The effect of interactions on the cellular uptake of nanoparticles

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Abstract
We present a simple two-state model to understand the size-dependent endocytosis of nanoparticles. Using this model, we elucidate the relevant energy terms required to understand the size-dependent uptake mechanism and verify it by correctly predicting the behavior at large and small particle sizes. In the absence of interactions between the nanoparticles, we observe an asymmetric distribution of sizes with maximum uptake at intermediate sizes and a minimum size cut-off below which there can be no endocytosis. Including the effect of interactions in our model has remarkable effects on the uptake characteristics. Attractive interactions shift the minimum size cut-off and increase the optimal uptake while repulsive interactions make the distribution more symmetric lowering the optimal uptake.

1. Introduction
The endocytic process [1, 2] is crucial to the understanding of the cellular uptake of nano-materials which are used extensively for drug delivery purposes. A key feature in the development of drug delivery tools is to achieve effective cytosolic delivery. To this purpose there have been experiments using liposomes [3], nanoparticles (NPs) [4], polymerosomes [5–7], nanotubes [8, 9], electroporation [10] and ultrasonic treatments [11]. Several of these techniques also suffer from the problem of high levels of cytotoxicity although recent experiments using polymerosomes overcome this shortcoming.

In several of these experiments using gold and silver nanoparticles, nanotubes and polymerosomes [5–7, 9, 12–17], particle size plays an important role in the cellular uptake. These experiments suggest that endocytosis of NPs is receptor mediated and that there is an optimal size where the uptake is maximum. Most theoretical approaches [18–21] to study the effect of NP geometry on cellular uptake predict a threshold radius below which there can be no cellular uptake, and an asymmetric distribution of the uptake which decays with particle size. Although these approaches correctly predict the size where the uptake is optimal (∼20–30 nm), experimentally the distribution is symmetric and does not seem to agree with the lower bound as predicted from the theories [15, 16]. The answer to this anomaly could be hidden in the highly complex endocytic mechanism itself.

The endocytic process involves the selection and segregation of the cargo at the cell surface, subsequent invagination and pinching off from the cell membrane, and, finally, the transport of these vesicles into intracellular compartments where they fuse with the target membrane. The mechanisms by which specific cargo are internalized differ in their morphological and biochemical details [1, 2, 22]. However, recent evidence [23] suggests the need to consider the sharing of molecular machinery depending on the nature of the cargo and to understand the basic physical principles common to these different uptake mechanisms.

A key step in the endocytic process is the segregation and clustering of cargo into domains on the cell membrane which are believed to be the sites where molecular machinery is recruited for subsequent processes like membrane curvature, invaginations and finally scission to occur [1, 23]. The mechanisms of formation of these nanodomains can be both passive and active. Although passive clustering which does not involve ATP hydrolysis can occur via direct interactions between cargo molecules, Reyner et al. [24] showed using coarse-grained simulations the existence of attractive interactions between curvature-inducing proteins on the cell
surface that occur purely due to curvature of the membrane. These interactions could lead to clustering and subsequent invaginations. An example of such passive clustering is the binding of Shiga toxin—a bacterial toxin—to glycolipid receptors, Gb3, in the cell membrane of certain cell types [25, 26]. Although the Shiga toxin molecules do not interact directly, they induce the clustering of the Gb3 lipids, thereby causing local membrane curvature.

The active mechanisms for cell surface clustering require ATP and are therefore energy-dependent processes. An example of active clustering is the formation of nanoscale clusters of glycosylphosphatidylinositol anchored proteins (GPI-AP) on the cell membrane which are required for their subsequent endocytosis [27]. These proteins exist as monomers and nanoclusters (∼4–6 nm in size, consisting of <5 molecules) with the interconversion between the two being spatially heterogeneous, being coupled to an active cortical cytoskeleton. Perturbing the cortical actin activity affects the construction, dynamics and spatial organization of these nanoclusters [28]. This type of active segregation also occurs with gangliosides GM1 and GM3 in the exoplasmic (outer) leaflet [29] and Ras isoforms in the cytoplasmic (inner) leaflet of the plasma membrane [30].

