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RESEARCH ARTICLE

Optimal aspect ratio of endocytosed spherocylindrical nanoparticle

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Recent simulations have demonstrated that bioparticle size and shape modulate the process of endocytosis, and studies have provided more quantitative information that the endocytosis efficiency of spherocylindrical bioparticles is decided by its aspect ratio. At the same time, the dimensions of the receptor-ligand complex have strong effects on the size-dependent exclusion of proteins within the cellular environment. However, these earlier theoretical works including simulations did not consider the effects of ligand-receptor complex dimension on the endocytosis process. Thus, it is necessary to resolve the effects of ligand-receptor complex dimension and determine the optimal aspect ratio of spherocylindrical bioparticles in the process of endocytosis. Accordingly, we proposed a continuum elastic model, of which the results indicate that the aspect ratio depends on the ligand-receptor complex dimension and the radius of the spherocylindrical bioparticle. This model provides a phase diagram of the aspect ratio of endocytosed spherocylindrical bioparticles, the larger aspect ratio of which appears in the phase diagram with increasing ligand density, and highlights the bioparticle design.

Keywords cellular uptake, depletion effects, dimension of ligand-receptor complex, elasticity theory

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1 Introduction

Receptor-mediated endocytosis is a process by which cells engulf and internalize nanometer-sized targets that is driven by the specific binding of ligands on target surfaces with receptors on the cell membrane. The study of endocytosis has revealed several highlights about the design and synthesis of nanoparticles during the chemical synthesis process, in which some distinct physical properties can be tuned, so the foundation of nanotechnology research is based on nanostructure size or shape [1, 2], which is attracting increasing attention in simulations [3, 4] and other experiments [5, 6]. Two recent simulations [3, 4] examined the endocytosis of spherocylindrical bioparticles according to molecular dynamics. The common feature of these simulations is that, during endocytosis, the spherocylinder is initially oriented perpendicular to the membrane but then becomes parallel upon membrane binding and becomes further endocytosed. However, the effects of aspect ratio on such simulation results differ. Vàcha *et al.* [3] showed varied aspect ratios of the spherocylindrical particle and ligand-receptor interaction strength. Whether endocytosis occurred in their model system depended strongly on the ligand-receptor interaction strength; however, the authors did not report a definite dependence on the aspect ratio of the particles. They found that the efficiency of endocytosis for spherocylindrical particles was higher than that for spheres; in contrast, Huang *et al.* [4] found the endocytosis pathway of spherocylindrical bioparticles depends on its aspect ratio regardless of whether its efficiency is higher than that of spherical bioparticles. The spherical bioparticles take more time to be fully endocytosed than the spherocylindrical bioparticles with an aspect ratio of 1.5 (0.5 in Vàcha *et al.*'s study), but less time than the spherocylindrical bioparticles with an aspect ratio of 2 (1 in Vàcha *et al.*'s study). Recent stud-

ies [5, 6] also reported that the endocytosis of bioparticles depends on size and shape. The maximum endocytosis efficiency of spherical gold nanoparticles coated with transferrin occurred at an optimal size of 50 nm. The efficiency of endocytosis for spherocylindrical bioparticles depended on its aspect ratio,The efficiency of endocytosis for spherocylindrical bioparticles with a lower aspect ratio is greater than that of spherocylindrical bioparticles with a higher aspect ratio. The simulations mentioned above and previous theoretical works [3, 4, 7, 8] only considered the ligand-receptor energy and overlooked its dimension, but an experiment [9] based on quantum dots demonstrated that the ligand-receptor complex dimension has strong effects on the size-dependent exclusion of proteins. Our recent work also proved that the optimal size of 50 nm for spherical gold nanoparticles coated with transferrin depends on the ligand-receptor complex dimension [10].

After reviewing the works mentioned above, the effects of the aspect ratio of spherocylindrical bioparticles on endocytosis need to be revolved; at the same time, the dimension of the ligand-receptor complex deserves consideration in the endocytosis of spherocylindrical bioparticles. To accomplish this, a continuum model based on equilibrium mechanics is proposed that includes the effects of aspect ratio and ligand-receptor complex dimension in the endocytosis of spherocylindrical bioparticles.

