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Nanoparticle Design Optimization for Enhanced Targeting: Monte Carlo Simulations

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Abstract

Using computer simulations, we systematically studied the influence of different design parameters of a spherical nanoparticle tethered with monovalent ligands on its targeting efficiency of planar cell surfaces containing mobile receptors. We investigate how the nanoparticle affinity can be affected by changing the binding energy, the percent of functionalization by ligands, tether length, grafting density, and nanoparticle core size. In general using a longer tether length or increasing the number of tethered chains without increasing the number of ligands increases the conformational penalty for tether stretching/compression near the cell surface and leads to a decrease of targeting efficiency. At the same time using longer tethers or a larger core size allows ligands to interact with receptors over a larger cell surface area, which can enhance the nanoparticle affinity towards the cell surface. We also discuss the selectivity of nanoparticle targeting of cells with a high receptor density. Based on the obtained results, we provide recommendations for improving the nanoparticle binding affinity and selectivity, which can guide future nanoparticle development for diagnostic and therapeutic purposes.

Keywords

Nanoparticle Targeting; Computer Simulations; Nanoparticle Design; Binding Specificity; Targeting Efficiency; Ligand-Receptor Binding

Introduction

Targeted drug delivery by polymeric nanoparticles has attracted substantial attention for its potential applications in diagnosis and treatment of life-threatening diseases.^{1–6} Besides providing protection of therapeutic and imaging agents, allowing controlled release and offering high payload capacity, targeted nanoparticles can actively navigate to a diseased area and selectively interact with receptors on cell surfaces. Many experimental studies have reported that nanocarrier targeting can diminish the incidence of adverse side effects, allows reduction of drug dosage and achieves superior therapeutic responses compared to non-targeted drug delivery systems.^{1,4,5,7–10} To optimize the therapeutic efficiency of targeted nanoparticulate systems a better control of polymer nanoparticle design as well as the interactions within the biological media has to be established. A few recent studies follow this premise by systematically evaluating the influence of one or another design parameter and identifying important factors to be considered in nanoparticle targeting.^{11–13} The

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Supporting information Available: Details of the free energy calculation and the influence of different parameters on the number and fraction of bound ligands, compression free energy, reduced and total free energy. This information is available free of charge via the Internet at <http://pubs.acs.org>.

experimental exploration of the influence of a nanoparticle design on targeting efficiency is a rather difficult task due to both the complexity of the problem and the necessity to consider multiple factors. In this manuscript we apply computer simulations to assess the importance of different design parameters of a spherical polymer nanoparticle on its targeting efficiency and provide guidelines for the rational design of a nanoparticle to enhance its targeting affinity and selectivity.

The success of targeting cell surfaces by ligand-conjugated nanoparticles is largely determined by the nanoparticle physical and biochemical properties (such as nanoparticle size, polymer tether length and density, ligand properties (e.g. valence, architecture)) and cell surface characteristics (receptor size, density, and mobility). For example, it has been shown experimentally that an increase in nanoparticle size and number of ligands carried by nanoparticles or dendrimers leads to stronger binding of nanoparticles to cell surfaces.^{11,14–20} In order to enhance the interaction with target cells an appropriate tether length has to be selected to expose the ligands conjugated to the nanoparticle and span multiple receptors.²¹ Using ligands with a larger binding energy is also reported to increase the interaction of nanoparticles with the tumor endothelium.²² In addition to nanoparticle binding affinity, there is another factor to consider: specificity of targeting cells with overexpressed high density receptors compared to benign cells with low receptor density. Recent experimental and theoretical studies have suggested that increasing binding affinity might lead to a decrease in targeting selectivity and that using multivalent low-affinity ligands can be beneficial for selective cell targeting.^{7,22–26}

Evidently to design a polymer nanoparticle which can achieve both sufficient affinity and specificity is a very complicated task. A systematic variation of different nanoparticle design parameters experimentally is rather difficult and time-consuming. An alternative strategy is to apply computer simulation and theoretical modeling tools to analyze the influence of different factors on the thermodynamics of nanoparticle targeting. We are aware of only a few reports on this subject. Annapragada et al.¹⁷ adopted a mathematical model and proposed that the number of ligands bound to a receptor surface can be optimized by varying the tether length and the number of ligands per liposome. Using dissipative particle dynamics simulations Djohari et al.²⁷ have studied the influence of ligand-receptor binding energy, percent of functionalization and ligand distribution on the fraction of ligands bound to high-density stationary receptors on a cell surface. In the present paper we will consider the interaction of spherical polymeric nanoparticles carrying different densities of monovalent ligands with a planar cell surface containing mobile receptors, as shown in Figure 1. Using Monte Carlo simulations, we will systematically analyze the influence of different structural design parameters of the nanoparticle on its targeting affinity and will also briefly discuss the requirements to achieve targeting specificity. We will make predictions regarding the optimal nanoparticle design which can guide future experimental research on nanoparticle targeting.

Computational Details

Most nanoparticles, such as polymer micelles (with a kinetically frozen core), PEGylated gold nanoparticles or polymer-coated superparamagnetic iron oxide (SPIO) agents are composed of a hard core and a corona formed by hydrophilic polymers. Thus in our current simulations a nanoparticle is modelled as a hard impenetrable sphere of radius r with Q tethered linear polymers forming the protective layer. Each polymer chain is represented by a sequence of N chemically bonded monomers. We note that the number of monomers does not have to be equal to the actual number of atoms in a chain but can represent a number of repeat units in a polymer. In this study we consider the tether lengths comparable or larger than the nanoparticle core radius, as in the case of a large class of nanoparticles, such as copolymer micelles or polymer tethered gold nanoparticles.^{13,28,29} Since polymer tethers,

such as polyethylene glycol, are flexible biocompatible hydrophilic polymers for which the physiological media acts as a good solvent, in our simulations we apply the bond-fluctuation model (BFM) which accounts for the excluded volume interactions by imposing constraints on the positions of neighboring monomers (or ligands, described below) and bond lengths: $2a$, $\sqrt{5}a$, $\sqrt{6}a$, $3a$, or $\sqrt{10}a$ (a being the unit of cubic lattice spacing).^{30,31} In our simulations we consider monovalent targeting ligands which are modeled as end-monomers of tether chains and are capable of reversible interactions with the functional centers of receptors on a cell surface. The functionalized and non-functional polymers are homogeneously attached to the core surface. At the beginning of the simulations the nanoparticle is placed in the center of a 3-dimensional cubic periodic box of lateral size $200a$. The lateral box size is considerably larger than the overall nanoparticle size, so that there are no boundary effects. The equilibration of polymer chain conformation is achieved by implementing moving updates for all monomers and ligands on the 3-dimensional lattice: a new monomer/ligand position should satisfy the BFM rules regarding bond geometric constraints (and should not lie inside the core of the nanoparticle or the cell surface). After the system reaches equilibrium in about 2 million Monte Carlo steps, the distribution of monomers as well as the targeting ligands inside the protective polymer layer are measured with respect to their distance from the surface of the core and averaged over an additional 4 million MC time steps. One MC step includes a number of moving and binding updates on randomly selected monomers, ligands and receptors equal to the total number of monomers plus ligands and receptors in the system.

