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Optimization of Functionalized Polymer Layers for Specific Targeting of Mobile Receptors on Cell Surfaces

Matthew C. Hagy, Shihu Wang, and Elena E. Dormidontova*

Department of Macromolecular Science and Engineering Case Western Reserve University, Cleveland, Ohio

Abstract

The reversible binding between a planar polymer layer functionalized by targeting groups and a planar cell surface containing different densities of mobile receptors has been studied by Monte Carlo simulations. Using the acceptance-ratio method the distance-dependent profiles for the average number of ligands bound to receptors, the total free energy for the polymer layer-cell surface interaction and the interaction force were obtained. Four main design parameters for the polymer layer were considered: the degree of functionalization, chain degree of polymerization, polymer density of grafting and the binding energy for the targeting group-receptor interaction. We found that an increase in the degree of functionalization or in the absolute energy of ligand-receptor binding results in a larger number of ligands bound to the receptors, lower free energy and stronger attractive force. Polymer layers composed of shorter chains were found to exhibit a deeper and narrower free energy profile and a larger attractive force, while longer tethers can interact with the cell surface at a larger and broader range of separation distances, in agreement with experimental observations. Our simulation results show that the increase in polymer grafting density from the mushroom to brush regime enhances the ligand availability and results in a stronger attractive force, increases the maximum binding distance, but exhibits a shallower free energy minimum due to the smaller tolerance to compression for polymer layers with high grafting density. We used two measures of the polymer layer binding affinity to the cell surface: the free energy minimum, related to the equilibrium binding constant and the fraction of bound ligands. We found that the polymer layers with a smaller chain length and grafting density, larger degree of functionalization and larger absolute binding energy exhibit both a larger equilibrium binding constant to the cell surface and a larger average number of bound ligands, except for high binding energies when the maximum level of binding is reached independently of polymer length and grafting density. We showed that high binding specificity can be achieved by the polymer layers with intermediate ligand-receptor binding energies or an intermediate number of ligands, as a larger binding energy or number of ligands ensures a high binding affinity but lacks specificity while a smaller binding energy or number of ligands provides inadequate affinity. We found that the results for polymer layers with different properties follow a similar pattern when both high binding affinity to cells with high receptor density and high binding specificity are considered. As a result, the optimal design of the polymer layers can be achieved by using several different strategies, which are discussed.

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*corresponding author, email: eed@case.edu.

Supporting Information Available: Simulation data concerning the influence of binding energy on the equilibrium and maximum binding distance, the details of determination of the mushroom to brush transition, results addressing the influence of grafting density on equilibrium number of bound ligands and the details of estimation of the optimal range of E_{bind} for achieving high selectivity for Table 1. This information is available free of charge via the Internet at <http://pubs.acs.org>.

Introduction

Targeted drug and gene delivery is a rapidly growing field with a high potential for new advances in treating life-threatening diseases.¹⁻³ Polymer-based nanoparticles allow encapsulation and protection of hydrophobic drugs, imaging and diagnostic agents and gene/charged polymer complexes from undesirable non-specific interactions.^{1,3,4} In all cases site-specific delivery to a diseased area is a necessary requirement for designing a successful drug-delivery carrier.^{1,2} Polymer nanoparticles are able to satisfy this requirement by incorporating at the end of tethers biorecognition ligands having affinity to receptors overexpressed on diseased cell surfaces.^{1,4} While direct ligand-receptor interactions have been extensively studied,^{5,6} much less is known regarding the interaction of nanoparticle-conjugated ligands with receptors on cell surfaces. This paper serves to provide some insights on the influence of the polymer layer design on the affinity and specificity of interactions between a ligand-modified polymer layer and a cell surface carrying mobile receptors.