2 Theoretical model

As mentioned in the introduction, during endocytosis, the spherocylindrical bioparticle becomes parallel upon membrane binding and is then further endocytosed. At this time, four energies are considered in the continuum model (Fig. 1): (i) E_1, the favorable energy from depletion effects, which originates from entropy and is affected by ligand-receptor complex dimension; (ii) E_2, the favorable energy of the ligand-receptor complex, which is proportional to the number of ligand-receptor complexes in the adhesion zone; (iii) E_3, the unfavorable distortion energy of the biomembrane; and (iv) E_4, the unfavorable energy due to biomembrane deformation. The spherocylindrical bioparticle can be treated as a cylinder with hemispherical caps at both ends and cylindrical length L and radius R, and the aspect ratio can be defined as $m = \frac{L}{R}$, as described by Vàcha *et al.* [3] (in Huang's work [4], the aspect ratio is defined as $m = \frac{L+2R}{2R}$). The variables δ, h, and r in the following equation represent the receptor-ligand complex dimension, engulfment of a virus-like particle, and radius of a small bioparticle within the cellular environment, respectively.

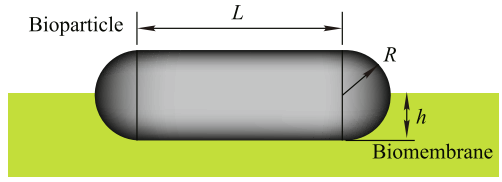

Fig. 1 Representation of the endocytosis process of a spherocylindrical bioparticle. The spherocylindrical bioparticle is a cylinder with hemispherical caps at both ends with cylindrical length L, radius R, and engulfment h. The green sheet is the biomembrane that includes the cytoskeleton.

2.1 Depletion effects in the endocytosis

The depletion effects of endocytosis result from a nonspecific interaction that originates from entropy. The free energy that results from the depletion effects can be described by the *AO* model (achieved by Asakura and Oosawa a half century ago [11]): $E_1 = -\int p\mathrm{d}V$ [12], where V is excluded volume and p is proportional to the concentration of the small particles in cellular environment (c) and can be expressed by the Van't Hoff relation [12] of $p = ck_{\mathrm{B}}T$. If the ligand-receptor complex dimension is not considered and the bioparticle is gradually endocytosed, the gap between the bioparticle and biomembrane will be reduced to a limit, and then the bioparticle will overlap with the biomembrane. The thickness of the limit gap is $2r$; at this time, the volume between the bioparticle and biomembrane is the excluded volume. If the hemispherical caps at both ends of a spherocylindrical bioparticle can be treated as a sphere, then the excluded volume caused by the caps should be the volume difference of two spherical crowns with a radius of $R+2r$ and R, respectively.

$$\frac{2\pi h}{3R}[(R+2r)^3 - R^3] \tag{1}$$

The excluded volume of the cylinder between the hemispherical caps should be

$$\pi mR[(R+2r)^2 - R^2]\arccos\frac{R-h}{R} \tag{2}$$

The total depletion volume including Eqs. (1) and (2) should be

$$\pi\left[\frac{2h}{3R}[(R+2r)^3 - R^3] + mR[(R+2r)^2 - R^2]\arccos\frac{R-h}{R}\right] \tag{3}$$

Once the ligand-receptor complex dimension is considered, the limit gap between the bioparticle and biomembrane can be reduced from $2r$ to $2r-\delta$, which reduces the excluded volume to

$$\pi[\frac{2h}{3(R+\delta)}[(R+2r-\delta)^3-R^3]+m(R+\delta)$$
$$[(R+2r-\delta)^2-R^2]\arccos\frac{R+\delta-h}{R+\delta}] \quad (3)$$

The total free energy from depletion effects upon consideration of the ligand-receptor complex is

$$E_1=-c\pi[\frac{2h}{3(R+\delta)}[(R+2r-\delta)^3-R^3]+$$
$$m(R+\delta)[(R+2r-\delta)^2-R^2]\arccos\frac{R+\delta-h}{R+\delta}] \quad (4)$$

2.2 Ligand-receptor interaction

The surface ligand allows the virus-like particles to interact specifically with the receptor on the biomembrane. At the adhesion zone, the favorable contact energy between the ligand and the receptor is proportional to the adhesion area, receptor-ligand binding energy f, and receptor-ligand complex density ρ, the favorable contact energy can be expressed as

$$E_2=-2\rho f[\pi h(R+\delta)+m(R+\delta)^2\arccos\frac{R+\delta-h}{R+\delta}] \quad (6)$$

On the right side of Eq. (6), two parts correspond to the interaction between the ligand on the two hemispherical caps and the cylindrical portion of the spherocylindrical bioparticle with the receptor on the biomembrane, respectively.

