Effects of particle size and ligand density on the kinetics of receptor-mediated endocytosis of nanoparticles

Hongyan Yuan and Sulin Zhang[a)]
Department of Engineering Science and Mechanics, Pennsylvania State University, Pennsylvania 16802, USA

(Received 27 October 2009; accepted 22 December 2009; published online 22 January 2010)

We elucidate, from thermodynamic arguments, the governing factors of receptor-mediated endocytosis of nanoparticles (NPs). We show that the endocytic energetics specifies a minimal particle size and a minimal ligand density below which endocytosis is not possible. Due to the entropic penalty involved in ligand-receptor binding, endocytosis may occur with a large fraction of ligands unbound with receptors. Our analyses suggest that the endocytic time depends interrelatedly on the particle size and ligand density. There exists an optimal condition at which the endocytic time minimizes. These findings may provide valuable guidance to the rational designs of NP-based biomarkers and anticancer bioagents. © *2010 American Institute of Physics*. [doi:10.1063/1.3293303]

The high efficiency of virus invasion of living cells through protein-mediated endocytosis has inspired the design of multi-functional nanoparticles (NPs) that are capable of simultaneous disease diagnosis and treatment.[1–5] These NPs are surface-coated with ligands (i.e., antibody, peptide, and aptamer) that are complementary to the transmembrane proteins (receptors) on type-specific cells. The ligand-receptor binding enables specific targeting, which enhances therapeutic efficacy and minimizes adverse side effects. Recent studies have revealed that the uptake properties are critically dependent on particle size: NPs of ~50 nm in diameter give rise to maximal uptake rate.[6–12] While the size effect provides valuable guidance to the design of NPs in the first dimension, an equally important question remains unanswered, how many binding sites (ligands) should be decorated onto an NP to achieve optimal uptake properties? On the one hand, therapeutic NPs require an antibiofouling surface to decrease the nonspecific uptake by liver.[13] A high ligand density may increase targeted delivery but also compromises the stealth surface of NPs. On the other, early experiments seeking to identify the role of only one of the parameters had often led to controversial conclusions,[14,15] which indicates that the effects of these parameters are likely interrelated. The interrelated effects present a great challenge in experiments *in vivo*, and call for theoretical understanding.

In this letter, we elucidate the effects of ligand density in addition to particle size on the energetics and kinetics of receptor-mediated endocytosis through thermodynamic analyses. We show that there exists a minimal particle size at given ligand density and a minimal ligand density at given particle size. Below these minimal values, endocytosis is energetically not possible. Due to the entropic penalty involved in receptor diffusing and binding to ligands, wrapping of an NP may proceed with many ligands unbound with receptors, where the binding ratio of the ligands by receptors is particle size dependent. We further show that there exists an optimal condition in terms of particle size and ligand density at which the endocytic time minimizes.

We consider a single spherical NP of radius R surface-coated with ligands of density ξ_l and wrapped by the cell membrane (Fig. 1). Here "single" means that we exclude the interactions among the NPs: the neighboring NPs are far away from each other so that their wrapping zones do not overlap and they do not compete for receptors. We assume that a finite number of receptors are distributed on the cell membrane with an average density ξ_0. We denote the cross-sectional area of the receptor A_0, and hereafter use it as unit area. Correspondingly $L=\sqrt{A_0}$ is taken as unit length. Typically $L\sim 15$ nm. The maximum number of receptors accessible by an NP is $K=4\pi R^2/A_0$; K is also the surface area of the NP in the unit of A_0. When binding occurs between a ligand-receptor pair, a chemical energy ε is released; ε is typically on the order of $15k_BT$, where k_BT is the thermal energy and from hereafter used as the energy unit. The binding may be assisted by other proteins, such as clathrin or caveolin,[16] which contributes extra binding energy. The binding energy drives the local wrapping of the membrane around an NP at the cost of the bending energy due to local membrane curvature formation and of stretching energy due to lateral membrane tension.[17,18] We denote the bending energy density (per unit area) of the cell membrane by $\hat{\kappa}$. For spherical NPs, the total curvature is $2/R$, and the bending energy density is $\hat{\kappa}=8\pi B/K$, where B is the membrane bending modulus, typically on the order of $\sim 20k_BT$. Note that in determining the bending energy, we neglect the effect of the

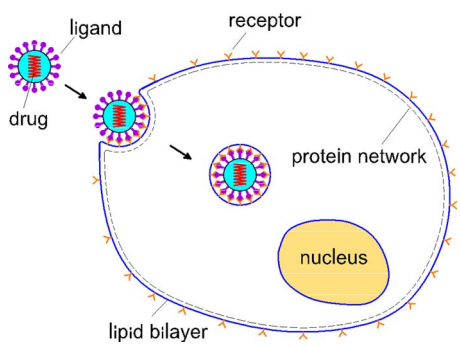

FIG. 1. (Color online) Schematics of receptor-mediated NP endocytosis.

a)Electronic mail: suz10@psu.edu.

spontaneous curvature and assume that the NP is small as compared to the cell and essentially interacts with a planar membrane. For each ligand-receptor binding to occur, the bending energy penalty is $\kappa=\hat{\kappa}/\xi_l$. The relative significance of membrane tension effect varies, depending not only on the particle size and the cell type, but also on whether certain processes, such as membrane reservoir release and lipid insertion from cytosol to membrane, are active. To simplify our analysis, membrane tension effect is here neglected.

