shiga and cholera), when binding to glycolipid receptors on a cell, induce tubule formation at low membrane tension but fail to do so at high membrane tension [84]. Numerical simulations have shown that two adsorbed nanoparticles induce a membrane tube on a vesicle at a small reduced volume but form a linear cluster on the membrane surface of a vesicle at a large reduced volume [40]. This is consistent with our analysis in view of the fact that the conjugated membrane tension is greater at a larger reduced volume. Based on similar geometries and induced wrapping configurations, the particle-chain induced membrane tubulation and linear aggregation of nanoparticles adhering on the surface can be considered as examples analogous to the near-perpendicular tip entry at small membrane tensions and surface adhering of 1D nanomaterials at large membrane tensions, respectively.

Another interesting phenomenon which can be understood from the theory of membrane tension-governed nanotube rotation is that the filopodia of distinct types of cells only display a certain range of radius ($a = 40\text{--}150\text{ nm}$) [85, 86]. As finger-like structures composed of bundled actin filaments without an explicit end, the (growing) filopodia serve as an ideal biological example of 1D nanomaterials. To move efficiently toward a target, the filopodia involved in cell migration typically grow perpendicular to the leading edge of the cell membrane which requires $\bar{\sigma} < \bar{\sigma}_c$ as suggested by our analysis. Considering a typical filopodial of radius ($a = 40\text{--}150\text{ nm}$) and membrane bending stiffness $\kappa = 20\ k_B T$, the critical value $\bar{\sigma}_c \approx 1.257$ predicted by Eq. (4.3) corresponds to membrane tension of $\sigma = 0.002\text{--}0.033\text{ mN/m}$, which is consistent with the reported range ($0.003\text{--}0.04\text{ mN/m}$) for the membrane tension of neurons [87, 88], a type of migrating cells displaying filopodia. This explanation also implies that the coalescence of neighboring filopodia cannot proceed constantly once the filopodial radius is above some critical size governed by the membrane tension. Microtubules in a vesicle also exhibit a similar tension-induced rotation behavior. At low membrane tension, a linear bundle of microtubules in a vesicle pushes the membrane vertically and deforms it into a tether; while at large membrane tension, the microtubules buckle into an equatorial ring attaching parallel to the membrane [89, 90].

In the scaling dependence of the normalized membrane tension parameter $\bar{\sigma} \equiv 2\sigma a^2/\kappa$ on tube radius a , the $\bar{\sigma}$ -dependent uptake behavior also implies a size-dependence that nanotubes with small (large) radii could rotate to configurations with high (low) entry angles. For example, while individual nanotubes could enter cells with a near-perpendicular mode, a bundle of nanotubes may lay parallel adhering to the membrane surface (e.g., Supplementary Figure S3(a) in Ref. [9]). Since reported values of membrane tension vary in a wide range from 0.003 mN/m

to 1 mN/m [57], this size-dependent rotation behavior may provide a new and valuable complementary approach to estimate membrane tension by observing rotation behaviors of 1D nanomaterials with different radii. This is particularly interesting as nanophotonic tweezers based on near-field technology allow manipulating the orientation of 1D nanomaterials [91].

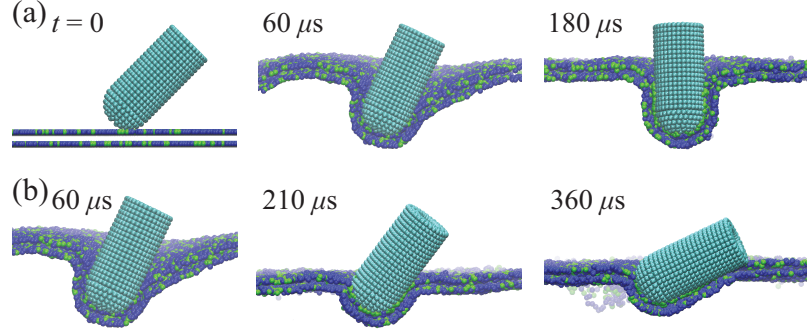


Figure 4.4: Time sequences of CGMD simulations showing a nanotube entering a membrane patch (blue) with an initial entry angle of 45° and diffusible receptors (green) under different membrane tensions. The receptor-to-lipid ratio is $1/3$. The nanotube with radius of $a = 10$ nm and length of 46 nm is seen to rotate to (a) 90° entry at a small membrane tension of $\sigma = 0.03436 k_B T/\text{nm}^2$, corresponding to $\bar{\sigma} \equiv 2\sigma a^2/\kappa = 0.3436$, and (b) a near-parallel orientation at the relatively large membrane tension $\sigma = 0.74 k_B T/\text{nm}^2$, corresponding to $\bar{\sigma} = 7.4$.

Finally, to demonstrate the role of membrane tension on the uptake pathway of 1D nanomaterials, we have also performed coarse grained molecular dynamics (CGMD) simulations by considering a multi-walled carbon nanotube binding to diffusible receptors (green) embedded in an initially flat, square patch of bilayer membrane (blue) at different membrane tensions (see Fig. 4.4). CGMD as one of the most popular computer simulation techniques has been demonstrated to be effective and efficient in studying biomembrane [9, 10, 15, 16, 19, 92, 93, 94, 95, 96, 97, 98, 99, 100] and other biological systems [101, 102]. Since the time scale associated with the equilibration of deformed lipid membranes is much smaller than the typical time for dynamic cellular uptake mediated by receptor diffusion, we focus on the shorter time scale and assume in the preceding theoretical analysis that the area of the contact region remains constant as the 1D nanomaterial adjust its entry angle to reduce the elastic energy of the membrane. That particular assumption is relaxed in the fol-

lowing CGMD simulations whose results further support its validity. The simulation setup here is similar to that used in our earlier study [9], where each lipid and receptor molecule is modelled as three connected beads with one hydrophilic head-bead and two hydrophobic tail-beads. The bead diameter, d_b , is set to 1 nm to construct a bilayer with an appropriate thickness. The CGMD simulations are first performed at constant temperature ($k_B T = 1.1\varepsilon$) and pressure with a dissipative particle dynamics (DPD) thermostat. A damping parameter $\Gamma = 1.0\varepsilon\tau/d_b^2$ with $\tau = 15$ ns and a cut-off distance $d_{cut} = 3.0d_b$ are used in the thermostat. In Fig. 4.4(a), the projected area of the membrane shrinks from $100 \times 100 \text{ nm}^2$ at $t = 0$ to $92 \times 92 \text{ nm}^2$ at $t = 60 \mu\text{s}$ as the membrane wraps around the nanotube. The lateral dimension of the bilayer is then fixed and the simulation system is adjusted to be at constant temperature and volume conditions. For a membrane with a fixed lateral dimension, the membrane tension can be effectively controlled by randomly deleting a certain amount of lipid molecules. Such a procedure, often referred to as the N-varied DPD method, has been widely used to investigate the interaction between nanoparticles and cell membrane [96, 97, 98, 99, 100]. In Fig. 4.4(a), no lipids has been deleted, which yields a membrane tension of $\sigma = 0.0378\varepsilon/d_b^2 = 0.03436 k_B T/\text{nm}^2$ or $\bar{\sigma} \equiv 2\sigma a^2/\kappa = 0.3436$ with $\kappa = 20 k_B T$, and the system equilibrates at about $t = 180 \mu\text{s}$ with an entry angle around 90° . In contrast, in Fig. 4.4(b) we simulate a larger membrane tension by adopting a similar procedure as that in Fig. 4.4(a), except that 13% lipid molecules are deleted from the membrane at $t = 60 \mu\text{s}$. In this case, the membrane tension is $\sigma = 0.814\varepsilon/d_b^2$ (or $\bar{\sigma} = 7.4$ with $\kappa = 20 k_B T$) and the nanotube rotates to a lower entry angle. These simulations clearly demonstrate the essential physics revealed by our theoretical analysis that the nanotube would prefer a low entry angle at a large tension and near-perpendicular entry at a small $\bar{\sigma}$.

A related but different phenomenon is the orientational changes of a short nanorod

wrapped around by a membrane which would occur in the middle stage of the wrapping process no matter how large or small the membrane tension is. Recent studies showed that short rigid adhesive nanorods including ellipsoidal nanoparticles with small aspect ratios orient their longer axes parallel to the membrane first and remain in that configuration until about a half of the particle surface area is wrapped, and then undergo a configurational transition by realigning their longer axes perpendicular to the membrane during further wrapped [10, 12, 13, 15]. This phenomenon is also observed in two-dimensions as discussed in Chapter 2 where a two-dimensional nanorod being parallel initially rotates to a perpendicular configuration as the membrane wrapping proceeds. After the transition moment, a short nanorod remaining parallel to the membrane with both highly curved ends covered by the membrane would cost more membrane bending energy than that the perpendicular configuration in which only one end needs to be covered by the membrane. Here, the membrane bending energy difference results in nanoparticle reorientation. This reorientation of short rigid nanorods caused by membrane wrapping has also been observed in cellular uptake of very soft nanoparticles in which the soft particle adheres on the membrane with its flattened surface at early stage of wrapping but then changes its configuration to one with longer axis perpendicular to the membrane during the late stage of wrapping (see Ref. [36] and Chapter 2). Compared with our present analysis on tension-controlled two modes of interaction between 1D nanomaterials and lipid membranes, the mode of interaction between short nanorods and lipid membranes consists of two stages distinguished by a particle reorientation regardless of the value of membrane tension.

