SPECIAL TOPIC — Soft matter and biological physics

Enhanced effect of dimension of receptor-ligand complex and depletion effect on receptor-mediated endocytosis of nanoparticles*

Ye Liu(刘野)[1,†], Qingqing Gao(高庆庆)[1,†], Yijun Liu(刘益军)[2,†], Chuang Zhao(赵闯)[1], Zongliang Mao(毛宗良)[1], Lin Hu(胡林)[1,‡], and Yanhui Liu(刘艳辉)[1,§]

[1] College of Physics, Guizhou University, Guiyang 550025, China

[2] CAS Key Laboratory for Mechanical Behavior and Design of Materials, University of Science and Technology of China, Hefei 230027, China

(Received 24 August 2017; revised manuscript received 10 October 2017; published online 20 November 2017)

We present an extended analytical model including the depletion effect and the dimension of ligand-receptor complex, aiming to elucidate their influences on endocytosis of spherocylindrical nanoparticles (NPs). It is found that the dimension of ligand-receptor complex (δ) and the depletion effect interrelatedly govern the optimal conditions of NP endocytosis. The endocytosis phase diagram constructed in the space of NP radius and relative aspect ratio indicates that the endocytosis of NP is enhanced evidently by reducing the optimal radius and the threshold radius of endocytosed NP. Meanwhile, through thermodynamic and kinetic analysis of the diffusion of receptors, the dependence of diffusion length on depletion effect and the dimension of ligand-receptor complex can be identified in great detail. For small aspect ratio, diffusion length decreases with increasing concentration c of small bioparticles in cellular environment. Endocytosis speed corresponding to large radius R and high concentration c of small bioparticles strongly depends on the increasing $(2r-\delta)$. These results may show some highlights into the conscious design of NPs for diagnostic agents and therapeutic drug delivery applications.

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

PACS: 87.10.Pq, 87.16.A–, 87.17.Rt **DOI:** 10.1088/1674-1056/26/12/128704

1. Introduction

Most matured animal viruses enter their host cells via receptor-mediated endocytosis,[1] in which the specific binding of a viral protein to some receptor protein on the outer cell membrane triggers the internalization of the virus inside an endosome. Inspired by the high specificity and efficiency of the above process, extensive effort has been devoted to develop biomimetic nanoparticle (NP)-based therapeutics. The design of synthetic nanoparticles (NPs) for biomedical and clinical applications, such as drug delivery and biomedical imaging,[2–5] requires a thorough understanding of the physical mechanisms of receptor-mediated endocytosis of the NPs. Since the endocytosis are driven by the NP-membrane adhesion while penalized by the cell membrane deformation,[6] both NPs properties[7] and cell surface mechanics, such as membrane curvature,[8] play important roles in determining endocytosis of NPs. Experimental studies have demonstrated particle size and shape as important factors in cellular uptake of nanomaterials, and there is an optimal size about 25 nm corresponding to the minimal endocytic time.[9,10] Several analytical models[11–14] based on thermodynamics and dynamics also endeavor to pursue the underlying mechanism on the receptor-mediated endocytosis, and predict that the optimal size for endocytosis is around 25 nm–30 nm.

However, few existing theoretical models concerning the endocytosis of single NP ever considered the influences of the depletion interaction as well as the dimension of receptor-ligand complex. The depletion effect has been demonstrated experimentally[15] that larger spheres among small colloidal particles inside lipid bilayer vesicles are to be pinned to the vesicle surface due to excluded-volume effect, which might be also significant factor in the cellular uptake of NPs that evidenced by experiments. Recent experiments have demonstrated that the dimension of receptor-ligand complex ranges from 10 nm to 42 nm and has strong effect on the size-dependent exclusion of proteins.[16] Size differences of membrane proteins can drastically alter their organization at membrane interfaces formed at cell-cell junctions, with as little as a 5nm increase in non-binding protein size driving its exclusion

*Project supported by the National Natural Science Foundation of China (Grant Nos. 11047022, 11204045, and 11464004), the Tracking Key Program of Social Development of Guizhou Province, China (Grant Nos. SY20123089 and SZ20113069), the General Financial Grant from the China Postdoctoral Science Foundation (Grant No. 2014M562341), the Research Foundation for Young University Teachers from Guizhou University (Grant No. 201311), and the College Innovation Talent Team of Guizhou Province, China (Grant No. (2014)32).

† These authors contribute equally to this work.

‡Corresponding author. E-mail: hulin53@sina.com

§Corresponding author. E-mail: ionazati@itp.ac.cn

© 2017 Chinese Physical Society and IOP Publishing Ltd

from the interface.[17] At the same time, a preliminary work including depletion effect, dimension of ligand-receptor complex has pointed out that the optimal radius of endocytosed NPs depends on the dimension of ligand-receptor complex[18] and a simulation work also demonstrated the effect of receptor length.[19] All of which highlight necessity that the depletion effect, dimension of ligand-receptor complex and their combined effect should be considered during the endocytosis process of NPs and detected further.