There are many experimental studies devoted to the investigation of specific targeting of polymer nanoparticles to cells or biofunctionalized surfaces.⁷⁻¹¹ As nanoparticle formation allows a large variation in design parameters, (e.g. size, tether length, degree of functionalization by targeting groups), individual experimental studies normally address the effect of only one such factor. For instance, the effect of degree of nanoparticle functionalization by ligands on the binding efficiency or extent of nanoparticle internalization was studied for block copolymer micelles,^{7,8} polymersomes⁹ and dendrimers.¹¹ It was found that although in general an increase in the degree of functionalization enhances the nanoparticle affinity, above some limit a further increase in the number of functional groups does not necessarily provide a substantial improvement,^{9,11} likely due to saturation of the number of bound receptors. Even though a high affinity of the targeted nanoparticles to cell surfaces can be achieved,^{7-9,11} it does not automatically guarantee selectivity of binding to cell surfaces with high density of receptors compared to normal cells. It has been shown that specific binding of a generation 5 poly(amidoamine) (PAMAM) dendrimers conjugated with an average of 5 folic acid targeting groups can be achieved to FAR+ cells, which overexpress folic acid receptors compared to FAR- cells with a low density of folic acid receptors.^{10,12} Kiessling et al.¹³ have shown that upon marking cells with high density of receptors one can stimulate the desired immune response by taking advantage of multivalent interactions between the antibodies and the markers. Since polymer nanoparticles are capable of multivalent binding to cell surfaces, even when monovalent ligands are used for targeting, understanding the nanoparticle design factors controlling selectivity of binding becomes an important goal. To accomplish this goal experimentally would take considerable time and resources, whereas analytical considerations and computer modeling such as considered in the current paper can considerably aid in this task.

To better understand the interactions between a polymer layer and a receptor-containing surface several analytic models have been developed.¹⁴⁻¹⁹ Wang et al. studied analytically the effect of the energy of ligand-receptor interactions, tether length and receptor density on the interaction between a single Gaussian polymer chain end-functionalized by a ligand and a planar cell surface containing randomly distributed mobile or immobile receptors.¹⁷ Their model predicts stronger binding for mobile compared to immobile receptors of the same density. A similar conclusion has been reached in a more recent approach that considered the interaction between a planar polymer layer functionalized with (mobile or immobile) ligands and a planar surface containing (mobile or immobile) tethered receptors.¹⁸ Assuming that the partition function of each tethered ligand can be considered independently of each other and separated into tether-dependent and ligand-dependent portions, the model predicts that the binding distance and the overall free energy is almost independent of tether length (if scaled by the tether radius of gyration) with a nearly linear decrease in the free energy of the system

with binding energy, when the latter is large.¹⁸ Interaction between end-functionalized polymer layers and free finite size receptors has also been studied analytically using Monte Carlo sampling of polymer chain conformations.^{20,21} Longo et al. found that there is an optimal polymer grafting density that maximizes the interaction with receptors and that the extent of binding increases with an increase of tether length. Karlstrom et al. have studied the effect of receptor size, polymer volume interactions and polymer-surface interactions on the binding between an end-functionalized polymer layer and free receptors.²¹ Application of analytical models has allowed the prediction of the influence of the surface radius of curvature, the strength of specific ligand-receptor pair interactions, tether length and grafting density on the overall range and force of attraction between two planar or curved surfaces, as in a surface force apparatus.^{14,15,19} In the recent decade the use of the surface force apparatus has emerged as a powerful technique to study the details of interactions between polymer layers functionalized by ligands and surfaces carrying receptors.^{14-16,22} Thus, direct surface force measurements of the biotin-streptavidin system demonstrated the increase of the binding distance and change in the attractive force between the surfaces with an increase of polymer tether length.^{14-16,22}

While there have been several analytical models developed to understand the polymer layer-receptor interactions, the application of computational methods has been much more limited.²³⁻²⁶ Some computer simulation efforts were applied to study either specific ligand-receptor pair interactions²⁴ or to understand the effect of receptor clustering upon interaction with uniformly distributed on a surface or clustered immobile²³ or mobile²⁶ ligands. In these studies the effect of the tether on ligand-receptor interactions has been ignored.^{23,24,26} At the same time this effect was shown to be rather important based on analytical predictions and experimental observations.^{14,15,17,18,20} Our previous Monte Carlo (MC) simulations of a functionalized polymer layer interacting with a surface containing immobile receptors, addressed the effect of chain length bidispersity, architecture and multivalence of ligands.²⁵ In the present paper we expand our previous simulation approach and apply the contact-distribution^{27,28} and acceptance-ratio²⁹ methods to address in more detail conformational changes in the planar polymer layer upon interaction with cell surface containing finite size mobile receptors.

This paper is arranged in the following way. In the next two sections, we will briefly discuss our simulation approach and data analysis methodology. Then the results of simulations for the distance-dependent extent of binding, free energy and force will be discussed for different design parameters for the polymer layer (specifically, polymer grafting density, tether length, the degree of functionalization and the ligand-receptor binding energy). This will be followed by an analysis of the comparative influence of these design parameters on the affinity of polymer layer to the receptor surface. We will continue with a discussion of the influence of receptor density on the equilibrium free energy and number of bound ligands. An analysis of the selectivity of binding and a discussion of the optimal design strategies for a polymer layer to achieve both high affinity and strong selectivity of binding will be presented. Finally we will summarize our main findings in the Conclusions section.