A planar cell surface impenetrable to polymer chains (or ligands) and containing mobile receptors was placed at a distance s from the nanoparticle core surface, as shown in Figure 1. Considering the large difference in the nanoparticle size (nm) compared to the cell size (μm), the details of the cell surface structure have been omitted and modeled as a plane. Each receptor occupies a 2-dimensional circular area (as shown in Figure 1) with an overall diameter of $12a$ such that any two receptor centers could not approach each other closer than the distance of $12a$. The density of receptors on the cell surface is controlled probabilistically: during each MC step, an attempt is made with a probability p_{add} to place randomly a new receptor on the cell surface that is free of receptors. The deletion of a free receptor (which is not bound to a ligand) is implemented with a probability of p_{del} during each MC step. Thus the number of receptors is controlled by the p_{add}/p_{del} ratio, which can be related to the receptor density (e.g. the highest density of receptors considered in our simulations was 0.6 receptors per $200a \times 200a$ surface area, i.e., $\rho = 1.5 \times 10^{-5}a^{-2}$). The moving update for a receptor is rather similar to that for a regular monomer but is restricted to the motion within the planar cell surface. The functional center of a receptor can interact reversibly with a ligand on the nanoparticle with the binding energy $-E_{bind}$ (below we will refer to the absolute values of binding energy as E_{bind}). To equilibrate the system with regard to the reversible interactions between ligands and receptors, binding updates were applied to the ligands following their moving updates. The binding update includes breaking an existing reversible bond with a receptor and forming a new reversible bond with a randomly selected receptor located within the bonding distance with a probability $\exp(E_{bind}/kT)/(1 + \sum \exp(E_{bind}/kT))$, where the summation is performed overall all ligand-receptor pairs within the binding distance. As a result of the binding update a previously existing bond can be broken, a new bond can be formed or no change made. The interaction of the nanoparticle with the mobile receptors on a planar cell surface (for a given separation distance s) is brought to equilibrium after at least 8 million of Monte Carlo time steps.

We firstly investigated the conformation of the tethers attached to the nanoparticle core in the presence of cell surface but without specific interactions between the ligands and receptors ($E_{bind} = 0$). When the nanoparticle is far away from the cell surface, tether conformation is undisturbed and the average radius of gyration of the tethers is R_{g0} . When

the nanoparticle approaches the surface, the tethers on the nanoparticle facing the cell surface start to feel the presence of the cell, as the space available to these tethers diminishes as does their radius of gyration R_g . As is seen from the inset in Figure 2, the ratio of the average square radius of gyration for tethers attached to nanoparticle separated from the cell surface by distance s to that for an isolated nanoparticle, $\langle R_g^2 \rangle / \langle R_g^2 \rangle_0$ becomes less than 1 indicating that some tethers are slightly compressed. As the nanoparticle moves closer to the cell surface, more chains become affected by the limitation of possible conformations and $\langle R_g^2 \rangle / \langle R_g^2 \rangle_0$ further decreases. At short separation distances the volume available for tethers becomes rather limited and some tethers start to stretch away from the narrow region between solid nanoparticle core and cell surface. This leads to an increase of the ratio $\langle R_g^2 \rangle / \langle R_g^2 \rangle_0$, which can become larger than 1 at very small separation distances, as is seen from Figure 2 (inset) indicating that tether stretching becomes a dominant factor. It is worthwhile to note that not all chains are equally affected by the presence of cell surface. Evidently tethers facing the surface are affected the most - indeed, the average ratio $\langle R_g^2 \rangle / \langle R_g^2 \rangle_0$ for the tethers attached to the half of the nanoparticle core facing the cell decreases more strongly and exhibits a deeper minimum than the average value for all tethers (Figure 2, inset).

In the presence of reversible ligand-receptor interactions, the influence of the cell surface on tether conformation becomes noticeable at somewhat larger separation distances, when a few tethers facing the cell surface stretch out allowing the ligands to reach receptors. At this point the radius of gyration R_g of the tethers carrying ligands bound to receptors reaches its maximum value (which depends on the overall nanoparticle properties, such as the number of tethered polymers and tether length), as is seen in Figure 2. As the nanoparticle approaches the cell surface, more tethers functionalized by ligands can reach the cell surface in a less stretched conformation and $\langle R_g^2 \rangle / \langle R_g^2 \rangle_0$ of the tethers carrying bound ligands decreases, as seen in Figure 2. We note that even in the very vicinity of the cell surface, bound tethers still experience little stretching as some of them spread along the cell surface for the ligands to bind more receptors (and to increase the space available for tethers, as discussed above). The conformation of polymer tethers carrying unbound ligands remains practically unaffected by the presence of cell surface (except for a rather small compression caused by the presence of the neighboring chains carrying bound ligands at short separation distances).

Based on these observations for the tether conformation in the vicinity of cell surface, one can conclude that different tethers are affected differently, depending on their position with respect to cell surface and whether the carried ligand is bound or not. The two main entropic factors which need to be accounted in the description of nanoparticle interaction with cell surface mediated by ligand-receptor binding are conformational limitations (mainly compression) for all (functionalized or nonfunctional) tethers facing the cell surface and tether stretching for tethers carrying ligands bound to receptors (Figure 2).