In this article, an extended analytical model including the depletion effect and the dimension of ligand-receptor complex is presented, aiming to elucidate their influences on endocytosis of spherocylindrical NPs. Based on thermodynamic analysis and kinetic diffusion of receptors, we revealed that the depletion effect and the dimension of ligand-receptor complex interrelatedly govern the optimal conditions of NPs endocytosis. Furthermore, through thermodynamic and kinetic analysis of the diffusion of receptors, the endocytosis phase diagram in the space of NP radius and relative aspect ratio were constructed. The results may provide a guidance to the design of NPs for diagnostic agents and therapeutic drug delivery applications.

2. Theoretical method or experimental setup

The engulfment of NP endocytosed by a cell is a complex process. Cell membrane deformation, including membrane bending and stretching at the wrapping area, the elastic energy of cell membrane including cytoskeleton, the diffusion of receptor towards the wrapping area from the remote area on the cell, and depletion effect determined by the dimension of ligand-receptor complex and radius of small particles in cellular environment are involved in this process. While the clathrin protein is restricted, the endocytosis can also be realized by ligand-receptor interaction,[20] so effect of clathrin protein was not included in current work.

2.1. Bending, stretching energy, and elastic energy of cell membrane

The bending and stretching energy of the cell membrane treated as an elastic sheet can be given by the Canham-Helfrich energy,[21] as shown in Fig. 1,

$$E = \iint_A \left[\frac{\kappa}{2}(c_1 + c_2 - c_0)^2 + \lambda\right] \mathrm{d}A, \tag{1}$$

where c_1 and c_2 are two principal curvatures of the bound membrane surface, c_0 is the spontaneous curvature (during the endocytosis, the NP is much smaller than the cell, so that the topology state of cell does not change, c_0 is considered to be zero in the following sections), κ is the bending modulus of the membrane and λ is the tensile stress on the membrane caused by the endocytosis (in our calculations, the parameters $\kappa = 20k_BT$, $\lambda = 0.001k_BT/\mathrm{nm}^2$, where k_B is the Boltzmann constant and T is the absolute temperature). As shown by Fig. 1, the NP is sketched as a cylindrical rod with hemispherical caps. While the dimension of ligand-receptor complex (δ) was considered, the bending energy caused by a full wrapping can be expressed as

$$E_{\mathrm{bend}} = \iint_{A_L} \frac{\kappa}{2}\left(\frac{1}{R+\delta}\right)^2 \mathrm{d}A + \iint_{A_R} \frac{\kappa}{2}\left(\frac{2}{R+\delta}\right)^2 \mathrm{d}A, \tag{2}$$

Where A_L and A_R denote the surface of cylindrical rod and hemispherical caps of the spherocylindrical particle. So that the bending and stretching energy per area can be defined as

$$\frac{E}{S} = \frac{A_0\kappa(m+8)}{2(R+\delta)^2(m+2)} + \lambda A_0, \tag{3}$$

where m is aspect ratio defined as $m = L/(R+\delta)$, L and R correspond to the length of cylindrical part and the radius of the hemispherical part, respectively. A_0 is the cross-sectional area of one receptor which will be taken as the unit area hereafter (in our calculations, the parameter $A_0 = 15\ \mathrm{nm} \times 15\ \mathrm{nm}$).

Under the condition that both NP and cell membrane are uniform and isotropic, the interaction between NP and cell membrane can be modelled as the interaction between a sphere and biomembrane, a cylindrical rod with biomembrane. The elastic energy for the interaction between a sphere and cell membrane can be expressed as $(2\sqrt{R}/5D)h^{2.5}$,[22] and its extended version for the interaction between a cylindrical rod with cell membrane $(3\pi m/16D)[Rh^2 - (1/3)h^3]$.[23] Once the dimension of ligand-receptor complex is considered, the total elastic energy can be written as

$$\frac{2\sqrt{R+\delta}}{5D}h^{2.5} + \frac{3\pi m}{16D}\left[(R+\delta)h^2 - \frac{1}{3}h^3\right], \tag{4}$$

where h is the engulfment depth,

$$D = \frac{3}{4}\left(\frac{1-\sigma_1^2}{\varepsilon_1} + \frac{1-\sigma_2^2}{\varepsilon_2}\right)$$

is related to the Young's modulus and Poisson ratio of spherocylindrical particle and cell membrane. σ_n and ε_n $(n = 1, 2)$ are the passion ratio and Young's modulus, and 1 and 2 represent NP and cell membrane, respectively. For the Young's modulus of the biomembrane is much less than that of virus-like particle, which leads to

$$\frac{1-\sigma_1^2}{\varepsilon_1} \ll \frac{1-\sigma_2^2}{\varepsilon_2}.$$

So that D is only determined by σ_2 and ε_2, and

$$D = \frac{3}{4}\left(\frac{1-\sigma_2^2}{\varepsilon_2}\right) = 256.25,$$

while the typical experimental data is assigned to σ_2 and ε_2, σ_2 is taken to be 0.5 and ε_2 is on the order of 10 kPa.