Simulation Model

The bond-fluctuation model^{30,31} was used to simulate a functionalized planar polymer layer interacting with a cell surface. Simulations were performed in a box with periodic boundary conditions in the x and y directions ($L_x = L_y = 64a$) and a fixed length ($L_z = 256a$) in the z direction, where each cubic lattice cell had sides of length a .

Polymer chains were modeled as linear sequences of N monomer beads joined through covalent bonds. In accordance with the bond-fluctuation model the bond length between two covalently

joined monomers is constrained to the following values: $2a$, $\sqrt{5}a$, $\sqrt{6}a$, $3a$, $\sqrt{10}a$, to avoid bonds crossing and ensure volume exclusion.^{30, 31} Thus our simulations correspond to good solvent conditions for the polymer chains. Some fraction of polymer chains was functionalized by replacing free-end terminal monomer with a ligand. In our current simulations we considered only monovalent ligands, i.e. containing a single targeting group. Such a targeting group was identical to regular monomer in size, but was capable of reversible binding to the center of a receptor on a cell surface with a corresponding energy of binding E_{bind} . All polymer chains (functional and non-functional) were uniformly tethered to a solid surface at $z = 0$ to create a homogeneous polymer layer with a specific polymer grafting density σ (Figure 1).

Another solid surface, corresponding to the cell surface was introduced at $z = s$ such that s was the separation distance between the polymer-tethered surface and the cell surface. The mobile receptors on the cell surface were modeled as 2-dimensional spherically-symmetric disks with a binding center and overall diameter $12a$, such that any two receptors could not approach each other closer than $12a$ (Figure 1). The receptor surface density was controlled probabilistically: An attempt was made to add a receptor at a random coordinate on the cell surface with probability p_{add} . A new receptor was added to this location if it does not overlap with already existing receptors. Additionally, we implemented a receptor deletion procedure such that each receptor would be deleted with probability p_{del} each MC step. We note that this probability was applied only to free receptors, i.e. receptors which were bound to ligands were not subjected to the deletion algorithm. In our simulations for the deletion probability we used $p_{\text{del}} = 0.1$ or 0.5 , while p_{add} was varied to control receptor density. Cell surface simulations showed that the average number of receptors on the (isolated) surface was equal to $p_{\text{add}}/p_{\text{del}}$ for $p_{\text{add}} < 0.5$. The absolute value of p_{add} or (p_{del}) was found to influence only the equilibration time (and/or the necessary box size to achieve the equilibrium conformation), but not the average density of receptors as long as $p_{\text{add}}/p_{\text{del}}$ was constant. It was also found that the maximum receptor density which could be achieved is 7.32×10^{-3} receptors/ a^2 or 30 receptors per $64a \times 64a$ cell surface. In the discussion below we study the effect of the average receptor density.

The equilibrium polymer conformation and distribution of receptors on cell surface was achieved via MC moving and binding updates. Receptor mobility was simulated through trial attempts to move a receptor in either the x or y directions on the cell surface. This move was accepted if the new position was not occupied by another receptor and the reversible bond length with a targeting group (if any) was in the range of permissible bond lengths (the same as for chemical bonds). For polymer monomers (or targeting groups) an attempt was made to move a bead by one lattice cell in one of the six primary directions. The move was accepted if it did not violate both volume exclusion (i.e. new position is not occupied by another monomer or is not a part of solid surface at $z = 0$ and $z = s$ and bond geometry (the new bond length with neighboring monomers or with receptor is in the range of permissible bond lengths). Following the moving attempts for targeting groups we also performed a reversible bond update, which included firstly breaking of any existing reversible bond with a receptor and then searching for free receptors on the cell surface within the bonding distance. If a receptor was found, a reversible bond was formed with the probability

$$\frac{\exp(-E_{\text{bind}}/kT)}{\exp(-E_{\text{bind}}/kT) + 1} \quad (1)$$

in accordance with Metropolis/Hastings algorithm for the direct sampling of the Boltzmann-weighted system configurations.^{32, 33} Each MC step corresponds to the number of moving attempts on randomly chosen monomers, targeting groups or receptors equal to the total number of monomers (including targeting groups) and receptors in the system.