Previous studies[6,9,19,20] assumed that every ligand coated on the NP binds with a receptor, and thus the receptor density is assumed to be the same as the ligand density in the wrapping zone. For a more general consideration, we here abandon this assumption, but argue from a thermodynamic point of view to elucidate the endocytic process. It should be noted that the thermodynamic treatment adopted here naturally leads to results of Bao and Bao[19] provided that the same assumption (one-to-one ligand-receptor binding in the wrapping zone) is made.

We consider a general stage of wrapping at which there are n_b receptors bound with ligands, which leads to a wrapped surface area of A_b. By definition, the receptor density in the wrapping zone is $\xi_b=n_b/A_b$. We identify an effective region of area A_+ in the immediate vicinity of the wrapping zone with an average receptor density is $\xi_+=n_+/A_+$, where n_+ is the number of receptors in the region A_+. The free energy in the area $A=A_b+A_+$ can be written as follows:

$$E_{\text{Total}}=A_b\xi_l[\hat{\xi}\ln\hat{\xi}+(1-\hat{\xi})\ln(1-\hat{\xi})]+A_+[\xi_+\ln\xi_+ + (1-\xi_+)\ln(1-\xi_+)]-n_b\varepsilon+\hat{\kappa}A_b, \tag{1}$$

where $\hat{\xi}=\xi_b/\xi_l$. The first two terms in Eq. (1) are the translational entropy of the receptors in the bound and free membrane regions, and the other two terms are adhesion and bending energies, respectively. Considering the constraints of conservation of membrane area $A=A_b+A_+$ and conservation of receptors $n=n_b+n_+$, the free energy functional features two independent variables as follows: n_b and A_b. Minimizing the energy functional with respect to these two variables subject to these constraints gives rise to

$$\ln\frac{\hat{\xi}}{1-\hat{\xi}}-\varepsilon-\ln\frac{\xi_+}{1-\xi_+}=0, \tag{2}$$

and

$$\hat{\xi}=1-e^{-\kappa}(1-\xi_+)^{1/\xi_l}. \tag{3}$$

Equations (2) and (3) can be solved numerically to obtain $\hat{\xi}$ and ξ_+. Due to the diffusion of the receptors toward the wrapping zone from its vicinity, the receptor density is always smaller than that of the far field, i.e., $\xi_+<\xi_0$. Under this condition, $1-\xi_+$ is sufficiently close to unity, and Eq. (2) can be approximated as:

$$\xi_+=\frac{\hat{\xi}}{1-\hat{\xi}}e^{-\varepsilon}. \tag{4}$$

Combining Eqs. (3) and (4), one has

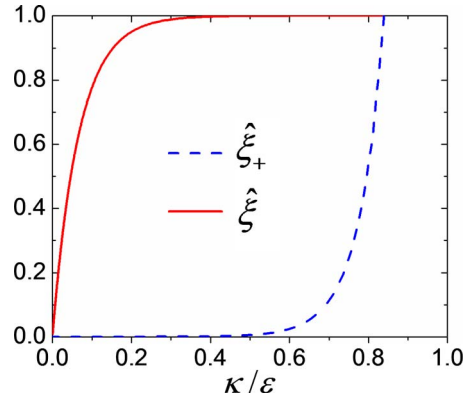

FIG. 2. (Color online) Receptor densities in the bound region and at the vicinity of the wrapping site as a function of κ/ε.

$$\frac{\xi_+}{\hat{\xi}}(1-\xi_+)^{1/\xi_l}=e^{\kappa-\varepsilon}. \tag{5}$$

Because $\xi_+<\hat{\xi}\leq 1$, one follows that wrapping is only possible when $\kappa\leq\varepsilon$, i.e., the binding energy should be sufficient to compensate the bending cost in order for wrapping to occur.

Clearly from Eq. (3) in order for $\hat{\xi}\sim 1$, the bending energy density κ has to be sufficiently large. Since κ is inversely proportional to R^2 and ξ_l, one expect that for sufficiently large particles, $\hat{\xi}$ will be significantly smaller than 1, and wrapping proceeds with many binding sites unbound by receptors. For example, for $\xi_l=1$, $B=20$, when $R=25$ nm, $K\approx 35$, $\kappa\approx 14.4$, and $\hat{\xi}\approx 1$; almost all the ligands in the wrapping zone are bound by receptors. When $R=100$ nm, $K=558$, $\kappa\approx 0.9$, and $\hat{\xi}\approx 0.59$; approximately half of the ligands in the wrapping zone are unbound by receptors.