2.2. Depletion effect

Depletion interaction originating from entropy can be described by the A–O model (achieved by Asakura and Oosawa a half century ago[24,25]) and expressed as $E_{\rm dep} = \int p{\rm d}V$, according to the Van't Hoff relation,[26] $p = ck_{\rm B}T$ is proportional to the concentration (c) of the small bioparticles within cellular environment. V corresponding to the excluded volume depends on the radius (r) of small bioparticle in cellular environment and dimension (δ) of ligand-receptor complex. Once the dimension of ligand-receptor complex is considered, which makes NP cannot overlap with cell membrane, as shown by the sketch in Fig. 1, a limit gap existing between the NP and cell membrane. While the diameter ($2r$) of small bioparticle is larger than the dimension of ligand-receptor complex, the thickness of excluded volume in radial direction is reduced from $2r$ to $2r-\delta$, so that the excluded volume as the function of the radius (r) of small bioparticle in cellular environment and the dimension (δ) of ligand-receptor can be written as

$$\frac{2\pi h((R+2r-\delta)^3-R^3)}{3(R+\delta)} + m\theta(R+\delta)((R+2r-\delta)^3),$$

where θ is the polar angle indicated in Fig. 1. In contrast with the condition that the dimension of ligand-receptor complex is not considered, obviously the depletion interaction is reduced. The free energy originated from depletion effect can be obtained

$$E_{\rm dep} = -c\left[\frac{2\pi h((R+2r-\delta)^3-R^3)}{3(R+\delta)} + m\theta(R+\delta)((R+2r-\delta)^2-R^2)\right]. \tag{5}$$

2.3. Ligand-receptor interaction and conformational entropy change of receptor

During the process of NP endocytosed by a cell, receptors on the cell membrane can diffuse to the wrapping area driven by a local reduction of free energy due to ligand-receptor chemical binding. Furthermore, owing to the change of receptors from free to bound states, the process can also lead to the loss of configurational entropy of the receptors. The local reduction of free energy and the loss of configurational entropy of the receptors can be expressed as $-\mu N_{\rm b}$ and $A_{\rm b}(\xi_{\rm b}\ln\xi_{\rm b} + (1-\xi_{\rm b})\ln(1-\xi_{\rm b})) + A_{\rm f}(\xi_{\rm f}\ln\xi_{\rm f} + (1-\xi_{\rm f})\ln(1-\xi_{\rm f}))$,[27] respectively. $A_{\rm b}$ represents the wrapping area bound by $N_{\rm b}$ receptors and the $A_{\rm f}$ is free membrane region with $N_{\rm f}$ receptors on it, thus the densities of receptors in the wrapping and free area are defined as $\xi_{\rm b} = N_{\rm b}/A_{\rm b}$, $\xi_{\rm f} = N_{\rm f}/A_{\rm f}$, respectively. μ is the chemical energy released from each ligand-receptor chemical binding (in our calculations, the parameter $\mu = 20k_{\rm B}T$).

All the energy contributions related to the endocytosis process take $k_{\rm B}T$ as unit, therefore the free energy can be rewritten as

$$\begin{aligned}\frac{\Delta G}{k_{\rm B}T} = {} & \frac{A_{\rm b}E}{S} - c\left[\frac{2\pi h((R+2r-\delta)^3-R^3)}{3(R+\delta)} + m\theta(R+\delta)((R+2r-\delta)^2-R^2)\right] \\ & + \frac{2\sqrt{R+\delta}}{5D}h^{2.5} + \frac{3\pi m}{16D}\left[(R+\delta)h^2 - \frac{1}{3}h^3\right] + A_{\rm b}\left[\xi_{\rm b}\ln\xi_{\rm b} + (1-\xi_{\rm b})\ln(1-\xi_{\rm b})\right] \\ & + A_{\rm f}\left[\xi_{\rm f}\ln\xi_{\rm f} + (1-\xi_{\rm f})\ln(1-\xi_{\rm f})\right] - \mu N_{\rm b}.\end{aligned} \tag{6}$$

The membrane area and the number of the receptors are constrained by the conservation condition $A = A_{\rm b} + A_{\rm f}$ and $N = N_{\rm b} + N_{\rm f}$, so that the free energy can be minimized with respect to two independent variables $A_{\rm b}$ and $N_{\rm b}$. The minimum of the free energy obtained from $\partial\Delta G/\partial A_{\rm b}$ and $\partial\Delta G/\partial N_{\rm b}$ satisfies,

$$\frac{\xi_{\rm b}}{1-\xi_{\rm b}} = {\rm e}^{\mu}\frac{\xi_{\rm f}}{1-\xi_{\rm f}}, \quad \frac{1-\xi_{\rm b}}{1-\xi_{\rm f}} = {\rm e}^{-W}. \tag{7}$$

The density of bound and free receptors elucidated by Eq. (7) can be obtained

$$\xi_{\rm b}^* = \frac{1-{\rm e}^{-W}}{1-{\rm e}^{-\mu}}, \quad \xi_{\rm f}^* = \frac{{\rm e}^{W}-1}{{\rm e}^{\mu}-1}. \tag{8}$$

The detailed expression of W in Eq. (7) and Eq. (8) is provided in Appendix A (supplementary information).