The cellular uptake of nanomaterials can usually be considered as a process limited by diffusion of receptors in the cell membrane toward the contact region [2, 9]. For the uptake of cylindrical nanoparticles staying parallel to the membrane, the

wrapping process is controlled by the one-dimensional diffusion of receptors and the wrapped area should scale as $\sim \alpha_{1d}L\sqrt{Dt}$ where D is the diffusivity of receptors in the membrane, t the wrapping time, L the length of the cylindrical particle, and α_{1d} a dimensionless constant, commonly referred to as the speed factor, which depends on the initial receptor density ξ_0 before contact with nanoparticles, the receptor-ligand bond density ξ_L in the contact region, the binding energy of a receptor-ligand bond $e_{RL}k_BT$, and the elastic energy of the membrane [2]. In contrast, the wrapping process of sphere-like nanoparticles involves two-dimensional diffusion of receptors and the wrapped area should scale as $\sim \alpha_{2d}^2Dt$, where α_{2d} is the corresponding speed factor. Taking typical parameter values as $e_{RL} = 15$, $\xi_L = 5 \times 10^3 \mu\text{m}^{-2}$, $r = 20 \text{ nm}$, $\kappa = 20 k_BT$ and $\xi_0/\xi_L = 0.1$, we estimate $\alpha_{1d} \approx 0.0603$ and $\alpha_{2d} \approx 0.185$ from our previous study [2], meaning that membrane wrapping regulated by two-dimensional diffusion of receptors is much faster than that regulated by one-dimensional receptor diffusion. For 1D nanomaterials, membrane wrapping of their curved tip should be governed by two-dimensional diffusion of receptors, while wrapping of their cylindrical wall is regulated by one-dimensional diffusion of receptors. This means that the tip, rather than the lateral wall, of a 1D nanomaterial should be wrapped first, and the subsequent interaction is governed by the two interaction modes controlled by the normalized membrane tension $\bar{\sigma} \equiv 2\sigma a^2/\kappa$. For short nanorods with two curved tips and a much smaller lateral wall, the difference between receptor diffusion between the tip and wall regions may not be evident. Therefore, the initial wrapping of short nanorods would be mainly governed by the elastic energy of the deformed membrane. Such nanorods would align with their long axis parallel to the membrane first and reorient perpendicular to the membrane during the late stage of wrapping regardless of the value of membrane tension. As the length of the nanorod increases, the receptor diffusion in the early stage of wrapping and membrane tension become more important and begin to dominate the wrapping process beyond a critical length. A

detailed study on such length effect of rod-shaped nanoparticles on cellular uptake is out of the scope of the current study and deserves further investigations.

Some exceptional cases that are somewhat related to the present study are worth mentioning. First, in a theoretical limiting but physiologically irrelevant case in which the cell membrane is fully covered with receptors, the interaction between the membrane and adhesive nanomaterials becomes non-specific and a 1D nanomaterial would adhere parallel to the membrane, as demonstrated in our previous study (see Fig. 3(c) in Ref. [9]). Second, we have focused on clathrin-free endocytosis where the membrane deformation serves as the dominant resistance to nanoparticle entry. In clathrin-mediated endocytosis, the cell membrane and the underlying cortical actin cytoskeleton form a composite physical barrier to the entry of nanomaterials, which would involve both membrane deformation and remodeling of actin cytoskeleton [65, 103, 104, 105]. Recent theoretical study indicates that the relative contribution of membrane deformation and cytoskeleton remodeling to the entry resistance depends nonlinearly on the size of nanomaterials, the elastic and viscous properties of actin cytoskeleton, and the stage of endocytosis [65]. A thorough understanding of these effects in cellular uptake would call for more sophisticated models.

4.3 Summary

In summary, we have performed both theoretical analysis and molecular dynamics simulations to study the cellular uptake of one-dimensional nanomaterials such as nanotubes, nanowires, nanofibers, and certain nanoparticle-chains. First, we have established exact solutions to the problem in the limiting cases of vanishing bending stiffness or vanishing membrane tension, revealing two fundamental modes of

interaction between one-dimensional nanomaterials and lipid membranes: a perpendicular entry mode and a parallel adhering mode. Based on the limiting solutions, we postulated that there exists a transition between these two modes as the normalized membrane tension parameter $\bar{\sigma} \equiv 2\sigma a^2/\kappa$ increases. This hypothesis is then confirmed through a numerical analysis based on a Monte Carlo method. In the numerical analysis, we have calculated the variations of the system energy as a nanotube is wrapped into a patch of membrane at a prescribed entry angle. We conclude that perpendicular tip entry and parallel surface adhering are two basic modes of cell interaction with one-dimensional nanomaterials, controlled by a single dimensionless parameter, the normalized membrane tension $\bar{\sigma} \equiv 2\sigma a^2/\kappa$ which depends on the nanomaterial radius a , membrane tension σ , and bending stiffness κ of the membrane. This $\bar{\sigma}$ -dependent uptake behavior is ubiquitous in the interplay between cell membranes and one-dimensional nanomaterials. The B-spline based simulation method for evolving lipid membrane surfaces used in the present analysis can be extended to membrane wrapping around nanomaterials of more complex geometries, such as ellipsoidal nanoparticles and cylinders with elliptic cross sections. Our results can also serve as a foundation for future studies taking into account membrane fluctuation [106, 107], receptor diffusion [2, 108], receptor-ligand binding [109], deformation of cytoskeleton [64, 65], elasticity of nanomaterials [36], and simultaneous interaction with multiple nanoparticles [37, 38, 39, 40, 59, 94, 110].

CHAPTER FIVE

Interaction between Cell Membranes and Microsized Two-Dimensional Nanomaterials

Understanding how two-dimensional (2D) nanomaterials interact with cells is essential for biomedical diagnostics, therapeutics, and nanotoxicity [24, 111]. Typical 2D nanomaterials are plate-like materials with thickness at the nanoscale but lateral dimensions at a much larger scale, such as talc, mica nanosheets, boron nitride nanosheets, and graphene-family nanomaterials (GFNs) including graphene, graphene oxide (GO), reduced graphene oxide (rGO) and other related materials. Recent experiments, most related to GFNs, have indicated that size and surface properties of 2D nanomaterials play a critical role on their biological behavior [112, 113, 114, 115, 116, 117]. For example, the uptake of ultra-small polyethylene glycol (PEG) functionalized GO by liver and spleen is significantly higher than that of large rGO functionalized with PEG [112]. Much more cell viability loss is induced by small GO than large ones [113]. After attachment to cell membranes, small (500 nm lateral dimension) and large (1 μm) protein-coated GO microsheets are internalized through clathrin-mediated endocytosis and phagocytosis, respectively [114]. Murine macrophages treated with graphene undergo a dose-dependent apoptosis [115]; while these incubated with GO elicit autophagy and toll-like receptor signaling cascades associated innate immunity [116]. Graphene shows little hemolysis of red blood cells which implies a low risk of thrombosis; while GO nanosheets are thrombogenic and these with smaller size exhibit higher hemolytic activity [117]. In addition, coating GO with chitosan, a biocompatible polymer, can eliminate such high hemolysis [117].

Compared with extensive experiments on the biological behavior of 2D nanomaterials, few studies, most employing molecular simulations and targeting on small graphene and GO platelets [16, 118, 119, 120, 121], are focused on the modes of interaction between 2D nanomaterials and cell membranes. For example, it has been demonstrated by coarse-grained molecular dynamics simulations that a graphene

nanoflake spontaneously pierce into the lipid bilayer membrane at its corners or asperities [16, 119] and, depending on its lateral size, it either spans the membrane as a transmembrane nanostructure or aligns parallel at the interface between the two lipid monolayers [16, 118, 119]. Further investigations show that graphene nanoflakes with 10% carbon atoms oxidized at edges form a transmembrane nanostructure; while these with 5% oxidized edge atoms stay parallel between two monolayers [119]. These different phenomena result from the interplay of the hydrophobic attraction between the pristine graphene and lipid hydrocarbon chains, the hydrophilic attraction among oxidized regions, lipid heads and water, and the repulsion between the hydrophobic and hydrophilic parts.