The above examples of passive and active clustering do not involve the clathrin-mediated endocytic pathway where specific adaptor molecules recruit cargo. However, even in the clathrin coat-mediated endocytic pathway, the clathrin lattice organizes the epsins or BAR domain proteins into domains, which then locally deform the membrane [31, 32]. Therefore, the segregation and clustering of cargo on the cell surface is highly important in the endocytic process. This naturally raises the following questions: could cell surface clustering affect the size-dependent cellular uptake of NPs and if so how could we model the clustering process? Recent experiments [9] using single-walled carbon nanotubes (SWNT) have shown evidence of NP surface clustering on the cell membrane. Although they model NP complex formation on the cell membrane, the clustering of NPs is not accounted for by including interactions systematically and it is important to investigate this in some detail.

Here, we study systematically for the first time the effect of interactions on the cellular uptake of NPs using a thermodynamic model first proposed by Tzlil et al [18, 33] in the context of budding of viral capsids at the cell membrane and subsequently studied by Zhang et al [19] and others [34–36] in the NP context. We develop our model by incorporating interactions between NPs. Using a simplified two-state version of the model, we then elucidate the relevant energy terms which affect the uptake of NPs in the absence of interactions. We then show that interactions between NPs indeed affect the minimum radius of uptake as well as the distribution. We note that this model is closely related to other models of viral entry [37, 38] and has a one-dimensional analog of adsorption and wrapping of DNA around histones [39].

2. The model

In this model, the system, which consists of a cell and ligand-coated spherical NPs in a solution, is in a thermodynamic equilibrium. At this equilibrium state, N NPs adhere to the cell surface that contains L receptors via ligand–receptor binding and are wrapped to different extents by the cell membrane (see figure 1). The receptors diffuse freely on the cell surface and are segregated into $L_p$ free receptors in the planar membrane and $L_b$ bound receptors in the curved regions. Let A be the cross-sectional area of a receptor. For a given NP radius R, the number of receptors that can attach to the NP is $K = 4\pi R^2/A$. For convenience, we shall choose A as our unit of area and $\sqrt{A} = R\sqrt{4\pi/K}$ as our unit of length. The total membrane area is denoted by $MA$ and the surface concentration of NPs is $c = N/M$.

We have assumed that the time scale for endocytosis is much larger than the time for the receptors to diffuse and segregate into the curved and planar regions. Therefore, we can treat the distribution of wrapping sizes and receptor densities using a thermodynamic formalism.

Using the notations by Tzlil et al [18] we denote the number of NPs wrapped by a membrane section of area k by
where \( k \) varies discretely between \( k = 0 \) (unwrapped state) and \( k = K \) (completely wrapped state). Then, we have

\[
N = \sum_{k=0}^{K} n_k
\]

and

\[
M_B = \sum_{k=0}^{K} k n_k,
\]

where \( M_B A \) is the total membrane area associated with the wrapped NPs and \( M_B A = (M - M_b) A \) is the total area of the planar regions. The binding of a ligand and a receptor releases chemical energy, \( \epsilon \), which drives the wrapping at the cost of the energy required to bend the membrane. Therefore, the diffusion of receptors inside the curved regions should lower the energy of the system facilitating wrapping. However, this leads to the segregation of receptors between planar and curved regions, which costs entropy. Also diffusion of free receptors into the curved regions increases the total curved area (more ligand–receptor bonds), therefore increasing the total membrane bending energy. Furthermore, attractive (repulsive) interactions between the NPs could lead to clustering (anti-clustering) and therefore affect the wrapping size distribution of NPs. To determine the size distribution of the varyingly wrapped NPs, we first write down the free energy of the system as

\[
\frac{F}{k_B T} = M_p [\phi_p \ln \phi_p + (1 - \phi_p) \ln (1 - \phi_p)] + M_b [\phi_b \ln \phi_b + (1 - \phi_b) \ln (1 - \phi_b)] + \sum_k n_k \ln (n_k / M) - 1 - \epsilon L_b + \kappa M_b + \sum_k n_k \Lambda_k + \sum_{k,k'} k' n_k n_{k'},
\]

where \( \phi_p = L_p / M_p \) and \( \phi_b = L_b / M_b = (L - L_p) / M_b \) denote the densities of the receptors in the planar membrane and the wrapped regions, respectively, \( k_B \) denoting the Boltzmann constant and \( T \) being the temperature.