To drive the receptors on free membrane towards the wrapping area, the highest possible density of receptors on free membrane should be less than the initial density of receptors on the membrane surface, i.e., $\xi_{\rm f}^* < \xi_0$, so the minimum radius of wrapping NP is given by setting $\xi_{\rm f}^* = \xi_0$ (in our calculations, the parameter $\xi_0 = 0.05$) and using Eq. (8), and satisfies the following equation,

$$\frac{A_0k(8+m)}{2(2+m)(R_{\rm min}+\delta)^2} + \lambda A_0 + \frac{cA_0((R_{\rm min}+2r-\delta)^2 - R_{\rm min}^2)}{2(R_{\rm min}+\delta)} = \mu + \ln\xi_0, \tag{9}$$

which is a nonlinear equation, and the threshold NP size ($R_{\min}$) depending on the dimension of ligand-receptor and the concentration of small bioparticle in cellular environments, has been solved numerically. The wrapping NP with radius below the threshold NP size ($R_{\min}$) can not be internalized through receptor-mediated endocytosis.

The continuous binding of ligand with receptors diffused from the vicinity of the NP triggers the endocytosis, conservation of the receptors in the wrapping area and its vicinity specifies a characteristic length l as shown by Fig. 1, defined by

$$l_{\text{diff}} = \sqrt{\frac{S(\xi_{\text{b}}^* - \xi_0)}{\pi(\xi_0 - \xi_{\text{f}}^*)}}, \tag{10}$$

which is called diffusion length and defined as the distance for receptor diffusion from free region to bound region, the time scale for full wrapping of an NP can be estimated by considering the diffusion length, and then approximated as $t \sim l_{\text{diff}}^2/D$, where D is the diffusivity of the receptors. Clearly, the faster endocytosis appears to have a smaller diffusion length. The theoretical optimal NP size can be obtained numerically by minimizing the diffusion length with respect to R.

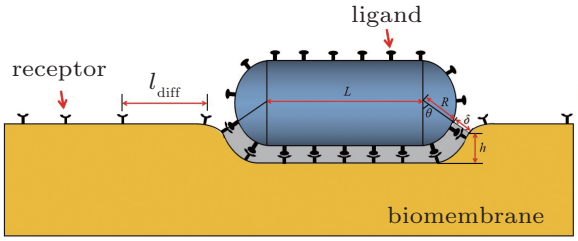


Fig. 1. (color online) Receptor-mediated endocytosis of NP. l_{diff}, R, h, and δ represent diffusion length, radius of NP, engulfment of NP, and the dimension of ligand-receptor complex, respectively.

3. Results and discussion

By analyzing the dependence of endocytosis speed ($l_{\text{diff}}/t \propto D/l_{\text{diff}} \propto 1/l_{\text{diff}}$) on the radius of NPs, phase diagrams are constructed on R–m plane as shown by Fig. 2. Wherein phase diagram figure 2(a) is constructed under the conditions that the depletion effect, the dimension of ligand-receptor complex and membrane tension are not considered, namely, the parameters c, δ, and λ are set to be zero. The solid dark line and white line represent the theoretical optimal NP size and the threshold NP size for endocytosis, respectively. The optimal radius for spherical NP with aspect ratio $m = 0$ is 25 nm, which is well consistent with previous experimental and theoretical results[9,11,28] that the NPs with radius about 25 nm have a maximal endocytosis rate. Effect of NP size on endocytosis speed can also be identified from the phase diagram, the color bar indicates the values of endocytosis speed $1/l_{\text{diff}}$, the high and low value of $1/l_{\text{diff}}$ correspond to the fast and slow endocytosis, respectively. It is impossible for the NPs to be endocytosed in the dark blue region.

After the depletion effect, the dimension of ligand-receptor complex and membrane tension are considered, they make the phase diagram Fig. 2(b) different from the phase diagram Fig. 2(a). The optimal NP size and the threshold NP size for endocytosis as compared to the one in Fig. 2(a) scrolled down. As an example, the optimal radius for NP with aspect ratio $m = 0$ is reduced from ~ 25 nm to ~ 17 nm.

During its endocytosis process, the bending energy density $2k/R^2$ is proportional to $1/R$, and the local energy balance between adhesion energy and bending energy determine a optimal radius of NP, $\sqrt{2k/\alpha}$. α is the local adhesion energy density, which is dependent on the strength of ligand-receptor interaction and depletion effect. The introduction of depletion effect make the local adhesion energy density increased and leads to the endocytosis of NPs with small optimal radius, so that the dark blue region impossible for NPs to be endocytosed is also reduced evidently.