Recently, mounting experimental studies have been conducted on the interaction between cell membranes and micro-sized GFNs [16, 24, 114]. It has been observed that graphene and few-layer graphene microsheets ($0.5\text{--}5\text{ }\mu\text{m}$ lateral dimension) orient their surfaces nearly orthogonal to the membrane surfaces of human lung epithelial cells, keratinocytes and mouse macrophages, after the spontaneous membrane penetration at edge asperities and corner sites [16]; while human THP-1 macrophages exposed with $25\text{ }\mu\text{m}$ few-layer graphene sheets adhere to, then spread on and finally cover the graphene surfaces [24]. Similar attachment of 2D nanomaterials onto cell membranes is observed in the cell uptake of protein-coated graphene oxide microsheets [114]. These experiments suggest that there exist two basic modes of interaction between micro-sized 2D nanomaterials and cell membranes: near-orthogonal membrane translocation after spontaneous membrane penetration at corners and parallel attachment onto the cell membranes. However, the underlying mechanisms of these two modes of interaction are not clear. In this Chapter, we present the first theoretical model on the interaction between cell membranes and rigid micro-sized 2D nanomaterials with thickness at the nanoscale comparable to the membrane thick-

ness but lateral dimension at a much larger scale. For simplicity but without loss of generality, we restrict our analysis on a symmetric lipid bilayer membrane composed of two lipid monolayers of the same composition.

5.1 Mode of transmembrane penetration

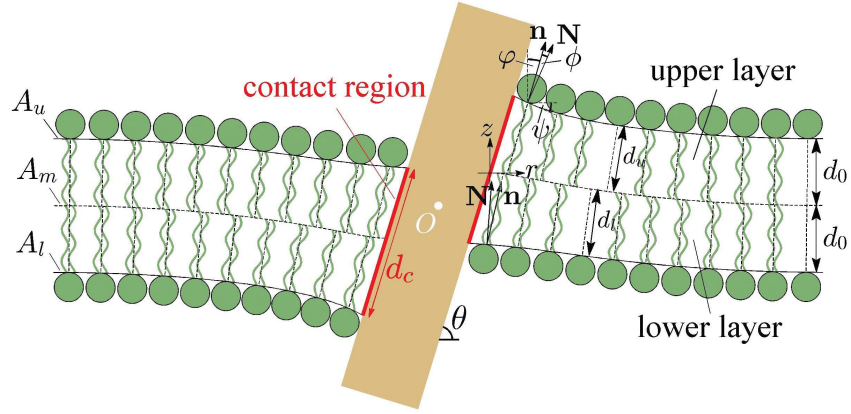


Figure 5.1: Schematics of the configuration of a rigid transmembrane 2D nanomaterial with an orientational angle θ . A_m is the midplane along the interface between two monolayers, A_u and A_l are the upper and lower dividing surfaces locating along the interface between the lipid heads and tails. The origin of the two-dimensional Cartesian coordinate rz is located at the point of intersection of the right-hand midplane and contact region. ψ is the tangent angle of the dividing surface whose unit normal vector is denoted by $\mathbf{N}$. In our notation, the angle φ between the unit tail director $\mathbf{n}$ and z -axis is positive as it is measured clockwise from the positive z -axis; angle ϕ between $\mathbf{n}$ and $\mathbf{N}$ and tangent angle ψ are positive as measured counterclockwise from $\mathbf{n}$ and the positive r -axis, respectively, for both upper and lower monolayers; accordingly here the angle on top marked by φ has a positive value, and angles marked by ϕ and ψ have negative values. d_c is the length of the contact region, d_0 denotes the length of unperturbed tail chains, and d_u and d_l are the lengths of perturbed tail chains in the upper and lower monolayers, respectively.

We first focus on the mode of transmembrane penetration. On the scale of membrane deformation much larger the thickness of bilayer, the cell membrane can be modeled as a smooth and structureless elastic fluid surface with the membrane bending and tension energies [41]. For the membrane penetration by 2D nanostructures where the length scale of concern is comparable with the bilayer thickness, a model with the molecular details of each monolayer is required. Here we adopt an elastic continuum framework proposed in the studies of the lipid mesophase construction

[122, 123] and further developed in the studies of lipid membrane fusion [124, 125] and membrane-protein interaction [126], where the effects of membrane bending, compression, and lipid orientation are considered. The structure of the lipid bilayer perturbed by the 2D transmembrane nanomaterial is characterized by the shape of the midplane A_m defined as the interface between the lipid tail groups of the upper and lower monolayers, and the length d and orientation $\mathbf{n}$ of the tail region of each lipid molecule (see Fig. 5.1). Hereafter we use subscripts m , u , and l to identify quantities associated with the midplane, upper, and lower monolayers, respectively. For the upper monolayer, the dividing surface A_u locating along the interface between the hydrophilic lipid heads and hydrophobic lipid tails (hydrocarbon chains) can be represented by a position vector $\mathbf{x}_u = \mathbf{x}_u(r_u, z_u)$ whose Cartesian coordinate (r_u, z_u) is determined as $r_u = r_m + d_u \sin \varphi_u$ and $z_u = z_m + d_u \cos \varphi_u$, where (r_m, z_m) is the coordinate of the corresponding position vector $\mathbf{x}_m = \mathbf{x}_m(r_m, z_m)$ along the midplane A_m , d_u is the length of the lipid tail, and φ_u is the angle between the unit tail director $\mathbf{n}_u$ and the z -axis with a geometric relation $\mathbf{n}_u = \sin \varphi_u \mathbf{e}_r + \cos \varphi_u \mathbf{e}_z$ [Fig. 5.1(a)]. Here $\mathbf{e}_r$ and $\mathbf{e}_z$ denote the unit base vectors along the coordinates r and z , respectively. The unit normal vector $\mathbf{N}_u$ to the dividing surface A_u is determined by the tangent angle ψ_u of A_u as $\mathbf{N}_u = -\sin \psi_u \mathbf{e}_r + \cos \psi_u \mathbf{e}_z$, where $\sin \psi_u = (dz_u/dr_u)/\sqrt{1 + (dz_u/dr_u)^2}$ and $\cos \psi_u = 1/\sqrt{1 + (dz_u/dr_u)^2}$. In general, $\mathbf{n}_u \neq \mathbf{N}_u$ in the perturbed membrane and the angle ϕ_u between them is $\phi_u = \varphi_u + \psi_u$. Both $\mathbf{n}_u$ and $\mathbf{N}_u$ point toward the exterior of the membrane. For the lower monolayer, $r_l = r_m - d_l \sin \varphi_l$, $z_l = z_m - d_l \cos \varphi_l$, and the unit tail director $\mathbf{n}_l$ and unit normal vector $\mathbf{N}_l$ to the dividing surface A_l are defined to point toward the interior of the membrane (see Fig. 5.1). Terms $\mathbf{n}_l$, $\mathbf{N}_l$, $\sin \psi_l$, $\cos \psi_l$, and ϕ_l have the same form as these for the upper monolayer with the subscript ‘ u ’ replaced by ‘ l ’.

The elastic energy of a monolayer mainly results from the deformation of hydro-

carbon chains [41, 127]. With the knowledge of the monolayer structure, the elastic free energy of a monolayer consisting of a certain amount of lipid molecules is $\int_A f dA$ with an integral over the dividing surface A , where f is the elastic free energy per unit length of the monolayer given as

$$f = \frac{1}{2}k(\text{div}\mathbf{n} - c_0)^2 - \frac{1}{2}kc_0^2 + \frac{1}{2}k_t\mathbf{t}^2 + \frac{K}{2}\left(\frac{d}{d_0} - 1\right)^2. \quad (5.1)$$

The first term in Eq. (5.1) accounts for the splay energy of the lipid tail molecules, where k and c_0 are the bending modulus and spontaneous curvature of the monolayer, respectively, and $\text{div}\mathbf{n} = \cos\psi \cos\phi d\varphi/dr$ is the divergence of the unit tail director $\mathbf{n}$ along the dividing surface. In the case of vanishing tilt ($\mathbf{t} = \mathbf{0}$ or $\phi = 0$), $\text{div}\mathbf{n} = -\cos\psi d\psi/dr$ reduces to the expression introduced in the classical Helfrich model [41]. The second term in Eq. (5.1) is the energy of ground state with vanishing splay and tilt ($\text{div}\mathbf{n} = 0$ and $\mathbf{t} = \mathbf{0}$) for the monolayer in a flat unperturbed membrane. The third term in Eq. (5.1) represents the tilt energy of lipid molecules where k_t is the tilt modulus of the monolayer and $\mathbf{t}$ is the tilt vector defined as

$$\mathbf{t} = \frac{\mathbf{n}}{\mathbf{n} \cdot \mathbf{N}} - \mathbf{N} = \tan\phi \mathbf{e}_\psi,$$