The first three terms in the free energy are entropic contributions written in terms of a two-dimensional lattice gas model.

- \( M_p \phi_p \ln \phi_p + (1 - \phi_p) \ln (1 - \phi_p) \) represents the configurational entropy of \( L_p \) free receptors distributed in the \( M_p \) sites of the planar parts of the membrane.
- \( M_b \phi_b \ln \phi_b + (1 - \phi_b) \ln (1 - \phi_b) \) represents the configurational entropy of distributing \( L_b \) receptors among the \( M_b \) sites of the curved regions.
- \( \sum_k n_k \ln (n_k / M) - 1 \) is the configurational entropy of a 2D mixture of wrapped NPs when treated as a multicomponent ideal gas. However, we are interested in interacting NPs and would therefore have to include interaction energy for this 2D mixture.

The next five terms are energetic.

- \( -\epsilon L_b = -M_b \phi_b \kappa = -\phi_b \epsilon \sum_k k n_k \) is the total chemical energy released upon the binding of \( L_b \) ligand–receptor pairs.
- \( \kappa M_B = \kappa \sum_k k n_k \) is the total membrane curvature energy in the budding regions. For a spherical geometry, the bending energy per unit area across a NP of radius \( R \) is \( \kappa = (\kappa A / 2k_B T)(2 / R - c_0)^2 \), where \( \kappa \) denotes the bending modulus. Note that the spontaneous curvature \( (c_0) \) of cell membranes is nonzero. In our analysis we consider a vanishing spontaneous curvature \( (c_0 = 0) \). Therefore, \( \kappa = 2 \kappa A / k_B T R^2 = 8 \pi \kappa / k_B T K \). We shall discuss the effect of spontaneous curvature on cellular uptake later.
- \( \sum_k n_k \Gamma_k \): total work of pulling excess membrane toward the wrapping sites against lateral tension \( \sigma \). For a single NP wrapping, the excess area pulled toward the wrapping site is \( 4 \pi R^2 k^2 / K^2 = (k^2 A / K) \) [40]. Thus, the excess energy is \( \Gamma_k = \sigma \times \) excess area = \( k^2 \sigma A / k_B T K \).
- \( \sum_k n_k \Lambda_k \): total line energy of the rim, where \( \Lambda (k) \) denotes the line energy of a \( k \)-bud. Assuming a spherical shape of the membrane at the rim of a partially wrapped NP, \( \Lambda_k \) is modeled as being proportional to the length, \( \ell_k \), of its rim, with a constant line energy per unit length \( \gamma \) [18]. Therefore,

\[
\Lambda_k = \gamma \ell_k = \gamma 2 \pi R \frac{L}{K} \left( 1 - \frac{k}{K} \right).
\]

Note that \( \ell_k \) vanishes for \( k = 0 \) and \( k = K \) and is maximum \((2 \pi R)\) for a half-wrapped NP \((k = K/2)\). However, the local wrapping behavior of the membrane to a NP is different from the assumption made above [40, 41]. We need to consider an additional bending energy for the unadsorbed membrane detaching from the NP at the rim. Although the \( k \)-dependence of this energy is different from the simple form assumed in equation (4), the general features of large energies for the half-wrapped state \((k = K/2)\) and very small energies for unwrapped and completely wrapped states are the same.

- \( w \sum_{k,k'} k' n_k n_{k'} = w \left[ \sum_k n_k \right] \left[ \sum_{k'} k' n_{k'} \right] = w M_B^2 \) is the interaction energy between the partially wrapped NPs, \( w \) denoting the strength of the interaction (or the second virial coefficient). We assume the interaction to depend on the degree of deformation and curvature of nearby curved membrane patches and therefore to the degree of wrapping of the cell membrane to individual NPs. Thus, the total interaction energy when summed over is proportional to the total curved area. Interaction between membrane inclusions or adsorbates could arise due to a variety of different mechanisms [42] including membrane fluctuations [43, 44].