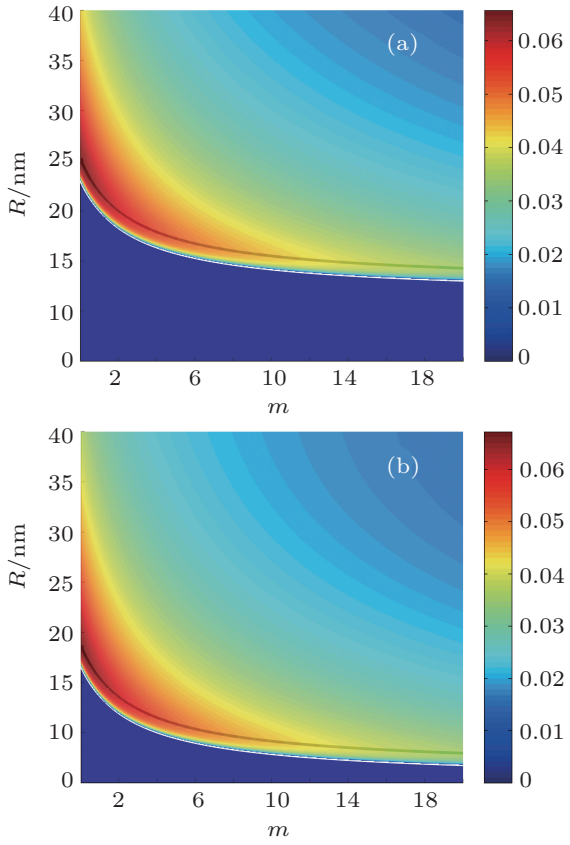


Fig. 2. (color online) The endocytosis phase diagram in the space of radius and aspect ratio m of NP based on Eq. (10). The color bar indicates the values of endocytosis speed $1/l_{\text{diff}}$, the endocytosis in the dark blue region is impossible. The solid dark line and white line represent the theoretical optimal NP size and the threshold NP size for endocytosis, respectively. (a) Depletion effect, the dimension of ligand-receptor complex and membrane tension are not considered, its corresponding parameters, c, δ, and λ, are set to be zero. (b) The corresponding parameters utilized in Fig. 2(b) are listed as $c = 0.0002/\text{nm}^3$, $\delta = 6$ nm, and $\lambda = 0.001 k_{\text{B}}T/\text{nm}^2$.

As demonstrated by the phase diagram Fig. 2(b), the dimension of ligand-receptor complex and depletion effect have obvious effect on the endocytosis speed. It is necessary to elucidate the effect of dimension of ligand-receptor complex

and depletion effect on the endocytosis speed through energetic analysis.

Figure 3 indicates that the values of diffusion length as the function of aspect ratio m at fixed R (15 nm), c ($0.0002/\text{nm}^3$), and r (10 nm) (a), and the function of R at fixed m (5), c ($0.0002/\text{nm}^3$), and r (10 nm) (b), respectively, change with the ligand-receptor complex δ. The curves in Fig. 3(a) have similar characters that with increasing aspect ratio m, the value of l_{diff} drops sharply to a bottom and then increases quickly. whereas for the dimension of ligand-receptor complex $\delta \geq 10$ nm, the value of l_{diff} increases gradually with increasing aspect ratio m. One can note that the sum of the fixed R and the dividing dimension of ligand-receptor complex δ between two features are equivalent to 25 nm (the optimal radius of spherical particle in Fig. 2(a) ($m = 0$)), which suggests that the ligand-receptor complex assists in the endocytosis of small NPs. As the same time, figure 3(b) provides direct evidences that the optimal radius of NP decreases gradually with increasing ligand-receptor complex δ.

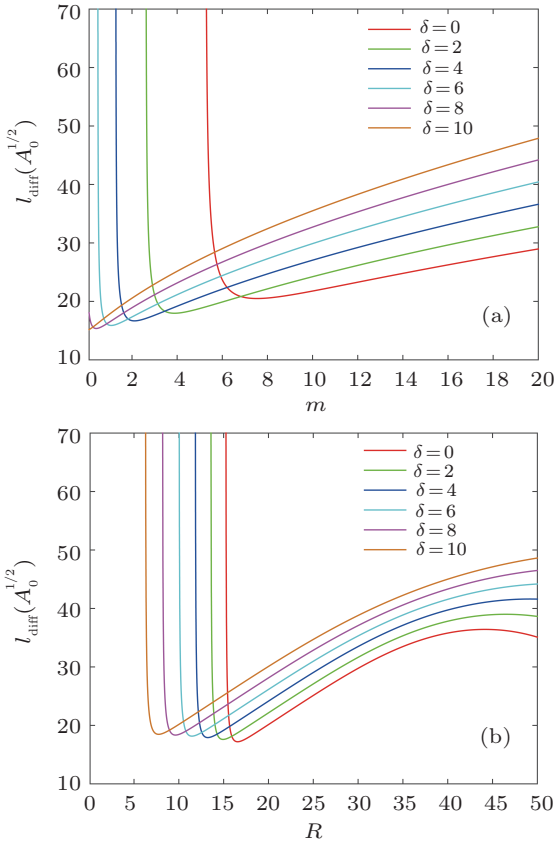


Fig. 3. (color online) The values of diffusion length as the function of aspect ratio m (at fixed R (15 nm), c ($0.0002/\text{nm}^3$), and r (10 nm)) (a), and the function of R (at fixed m (4), c ($0.0002/\text{nm}^3$), and r (10 nm)) (b), respectively, change with the ligand-receptor complex δ.