which is parallel to, $\mathbf{e}_\psi = \cos\psi \mathbf{e}_r + \sin\psi \mathbf{e}_z$, the unit tangent vector of the dividing surface, and characterizes the deviation of the tail director $\mathbf{n}$ from the surface normal $\mathbf{N}$. Here the quadratic form of tilt energy requires a small value of $\mathbf{t}$, that is, $|\mathbf{t}| < 1$. The last term in Eq. (5.1) denotes compression energy of the lipid molecules where d and d_0 are the lengths of perturbed and unperturbed tail chains, respectively, and K is the out-of-plane compressibility modulus of the monolayer being equivalent to its lateral area compressibility modulus. In general, the bending and compressibility moduli k and K of a monolayer can be taken approximately as half those of the bilayer. In the case of strong adhesive interaction, these lipid tails of either one or

both monolayers near the contact regions would be stretched theoretically as long as possible before they are broken or extracted out of the bilayer. Here the maximum length of a stretched lipid hydrocarbon chain is chosen as $\max\{d\} = 1.3d_0$ based on the thickness variation of a bilayer membrane induced by transmembrane proteins [128, 129]. In our notation, the angle φ between $\mathbf{n}$ and z -axis is positive as it is measured clockwise from the positive z -axis. Based on this notation, the spontaneous curvature c_0 in Eq. (5.1) for the upper monolayer is consistent with the conventional definition of monolayer spontaneous curvature; while for the lower monolayer c_0 in Eq. (5.1) takes the opposite value of the conventional spontaneous curvature. For example, the spontaneous curvature of a DOPC monolayer is about -0.11 nm^{-1} [130]. In our notation, $c_0 = -0.11 \text{ nm}^{-1}$ for the upper DOPC monolayer and $c_0 = 0.11 \text{ nm}^{-1}$ for the lower DOPC monolayer. It is also noted that the spontaneous curvature of the symmetric bilayer considered here is zero regardless of the value of c_0 . The energy associated with saddle-splay deformation of the membrane is not considered here due to a zero Gaussian curvature of the membrane. Here we assume that the cross-section area of each lipid molecule remain constant along the dividing surface, that is, the monolayer does not undergo lateral stretching [122, 124]. A constraint on the conservation of the molecular lipid volume is then relaxed without loss of accuracy, since small spontaneous curvature is considered here [125, 131]. Another implicit assumption is that no void is allowed to form within the hydrocarbon chain region.

Since the bilayers on both sides can slide freely along the 2D nanomaterial, the membrane would form an antisymmetric membrane configuration with respect to the point O at the minimum energy state (Fig. 5.1). Our analysis therefore is focused on the right-hand bilayer. Since the system energy at orientational angles θ and $\pi - \theta$ should be the same, it suffices to consider $\theta \in [0, \pi/2]$. The total free energy E_{tot}

accounting for the deformation of the perturbed bilayer membrane and its interaction with the penetrating nanomaterials is [122, 123, 124, 125, 126, 132]

$$E_{tot} = 2 \times \left(\int_{A_u} f_u dA_u + \int_{A_l} f_l dA_l + \sigma \Delta A_m - \gamma d_c \right). \quad (5.2)$$

The prefactor 2 in Eq. (5.2) results from the equal energy contribution from the right and left bilayers. The first two terms in the parentheses are the elastic free energy of the upper and lower monolayers each consisting of a given amount of lipid molecules. Physiologically, cells regulate the intrinsic membrane tension and maintain its value approximately as a constant by adjusting the membrane area through the lipid flow into or out of lipid reservoirs. This effect is characterized by the third term in Eq. (5.2) which denotes the membrane tension energy in terms of the membrane tension σ and the conjugated excess length ΔA_m of the midplane A_m , $\Delta A_m = \int_{A_m} \left[\sqrt{1 + (dz_m/dr_m)^2} - 1 \right] dr_m$. Last term in Eq. (5.2) represents the adhesive interaction between the 2D nanomaterials and adjacent lipid tail groups; γ is the effective adhesion energy in which the membrane line tension at the contact region is implicitly included and d_c is the length of the contact region. Since the thickness of lipid head groups can be assumed as a constant, the interaction energy between the 2D nanomaterial and adjacent lipid heads only results in an additive constant energy and is therefore neglected in Eq. (5.2). It is worth noting that although the repulsive interaction energy between lipid heads and 2D nanomaterial has no influence on the minimum energy state, it can become an energy barrier and play an important role when the 2D nanomaterial starts initial piercing into the membrane [16, 119].

Here we employ a numerical optimization technique to determine the minimum state of the total free energy at each given orientational angle θ . Recalling the geometric relations mentioned in the preceding paragraph before Eq. (5.1), the total

free energy in Eq. (5.2) can be represented as a function of the unknown variables $z_m(r_m)$, $d_u(r_m)$, $\varphi_u(r_m)$, $d_l(r_m)$, and $\varphi_l(r_m)$ which are approximated by cubic B-spline curves. For example, $z_m(r_m)$ can be parameterized as $z_m(r_m) = \sum_{i=0}^L a_i N_i(r_m)$, where the control points a_i are the coefficients of the basis functions $N_i(r_m)$ with L chosen as $L = 56$ in our calculations. The basis functions $N_i(r_m)$ are determined explicitly by specifying a knot vector $\{r_m^{(0)}, \dots, r_m^{(L+4)}\}$ [69]. A typical choice is taken here as $r_m^{(i)} = 0$ ($i = 0, \dots, 3$) and $r_m^{(i)} = r_{end}$ ($i = L + 1, \dots, L + 4$), where r_{end} is the length of the midplane projection on each side and is set to be $40d_0$ to approximate the remote boundary $r_m \rightarrow \infty$.

The boundary conditions provide either input parameters or equality constraints during energy minimization. At $r_m = 0$, $\varphi_u(0) = \varphi_l(0) = \pi/2 - \theta$ and $d_u(0) + d_l(0) = d_c$ ensure that no void exists between the 2D nanomaterial and adjacent lipid tails. At the remote boundary $r_m \rightarrow \infty$, the considered bilayer patch joins an flat and unperturbed membrane which requires $\varphi_u = \varphi_l = 0$ and $d_u = d_l = d_0$. It is noted that at $r_m \rightarrow \infty$ the condition $\varphi_u = \varphi_l = 0$ does not imply $\psi_u = \psi_l = 0$. z_m at $|r_m| \rightarrow \infty$ should have the same value for the left and right bilayers. To prevent a cross between adjacent lipid molecules, two inequality constraints are applied as $\cos \varphi_u + d_u(d\varphi_u/dr_m) - \sin \varphi_u(dz_m/dr_m) > 0$ for the upper monolayer and $\cos \varphi_l - d_l(d\varphi_l/dr_m) - \sin \varphi_l(dz_m/dr_m) > 0$ for the lower one. The total free energy E_{tot} as a function of z_m , d_u , φ_u , d_l , and φ_l under these constrains at a given d_c is minimized with respect to the control points using sequential quadratic programming [46]. Meanwhile the structure of the bilayer characterized by z_m , d_u , φ_u , d_l , and φ_l is determined at the state of minimum total free energy.

Taking typical parameter values as $k = 10 k_B T$, $c_0 = 0$, $k_t = 10 k_B T/\text{nm}^2$ [123], $K = 30 k_B T/\text{nm}^2$ [133], $d_0 = 1.4 \text{ nm}$, and $\max\{d\} = 1.3d_0$, the contour of total free energy E_{tot} in Eq. (5.1) at $\sigma = 1 k_B T/\text{nm}^2$ as a function of the penetration angle

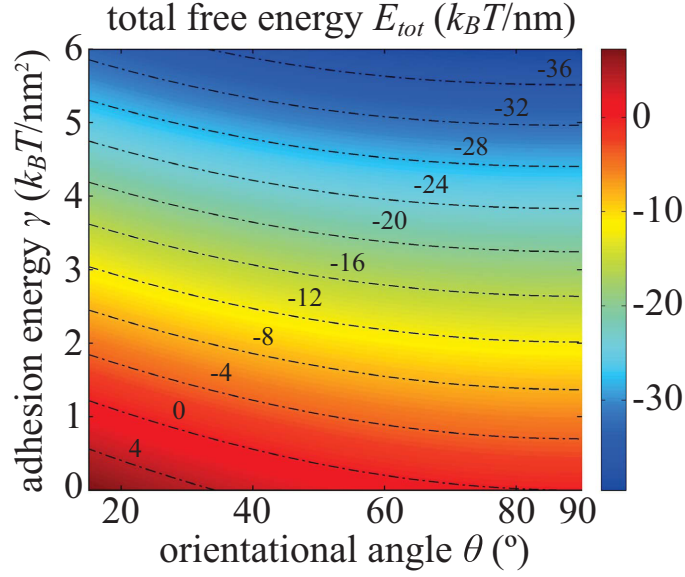


Figure 5.2: Contour of total free energy E_{tot} as a function of the orientational angle θ and adhesion energy γ .