In the absence of the reversible ligand-receptor binding, the free energy change (relative to the reference system with a nanoparticle being far away from the cell surface) $\Delta F_{compr}(s)$ due to the presence of a planar cell surface positioned at a separation distance s was calculated using the acceptance-ratio method.^{32,33} In the framework of this method, the free energy difference ΔF_d between any two canonical ensembles (0 and 1) that differ only in their potential functions (U_0 and U_1 , as discussed below) can be calculated using the following equation³²

$$\sum_0 g(U_1 - U_0 - \Delta F_d) = \sum_1 g(U_0 - U_1 + \Delta F_d) \quad (1)$$

where $g(p) = (1 + e^{p/kT})^{-1}$ is the Fermi-Dirac function and the summation (over different states) on the right and left hand side of eq 1 corresponds to the canonical ensemble 0 and 1 respectively. It is important to have a sufficient overlap of states between the ensembles 0 and 1. Thus, in our current simulations, we decrease step by step the separation distance between the nanoparticle core surface and cell surface from s_∞ to s (keeping $E_{bind} = 0$ kT) and sum up the free energy differences for all intermediate separation distances. Moreover, in order to decrease a separation distance s by one lattice spacing we introduce a fictitious repulsive potential E_{rep} acting on monomers that are in direct contact with the cell surface.³³ To ensure sufficient overlap between different states (and therefore accurate calculation of the free energy difference) we varied E_{rep} from 0 to 6 kT with a step of 1 kT for each separation distance s and calculated the ensemble probability of having i monomers being in direct contact with cell surface. We note that $E_{rep} = 6$ kT was sufficiently large to eliminate the direct monomer contacts with the cell surface. The compression free energy $\Delta F_{compr}(s)$ for moving the cell surface from s_∞ to s is obtained by summation of free energy difference

in all the intermediate separation distances: $\Delta F_{compr}(s) = \sum_{z=s_\infty}^{z=s} \sum_{E_{rep}=0}^{E_{rep}=6} \Delta F_d(s, E_{rep})$, where $\Delta F_d(s, E_{rep})$ is calculated by solving eq 1 using $U = iE_{rep}$ for two adjacent E_{rep} (see supporting information). $\Delta F_{compr}(s)$ accounts only for the loss of tether conformational entropy due to the presence of cell surface. As the separation distance s becomes smaller the number of available conformations for the polymer tethers decreases and the compression free energy $\Delta F_{compr}(s)$ increases (see Figure S1 in supporting information). As discussed above it is contributed mainly by tethers facing the surface (Figure 2, inset). We note that $\Delta F_{compr}(s)$ does not account for the additional conformational limitations for tethers caused by ligand-receptor binding.

The free energy change due to the reversible interactions (with binding energy $E_{bind} = m$ kT) between receptors and ligands tethered to the nanoparticle positioned at a separation distance s from cell surface was also calculated using the acceptance-ratio method.³² In this calculation, the absolute value of ligand-receptor binding energy E_{bind} was gradually increased from 0 to m kT with a step of 0.5 kT (and in some cases 1 kT) and the probability of having n bound ligands was calculated. The maximum absolute value of the binding energy considered was 15 kT. We note that the state with $E_{bind} = 0$ is the same as considered in the compression simulations discussed above. The free energy change upon increasing the absolute value of binding energy for ligand-receptor interactions from 0 to m kT for a given separation distance s is obtained by summation of all free energy differences for the

intermediate binding energies $\Delta F_{bind}(s, E_{bind}=m) = \sum_{E_{bind}=0}^{E_{bind}=m} \Delta F_d(s, E_{bind})$, where $\Delta F_d(s, E_{bind})$ is calculated by solving eq 1 using $U = -nE_{bind}$ for two adjacent binding energies (see supporting information). ΔF_{bind} accounts for both the enthalpic gain due to the reversible interaction between ligands and receptors and the additional loss of the conformational entropy of tethers carrying bound ligands (primarily stretching as shown in Figure 2) and the indirect effect of binding on conformation of nonfunctional tethers (or tethers carrying unbound ligands). The smaller is the separation distance between the nanoparticle and cell surface the larger is the number of bound ligands and smaller is the entropic penalty (stretching) for tethers carrying bound ligands (Figure 2) leading to the more negative values of ΔF_{bind} (see Figure S1 in supporting information).

The overall free energy (with respect to the reference state of non-interacting nanoparticle $E_{bind} = 0$ positioned at the separation distance s_{∞}), $\Delta F(s, E_{bind} = m kT)$ for a nanoparticle interacting with a cell surface with a ligand-receptor binding energy $E_{bind} = m kT$ at a separation distance s is the sum of $\Delta F_{compr}(s)$ (upon decreasing the separation distance from s_{∞} to s) and $\Delta F_{bind}(s, E_{bind} = m kT)$ (upon increasing the binding energy from $E_{bind} = 0 kT$ to $E_{bind} = m kT$):

$$\Delta F(s, E_{bind}=mkT)=\Delta F_{compr}(s)+\Delta F_{bind}(s, E_{bind}=mkT) \quad (2)$$

The first term in eq 2 increases with a decrease in s as the number of available conformations for tethers situated between nanoparticle core and cell surface decreases. The second term ΔF_{bind} , decreases (becomes more negative) due to increased number of ligands bound to receptors and decrease of corresponding tether stretching to reach the surface. As a result the overall free energy decreases (becomes more negative) with a decrease of s at larger separation distances where the second term dominates, exhibit a minimum at intermediate values of s and increases in a close vicinity of cell surface where the first term in eq 2 takes over (see Figure S1 in the supporting information).

Results

Number of Tethered Polymer Chains

One of the important design parameters of a nanoparticle is the number of tethered polymer chains forming the protective corona layer around the nanoparticle core. An increase in the number of tethered polymers for a given core size results in a monomer density increase in the corona and local stretching of the tethered polymers near the core surface. As a consequence, ligands become excluded (by the concentration gradient) from the core vicinity and are primarily expressed on the periphery of the nanoparticle. The number of bound ligands is shown in Figure 3a as a function of the separation distance s between the cell surface and the nanoparticle core surface for different numbers of tethered chains with the same tether length ($N = 32$) and degree of functionalization ($x = 100\%$). We note that a larger number of receptors on the cell surface is recruited from the rest of the cell by the presence of a larger number of ligands near the cell surface (which would not be possible in the case of immobile receptors unless the nanoparticle interacts with a part of the surface with a large concentration of clustered receptors). As is seen, nanoparticles with a larger numbers of tethered chains start to interact with the receptor surface at larger separation distances due to increased tether stretching and higher ligand density on the nanoparticle periphery. As the separation distance decreases an increasing number of ligands can reach the receptors on the cell surface and the number of bound ligands increases almost linearly. Correspondingly, the free energy of nanoparticle-cell surface interaction decreases with a decrease of the separation distance (Figure 3b). Upon approaching the cell surface the tether conformation becomes more limited and the entropic penalty for chain compression strongly increases leading to an increase in the free energy. The free energy minimum occurring at intermediate separation distances is related to the equilibrium binding constant as $K_{bind} = \exp(-\Delta F_{min}/kT)$ and characterizes how strongly the nanoparticle is bound to the cell surface. The separation distance s_{eq} corresponding to the free energy minimum is the equilibrium distance at which nanoparticle is bound to the cell surface and the corresponding number of bound ligands n_{eq} is the equilibrium number of bound ligands. We note that n_{eq} does not necessary correspond to the maximum number of bound ligands: at separation distances $s < s_{eq}$ more ligands can be bound to receptors, which, however does not compensate for the increased entropic loss for all tethers in the vicinity of the cell surface resulting in the free energy increase. A larger number of tethered chains Q implies a larger monomer density in the protective polymer layer, which results in a larger entropic loss for

tethers near the cell surface, so that the corresponding free energy minimum is reached at larger separation distance (Figure 3b). A consistently larger number of bound ligands at all separation distances for nanoparticles with a larger number of tethered chains leads to a larger equilibrium binding constant as long as the number of ligands is proportional to the number of chains.