Figure 4 demonstrates that the values of diffusion length as the function of aspect ratio m (at fixed R (15 nm), δ (2 nm), and r (10 nm)) (a), and the function of R (at fixed m (4), δ (6 nm) and r (10 nm)) (b), respectively, change with the concentration c of small bioparticles in cellular environment. For the summation of fixed R and the ligand-receptor complex δ less than 25nm, the diffusion length as the function of aspect ratio m in Fig. 4(a) shares similar features with the curves in Fig. 3(a), but the effect of the concentration c of small bioparticle in cellular environment on the diffusion length is dependent on the aspect ratio m. For small aspect ratio m, the minimum value of l_{diff} at different concentration c of small bioparticles is distinguishable and decreases gradually with increasing concentration c. Whereas for larger m, the value of l_{diff} overlaps together and increases gradually with increasing m. It is because the bending energy density decreases with increasing m (see Eq. (3)) and the adhesion energy including binding energy and depletion effect is always larger than the membrane deformation cost. Therefore, endocytosis of NPs under these conditions is always energetically possible with increasing m. Moreover, depletion effect is insufficient to endocytosis of NPs with larger aspect ratio,[29] which suggests that the binding energy in the adhesion energy is the dominant over the depletion effect, so that the value of l_{diff} is nominally dependent on the depletion effect and overlaps together for larger m.

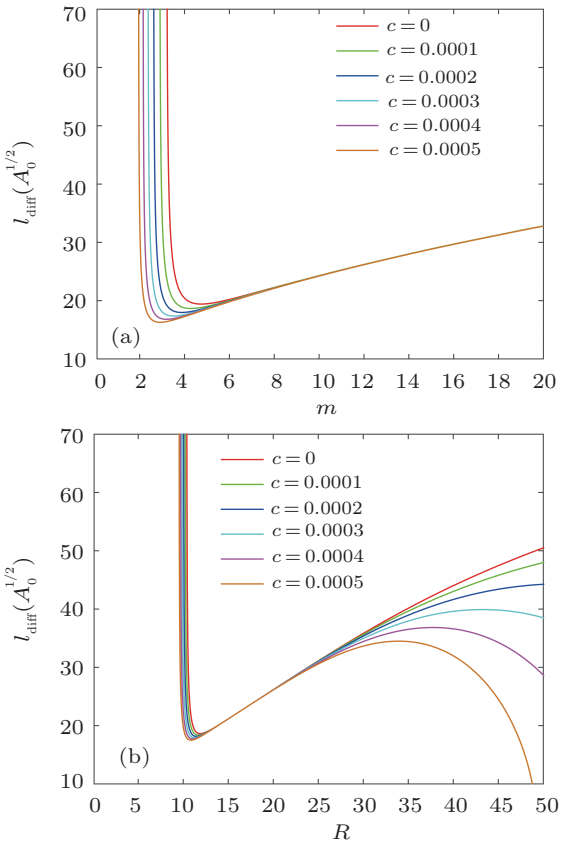


Fig. 4. (color online) The values of diffusion length as the function of aspect ratio m (at fixed R (15 nm), δ (2 nm), and r (10 nm)) (a), and the function of R (at fixed m (4), δ (6 nm), and r (10 nm)) (b), respectively, change with the concentration c of small bioparticles in cellular environment.

The curves in Fig. 4(b) demonstrate that the values of diffusion length as the function of R (at fixed m (4), δ (6 nm), and r (10 nm)) change with the concentration c of small bioparti-

cles in cellular environment. As compared to the diffusion length corresponding to small R, the one corresponding to larger R is strongly dependent on the concentration c of small bioparticles in cellular environment and decreases with the increasing concentration c.

These phenomena can be elucidated by re-expressing Fig. 4(b) by phase diagram in the space of radius R and the concentration c as shown in Fig. 5. From which, some important information about depletion effect on endocytosis speed can be revealed. The key result is that the values of endocytosis speed $(1/l_{\rm diff})$ corresponding to large radius R and high concentration c of small bioparticles take on evident change and strongly depend on the increasing $(2r-\delta)$ corresponding to Figs. 5(a)–5(c). Increasing $(2r-\delta)$ leads to enhanced depletion effect (see Eq. (5)), so that the endocytosis speed can be increased.