θ and adhesion energy γ is shown in Fig. 5.2. It is indicated that the configuration with $\theta = 90^\circ$ always corresponds to a minimum energy state. A rigid transmembrane 2D nanomaterial with θ deviating from 90° would tend to rotate itself toward an orthogonal orientation. The size of the contact region increases as adhesion energy γ increases, until around $\gamma = 5.05 k_B T/\text{nm}^2$ the stretched lipid tails in both upper and lower monolayers reaches the predefined limiting length $\max\{d\} = 1.3d_0$ at any θ . At larger γ , the size of contact region would remain as $d_c = 2.6d_0$. Therefore, further increment of γ would only contribute to the linear adhesion energy term $-2\gamma d_c$. The small energy gradient around $\theta = 90^\circ$ suggests that the micro-sized transmembrane 2D nanomaterial would spend much time in thermal oscillation around 90° during near-perpendicular entry. In this sense, the entry angle may not be strictly equal to 90° in experiments. This prediction is consistent with recent experimental and molecular simulation observation that few-layer graphene sheets adopt a nearly orthogonal surface orientation with respect to membranes of human lung epithelial cells, macrophages and primary human keratinocytes [16]. The choice of the specific value $\max\{d\} = 1.3d_0$ has only slight influence on the value of E_{tot} and does not

affect the conclusion that on a micro-sized rigid transmembrane 2D nanomaterials would rotate to a configuration with an orientational angle around $\theta = 90^\circ$. Further calculations indicate that spontaneous curvature has slight effects on the contour of total free energy. A similar energy contour is observed in the case $\sigma = 0$.

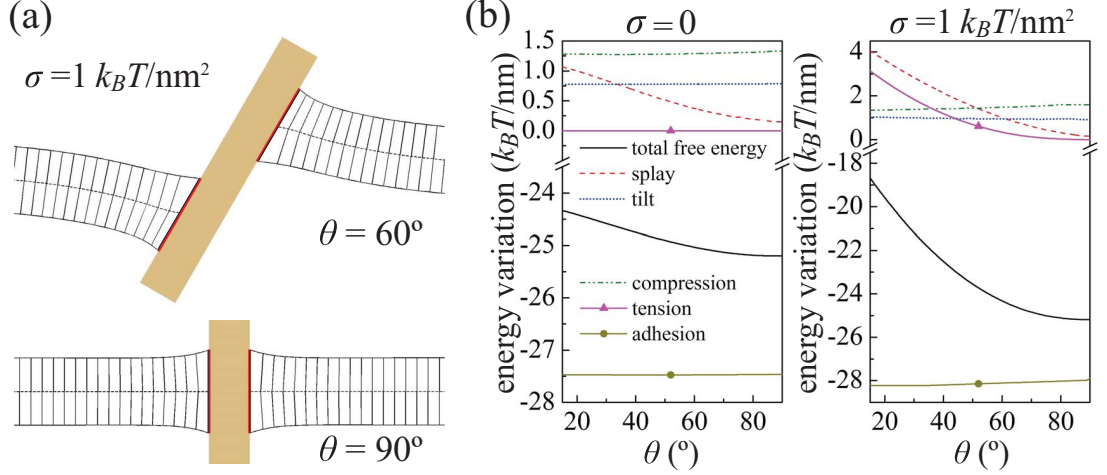


Figure 5.3: (a) Selective configurations of transmembrane 2D nanomaterial as well as cell membrane with $\sigma = 1 \text{ } k_B T / \text{nm}^2$ at $\theta = 60^\circ$ and 90° . (b) Variation of total free energy and its five components as a function of θ for different σ . In both (a) and (b), $\gamma = 4 \text{ } k_B T / \text{nm}^2$.

Selective configurations of a membrane with $\sigma = 1 \text{ } k_B T / \text{nm}^2$ as well as a transmembrane 2D nanomaterial at $\theta = 60^\circ$ and 90° are shown in Fig. 5.3(a). At $\theta = 90^\circ$, the membrane adopts a symmetric configuration with respect to the horizontal midplane. As θ decreases, the midplane becomes curved and the bilayers on the left and right sides slide along the nanomaterial surface in opposite directions. This results from the remote boundary conditions that $\varphi_u = \varphi_l = 0$ and z_m at $|r_m| \rightarrow \infty$ have the same value for the left and right bilayers. At a given θ , membranes with different σ have similar configurations but different relative sliding distances between the opposite bilayers whose value is inversely proportional to σ . A similar relative membrane sliding is observed in a recent molecular dynamics simulation where a folded graphene with a small flake glued at one end pierces and fully penetrates a lipid bilayer (see Fig. S7 in Ref. [16]). The total free energy given in Eqs. (5.1) and (5.2) can be decomposed into five components, including the splay energy, tilt,

compression, membrane tension, and adhesion parts. To find out how these energy parts rotate the nanomaterial and affect the bilayer structure, we plot the variations of the total free energy and its five components as functions of θ [see Fig. 5.3(b)]. It can be seen that the tilt, compression, and adhesion energies almost remain constant as θ varies, regardless of the value of membrane tension. The main driving force behind the rotation of the micro-sized rigid transmembrane 2D nanomaterial toward $\theta = 90^\circ$ generally comes from both splay and membrane tension energies. In a limit case $\sigma = 0$, tension energy vanishes and splay energy alone acts as the main driving force for the 2D nanomaterial rotation. The small energy gradient around $\theta = 90^\circ$ again suggests that the crossing 2D nanomaterial would spend much time in thermal oscillation around 90° as indicated in the preceding analysis. Relatively high compression and adhesion energies indicate a large contact region and large variation of lipid lengths; a relatively high splay energy means a rapid variation of lipid orientation along the upper and lower dividing surfaces; a relatively high tilt energy means a large difference between the lipid orientation and normal of the dividing surface; a relatively high tension energy associated with a large membrane tension results in a relatively flat midplane. Therefore, a rough structure of the perturbed bilayer can be derived from the relative magnitudes of these five energy components. A comparison of the magnitudes of these five energy components indicates that, beside the splay, compression, and tension energies [132, 134, 135], tilt energy plays an important role in the structural formation of the perturbed bilayer [see Fig. 5.3(b)]. As θ approaches 90° , the splay and tension energies become less important, and the lipid structure of membrane becomes dominated by the lipid length variation and lipid tilt. Therefore, although it has ignorable influence on the rotation of transmembrane nanomaterial, tilt energy has significant influence on the bilayer structure, especially as θ approaches 90° [see Fig. 5.3(b)]. The minor effect of splay and tension energies on the bilayer structure at $\theta = 90^\circ$ is illustrated in Fig. 5.3(a) where lipid chains

near the contact region are almost perpendicular to the horizontal midplane.

The orthogonal configuration ($\theta = 90^\circ$) of a micro-sized transmembrane 2D nanomaterial corresponds to the minimum energy state. It would be useful to have an analytical estimation of scaling behavior for such an important case. Due to the configurational symmetry with respect to the r - and z -axes, we focus on the right upper monolayer and $E_{el} \equiv E_{tot} + 2\gamma d_c = 4 \int_{A_u} f_u dA_u$ is four times of the elastic energy of the right upper monolayer. At $\theta = 90^\circ$, tension energy vanishes as the midplane is horizontal. Inspired by the slight orientation of lipid chains and minor energy contribution from splay energy at $\theta = 90^\circ$, we first develop a very simple estimation completely neglecting splay energy. We assume that all lipid chains are perpendicular to the horizontal midplane ($\varphi = 0$) and that the variation rate of monolayer thickness is so small that $dz_u/dr_m \ll 1$ and $\cos \phi \approx 1$. Then f_u given in Eq. (5.1) reduces to

$$f_u = \frac{k_t}{2} \left(\frac{dz_u}{dr_m} \right)^2 + \frac{K}{2} \left(\frac{z_u}{d_0} - 1 \right)^2.$$

Variation of E_{tot} with respect to z_u leads to $z_u = \exp[-\sqrt{K/(k_t d_0^2)} r_m] (d_c/2 - d_0) + d_0$, where the boundary conditions $z_u(r_m = 0) = d_c/2$ and $z_u(r_m \rightarrow \infty) = d_0$ are used. With the knowledge of z_u , E_{el} is given as

$$E_{el} = 2d_0 K \left(\frac{k_t}{K} \right)^{1/2} \left(\frac{d_c}{2d_0} - 1 \right)^2. \quad (5.3)$$

Our assumption on the perpendicular orientation of lipid chains can be regarded as an extra constraint which would result in a higher E_{el} expressed in Eq. (5.3).