In the final stages of endocytosis the membrane wrapped NP pinches off which results in a topology change. This severing mechanism of the wrapped NP from the membrane is brought about by proteins such as dynamin and C-terminal binding protein 3/brefeldin A-ribosylated substrate (CtBP3/BARS). According to the Gauss–Bonnet theorem [45], this leads to an increase of \( 4 \pi \kappa \) in Gaussian bending energy, with \( \kappa \) representing the Gaussian bending rigidity of the cell membrane. In our study we are interested in events prior to the final scission process and will therefore safely ignore this term.
The length scale is set by the length of the receptor energy where we have defined the conservation condition for the receptors gives terms that change with the wrapping of the nanoparticles.

Table 1. A summary of the energy terms that are dependent on the degree of wrapping of a nanoparticle.

| Energy terms | Physical definition | Mathematical expressions |
|--------------|---------------------|--------------------------|
| \( \varepsilon \) | Bending energy per unit area | \( 8\pi \kappa/k_B T K \) |
| \( \Gamma_k \) | Excess energy for wrapping | \( k^2 \sigma A/k_B T K \) |
| \( \Lambda_k \) | Total line energy of a \( k \)-bud | \( \gamma 2\pi R \sqrt{4k(1-k/K)}/K \) |
| \( wM_b^2 \) | Interaction energy between NPs | \( w \sum_{k,k'} k' n_k n_{k'} \) |

In fact, all partially wrapped NPs for which \( k \geq 0.9K \) will be assumed to be endocytosed. In table 1 we list all the energy terms that change with the wrapping of the nanoparticles.

To find the equilibrium state of the system we minimize the free energy with respect to \( L_b \) and \( n_k \). From \( \partial F/\partial L_b = 0 \), we have

\[
\phi_p \left( 1 - \phi_p \right) = \phi_b \left( 1 - \phi_b \right) e^{-\alpha}.
\]

Minimizing \( F \) subject to the constraint of equation (1), we get the normalized wrapping size distribution as

\[
p_k = \frac{n_k}{N} = \frac{e^{-\beta_k \alpha}}{\sum_{k=0}^{K} e^{-\beta_k \alpha} k},
\]

where we have defined

\[
\alpha = (\phi_p/\phi_b) e^{-\varepsilon/k}
\]

and

\[
\beta_k = \Lambda_k + \Gamma_k + 2wK \sum_{k'} k' n_{k'}
\]

\[
= \Lambda_k + \Gamma_k + 2wcMk \sum_{k'} k' p_{k'}.
\]

The conservation condition for the receptors gives

\[
\phi_p \left( 1 - c \sum_k k n_k \right) + \phi_b c \sum_k k n_k = \phi_b.
\]

The densities of the receptors \( \phi_p \) and \( \phi_b \) can be obtained by numerically solving equations (5)–(9). Substituting \( \phi_p \) and \( \phi_b \) back into equation (6) gives the wrapping size distribution. Then, the number of completely wrapped NPs is given by

\[
n_K = cMn_K.
\]

Since we assume that fully wrapped NPs are irreversibly internalized, \( n_K \) gives the cellular uptake of nanoparticles.

We first study the effect of particle size on the cellular uptake of nanoparticles. To analyze the size-dependent uptake of NPs we make a major simplification in the model. We assume that the NPs upon arrival to the cell surface are either endocytosed completely or remain free without there being any intermediate wrapped state. Then the model essentially reduces to a two-state model with the two states being \( k = 0 \) and \( k = K \). Our goal is to come up with the minimal model to understand the experimental uptake behavior and to find the only relevant energetic contributions. Note that the line energy term (\( \Lambda_k \)) automatically vanishes with this simplifying assumption. We now study the uptake behavior (i) in the absence of interactions and (ii) when the NPs interact.

3. Two-state model

To analyze the size-dependent uptake of NPs we make a major simplification in the model. We assume that the NPs upon arrival to the cell surface are either endocytosed completely or remain free without there being any intermediate wrapped state. Then the model essentially reduces to a two-state model with the two states being \( k = 0 \) and \( k = K \). Our goal is to come up with the minimal model to understand the experimental uptake behavior and to find the only relevant energetic contributions. Note that the line energy term (\( \Lambda_k \)) automatically vanishes with this simplifying assumption. We now study the uptake behavior (i) in the absence of interactions and (ii) when the NPs interact.