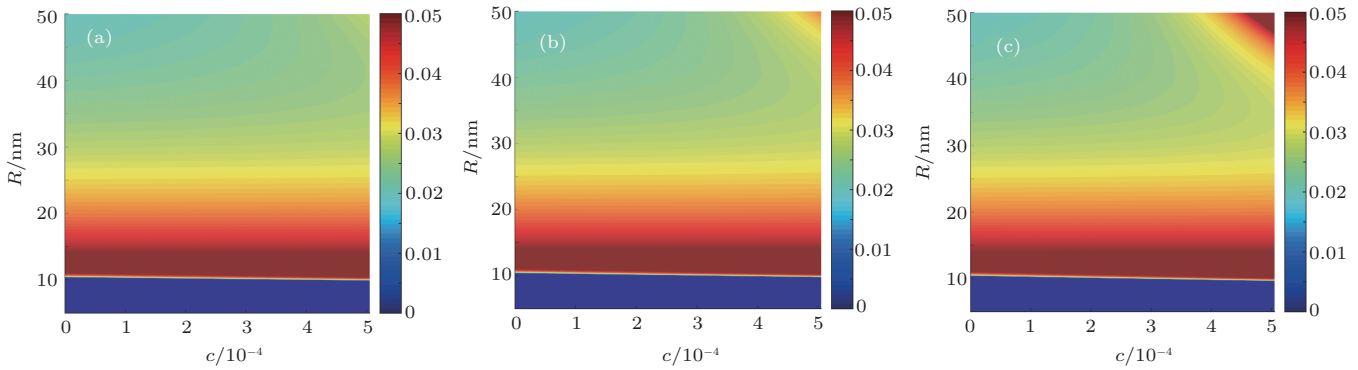


Fig. 5. (color online) The re-expression of Fig. 4(b) by the endocytosis phase diagram constructed in the space of radius and the concentration c of small particle in cell environment. $(2r-\delta)$ corresponding to panels (a)–(c) increases gradually (10 nm (a), 12 nm (b), and 14 nm (c)). The color bar indicates the values of endocytosis speed $(1/l_{\rm diff})$, the endocytosis in the dark blue region is impossible.

4. Conclusions

Nps designed for diagnostic agents and drug delivery is covered by protein corona as ligand, binding with receptor on cell membrane as a complex. It has been demonstrated that the dimension of ligand-receptor complex and the depletion effect have evident effect on NP endocytosis. The extended analytical model including the depletion effect and the dimension of ligand-receptor complex is utilized to elucidate their influences on endocytosis of spherocylindrical NPs. The dimension of ligand-receptor complex and the depletion effect interrelatedly govern the optimal conditions of NP endocytosis. As demonstrated by the endocytosis phase diagram constructed in the space of NP radius and relative aspect ratio, the endocytosis of NP is enhanced evidently by reducing the optimal radius and the threshold radius of endocytosed NP. Meanwhile, through thermodynamic and kinetic analysis of the diffusion of receptors, the dependence of diffusion length on depletion effect and the dimension of ligand-receptor complex can be identified in great detail. For small aspect ratio, diffusion length decreases with increasing concentration c of small bioparticles in cellular environment. The values of endocytosis speed corresponding to large radius R and high concentration c of small bioparticles strongly depend on the increasing $(2r-\delta)$. These results highlight the importance of the dimension of ligand-receptor complex and the size of small particle in cellular environment, on which more attention should be paid during the process of designing the NPs for diagnostic agents and therapeutic drug delivery applications.

Appendix A

All the energy contributions to the endocytosis process take $k_{\rm B}T$ as unit, therefore the free energy can be written as

$$\begin{aligned}\frac{\Delta G}{k_{\rm B}T} &= \frac{A_{\rm b}E_D}{S}+\frac{2\sqrt{R+\delta}}{5D}h^{2.5}+\frac{3\pi m}{16D}\left[h^2(R+\delta)-\frac{1}{3}h^3\right]\\ &+A_{\rm b}\left[\xi_{\rm b}\ln\xi_{\rm b}+\left(1-\xi_{\rm b}\right)\ln\left(1-\xi_{\rm b}\right)\right]+A_{\rm f}\left[\xi_{\rm f}\ln\xi_{\rm f}+\left(1-\xi_{\rm f}\right)\ln\left(1-\xi_{\rm f}\right)\right]\\ &-\mu N_{\rm b}-\frac{2c\pi h\left[\left(R+2r-\delta\right)^3-R^3\right]}{3\left(R+\delta\right)}-cm\theta\left(R+\delta\right)\left[\left(R+2r-\delta\right)^2-R^2\right],\end{aligned}\tag{A1}$$

where

$$A_{\rm b}=\frac{2\pi(R+\delta)^2(1-\cos\theta)+2m\theta(R+\delta)^2}{A_0}.$$

Considering the constraints of conservation of membrane area and conservation of receptors, the free energy is the function of two independent variables $N_{\rm b}$ and $A_{\rm b}$, minimizing the free energy with respect to these two independent variables, $\partial\Delta G/\partial N_{\rm b}=0$

and $\partial\Delta G/\partial A_{\rm b}=0$, giving rise to

$$\frac{\xi_{\rm b}}{1-\xi_{\rm b}}=\mathrm{e}^{\mu}\frac{\xi_{\rm f}}{1-\xi_{\rm f}}, \tag{A2}$$

$$\ln(1-\xi_{\rm b})-\ln(1-\xi_{\rm f})+W=0, \tag{A3}$$

where

$$W=\frac{kA_0(8+m)}{2(R+\delta)^2(2+m)}+\lambda A_0+\frac{1}{2D}h^{1.5}\frac{A_0\sin\theta}{\pi(R+\delta)^{0.5}\sin\theta+m(R+\delta)^{0.5}}+\frac{3m\pi}{32D}\frac{A_0\sin\theta\left[2h(R+\delta)-h^2\right]}{\pi(R+\delta)\sin\theta+m(R+\delta)}$$
$$-\frac{\pi c}{3}\left[(R+2r-\delta)^3-R^3\right]\frac{A_0\sin\theta}{\pi(R+\delta)^2\sin\theta+m(R+\delta)^2}-\frac{cmA_0\left[(R+2r-\delta)^2-R^2\right]}{2\pi(R+\delta)\sin\theta+2m(R+\delta)}. \tag{A4}$$