We now turn to another simplified model neglecting the tilt energy ($k_t = 0$), which is widely employed in the studies of protein-membrane interactions [132, 134, 135]. We further take $c_0 = 0$ for simplicity. Since the tilt energy is ignored in this case,

the lipid chains can adopt their orientation freely and a configuration perpendicular to the midplane is favorable due to its minimum requirement of compression energy. With assumptions of $dz_u/dr_m \ll 1$ and $\cos \phi \approx 1$, f_u reduces to

$$f_u = \frac{k}{2} \left(\frac{d^2 z_u}{dr_m^2} - c_0 \right)^2 - \frac{k}{2} c_0^2 + \frac{K}{2} \left(\frac{z_u}{d_0} - 1 \right)^2.$$

Variation of E_{el} with respect to z_u and boundary conditions $z_u(r_m = 0) = d_c/2$ and $z_u(r_m \rightarrow \infty) = d_0$ result in $z_u = z_u(\eta)$ and then $E_{el} = E_{el}(\eta)$. The unknown parameter η is determined by $dE_{el}(\eta)/d\eta = 0$. After that, E_{el} can be represented as

$$E_{el} = \sqrt{2} d_0 K \left(\frac{k}{K d_0^2} \right)^{1/4} \left(\frac{d_c}{2d_0} - 1 \right)^2. \quad (5.4)$$

A similar method has been used to study the protein-induced bilayer deformations in axisymmetric configuration, where the deformation energy can also be expressed as a quadratic function of the lipid length variation [134, 135]. E_{el} in Eq. (5.4) is expected to be underestimated due to the omission of tilt energy.

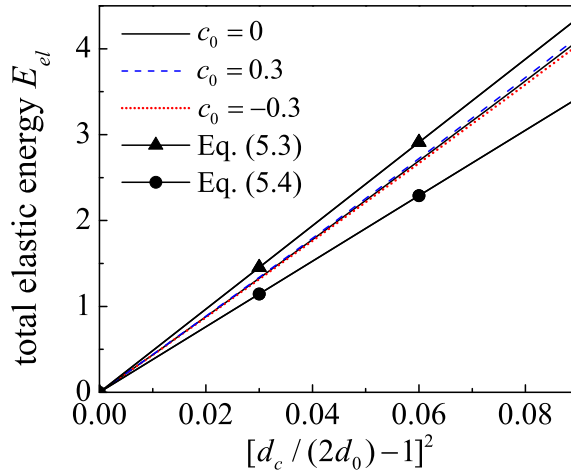


Figure 5.4: Total elastic energy E_{el} as a function of the lipid length variation at $\theta = 90^\circ$.

Combination of Eqs. (5.3) and (5.4) suggests E_{el} scaling as

$$E_{el} \sim H d_0 K [(d_c/2d_0) - 1]^2, \quad (5.5)$$

where the prefactor $H = H(k_t K^{-1}, k K^{-1} d_0^{-2})$ is a function of dimensionless parameters k_t/K and $k/(K d_0^2)$. Figure 5.4, showing that E_{el} scales as $\sim [(d_c/2d_0) - 1]^2$ and is bounded by Eqs. (5.3) and (5.4), confirms the validity of Eq. (5.5) and our preceding analysis on the overestimation of E_{el} in Eq. (5.3) and underestimation in Eq. (5.4). The relatively large deviation between Eq. (5.4) and the exact solution indicates again that the lipid tilt plays an important role in the membrane deformation. The spontaneous curvature has slight influence on the deformation energy. In Fig. 5.4 we only consider the case $d_c/(2d_0) \geq 1$, and the results show that the elastic energy profile corresponding to a larger spontaneous curvature has a larger slope. For readers who is interested in the case of $d_c/(2d_0) \leq 1$, E_{el} still scales as $\sim [(d_c/2d_0) - 1]^2$ but with a slightly smaller slope compared with the case of $d_c/(2d_0) \leq 1$, and the energy profile at a larger spontaneous curvature has a smaller slope.

Our analysis indicates that a micro-sized transmembrane 2D nanomaterial with θ deviating from 90° would always tend to rotate itself toward an orthogonal configuration ($\theta = 90^\circ$) with respect to the cell membrane. The main driving force of such a rotation toward $\theta = 90^\circ$ generally comes from both splay and membrane tension energies. Although tilt energy has ignorable influence on the rotation of transmembrane 2D nanomaterial, it is important to the structure formation of a perturbed bilayer.

5.2 Mode of parallel attachment onto cell membranes

We now study the other important mode of interaction between micro-sized 2D nanomaterials and cell membranes—parallel attachment onto the cell membranes. In this

case, the scale of membrane deformation is much larger than the membrane thickness and the cell membrane can be simply modeled as an elastic fluid surface with consideration of the membrane bending and tension energies [41]. Without loss of generality, we consider an initially flat membrane wrapping around a semi-infinite 2D nanomaterial with a rounded end (Fig. 5.5).

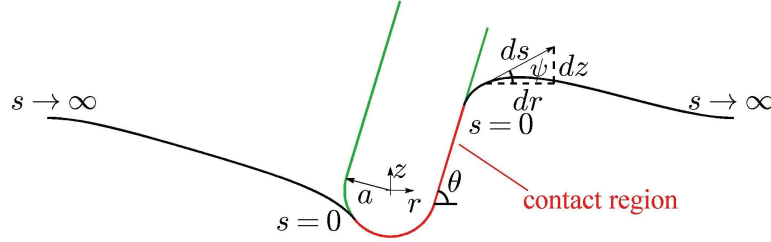


Figure 5.5: Schematics of adhesive attachment of a 2D nanomaterial onto the cell membrane. The 2D nanostructure has a thickness $2a$ with an orientational angle θ . The rounded cap occupies the region with $r^2 + z^2 \leq a^2$ and $z \sin \theta + r \cos \theta \leq 0$ in the adopted coordinate rz . ψ is the tangent angle and s denotes the arclength of the free membrane part from the contact edges $s = 0$ to outer boundaries $s \rightarrow \infty$.

The total energy of the system per unit length can be described by the Canham-Helfrich functional as [41, 49]

$$E_{tot} = \frac{\kappa}{2} \int \dot{\psi}^2 ds + \sigma \Delta l - \gamma l_c, \quad (5.6)$$

where $E_b = \frac{\kappa}{2} \int \dot{\psi}^2 ds$ is the bending energy, γ is the adhesion energy, and l_c is the length of the contact region; σ is the membrane tension which is conjugated to the excess length $\Delta l = \int (1 - \cos \psi) ds$ induced by wrapping; ψ , s , and κ are the tangent angles, arclength, and bending stiffness of the membrane (see Fig. 5.5). The superimposed dot in the first term in Eq. (5.6) denotes derivative with respect to the arclength s . For a bilayer membrane composed of two chemically identical monolayers as considered here, its bending stiffness κ is twice as much as that of each monolayer ($\kappa = 2k$ with k as the monolayer bending stiffness) and its spontaneous curvature vanishes. All lengths in this wrapping of a 2D nanomaterial are scaled by, a , a half of the nanomaterial thickness (Fig. 5.5). Other dimensionless parameters

are $\bar{\sigma} \equiv 2\sigma a^2/\kappa$ and $\bar{\gamma} \equiv 2\gamma a^2/\kappa$.

We employ sequential quadratic programming [46] to determine the minimum energy state at a given size of the contact region. The shape of the attached membrane is determined by that of the contact region. With geometric relations $dr/ds = \cos \psi$ and $dz/ds = \sin \psi$, the shape of the outer free membrane on the left or right side can be determined by ψ which is approximated by a Fourier series as [33, 49]

$$\psi(s) = \psi_0 + (\psi_l - \psi_0)\frac{s}{l} + \sum_{i=1}^N a_i \sin\left(\pi i \cdot \frac{s}{l}\right). \quad (5.7)$$

Here N is the number of Fourier modes chosen as $N = 80$ in our calculations, aa_i are the Fourier amplitudes, s is the arclength, and l is the membrane length on the considered side. ψ_0 and ψ_l are the tangent angles at $s = 0, l$, respectively. To approximate the condition $s \rightarrow \infty$, the length l of each outer membrane region is treated as an unknown parameter determined by a large prescribed length of the membrane projection, which is set to be $35a$.

The boundary and constraint conditions provide either input parameters or equality constraints during energy minimization. At $s \rightarrow \infty$, the outer free membrane becomes asymptotically flat which requires $\psi_l = 0$. The continuity of the coordinate (r, z) and tangent angles ψ at the left and right contact edges are enforced as equality constraints. The elastic energy as a function of $\psi(s)$ under these constraints at a given l_c is minimized with respect to the Fourier amplitudes a_i , tangent angles ψ_0 and ψ_l at both contact edges, and l for both outer free regions. Once the tangent angles ψ given by Eq. (5.7) are known, the elastic energy and corresponding shapes of the membrane can be determined. We here choose $\psi = \psi(s)$ as the membrane parameterization to handle the case where one value of r might correspond to several values of z . That kind of case, however, is not observed in our results shown below,

which means that a form based on the Cartesian coordinate $(r, z(r))$ can be adopted here as we do in the preceding analysis on the rotation of micro-sized transmembrane 2D nanomaterials.

It is known that the equilibrium time scale of a lipid membrane subjected to various types of loading is less than one second, much shorter than that of cell uptake of nanomaterials varying from hundreds of seconds to tens of minutes. Therefore, cellular uptake can usually be regarded as a process limited by receptor diffusion toward the contact region [2, 9, 18]. Due to such a limited rate of receptor diffusion, we can focus on a short time scale and assume that the total length l_c of the contact region is fixed as the micro-sized 2D nanomaterials adjust the orientational angle θ to reduce the total elastic energy $E_{el} \equiv E_{tot} + \gamma l_c$.