3.1. (i) Non-interacting case \((w = 0)\)

With the two-state model, in the absence of interactions and with \( \Delta_k = 0 = \Gamma_k \), we observe (figure 2(a)) that below a critical radius, \( R_{\text{min}} \), there is hardly any uptake. Above \( R_{\text{min}} \), the uptake increases sharply to reach a maximum and then decays as a power law with increasing radius. Thus, the two-state model correctly reproduces the optimal uptake behavior at intermediate radii seen in experiments. We compare our results for the two-state model with the full \( K \)-state model both in the presence and absence of the \( \Gamma_k \) term. We find that for \( \gamma = 1 \) and \( \sigma = 0.001 \) the surface tension term does

\[
A \sim 225 \text{ nm}^2. \quad \text{Experimental information suggests that the number of receptors varies from 50 to 500 \mu m^{-2} \cite{18, 19, 50, 51}. This implies that \( \phi_b \) could vary from 0.01 to 0.1. The concentration of NPs, \( c \), can vary between 0.001 and 0.005. The diameter of the cell being \( \approx 15 \mu m \), the surface area of the cell is \( \approx 701 \mu m^2 \). Therefore, \( M = 3.14 \times 10^6 \). In our numerical analysis, we choose \( \kappa = 20 \, k_B T, \epsilon = 25, c = 0.003, \phi_b = 0.05 \), and \( M = 3.14 \times 10^6 \) \cite{18, 19}. In what follows, we choose \( \sigma = 0 \) and consider the effect of \( \sigma \) on uptake in a later section. Both \( \gamma \) and \( w \) are free variables and we choose \( \gamma = 1.0 \) (in units of \( k_B T/pert unit length, \sqrt{A} \)). \( w \) is varied from zero (non-interacting) to positive (repulsion) and negative (attraction) values. Table 2 summarizes the variables and parameters used in our model.

Table 2. Physical constants and the range of values used in our study.

| Physical constants | Definition | Typical values |
|--------------------|------------|---------------|
| \( \epsilon \) | Chemical energy per ligand–receptor pair | 15–30 |
| \( \kappa \) | Bending modulus | 20 \( k_B T \) |
| \( \sigma \) | Surface tension | 0.001 \( k_B T/A \) |
| \( \gamma \) | Line energy per unit length | 1.0 \( k_B T/\sqrt{A} \) |
| \( c \) | Surface concentration of NPs | 0.001–0.005 |
| \( \phi_b \) | Surface density of receptors | 0.01–0.1 |
| \( M \) | Total membrane area | \( 3.14 \times 10^6 \) |
| \( w \) | Strength of interaction \( w > 0 \) or \( w < 0 \) |

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not affect the size-dependent distribution significantly. We shall briefly consider the effect of σ on cellular uptake for the two-state model in a later section. Thus, we conclude that the only relevant energy terms in understanding the size-dependent endocytosis of NPs are the energy released on ligand–receptor binding and the energy cost in bending the membrane. We verify this further by analyzing the behavior at large and small radii.

**Behavior at large R.** For the two-state model, equation (9) (the conservation condition) reduces to give the completely wrapped particle size distribution as

\[ p_K = \left[ \frac{\phi_0 - \phi_p}{\phi_0 - \phi_p} \right] \frac{1}{cK}. \]  

(11)

In the large R limit (or large K limit), the receptor density in the fully enveloped NPs is almost saturated, \( \phi_0 \approx 1 \), whereas the free receptor density is negligible \( \phi_p \approx 0 \). Then, \( p_K \approx \phi_0/cK = \phi_0 A/4\pi cR^2 \), giving the cellular uptake at large \( R \) as

\[ n_K = c M p_K = M \phi_0 A/4\pi R^2. \]  

(12)

Therefore, cellular uptake for larger NPs is inversely proportional to the square of the size of the NPs and reproduces the numerically predicted behavior at large radius exactly (figure 2(b)).