2.3 The elastic recoil energy of the biomembrane

These cooperative interactions mentioned in Eqs. (5) and (6) generate sufficient thermodynamic energy to overcome the elastic recoil of the biomembrane, which includes the bending and stretching energy and the elasticity energy of biomembrane. The bending and stretching energy can be expressed by Hefrich energy [14] as follows:

$$E_3=\frac{4\pi kh}{R+\delta}+\pi\lambda h^2+2m\lambda(R+\delta)[(R+\delta)$$
$$\cdot\arccos\frac{R+\delta-h}{R+\delta}-\sqrt{2(R+\delta)h-h^2}]+mk(R+\delta)$$
$$\cdot\arccos\frac{R+\delta-h}{R+\delta} \quad (7)$$

where k and λ are bending rigidity and surface tension of the biomembrane, respectively. At the adhesion zone, the spherocylindrical bioparticle and biomembrane are uniform and isotropic. Thus, the final resistive energy from the elastic energy of the biomembrane [14] can be written as

$$E_4=\frac{2(R+\delta)^{0.5}h^{2.5}}{5\mu}+\frac{3m\pi}{16\mu}[(R+\delta)h^2-\frac{1}{3}h^3] \quad (8)$$

Here, $\mu=\frac{3}{4}(\frac{1-\sigma_1^2}{\varepsilon_1}-\frac{1-\sigma_2^2}{\varepsilon_2})$ is related to the Young's modulus and Poisson ratio of spherocylindrical bioparticle and biomembrane, σ_1 and ε_1 are the passion ratio and Young's modulus of the virus-like particle, respectively, while σ_2 and ε_2 are those of the biomembrane. The Young's modulus of the biomembrane is much less than that of virus-like particle, which makes $\frac{1-\sigma_1^2}{\varepsilon_1}\ll\frac{1-\sigma_2^2}{\varepsilon_2}$, so μ is only determined by σ_2 and ε_2. The Young's modulus of the biomembrane is on the order of 10 kPa or less and the Poisson ratio of the biomembrane is 0.5, which makes $\mu=\frac{3}{4}\frac{1-\sigma_1^2}{\varepsilon_1}=256.25$.

The variable k_BT is taken as the unit of all the energies mentioned above, while the choice of the physical constants in the energies is guided by the experimental data. The bending modulus of the biomembrane is typically of the order of 10–20 and the surface tension of the biomembrane is approximately 0.005/nm^2 [7]. The receptor-ligand binding energy is estimated to be on the order of 10–25 [15].

3 Results and discussion

3.1 Phase diagram of δ, m and R

All of the energies mentioned above can be combined as follows:

$$E=-c\pi[\frac{2h}{3(R+\delta)}[(R+2r-\delta)^3-R^3]$$
$$+m(R+\delta)[(R+2r-\delta)^2-R^2]\arccos\frac{R+\delta-h}{R+\delta}]$$
$$-2\rho f[\pi h(R+\delta)+m(R+\delta)^2\arccos\frac{R+\delta-h}{R+\delta}]$$
$$+\frac{4\pi kh}{R+\delta}+\pi\lambda h^2+2m\lambda(R+\delta)[(R+\delta)$$
$$\cdot\arccos\frac{R+\delta-h}{R+\delta}-\sqrt{2(R+\delta)h-h^2}]$$
$$+mk(R+\delta)\arccos\frac{R+\delta-h}{R+\delta}+\frac{2(R+\delta)^{0.5}h^{2.5}}{5\mu}$$
$$+\frac{3m\pi}{16\mu}[(R+\delta)h^2-\frac{1}{3}h^3] \quad (9)$$