A larger number of bound ligands does not automatically guarantee the maximum efficiency for the ligand use.³⁴ Indeed, doubling the number of tethered chains (and hence ligands) considered in Figure 3a results in less than a factor of two increase in the number of bound ligands. Depending on the core size and tether length some ligands simply cannot reach the surface¹⁷ or the energy of ligand-receptor interaction E_{bind} is too low to overcome the entropic penalty for tether stretching (Figure 2) and the translational entropy of the receptors (which would not be a factor for immobile receptors). Thus a considerable fraction of ligands remains free. If one normalizes the free energy of nanoparticle-cell surface interaction by the number of ligands attached to the nanoparticle (which is equal to the number of tethered chains), the corresponding free energy minimum would be deeper for the nanoparticles with a smaller number of tethered chains, as the fraction of bound ligands is higher in this case (see supporting information). Similarly, if one compares the interaction with a cell surface of two nanoparticles with different numbers of tethered chains and the same number of ligands one can find that the equilibrium binding constant will be higher (and free energy is lower, as is seen in Figure 4) for the nanoparticle with a smaller number of tethered chains. Indeed, a nanoparticle with a larger number of tethered chains experiences a larger compression penalty for the chains in the vicinity of the cell surface. As a result the corresponding free energy minimum is higher and is reached at larger separation distance, even though the number of bound ligands is slightly larger in this case (due to a larger fraction of ligands situated further away from nanoparticle core, see Figure S3 in supporting information). We note that the nanoparticle with a larger number of tethered chains can interact with the cell surface at larger separation distances with the corresponding free energy being lower in this range of s compared to a nanoparticle with smaller number of tethered chains (Figure 4).

Effect of Binding Energy and Percent of Functionalization by Ligands

The percent of functionalization by ligands (x) plays an important role as is seen comparing the free energy of nanoparticle-cell surface interaction for 100% (Figure 3) and 50% (Figure 4) functionalization by ligands for $Q = 128$: the former is noticeably lower than the latter. In general, an increase in the percent of nanoparticle functionalization by ligands significantly enhances the density of ligands on the periphery of nanoparticle and leads to an increase in the number of bound functional groups (provided there is a sufficient number of receptors available and the cell surface underneath the nanoparticle is not saturated by receptors).²⁷ Correspondingly the free energy of the nanoparticle-receptor surface interaction decreases. The free energy minimum ΔF_{min} (related to the equilibrium binding constant) is presented in Figure 5 as a function of the percent of nanoparticle functionalization by ligands, x and the ligand-receptor binding energy E_{bind} . As is seen ΔF_{min} decreases with an increase of x , similar to a planar functional polymer layer.³⁵ An increase in the ligand-receptor binding energy E_{bind} enhances the enthalpic contribution to the free energy and leads to the decrease in the free energy of nanoparticle-cell surface interaction, as has been predicted for planar functionalized polymer layer.^{35,36} A higher E_{bind} overcomes the entropic penalty for tether compression near the cell surface and tether stretching, allowing ligands to reach receptors further away from the nanoparticle center. The free energy minimum ΔF_{min} considerably deepens with an increase in binding energy E_{bind} as is seen in Figure 5. Correspondingly the equilibrium binding constant K_{bind} as well as the number of bound functional groups at the free energy minimum increases. Thus, an increase in binding energy or the percent of

functionalization by ligands leads to a larger number of bound ligands and a higher affinity of nanoparticle to the cell surface, as is seen from Figure 5. To achieve a desired value of the equilibrium binding constant for a nanoparticle, determined by ΔF_{min} one can either vary the percent of functionalization for a selected ligand or choose a different ligand, thereby altering the ligand-receptor binding energy E_{bind} , for a given degree of functionalization, as indicated by the ΔF_{min} contour plot in Figure 5.

A noticeable increase in the binding association constants and in the number of bound ligands with an increase in percent of functionalization has been observed experimentally for ligand-conjugated micelles,^{9,16} dendrimers^{15,19} and diblock copolymer modified poly(D,L-lactic acid) (PLA) nanoparticles.²⁰ For example, for cRGD-functionalized polymer micelles, a high density of cRGD groups leads to a higher level of cell internalization of these micelles.¹⁶ For dendrimers with folate ligands¹⁹ (or sugar groups¹⁵), the amount of ligand binding (as well as the association constant) is seen to increase with the number of ligands per dendrimer molecule. It is interesting to note that in certain cases for nanoparticles with a high degree of functionalization^{15,19} the number of bound ligands reaches a plateau level, which is consistent with the maximum number of receptors that can gather on the cell surface underneath the nanoparticle. The saturation level depends on the intrinsic properties of the receptors, such as their size and clustering capability.^{15,19} In our simulations we also observe that the equilibrium fraction of bound ligands can reach a saturation value for larger values of binding energy (see supporting information). Interestingly, the fraction of bound ligands (with respect to the total number of ligands conjugated to the nanoparticle) firstly increases with the percent of functionalization x , reaches a maximum around $x = 50\%$ and then decreases with further increase in the percent of functionalization (see Figure S4 in supporting information). This implies that the optimal use of ligands occurs at some intermediate ligand density - if the number of ligands is too small, they cannot attract receptors on the cell surface, if it is too high then most of them will not be used as there is a limited number of receptors which can be gathered around the nanoparticle. This behavior persists for a range of binding energies with the position of the maximum of bound ligand fraction slightly shifting to a smaller percent of functionalization as E_{bind} increases.