Free Energy Calculations

To calculate the free energy of functionalized polymer layer interacting with a cell surface as a function of the distance between these two surfaces we employed the *acceptance-ratio method*.²⁹ This method allows calculation of the Helmholtz free energy difference between two canonical ensembles that differ only in their potential functions and have significant configuration overlap. Bennet has demonstrated the following relationship for the free energy difference F between two ensembles, 0 and 1, with corresponding potential functions u_0 and u_1 and resulting configuration energies U_0 and U_1 :²⁹

$$\sum_0 f(U_1 - U_0 - F) = \sum_1 f(U_0 - U_1 + F) \quad (2)$$

where f is the Fermi-Dirac function, which precise form used in our calculations is discussed below. On the left-hand side, the configuration energies U_0 and U_1 are calculated for the system 0 using corresponding potentials u_0 and u_1 , while the right-hand side corresponds to system 1. The *acceptance-ratio method* requires good overlap between these two ensembles. If this is not the case, one can consider a series of adjacently well-overlapping intermediate ensembles between the two ensembles of interest and the free energy differences can be calculated as a sum of the corresponding free energy difference between the intermediate ensembles.²⁹

In this study the free energy of a polymer layer interacting with cell surface at a separation distance s with binding energy E_{bind} was calculated relative to the reference system at s_∞ and $E_{\text{bind}} = 0$, where s_∞ is a separation distance sufficiently large to prevent any interaction or influence of the cell surface on the polymer layer. To determine the free energy difference we firstly decrease the separation distance from s_∞ to s considering no attractive interactions between the polymer layer and cell surface - this allowed us to calculate the compression free energy (entropy) for a non-functional polymer layer. Then for each intermediate separation distance the energy of interaction was incrementally increased from 0 to E_{bind} - this gave us the free energy difference for binding free energy at a constant distance. By summing the free energy differences between all adjacent simulations for different interaction energies E_{bind} and different separation distances s (as discussed below) one arrives at the desired free energy of the system calculated with respect to the reference state.

To calculate the free energy of polymer layer compression we used the modification of the *acceptance-ratio method*, the *contact-distribution method*, developed by Rajagopalan and Jimenez.^{27,28} This method allows the calculation of the free energy for a polymer layer compression by introducing a fictitious repulsion potential, $E_{\text{wall}} > 0$, at $z = s - a$ so that any bead at this z coordinate would incur an energetic penalty of E_{wall} , i.e. in accordance with the Metropolis/Hastings algorithm a bead movement attempt from $z = s - a$ to $z = s - 2a$ was accepted with probability proportional to $\exp(E_{\text{wall}}/kT)$. Therefore, if E_{wall} is sufficiently high, it will stimulate all monomers at $z = s - a$ to move to the neighboring ($z = s - 2a$) layer and the result of application of this potential will be analogous to introducing a solid wall at $z = s - a$. Consequently the free energy difference for the system with and without the potential (E_{wall}) gives us the free energy for polymer layer compression upon moving a solid wall by one lattice site (from $z = s$ to $z = s - a$), F_{wall} . The compression free energy, F_{wall} , is found by solving eq 2 in the form:

$$\sum_{i=0}^{n^{(\text{wall})}} \frac{P_{\text{contact}, i^{(\text{wall})}}}{1 + \exp\left(\frac{-iE_{\text{wall}} + F_{\text{wall}}}{kT}\right)} = \sum_{i=0}^{n^{(0)}} \frac{P_{\text{contact}, i^{(0)}}}{1 + \exp\left(\frac{iE_{\text{wall}} - F_{\text{wall}}}{kT}\right)} \quad (3)$$

where the configuration energy U_1 is simply the number of beads i at $z = s - a$ multiplied by E_{wall} and $U_0 = 0$, correspondingly. $p_{\text{contact},i}^{(\text{wall})}$ is the ensemble average probability to have i beads at $z = s - a$ in the presence of the potential E_{wall} and $p_{\text{contact},i}^{(0)}$, is that in the absence of potential. The summation in eq 3 goes from zero to the maximum number of monomers at $z = s - a$ in the presence ($n^{(\text{wall})}$) or absence of potential ($n^{(0)}$).