Figure 2 shows the variations in $\hat{\xi}$ and $\hat{\xi}_+=\xi_+/\xi_0$ with respect to κ/ε, where $\xi_0=0.1$. Note that the energetic requirement of wrapping $\kappa/\varepsilon\leq 1$, we thus vary κ/ε from 0 to 1. With decreasing κ/ε, $\hat{\xi}$ rapidly decreases from 1 to 0. Oppositely, with increasing κ/ε, $\hat{\xi}$ rapidly increases to 1. One notes the dependence of $\hat{\xi}$ and ξ_+ on the design parameters R and ξ_l because of their relations to κ.

Combining Eqs. (3) and (4), and assuming that $\xi_+\ll 1$ and $e^{\varepsilon}\hat{\xi}\gg 1$, one has

$$R=\sqrt{2B/[\xi_l(\varepsilon+\ln\xi_+)]}. \tag{6}$$

Note that $\ln\xi_+$ is the energy penalty due to the entropy of the receptors when diffusing to the wrapping zone from its immediate vicinity. This suggests the dual role of receptors as follows: adhesion energy provider carry entropy. When $\ln\xi_+$ reaches a critical value $\xi_+=e^{-\varepsilon}$, $R\to\infty$; the NP cannot be internalized regardless of its particle size. The highest possible receptor density in the immediate vicinity of the wrapping zone governed by the thermodynamics is ξ_0. This specifies a minimal particle size

$$R_{\min}=\sqrt{2B/[\xi_l(\varepsilon+\ln\xi_0)]}, \tag{7}$$

at given ligand density, and a minimal ligand density at given particle radius

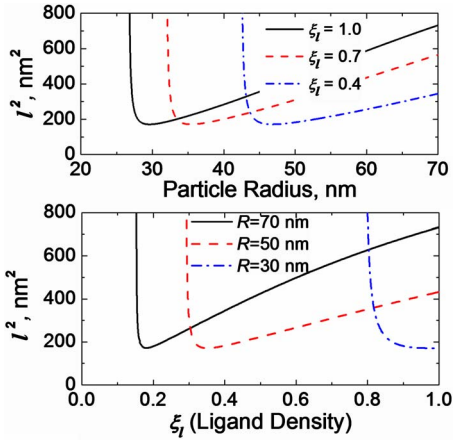

FIG. 3. (Color online) The characteristic length scale impacted by the NP wrapping depends on the interrelated effects of particle size and ligand density.

$$\xi_{l,\min} = 2B/[R^2(\varepsilon + \ln \xi_0)]. \quad (8)$$

Below these minimal values, the particle cannot be internalized through receptor-mediated endocytosis.

Conservation of the receptors in the wrapping zone and its vicinity specifies a characteristic length l, defined by

$$l^2 = \frac{K\hat{\xi}\xi_l}{\pi(\xi_0 - \xi_+)}. \quad (9)$$

The time scale of full wrapping an NP is then $t \sim l^2/D$, where D is the diffusivity of the receptors. Figure 3 plots l^2 as a function of particle radius (top panel, at fixed ligand density) and ligand density (bottom panel, at fixed particle radius). These curves share similar features; with increasing R (top panel) or ξ_l (bottom panel), l^2 decreases sharply, reaches a minimum, and thereafter monotonically increases. The dependence can be understood as follows. Starting from the minimal particle size or ligand density, when the particle size or ligand density is small, ξ_+ is on the same order of ξ_0 (see Fig. 2) and thus $\xi_0 - \xi_+$ is small. As a result, the receptors required to wrapping the NP comes from a wide membrane region (large A_+), leading to large l^2. With increasing particle size or ligand density (thus decreasing κ), ξ_+ decreases rapidly according to Fig. 2, leading to rapid decrease of l^2. As the particle size or the ligand density exceeds a certain value, ξ_+ becomes negligibly small as compared to ξ_0. Further increasing the particle size or ligand density only increases the number of receptors to envelope the NP, which leads to monotonic increase of the impacted membrane area. From Fig. 3 (top panel), the model predicts that the wrapping time reaches a global minimum when particle radius $R \sim 28$ nm and ligand density $\xi_l = 1$, which seems in good agreement with recent experimental findings[7,8,10–12] that NPs of ~ 25 nm in radius give rise to maximal uptake rate.

In conclusion, our analysis shows that, in addition to particle size, ligand density plays an important role governing the endocytic kinetics and energetics of NPs. As receptors are adhesion energy providers carry entropies, concentrating receptors at the wrapping sites is entropically unfavorable. At large particle size, endocytosis may occur with a large fraction of ligands unbound with receptors. The wrapping energetics specifies a minimal particle size at fixed ligand density and a minimal ligand density at fixed particle size below which endocytosis is energetically not possible. Our analysis reveals that there exists an optimal condition (in terms of particle size and ligand density) at which the endocytic time minimizes. These findings suggest that ligand density can be regarded as an additional dimension for the design of NP-based biomarkers and anticancer bioagents.

We acknowledge the support from National Science Foundation under Award Nos. 0826841 and 0600642.

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