Effect of Tether Length

An increase in the tether length (for a given number of tether chains) results in a thicker protective layer around the nanoparticle core. As a result, ligands are distributed over a larger volume around the nanoparticle core and their density decreases. Since some of the ligands extend far away from the nanoparticle core surface, an increase of tether length results in an increase in the separation distance at which ligand-receptor binding can occur, as is seen in Figure 6 where the free energies of nanoparticle-cell surface interaction are compared for two nanoparticles with different tether chain lengths ($N = 64$ and $N = 32$). As is seen, the nanoparticle with the longer tether length has lower free energy at larger separation distances, when a nanoparticle with the shorter tether length cannot reach the cell surface unless the tether chains are strongly stretched. At the same time a thicker protective layer implies a larger entropic penalty for chain compression/conformational change near the cell surface (see Figure S5a in supporting information) and the free energy minimum is less negative despite the larger number of bound ligands (for a given s , see Figure S5b in supporting information) and is achieved at a larger separation distance (Figure 6). The increase in the number of bound ligands is due to the larger cell surface area accessible to longer tethers, which increases the probability of binding to receptors.³⁷ It is interesting to note that the nanoparticle with a longer tether length shows a more gradual decrease in the free energy with a decrease in s than the nanoparticle with shorter tethers. This implies that the attraction force between the nanoparticle and cell surface will be lower (but acting over a

longer range) in the former case. Correspondingly a nanoparticle with shorter tethers has a stronger (but short-range) attractive force of interaction with cell surface, which is related to a more abrupt increase in the number of bound functional groups and a smaller tether compression zone near the surface.

The interplay between the increased entropic penalty for compression of longer tethers near the cell surface and an increase in enthalpy of ligand-receptor interactions due to the larger surface area accessible for a longer tether does not always resolve in the favor of the former. Figure 7 shows the difference in the free energy minima of nanoparticle-cell surface interactions (which corresponds to the ratio of the equilibrium constants) for nanoparticles with different tether lengths $N = 64$ and $N = 32$ as a function of binding energy E_{bind} for different numbers of tethered chains (Q). As is seen, the free energy difference firstly increases with binding energy, reaches its maximum and decreases with further increase in E_{bind} (Figure 7). The positive value of the free energy difference implies that the nanoparticle with shorter tether length has a larger affinity (lower free energy) towards the cell surface. At low binding energies there are only a few ligands bound and since the loss of conformational entropy is larger for longer chains, the nanoparticles with shorter tethers have the advantage. With an increase of E_{bind} more ligands are bound and now the main conformational penalty is due to chain compression (or conformational limitations) in the vicinity of the surface. This entropic penalty is larger for longer tether chains which results in an increase in the free energy difference, implying even larger advantage of nanoparticles with shorter tethers in cell targeting. Comparing nanoparticles with different numbers of tethered chains (for 100% of functionalization) one can notice that at a relatively low binding energies the free energy difference (and hence the advantage for nanoparticles with short tethers) increases with an increase in the number of tethered chains due to an increase in the compression penalty. When the binding energy becomes sufficiently large to attract a large number of receptors on the cell surface to the nanoparticle vicinity, the situation starts to change. Indeed, a nanoparticle with longer tethers can reach a considerably larger area on the cell surface with a resulting increase in the number of bound receptors. If the increase in the enthalpic advantage of ligand-receptor interactions for the nanoparticle with longer tethers is sufficient to overcome the larger entropic penalty compared to nanoparticle with short tethers, then the former gains advantage compared to the latter. As is seen from Figure 7, the free energy difference becomes negative above $E_{bind} \approx 14 \text{ kT}$ for the largest number of tethered chains considered. For the lower tether grafting densities it would take a larger binding energy to reach the limit when longer tether length ($N = 64$) would result in a higher nanoparticle affinity toward receptor surface (e.g. $E_{bind} \gtrsim 16 \text{ kT}$ for $Q = 64$). The lower is the number of ligands (or lower is the receptor density on a cell surface) the higher is the binding energy required to achieve a sufficiently large number of bound receptors to see the advantage of longer tether length.

Effect of Nanoparticle Core Size

To investigate the influence of nanoparticle core size on the efficiency of nanoparticle targeting we will consider an increase in the core size in the following cases: a) while keeping constant the grafting density of functionalized (and nonfunctional) tethers, b) keeping constant the number of ligands and overall grafting density of tethers and c) keeping constant both the number of tethers and ligands.

In general, increasing the nanoparticle core size while keeping the tether grafting density constant decreases the relative volume available for tether chains as the core radius of curvature increases. As a result, the monomer density of tethers in the protective layer increases and tether chains may experience some local stretching near the core surface. Due to the larger number of tethers attached to the larger core (to maintain constant grafting density) the entropic penalty for chain compression (or decrease in the number of possible

conformations) near the cell surface considerably increases with an increase in the core radius of curvature (see Figure S6a in supporting information). On the other hand, if the number of ligands increases together with the number of tethered chains (to achieve the same grafting density) on a larger nanoparticle core, the density of ligands on the nanoparticle periphery increases as well (see Figure S6b in supporting information). Analyzing the density of bound receptors beneath the nanoparticle at a given separation distance s between a nanoparticle and core surface one can notice that the receptor density is somewhat higher for the nanoparticles with a larger core size, as is seen from Figure 8 where the density of bound receptors is plotted as a function of the radial distance from the projection of nanoparticle center on the cell surface. More importantly, a noticeably larger difference in the density of bound receptors is seen on the periphery of the nanoparticle - ligands attached to a nanoparticle with a larger core size can interact with receptors over a larger cell surface area (Figure 8). As a consequence of both, the ligand density increase and especially an increase in the interaction surface area, the nanoparticles with a larger core size demonstrate a noticeably larger number of bound ligands compared to the nanoparticles with a smaller core size (for a constant grafting density of functionalized tethers), as is seen in Figure S7a in supporting materials. The considerable enhancement of the enthalpic gain of ligand-receptor interactions for nanoparticles with a larger core size overcomes the increase in the entropic penalty for chain compression near the cell surface leading to a noticeably deeper free energy of nanoparticle-cell surface interactions, as is seen in Figure 9. Doubling the core size results in about 2 times decrease in the free energy minimum of nanoparticle-cell surface interactions for the case considered in Figure 9. The separation distance at which the free energy reaches its minimum is slightly larger for nanoparticle with a larger core size due to the larger entropic penalty for chain compression. An increase in the binding affinity with an increase in a nanoparticle size (accompanied by the increase in the number of functional groups) has also been observed experimentally for dendrimers of different generations and functionalized colloidal gold nanoparticles of different sizes.^{11,15}

As discussed above, depending on tether length relative to the core size, some ligands simply cannot reach the surface¹⁷ and a considerable fraction of ligands remains free. The fraction of such ligands will become only larger with an increase of core size. Thus, if one would normalize the free energy of nanoparticle-cell surface interaction by the number of ligands attached to the nanoparticle (which is equal to the number of tethered chains in the case considered in Figure 9), the corresponding free energy minimum would be deeper for the nanoparticles with a smaller core size (see Figure S7b in supporting information), as the fraction of bound ligands is higher in this case. Similarly, if one compares the interaction with a cell surface of nanoparticles with different core sizes carrying the same number of ligands and having the same grafting density of tethers, one can find that the equilibrium binding constant will be higher and free energy lower for the nanoparticles with a smaller core size, as is seen in Figure 10. Indeed, for a nanoparticle with a larger core size the same number of ligands will be distributed over the larger volume thus decreasing their average density. We note that even though the ligands attached to a larger core size can reach a larger cell surface area, this effect is minor compared to the overall decrease in the ligand density. The penalty for chain compression near the cell surface is also larger for the nanoparticles with larger core size (since the total number of tethered chains is larger) resulting in a less negative free energy of nanoparticle-cell surface interaction and hence weaker nanoparticle affinity to a cell surface.