The densities of bound and free receptors and derived from Eq. (A2) and Eq. (A3),

$$\xi_{\rm b}^{*}=\frac{1-\mathrm{e}^{-W}}{1-\mathrm{e}^{-\mu}},\qquad \xi_{\rm f}^{*}=\frac{\mathrm{e}^{W}-1}{\mathrm{e}^{\mu}-1}.$$

For full wrapping of an NP ($\theta=\pi$), receptor densities in the bound and free membrane regions governed by the thermodynamics should satisfy $\xi_{\rm f}^{*}<\xi_0<\xi_{\rm b}^{*}$, where ξ_0 is the initial receptor densities. From the critical condition $\xi_{\rm f}^{*}=\xi_0$, the minimum radius of NP for endocytosis meets

$$\frac{A_0k(8+m)}{2(R_{\rm min}+\delta)^2(2+m)}+\lambda A_0+\frac{cA_0[(R_{\rm min}+2r-\delta)^2-R_{\rm min}^2]}{2(R_{\rm min}+\delta)}=\mu+\ln\xi_0,$$

which is nonlinear and can be numerically solved.

References

[1] Sieczkarski S B and Whittaker G R 2002 *J. Gen. Virol.* **83** 1535
[2] Elghanian R, Storhoff J J, Mucic R C, Letsinger R L and Mirkin C A 1997 *Science* **277** 1078
[3] Klostranec J M and Chan W C W 2006 *Adv. Mater.* **18** 1953
[4] Cobley C M, Chen J, Cho E C, Wang L V and Xia Y 2011 *Chem. Soc. Rev.* **40** 44
[5] Moon G D, Choi S W, Cai X, Li W, Cho E C, Jeong U, Wang L V and Xia Y 2011 *J. Am. Chem. Soc.* **133** 4762
[6] Deserno M and Bickel T 2003 *Europhys. Lett.* **62** 767
[7] Wang J J, Feng J W and Ren C L 2015 *Chin. Phys. B* **24** 088701
[8] Bahrami A H, Lipowsky R and Weikl T R 2016 *Soft Matter* **12** 581
[9] Saki F, Kanamori T, Sando T, Sera T and Aoyama Y 2004 *J. Am. Chem. Soc.* **126** 6520
[10] Chithrani B D, Ghazani A A and Chan W C 2006 *Nano Lett.* **6** 662
[11] Gao H, Shi W and Freund L B 2005 *Proc. Natl. Acad. Sci. USA* **102** 9469
[12] Zhang S, Li J, Lykotrafitis G, Bao G and Suresh S 2009 *Adv. Mater.* **21** 419
[13] Yuan H, Huang C and Zhang S 2010 *PLoS ONE* **5** e13495
[14] Shi W, Wang J, Fan X and Gao H 2004 *Phys. Rev. E* **78** 061914
[15] Dinsmore A D, Wong D T, Nelson P and Yodh A G 1998 *Phys. Rev. Lett.* **80** 409
[16] Alakoskela J M, Koner A L, Rudnicka D, Kohler K, Howarth M and Davis D M 2011 *Biophys. J.* **100** 2865
[17] James J R and Vale R D 2012 *Nature* **487** 64
[18] Liu Y H, Chen Y B, Mao W, Hu L, Deng L H and Xu H Q 2014 *Front. Phys.* **9** 519.
[19] Zhang H, Wang L, Yuan B, Yang K and Ma Y Q 2014 *Materials* **7** 3855
[20] Yang T and Zaman M H 2007 *J. Chem. Phys.* **126** 045103
[21] Ou-Yang Z C, Liu J X and Xie Y Z 1999 *Geometric methods in the elastic theory of membranes in liquid crystal phases* (Singapore: World Scientific) p. 54
[22] Landau L D and Lifshitz E M 1986 *Theory of Elasticity* (Oxford: Butterworth-Heinemann) p. 31
[23] Chen Y B, Liu Y H, Zeng Y, Mao W, Hu L, Mao Z L and Xu H Q 2015 *Front. Phys.* **10** 116
[24] Asakura S and Oosawa F 1954 *J. Chem. Phys.* **220** 1255
[25] Asakura S and Oosawa F 1958 *J. Polym. Sci.* **33** 183
[26] Nelson P 2007 *Biological Physics: Energy, Information and Life* (New York and Basingstoke: W H Freeman) p. 247
[27] Tzlil S, Deserno M, Gelbart W M and Ben-Shaul A 2004 *Biophys. J.* **86** 2037
[28] Chithrani B D and Chan W C 2006 *Nano Lett.* **7** 1542
[29] Zeng Y, Liu Y H, Chen Y B, Mao W, Hu L, Mao Z L, Song X Y and Xu H Q 2015 *J. Biol. Syst.* **23** 49