The equilibrium state can be identified using Eq. (9). The total energy satisfies $\frac{\partial E}{\partial h}\,|_{h_0}=0$, where the engulfment depth h_0 is the root of Eq. (9) at the equilibrium state:

$$
\begin{aligned}
&-c\pi\Big[\frac{2}{3(R+\delta)}[(R+2r-\delta)^3-R^3] \\
&\quad +m(R+\delta)[(R+2r-\delta)^2-R^2]\frac{1}{\sqrt{2h(R+\delta)-h^2}}\Big] \\
&\quad -2\rho f\Big[\pi(R+\delta)+m(R+\delta)^2\frac{1}{\sqrt{2h(R+\delta)-h^2}}\Big] \\
&\quad +\frac{4\pi k}{R+\delta}+2\pi\lambda h+2m\lambda(R+\delta) \\
&\quad \cdot\Big[(R+\delta)\frac{1}{\sqrt{2h(R+\delta)-h^2}} \\
&\quad -\frac{R+\delta-h}{\sqrt{2h(R+\delta)-h^2}}\Big]+mk(R+\delta)\frac{1}{\sqrt{2h(R+\delta)-h^2}} \\
&\quad +\frac{(R+\delta)^{0.5}h^{1.5}}{\mu}+\frac{3m\pi}{16\mu}[2(R+\delta)h-h^2]=0 \quad (10)
\end{aligned}
$$

When the spherocylindrical bioparticle is fully engulfed, the engulfment should be satisfied with $h = 2R$. Under this condition, the correlation among the three parameters (ligand-receptor complex dimension (δ), aspect ratio (m), and radius (R)) can be obtained by Eq. (10) and shown as the phase diagrams (Fig. 2). The ligand-receptor densities have strong effects on the aspect ratio. All of the other parameters are same as those corresponding to the upper phase diagram in Fig. 2, and only the ligand-receptor density ρ increases from $0.007/\text{nm}^2$ to $0.008/\text{nm}^2$, while the aspect ratio takes on an obvious change. The larger aspect ratio appeared in the lower phase diagram in Fig. 2 with the ligand-receptor density increasing. Huang *et al.* [4] revealed a universal pathway (lying-down-then-standing-up sequence) in the endocytosis process of spherocylindrical bioparticles and observed that the standing-up process is absent provided that very high ligand-receptor densities are prescribed. They attributed the absence of the standing-up process to the loss of interaction specificity at high ligand-receptor densities.

In the simulation work [4], the ligand density on the bioparticle and the receptor density on the biomembrane are set at $0.01475/\text{nm}^2$ and $0.00095/\text{nm}^2$, respectively, and the diffusion of receptor on the biomembrane is considered, which will make the ligand-receptor densities in the endocytosed area near the ligand density on the bioparticle ($0.01475/\text{nm}^2$). Vàcha *et al.* [3] also reported high ligand-receptor density. Obviously, both simulations demonstrated the effects of ligand-receptor densities, which are also shown by the phase diagrams in Fig. 2. The highlights from Fig. 2 show that the higher ligand-receptor densities should correspond to the larger aspect ratio. The ligand densities in the simulations mentioned above are greater than those reported in our study, but the aspect ratio considered in the simulation mentioned above is limited.