Finally we can also compare the affinity towards the cell surface for nanoparticles with different core sizes carrying the same number of ligands and tether chains. In this case, for a larger nanoparticle core size both the ligand and tether monomer density will be lower as they are distributed over a larger volume (and surface area). Correspondingly bound receptors on the cell surface will also be distributed over a large area, as discussed above.

Additionally, a large average distance between ligands in the corona of a nanoparticle with a larger core size results also in a relatively larger average separation distance between bound receptors (see Figure S8 in supporting information). In general a smaller density of ligands implies fewer bound receptors and a smaller ligand-receptor interaction enthalpy. At the same time, since the grafting density of tethers decreases with an increase of core size, the entropic penalty for chain compression in the vicinity of the surface decreases as well. Correspondingly, the overall free energy profile as well as its minimum shifts to a smaller separation distance s , as shown in Figure 11. The free energy minimum slightly decreases with an increase of core size as the decrease in the compression entropy exceeds the decrease in the enthalpy of ligand-receptor interactions. As the absolute value of the free energy minimum $\Delta F_{\min}$ (as well as the equilibrium binding constant K_{eq}) is defined by the competition between the enthalpy of ligand-receptor binding and chain compression entropy, both of which decrease with an increase of core size. The outcome of this competition (and the change in $\Delta F_{\min}$) will be influenced by the binding energy, tether length, grafting density and percent of functionalization by ligands. In general the change in core size for a given number of ligands and tethers does not result in a dramatic change in nanoparticle affinity to the cell surface.

Receptor Density Effect and Binding Specificity

Above we discussed the influence of different nanoparticle design parameters on the binding affinity of a nanoparticle to a given cell surface. The results of nanoparticle targeting will also be dependent on the cell surface properties, such as receptor density, mobility, size, capability of clustering, etc. In particular understanding the effect of receptor density is important for achieving selectivity of targeting, i.e. nanoparticle adsorption on cells with large densities of receptors but not on cell surfaces with low receptor densities. To illustrate this effect we selected another cell surface with a receptor density $\rho_L = 7.5 \times 10^{-7} a^{-2}$, 20 times smaller than considered above ($\rho_H = 1.5 \times 10^{-5} a^{-2}$), and compared the obtained results for nanoparticle-cell surface interactions. In general, the reduction in receptor density results in a decrease of the number of bound ligands and an increase in free energy, with a considerably more shallow separation distance dependence in a more narrow range of s (see Figure S9 in supporting information). Figure 12 shows the equilibrium number of bound ligands (i.e., number of bound ligands corresponding to the free energy minimum) and the free energy minimum as a function of binding energy E_{bind} for a nanoparticle interacting with these two cell surfaces. As is seen, the number of ligands bound to the cell surface with a smaller receptor density is noticeably smaller and the free energy is considerably less negative compared to the case of a high density of receptors on a cell surface. The difference in the equilibrium number of bound ligands and especially in the free energy minima (shown as shaded area in Figure 12) becomes only larger with an increase in the binding energy.

The implication of these results for selectivity of nanoparticle targeting is the following. For an illustration, let's consider that in order for a nanoparticle to be stably bound to cell surface and consequently internalized, e.g. at least 5 ligands should be bound to receptors - as would be the case when internalization is enhanced by receptor clustering in response to multivalent binding.^{11,16,38} Based on Figure 12a, if the nanoparticle is conjugated with ligands interacting with receptors with binding energy $E_{bind} < 10.5 kT$, then the number of bound ligands will be insufficient to achieve stable nanoparticle attachment to cells with either low or high receptor densities. If the ligand-receptor binding energy is in the range of $10.5 kT < E_{bind} < 12 kT$ then a nanoparticle will be bound to the cell with high density of receptors (as there is a sufficient number of bound ligands), but there are not any bound ligands on the cell with a low density of receptors. This would be the optimal range of binding energies in our example to achieve specific targeting. In a range of binding energies $12 kT < E_{bind} < 13.7 kT$ the nanoparticle will be stably attached to a cell with high receptor

density, but not to the cell with low receptor density, even though there will be some bound ligands on the latter. Finally for $E_{bind} > 13.7 \text{ kT}$, nanoparticles will be stably bound to both cells. Thus in this example too low ($E_{bind} < 10.5 \text{ kT}$) or too high ($E_{bind} > 13.7 \text{ kT}$) binding energy results in either insufficient nanoparticle attachment to cause internalization or high attachment leading to internalization by both cells, respectively. This result is in agreement with analytical and computer simulation predictions that increasing nanoparticle binding affinity does not necessarily enhance its targeting specificity^{23,26,35} Only when the ligand-receptor binding energy is in the range of $10.5 \text{ kT} < E_{bind} < 13.7 \text{ kT}$ does the nanoparticle show specific targeting of cells with high receptor density.

One can also consider a different example when the strength of nanoparticle attachment, as described by the equilibrium binding constant $K_{eq} = \exp(-\Delta F_{min}/kT)$, can serve as a criterion of nanoparticle attachment/internalization. This situation would be relevant to the experimental case when a nanoparticle would eventually get internalized as long as it is stably attached to the cell surface. Based on Figure 12b if one assumes that a stable attachment is achieved when $\Delta F_{min} < -10kT$, then selective targeting of a nanoparticle will occur in the range $11.2 \text{ kT} < E_{bind} < 14.6 \text{ kT}$ with zero nanoparticle attachment (i.e. $\Delta F_{min} \approx 0$) to the cell surfaces with low receptor densities for $E_{bind} < 12 \text{ kT}$. For low ($E_{bind} < 11.2 \text{ kT}$) or high ($E_{bind} > 14.6 \text{ kT}$) ligand-receptor binding energies there is either no attachment or stable attachment (leading to internalization) to both cell types, respectively. We note that the range of binding energies corresponding to selective nanoparticle targeting will ultimately depend on the experimental situation dictating the criterion of nanoparticle attachment/internalization to the cell. Based on the above examples, using the number of bound ligands or the equilibrium binding constant will lead to somewhat different windows of binding energies required to achieve selective nanoparticle targeting.