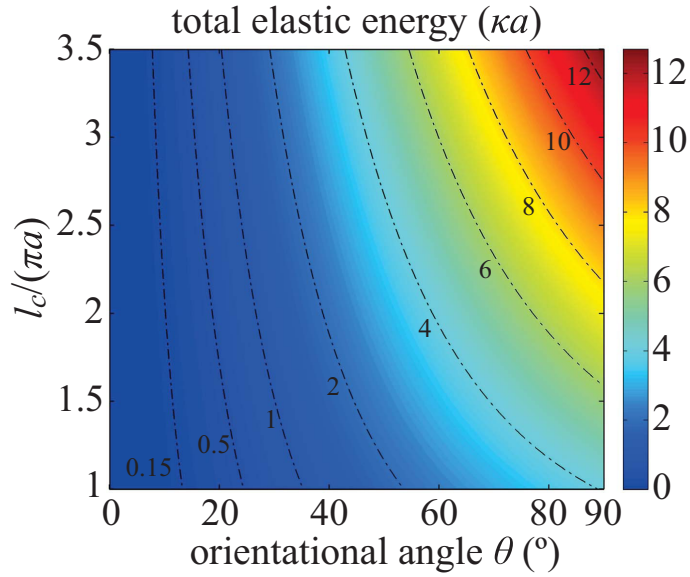


Figure 5.6: Contour of total elastic energy $E_{el} \equiv E_{tot} + \gamma l_c$ as a function of the orientational angle θ and normalized contact length $l_c/(\pi a)$ at $\bar{\sigma} = 2$.

Figure 5.6 shows the contour of total elastic energy $E_{el} \equiv E_{tot} + \gamma l_c$ in Eq. (5.6) as a function of the orientational angle θ and normalized contact length $l_c/(\pi a)$. A short time period of membrane wrapping immediately after the initial contact between the 2D nanomaterial and cell membrane is a highly dynamic process and

does not principally apply to our assumption that l_c could be regarded as a constant. The contact length therefore in Fig. 5.6 is restricted to relatively large values. It is shown that the configuration with $\theta = 0^\circ$ always corresponds to a minimum energy state regardless of the value of contact length. This result is also observed at very small l_c if the assumption of a fixed l_c is persisted in recklessly.

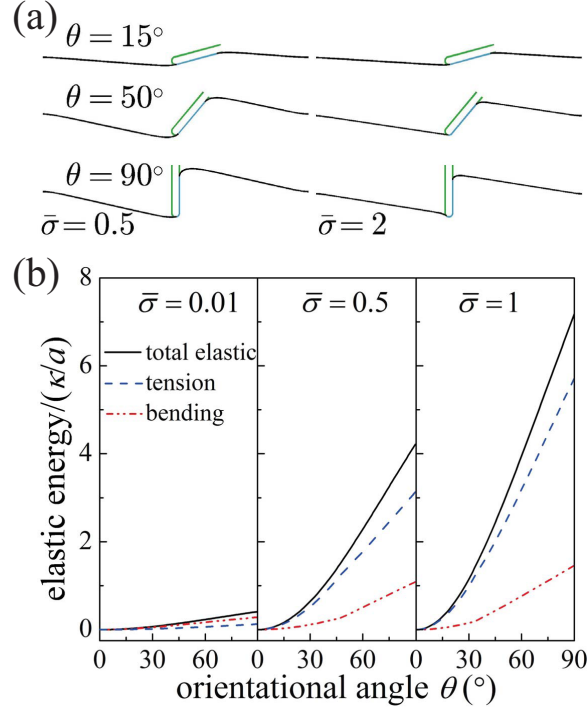


Figure 5.7: (a) Selective configurations of 2D nanomaterials wrapped by cell membranes with $\bar{\sigma} = 0.1$ and 2 at $\theta = 30^\circ$, 60° and 90° . (b) Variation of rescaled total elastic energy and its two components as a function of θ with $l_c = 3.5\pi a$ for different $\bar{\sigma}$. The outer free membrane boundaries are taken as $\pm 35a$ in (a) and (b).

Configurations of a 2D nanomaterial wrapped by a membrane with $\bar{\sigma} = 0.1$ and 2 at $\theta = 30^\circ$, 60° and 90° are shown in Fig. 5.7(a). The asymmetry of the deformed membrane at $\theta = 90^\circ$ results from the fact that a symmetric configuration would have much higher bending energy than the asymmetric configuration, which results in higher total elastic energy. Surface tension tries to flatten the cell membrane to reduce the excess length which results in the regions near contact edges having a higher curvature at larger surface tension than at lower surface tension, as demonstrated in Fig. 5.7(a). The rescaled elastic energy $(E_{tot} + \gamma l_c) / (\kappa/a)$ is split into two

parts as rescaled tension energy $\bar{\sigma}\Delta l/(2a)$ and rescaled bending energy $E_b/(\kappa/a)$ with $E_b = \frac{\kappa}{2} \int \dot{\psi}^2 ds$. Figure 5.7(b) shows the total elastic energy and its two parts as a function of θ for $\bar{\sigma} = 0.01, 0.5$ and 1 . For a large normalized surface tension $\bar{\sigma} \geq 1$, the elastic energy mostly gains from the surface tension energy; for an extremely low $\bar{\sigma}$ ($\bar{\sigma} \sim 0.01$), bending energy dominates the elastic energy; and for an intermediate $\bar{\sigma}$ ($\bar{\sigma} \sim 0.1$), the bending and surface tension energies make comparable contributions to the elastic energy. There is slight difference between the bending energies for different $\bar{\sigma}$. Different from the case of transmembrane 2D nanomaterials, the total elastic energy as well as its two components increases with an increasing entry angle θ in the case of membrane attachment, and the configurational torque $dE_{tot}/d\theta$ at large θ is larger than that at small θ . This result, independent of $\bar{\sigma}$, points out that adhesive 2D nanomaterials are in favor of a parallel position with the cell membrane. Recent experiments show that human THP-1 macrophages exposed with $25 \mu\text{m}$ few-layer graphenes adhere to, then spread on and finally cover the graphene surface as our analysis predicts [24].

5.3 Summary

We have performed theoretical analysis to study the two modes of interaction between micro-sized rigid two-dimensional (2D) nanomaterials such as graphene-family nanomaterials. Using the optimization method, we have calculated the energy profiles as functions of the orientational angle of 2D nanomaterials. We find that the micro-sized 2D nanomaterials undergo the transmembrane penetration mode correspond to an orthogonal configuration with respect to the cell membrane; while these undergo the adhesive attachment mode correspond to a parallel configuration. Both modes are independent of the bending stiffness and tension of the cell membrane.