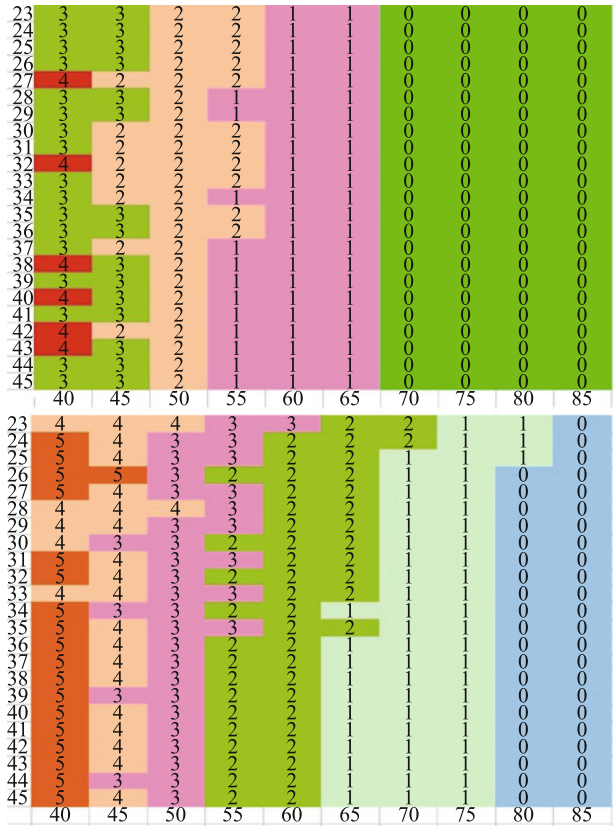

Fig. 2 The phase diagram of the three parameters, the ligand-receptor complex dimension δ, aspect ratio m, and radius R.The left column and the lowest line correspond to the ligand-receptor complex dimension δ and radius R, respectively. The numbers marked in different colors represent the aspect ratio corresponding to the ligand-receptor complex dimension δ and radius R. The ligand-receptor densities ρ are $0.007/\text{nm}^2$ for the upper phase diagram and $0.008/\text{nm}^2$ for the lower phase diagram,respectively, and the ligand-receptor binding energy for both phase diagrams is 20.

3.2 Effects of concentration of small particle (c) and the competition between depletion effects and dimension of ligand-receptor complex (δ)

According to the parameters in the phase diagrams in Fig. 2, the effective endocytosis and depletion effects can be highlighted by the correlation between total energy and engulfment (Fig. 3). Some important information can be derived from Fig. 3. First, with the concentration of the small particles (c) ranging from $0.0008/\text{nm}^3$ to $0.0012/\text{nm}^3$, the concentration (c) distinguishes the correlation between total energy and engulfment; second, while the effects of the ligand-receptor complex dimension are considered, once the diameter of the small particle is less than that of the ligand-receptor complex ($R+2r-\delta < R$), the depletion effect does not work, which shifts the free energy created by the depletion effects [Eq. (5)] from negative to positive, so that the total energy [Eq. (9)] increases as the concentration of

small particles (c) increases. As long as the condition $R+2r-\delta > R$ is satisfied, the depletion effect will work. The total energy [Eq. (9)] decreases as the concentration of small particles (c) increases.

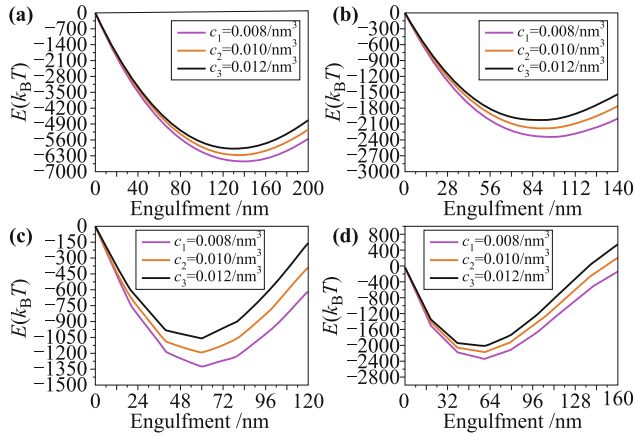

Fig. 3 Effects of depletion on endocytosis. The parameters in **(a–d)** are prescribed based on the phase diagrams in Fig. 2: aspect ratio, spherocylinder radius and ligand-receptor complex dimensions, from **(a)** to **(d)**, the values of these parameters are i) 65 nm and 40 nm, ii) 50 nm and 25 nm, iii) 45 nm and 27 nm, iv) 40 nm and 43 nm, respectively.

In conclusion, the continuum mechanics model proposed in this work considers not only the biomembrane elasticity and the ligand-receptor interaction included in previous theoretical works but also the depletion effects and ligand-receptor complex dimension not mentioned in previous works. The phase diagrams on the aspect ratio, spherocylindrical radius, and ligand-receptor complex dimension have been provided, which implies that the aspect ratio of the spherocylindrical bioparticle should match the ligand-receptor densities. The small particle diameter and the ligand-receptor complex dimension determines whether the depletion effects work in endocytosis.When the small particle diameter becomes smaller than the ligand-receptor complex dimension,the depletion effects are not favorable for bioparticle endocytosis. All of these conclusions will provide insights for future bioparticle design experiments.

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