The percent of functionalization by ligands is another factor which has a strong influence on nanoparticle binding affinity (Figure 5) and it has been predicted to influence the binding specificity for functionalized polymer layers.³⁵ In Figure 13 we compare the equilibrium number of bound ligands and free energy minimum as a function of the percent of functionalization by ligands for the nanoparticle interaction with cell surfaces with low or high receptor densities. As expected, the equilibrium number of ligands bound to the cell with high receptor density is considerably higher than that to the cell with low receptor density. Moreover, the shape of the dependence is different as well - the number of ligands bound to the cell with lower receptor density shows some tendency to saturation at large percent of functionalization, in contrast to the case for the cell with high receptor density (for the considered ligand-receptor binding energy $E_{bind} = 15kT$). If we apply the same exemplary criterion for the number of bound ligands (5) required for nanoparticle stable attachment leading to internalization, as discussed above, we can conclude that if the percent of functionalization is less than 14% no nanoparticle attachment to either cell will occur. In the range $14\% < x < 25\%$ nanoparticles will be exclusively bound to the cell with a high density of receptors while if the percent of functionalization is larger than 47% nanoparticles will be stably attached to (and can be internalized by) both cell types. Thus the selectivity of nanoparticle targeting can be achieved in this example in the range $14\% < x < 47\%$. If the critical number of bound ligands needed for stable nanoparticle attachment (and consequent internalization) is larger or smaller the corresponding range of the percent of functionalization by ligands to achieve selective targeting (indicated by shaded area in Figure 13a) will expand (and shift to larger values of x) or contract (and shift to smaller values of x), correspondingly. Similar conclusions can be derived if one uses equilibrium binding constant (using the free energy minimum shown in Figure 13b) as a criterion of nanoparticle attachment/internalization. In such a case, the selective nanoparticle targeting will occur in the range $13\% < x < 68\%$ (for the selected criterion $\Delta F_{min} < -10kT$ for stable attachment).

As is seen from the examples considered above, the selectivity of nanoparticle targeting is influenced by both binding energy and percent of functionalization. Moreover, both will also depend on the specified receptor densities and other nanoparticle design parameters (nanoparticle core size, tether grafting density and chain length) as well as receptor properties (size, mobility, capability of clustering). A detailed analysis of this complex problem is evidently needed and we plan to address it in our future work.

Discussion and Conclusions

Binding affinity and specificity are two important aspects of nanoparticle targeting that characterize the performance of nanoparticulate delivery systems. A larger nanoparticle binding affinity is usually desired in order to prolong the residence time of nanoparticles on cell surfaces and reduce the concentration of nanoparticles needed to maximize physiological responses. Specific targeting of nanoparticles to cell surfaces with over-expressed receptors, on the other hand, is a necessary requirement for diagnostic applications and for achievement of desired therapeutic responses using a smaller drug dosage, thereby reducing the incidence of adverse side effects. In this paper using computer simulations we analyzed the influence of different design parameters of a monovalent ligand conjugated-spherical nanoparticle on binding affinity and specificity of targeting mobile receptors on a planar cell surface.

The nanoparticle binding affinity to a cell surface has been assessed in our study by analyzing the free energy minimum which is related to the equilibrium binding constant or by considering the corresponding equilibrium number of bound ligands. Both criteria show qualitatively similar trends. As discussed above, the binding affinity is largely determined by the delicate balance between the enthalpic gain due to the ligand-receptor binding and the entropic penalty for tether chain stretching or compression in the vicinity of the cell surface (Figure 2). The influence of individual nanoparticle design parameters on the binding affinity can be summarized as the following. 1. An increase in the ligand-receptor binding energy or nanoparticle percent of functionalization results in a noticeable increase in the number of bound ligands (with a corresponding decrease in the free energy) and increases the nanoparticle affinity for a cell surface. This enhancement of nanoparticle affinity has a purely enthalpic origin. 2. An increase in the number of tethered polymers, which is accompanied by a corresponding increase in the number of ligands (for a constant nanoparticle core size) also increases nanoparticle binding affinity. In this case the increased ligand density strongly enhances the enthalpic contribution to the free energy leading to a larger affinity. However an increase in the number of tethered polymers for a fixed number of ligands results in a decrease in nanoparticle affinity due to the enhanced entropic penalty for chain compression near the cell surface. 3. An increase in tether chain length in most cases does not improve the nanoparticle affinity toward the cell surface, as longer polymer chains have a larger conformational entropy which results in a higher entropic penalty for chain stretching/compression. At the same time, longer tether chains can reach a larger area on the cell surface thus enhancing the probability of interactions with cell receptors. As a result, when the degree of functionalization of nanoparticle by ligands is large and there is a sufficient number of receptors on the cell surface which can cluster around the nanoparticle, stimulated by a high ligand-receptor binding energy E_{bind} , then the nanoparticle affinity towards cell surface can be enhanced by using longer tether chains. The attractive interaction force between a nanoparticle with longer tethers and cell surface extends over a larger separation range than that for nanoparticles with short tethers. 4. An increase in the core size for a constant tether grafting density (i.e. increasing number of tethered chains) results in a larger entropic penalty for chain compression near the cell surface, which decreases nanoparticle affinity if the number of ligands kept constant (i.e. their density decreases). However an increase in the number of ligands on a nanoparticle with a larger

core size results in a higher ligand density and, more importantly, allows ligands to reach a larger cell surface area leading to a considerable enhancement of the enthalpy of ligand-receptor interactions and nanoparticle affinity compared to a nanoparticle with a smaller core size (for a constant grafting density of functionalized tethers). For nanoparticles with a given number of ligands and tethers an increase in the core size results in a marginal change in nanoparticle affinity, as both ligand density and chain compression entropy decrease.

To illustrate the influence of binding energy and percent of functionalization on the specificity of targeting, we compared the nanoparticle interaction with two cell surfaces containing different receptor densities. We found that a small ligand-receptor binding energy or a small percent of nanoparticle functionalization by ligands results in a rather weak nanoparticle attraction to either cell surface. In the opposite limit, a very large binding energy or percent of functionalization by ligands results in strong nanoparticle attachment (judged by the number of bound ligands or free energy minimum of nanoparticle-cell surface interaction) to both cell surfaces. Thus specific targeting of cells with high receptor density can be achieved in an intermediate range of binding energies and/or percent of functionalization by ligands. We note that the precise parameter range to achieve specificity will depend on the biophysical/biochemical conditions of nanoparticle attachment/internalization by cells as well as the properties of benign cells. In general all different design parameters of a nanoparticle will collectively influence the specificity of targeting, which represents one of the challenging problems in modern imaging and therapeutic applications of nanoparticles. We hope to contribute to the solution of this problem in our future work.