APPENDIX A

Appendix

A.1 Supplement to Figure 2.2

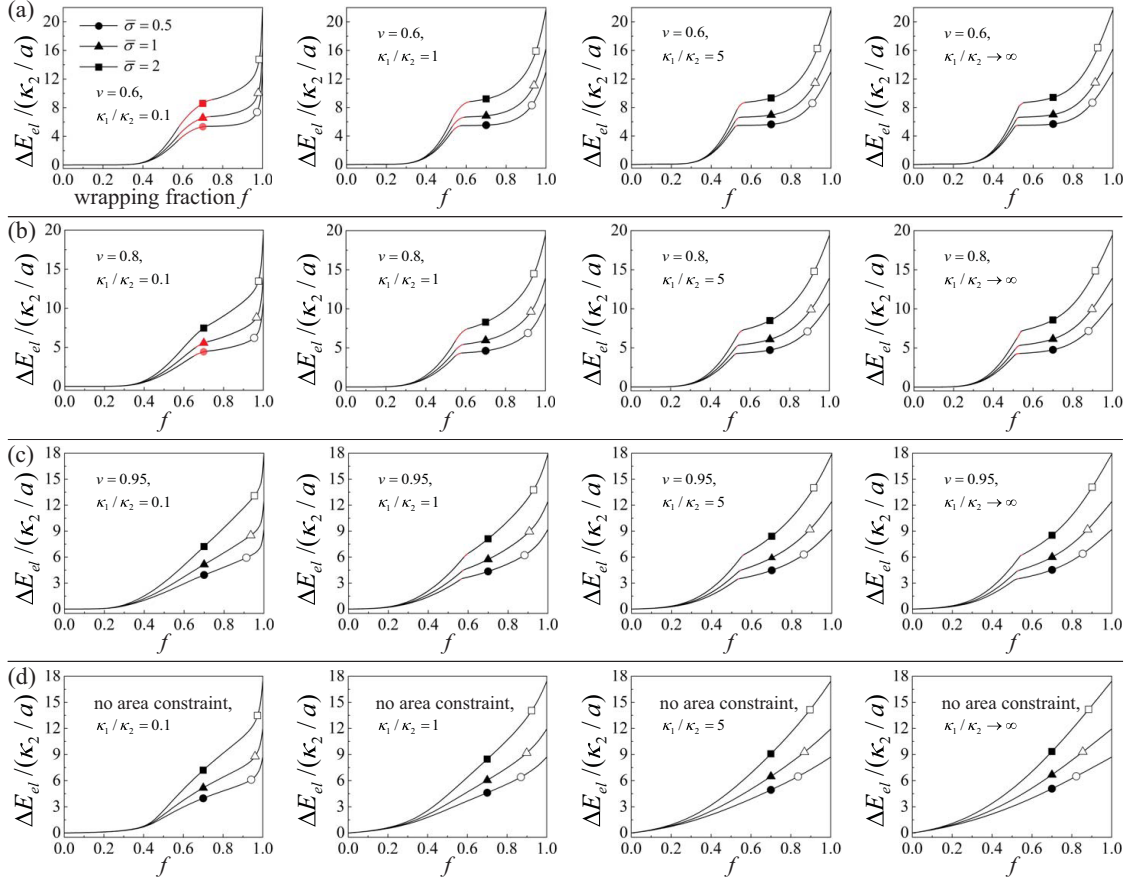


Figure A.1: Elastic energy ΔE_{el} as a membrane wraps around an elastic nanoparticle with wrapping degree f for different reduced areas v and κ_1/κ_2 at $\bar{\sigma} = 0.5, 1$, and 2 . (a-c) The cases with reduced areas of $v = 0.6, 0.8$, and 0.95 , and (d) the case without an area constraint. The open symbol on each curve corresponds to the state of full wrapping. The red regions on some curves indicate the situations involving asymmetric deformation.

Figure 2.2 in Chapter 2 shows elastic energy as a membrane wraps around a nanoparticle of different reduced areas and bending stiffness at $\bar{\sigma} = 1$. As a supplement to Fig. 2.2, Fig. A.1 here shows the elastic energy change $\Delta E_{el} = E_{el} - E_{el}^0$ as a function of the wrapping degree f at different membrane tensions $\bar{\sigma} = 0.5, 1$, and 2 , where E_{el} is the elastic energy of the system, and E_{el}^0 is the ground state energy before the particle contacts the membrane. The open symbol on each curve corresponds to full wrapping. The red segments on some curves indicate that the particle/membrane system has an asymmetric configuration. For most cases, smaller

bending rigidity ratio and larger membrane tension lead to larger asymmetric regions; while for very soft particles ($\kappa_1/\kappa_2 = 0.1$) with relatively large reduced areas, larger membrane tension can also inhibit asymmetric deformation (e.g., $v = 0.8$ at $\bar{\sigma} = 2$ [Fig. A.1(b)] and $v = 0.95$ [Fig. A.1(c)]). For the case where the enclosed area is not specified and can be adopted freely during the wrapping process, only symmetric configuration (no solid segments) is observed as indicated in Fig. A.1(d).

A.2 Cubic B-spline basis functions

$$N_i(x) = \begin{cases} (x_{i+2} - x_{i-2}) \left[\begin{aligned} & \frac{(x_{i-1}-x)^3}{(x_{i-1}-x_{i-2})(x_{i-1}-x_i)(x_{i-1}-x_{i+1})(x_{i-1}-x_{i+2})} \\ & + \frac{(x_i-x)^3}{(x_i-x_{i-2})(x_i-x_{i-1})(x_i-x_{i+1})(x_i-x_{i+2})} \\ & + \frac{(x_{i+1}-x)^3}{(x_{i+1}-x_{i-2})(x_{i+1}-x_{i-1})(x_{i+1}-x_i)(x_{i+1}-x_{i+2})} \\ & + \frac{(x_{i+2}-x)^3}{(x_{i+2}-x_{i-2})(x_{i+2}-x_{i-1})(x_{i+2}-x_i)(x_{i+2}-x_{i+1})} \end{aligned} \right], & x \in [x_{i-2}, x_{i-1}); \\ (x_{i+2} - x_{i-2}) \left[\begin{aligned} & \frac{(x_i-x)^3}{(x_i-x_{i-2})(x_i-x_{i-1})(x_i-x_{i+1})(x_i-x_{i+2})} \\ & + \frac{(x_{i+1}-x)^3}{(x_{i+1}-x_{i-2})(x_{i+1}-x_{i-1})(x_{i+1}-x_i)(x_{i+1}-x_{i+2})} \\ & + \frac{(x_{i+2}-x)^3}{(x_{i+2}-x_{i-2})(x_{i+2}-x_{i-1})(x_{i+2}-x_i)(x_{i+2}-x_{i+1})} \end{aligned} \right], & x \in [x_{i-1}, x_i); \\ (x_{i+2} - x_{i-2}) \left[\begin{aligned} & \frac{(x_{i+1}-x)^3}{(x_{i+1}-x_{i-2})(x_{i+1}-x_{i-1})(x_{i+1}-x_i)(x_{i+1}-x_{i+2})} \\ & + \frac{(x_{i+2}-x)^3}{(x_{i+2}-x_{i-2})(x_{i+2}-x_{i-1})(x_{i+2}-x_i)(x_{i+2}-x_{i+1})} \end{aligned} \right], & x \in [x_i, x_{i+1}); \\ \frac{(x_{i+2}-x)^3}{(x_{i+2}-x_{i-1})(x_{i+2}-x_i)(x_{i+2}-x_{i+1})}, & x \in [x_{i+1}, x_{i+2}); \\ 0, & \text{otherwise.} \end{cases} \quad (\text{A.1})$$

A cubic B-spline curve $y = y(x)$ is defined as a linear combination of cubic

B-spline basis functions $N_i(x)$ as

$$y = \sum_{i=0}^L c_i N_i(x),$$

where control points c_i are the coefficients of the basis functions $N_i(x)$ with integer indices $0 \leq i \leq L$. The basis function $N_i(x)$ defined on a knot vector $\{x_0, x_1, \dots, x_{i-1}, x_i, x_{i+1}, \dots, x_{L+4}\}$ ($x_i \leq x_{i+1}$) is given in Eq. (A.1).

In the case of a uniform knot vector $x_{i+1} - x_i = \Delta x > 0$, Eq. (A.1) reduces to

$$N_i(x) = \begin{cases} \frac{1}{2}(x - x_i)^2(\Delta x)^{-2}, & x \in [x_i, x_{i+1}); \\ \frac{1}{2} - (x - x_{i+1})(x - x_{i+2})(\Delta x)^{-2}, & x \in [x_{i+1}, x_{i+2}); \\ \frac{1}{2}(x - x_{i+3})^2(\Delta x)^{-2}, & x \in [x_{i+2}, x_{i+3}); \\ 0, & \text{otherwise.} \end{cases}$$

A.3 Differential geometry of two-dimensional surfaces

Consider a two-dimensional surface S whose position vector $\mathbf{r} = \mathbf{r}(u, v)$ can be parametrized by a set of curvilinear coordinates (u, v) on a domain Ω of the u, v -plane as

$$\mathbf{r}(u, v) = [x(u, v), y(u, v), z(u, v)],$$

where the Cartesian coordinates (x, y, z) are differential functions of (u, v) .

The unit normal vector $\mathbf{n}$ to S is then given by

$$\mathbf{n} = \frac{\mathbf{r}_u \times \mathbf{r}_v}{|\mathbf{r}_u \times \mathbf{r}_v|},$$

where $\mathbf{r}_u \equiv \partial \mathbf{r} / \partial u$ and $\mathbf{r}_v \equiv \partial \mathbf{r} / \partial v$ are two tangent vectors spanning the local tangent plane of S .

The surface area A of S is given by

$$A = \int_{\Omega} |\mathbf{r}_u \times \mathbf{r}_v| du dv = \int_{\Omega} \sqrt{EG - F^2} du dv,$$

where $E = \mathbf{r}_u \cdot \mathbf{r}_u$, $F = \mathbf{r}_u \cdot \mathbf{r}_v$, and $G = \mathbf{r}_v \cdot \mathbf{r}_v$.

The local mean curvature H and Gaussian curvature κ^g are given by

$$H = \frac{EN + GL - 2FM}{2(EG - F^2)} \quad \text{and} \quad \kappa^g = \frac{LN - M^2}{EG - F^2},$$

respectively, where $L = \mathbf{r}_{uu} \cdot \mathbf{n}$, $M = \mathbf{r}_{uv} \cdot \mathbf{n}$, and $N = \mathbf{r}_{vv} \cdot \mathbf{n}$. Here $\mathbf{r}_{uu} \equiv \partial^2 \mathbf{r} / \partial u^2$, $\mathbf{r}_{uv} \equiv \partial^2 \mathbf{r} / \partial u \partial v$, and $\mathbf{r}_{vv} \equiv \partial^2 \mathbf{r} / \partial v^2$.

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