**Behavior at small R.** To understand the low cellular uptake at smaller radii we follow Tzlil et al to make the macroscopic (bud) phase approximation, i.e. assume that instead of the curved regions being made up of several NPs wrapped to different extents, there is a single NP with wrapped area \( M_k \) that coexists with the planar membrane phase. This approximation causes the configurational entropy of the NPs in the free energy expression to drop off. Also \( \Lambda_k = 0 \) for all \( k \). Minimizing the resulting free energy with respect to \( L_0 \) gives equation (5) and minimizing it with respect to \( M_k \) yields

\[ \frac{1}{1 - \phi_p} = \frac{1}{1 - \phi_0} e^{-\kappa}. \]  

(13)

Solving equations (5) and (13) we can determine the receptor densities in the two coexisting phases as [18]

\[ \phi_0 = \frac{1 - e^{-\kappa}}{1 - e^{-\kappa}} \quad \text{and} \quad \phi_p = \frac{e^\kappa - 1}{e^\kappa - 1}. \]  

(14)

Therefore, we can have coexistence between the planar and wrapped phases only if \( \epsilon \gtrsim \hat{k} \gtrsim 0 \). Thus, for a single wrapped NP, \( \epsilon = \hat{k} \) is the critical value below which we cannot have wrapping. Substituting for \( \hat{k} \), we get the critical radius for the onset of wrapping as,

\[ R_{\text{min}} = \sqrt{\frac{2\kappa A}{\epsilon k_BT}}. \]  

(15)

This lower bound has been derived variously by [19,20,34,35,52]. For the values of \( \kappa \) and \( \epsilon \) used in the numerical estimates we get \( R_{\text{min}} \approx 19 \text{nm} \).

**Scaling at the optimal uptake radius.** Let \( R^* \) denote the radius when cellular uptake is maximum. We make two simplifying assumptions:

- \( R^* \sim a R_{\text{min}} \) and
- optimal cellular uptake is given by the uptake at large \( R \), i.e. \( n_k^* \sim M \phi_0 A/4\pi R^2 \)

where \( a \) is a constant. We verify our first assumption by looking at the variation of \( R^*/R_{\text{min}} \) as a function of \( \epsilon \) and \( \kappa \). Remarkably, we find that the assumption holds true (figure 3(a), insets (i) and (ii)). Therefore, the optimal uptake radius seems to depend on the relative values of \( \kappa \) and \( \epsilon \) in the same way as \( R_{\text{min}} \). The second assumption does not have much basis apart from the fact that the behavior at large \( R \) seemed to obey the numerical behavior quite well even close to the optimal uptake radius, \( R^* \). Coupling the two assumptions together gives us an expression for the optimal cellular uptake,

\[ n_k^* \approx \frac{M \phi_0}{4\pi (a R_{\text{min}})^2} \left( \frac{M \phi_0 k_BT}{8\pi a^2 A} \right) \frac{\epsilon}{\kappa}. \]  

(16)

Rescaling \( R \) by \( R_{\text{min}} \) and the cellular uptake by the optimal cellular uptake, \( n_k^* \), we find extremely good collapse for a wide range of \( \epsilon \) and \( \kappa \) values (figure 3(a)).
At high values of $\varepsilon$ and $\kappa$ show that $R_\text{opt}$ increases. (Figure 3.) The curves are for different values of $w = 0.00001$ (red, □), $0.00005$ (green, ◦), $0.0001$ (blue, △), $0.01$ (pink, ▽) and $1.0$ (aqua, ⋄). Collapse of data is observed only at high values of $w$.

**3.2. (ii) Interacting case ($w \neq 0$)**

As observed above, the uptake of NPs in the absence of interactions is highly asymmetric and also predicts a lower radius cut-off. Experimentally, the distribution has been found to be rather symmetric both for Au nanoparticles [15, 16] and DNA-wrapped single-walled carbon nanotubes (DNA-SWNT) [9]. Moreover, there is a significant internalization of particles below the minimum radius predicted by the model. Experiments using DNA-SWNT show an increase in near-infrared fluorescence from SWNT concentrated at the external cell membrane during the early stages of endocytosis mechanism [9], indicating possible clustering of nanotubes on the cell surface prior to uptake. To incorporate clustering in our model, we included an effective interaction in our model. The idea is that interactions could lead to clustering which could drive wrapping of NPs of smaller sizes. In our model, the interaction between NPs is controlled by the interaction parameter $w$. Negative $w$ implies attraction while positive $w$ implies repulsion. We do our analysis for the two-state model.