In conclusion, the presented research contributes to the fundamental understanding of the interactions between a ligand-conjugated spherical polymer nanoparticle with cell surfaces containing mobile receptors and makes specific predictions regarding the rational design of polymer nanoparticles to achieve high affinity and specificity of targeting, which can guide further experimental development of targeted nanoparticulate delivery systems for molecular-based diagnostic and therapeutic purposes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Schematic representation of ligand-tethered polymer nanoparticle interacting with the cell surface containing mobile receptors. The smallest separation distance between the nanoparticle core surface and the cell, s , is shown.

Figure 2.

The ratio of the average square radius of gyration for polymer tethers carrying ligand bound to a receptor (circles) and tethers with unbound ligands (squares) for a nanoparticle separated from the cell surface by distance s to the average square radius of gyration for polymer tethers on isolated nanoparticle, $\langle R_g^2 \rangle / \langle R_g^2 \rangle_0$. The inset shows $\langle R_g^2 \rangle / \langle R_g^2 \rangle_0$ in the absence of ligand-receptor interactions ($E_{bind} = 0 kT$) for all tethers (triangles) and for tethers attached to the half of nanoparticle core facing the cell surface (diamonds). The nanoparticle core size is $r = 20a$, the number of tethered polymers chains $Q = 128$, the percent of functionalization by ligands $x = 100\%$, tether length $N = 32$, binding energy $E_{bind} = 15 kT$ and receptor density on cell surface $\rho = 1.5 \times 10^{-5} a^{-2}$.

Figure 3.

a) Number of bound ligands and b) free energy of nanoparticles with different numbers of tethered chains (Q) interacting with a cell surface with density of mobile receptors $\rho = 1.5 \times 10^{-5} a^{-2}$ and binding energy $E_{bind} = 15kT$ as a function of separation distance s . The nanoparticle core size is $r = 20a$, the percent of functionalization by ligand $x = 100\%$, tether length $N = 32$.

Figure 4.

Free energy as a function of separation distance s for nanoparticles with different numbers of tethered chains (Q) and the same number of ligands $n_L = 64$ (i.e., different percent of functionalization x) interacting with cell surface with density of mobile receptors $\rho = 1.5 \times 10^{-5} a^{-2}$ and binding energy $E_{bind} = 15 kT$. The nanoparticle core size is $r = 20a$, tether length is $N = 32$.

Figure 5.

Free energy minimum of nanoparticle-cell surface interaction as a function of ligand-receptor binding energy E_{bind} and percent of functionalization by ligands x . Color bands mark different free energy ranges (with a step of approximately $-16 kT$) with the corresponding contour plot projection. Connected symbols (spheres) correspond to $\Delta F_{min} = 0$ with the projection shown as dashed line. Nanoparticle core size is $r = 20a$, number of tethered chains is $Q = 128$, tether length is $N = 32$ and the receptor density on the cell surface is $\rho = 1.5 \times 10^{-5} a^{-2}$.

Figure 6.

Free energy for a nanoparticle containing $Q = 32$ tether chains interacting with a cell surface with ligand-receptor binding energy $E_{bind} = 15kT$ as a function of separation distance s for two different tether lengths $N = 64$ and $N = 32$. The nanoparticle core size is $r = 20a$, percent of functionalization is $x = 100\%$ and density of mobile receptors is $\rho = 1.5 \times 10^{-5}a^{-2}$.

Figure 7.

The difference between the free energy minima for nanoparticles with longer, $N = 64$ and shorter, $N = 32$ tethers as a function of ligand-receptor binding energy E_{bind} for different numbers of tethered chains $Q = 32, 64$ and 128 . The nanoparticle core size is $r = 20a$, percent of functionalization is $x = 100\%$ and density of mobile receptors $\rho = 1.5 \times 10^{-5}a^{-2}$.

Figure 8.

Density of bound receptors as a function of the radial distance from the projection of nanoparticle center on the cell surface for nanoparticles with different core sizes ($r = 20a$ squares, $r = 30a$ circles, $r = 40a$ triangles), the same percent of functionalization by ligands ($x = 100\%$) and the same tether grafting density. The separation distance between nanoparticle and cell surface is $s = 16a$, tether length is $N = 32$, the density of mobile receptors is $\rho = 1.5 \times 10^{-5}a^{-2}$ and binding energy is $E_{bind} = 15kT$.

Figure 9.

Free energy of nanoparticle-cell surface interaction as a function of separation distance s for nanoparticles with different core sizes ($r = 20a$ squares, $r = 30a$ circles, $r = 40a$ triangles), the same percent of functionalization by ligands $x = 100\%$ and the same tether grafting density. The tether length is $N = 32$, the density of mobile receptors is $\rho = 1.5 \times 10^{-5} a^{-2}$ and binding energy is $E_{bind} = 15kT$.

Figure 10.

Free energy of nanoparticle-cell surface interaction as a function of separation distance s for nanoparticles with different core sizes ($r = 20a$ squares, $r = 30a$ circles, $r = 40a$ triangles), the same number of ligands $n_L = 64$ (i.e., different percent of functionalization x) and the same tether grafting density. The tether length is $N = 32$, the density of mobile receptors is $\rho = 1.5 \times 10^{-5} a^{-2}$ and the binding energy is $E_{bind} = 15kT$.

Figure 11.

Free energy of nanoparticle-cell surface interactions for 100% functionalized nanoparticles with different core sizes ($r = 20a$ squares, $r = 30a$ circles, $r = 40a$ triangles) containing 128 tethers and ligands for binding energy $E_{bind} = 15kT$ as a function of separation distance s . The tethers of length is $N = 32$ and the density of mobile receptors on the cell surface is $\rho = 1.5 \times 10^{-5}a^{-2}$.

Figure 12.

The equilibrium number of bound ligands (a) and the corresponding minimum of free energy (b) of nanoparticle-cell surface interaction for the cell surfaces containing different receptor densities ($\rho_L = 7.5 \times 10^{-7} a^{-2}$, circles and $\rho_H = 1.5 \times 10^{-5} a^{-2}$, squares) as functions of binding energy E_{bind} . The nanoparticle core size is $r = 20a$, tether length $N = 32$, aggregation number $Q = 128$ and percent of functionalization by ligands $x = 100\%$.

Figure 13.

The equilibrium number of bound ligands (a) and the minimum of the free energy (b) for nanoparticle interaction with cell surfaces with high ($\rho_H = 1.5 \times 10^{-5} a^{-2}$, squares) and low ($\rho_L = 7.5 \times 10^{-7} a^{-2}$, circles) receptor densities as functions of the percent of functionalization by ligands, x . The nanoparticle core size is $r = 20a$, tether length $N = 32$, aggregation number $Q = 128$ and ligand-receptor binding energy $E_{bind} = 15 kT$.