**Results for attraction ($w < 0$).** Figure 4(a) shows the results for size-dependent uptake of NPs in the presence of attractive interaction. Similar to the behavior in the absence of interactions, we find that below $R_{\text{min}}$, there is hardly any endocytosis. Above $R_{\text{min}}$, the uptake increases rapidly and subsequently reaches a maximum and then decays slowly. We note, however, that the uptake mechanism is strongly dependent on the value of $w$. With the increase in the strength of the attractive interaction (increasing $|w|$), the minimum radius for uptake, $R_{\text{min}}$, decreases substantially. Also the optimal uptake decreases with increasing repulsion.
Results for repulsion ($w > 0$). Figure 4(b) shows the results for size-dependent uptake of NPs in the presence of repulsive interaction. We find that the behavior is distinctly different from that with attractive interactions. Although the uptake mechanism strongly depends on the interaction strength $w$, the characteristics differ significantly. Interestingly, the lower radius cut-off $R_{\text{min}}$ does not shift with increasing $w$ and is the same for all $w$ values. The maximum uptake however decreases with increasing $w$. The uptake behavior at large $R$ is also affected. Although the uptake decreases at large $R$, the decay is much slower as $w$ increases. This behavior can again be explained by looking at the energetics. Repulsion between NPs (positive $w$) pushes them apart and the energetics at lower radius are governed by the same single NP wrapping mechanism strongly depends on the interaction strength $w$. The optimal uptake of NPs for the two-state model. In figure 5, we changed the relative values of $\sigma$ and $\kappa$. For fixed $\kappa$ and $\epsilon$, we see that repulsive interactions also have a significant impact, in that they cause the cellular uptake to be reduced and modify uptake characteristics towards being more symmetric.

4. Summary and conclusion

In this paper, we have studied the effect of interactions between surface-bound NPs on their subsequent endocytosis in the context of a statistical thermodynamic model. One of our first results is to clearly show the relevant energy terms required to understand the uptake mechanism qualitatively. To do so, we simplified our model to a two-state model, which captures the essential features of the uptake behavior. We showed that apart from the important entropic contributions coming from the distribution of the receptors and the NPs on the cell surface, the energy terms that dictate the uptake characteristics are the energy released via ligand-receptor binding and the energy cost in bending the membrane. Although we show that the line energy and energy in pulling excess membrane area are not significant when the aim is to understand the specific uptake behavior, we do not rule out the importance of these terms in the endocytic process. These energy terms, as well as the Gaussian bending energy during the pinch-off, are relevant for the studies of membrane invaginations and wrapping. However, they may be dispensable when addressing the question of the number of NPs endocytosed at a given time.

Beyond the two-state model, our results show that interactions between NPs could have a drastic effect on the uptake process. Attractive interactions lead to clustering of NPs, which effectively lowers the free energy threshold for wrapping and therefore shifts the lower cut-off radius. This is not possible in the absence of interactions unless, of course, we changed the relative values of $\kappa$ and $\epsilon$. For fixed $\kappa$ and $\epsilon$, we see that repulsive interactions also have a significant impact, in that they cause the cellular uptake to be reduced and modify uptake characteristics toward being more symmetric.

In our model we use equilibrium statistical mechanics to determine the size distribution of membrane wrapped NPs assuming that the diffusion of the receptors is fast enough to equilibrate receptor densities in the curved and planar regions of the cell membrane. Moreover, we also assume that the time scales of interactions between wrapped NPs is faster than the time taken for a NP to be endocytosed. This is a reasonable approximation as shown by Reynwar et al. [24] in their numerical study where curvature-mediated attractive interactions lead to clustering of NPs on the membrane before their endocytosis.

Finally, interactions are assumed to be proportional to the wrapped area and the strength of the interaction, $w$, is varied freely. Therefore, $w$ could be thought of as an effective parameter which models the membrane-mediated forces [42]. Müller et al. [53, 54] studied the curvature-mediated interaction between particles on the cell surface. From a purely geometric analysis, they showed that the net force between the particles...
is due to a competition between the force associated with the curvature along the direction joining the particles (which leads to repulsion) and the force associated with the curvatures perpendicular to it (which leads to attraction). It will be interesting to use this kind of model for membrane-mediated interaction in a more detailed study of receptor-mediated endocytosis of interacting NPs.

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