We note that in the polymer layer compression simulations no interactions with receptor wall was assumed, i.e. $E_{\text{bind}} = 0$, receptors were neglected ($p_{\text{add}} = p_{\text{del}} = 0$) and functional groups were indistinguishable from regular monomers. For these simulations it was important to chose an optimal value for E_{wall} . Too low a value would not reproduce the effect of a solid surface, while too high a potential would result in inefficient sampling. From a set of trial simulations we found that all potentials with $E_{\text{wall}} \geq 3kT$ yield nearly identical results, but with poorer sampling for higher values of the potential. Therefore, we used $E_{\text{wall}} = 3kT$ for most simulations presented in this paper, except for the high polymer grafting density case where changing E_{wall} from 0 to $6kT$ (with interval $1kT$) was necessary to obtain good data statistics. In our compression simulations, the equilibrium configuration for the wall separation distance s in the presence of potential E_{wall} was used as the initial configuration for $s - a$. As a result of the compression simulations we obtained the free energy of compression of non-functional polymer layer as a function of the separation distance to the cell surface (with respect to the state with infinite separation between the walls):

$$F_{\text{compr}}(s) = \sum_{z=\infty}^{z=s-a} F_{\text{wall}}(z) \quad (4)$$

A similar method was used to calculate the free energy difference between two simulations (with the same separation distance between the walls $z = s$) differing only in binding energies: $E_{\text{bind}}^{(0)}$ and $E_{\text{bind}}^{(1)}$. For these binding ensembles the configuration energy U_1 of a single sample is $n_{\text{bind}} E_{\text{bind}}^{(1)}$ and correspondingly $U_0 = n_{\text{bind}} E_{\text{bind}}^{(0)}$ where n_{bind} is the number of reversible bonds between functional groups and (mobile) receptors. The difference of the free energy of binding F_{bind} between these two ensembles is found by solving eq 2 in the form:

$$\sum_{n_{\text{bind}}=0}^{n^{(1)}} \frac{p_{n_{\text{bind}}}^{(1)}}{1 + \exp\left[\frac{n_{\text{bind}}(E_{\text{bind}}^{(0)} - E_{\text{bind}}^{(1)}) + F_{\text{bind}}}{kT}\right]} = \sum_{n_{\text{bind}}=0}^{n^{(0)}} \frac{p_{n_{\text{bind}}}^{(0)}}{1 + \exp\left[\frac{-n_{\text{bind}}(E_{\text{bind}}^{(0)} - E_{\text{bind}}^{(1)}) - F_{\text{bind}}}{kT}\right]} \quad (5)$$

where $p_{n_{\text{bind}}}^{(1)}$ is the probability of having n_{bind} reversible bonds between functional groups and receptors for the system with the energy of binding $E_{\text{bind}}^{(1)}$ and likewise $p_{n_{\text{bind}}}^{(0)}$ is that for the system with the energy of binding $E_{\text{bind}}^{(0)}$.

The first binding simulation with $E_{\text{bind}} = -3kT$, was initialized from the compression simulation configuration at the same $z = s$ and the free energy difference $F_{\text{bind}}(z, E_{\text{bind}}^{(1)} - E_{\text{bind}}^{(0)})$ between the system with $E_{\text{bind}} = -3kT$ and that with $E_{\text{bind}} = 0$ was calculated. We were interested in studying the range of binding energies up to $E_{\text{bind}} = -15kT$, which corresponds to the average ligand-receptor binding energy.¹⁵ For this end we performed simulations with the following E_{bind} energies: $-3kT$, $-6kT$, $-9kT$, $-10kT$, $-11kT$, $-12kT$, $-13kT$, $-14kT$, $-15kT$. For the initial configuration of the system with higher absolute binding energy E_{bind} we used the equilibrated configuration of the system (with the same overall properties including wall separation $z = s$)

with slightly smaller absolute energy E_{bind} . For the test simulations E_{bind} was varied with interval $\Delta E_{\text{bind}} = -kT$. Having a very low probability of binding for energies $|E_{\text{bind}}| \leq 9kT$ (and hence considerable overlap in the corresponding configurations), we concluded that for all further simulations it will be sufficient to use $\Delta E_{\text{bind}} = -3kT$ interval for the free energies up to $-9kT$ and $\Delta E_{\text{bind}} = -1kT$ interval for the higher absolute binding energies (where the difference in binding becomes more significant). The free energy difference relative to the reference state $E_{\text{bind}} = 0$ (at the same z position) was calculated as a sum of free energy differences between a series of incrementally higher binding energies to ensure good configuration overlap:

$$F_{\text{bind}}(s, E_{\text{bind}}) = F_{\text{bind}}(z, E_{\text{bind}} - E_{\text{bind}}^i) + F_{\text{bind}}(z, E_{\text{bind}}^i - E_{\text{bind}}^j) + \dots + F_{\text{bind}}(z, E_{\text{bind}}^m - 0) \quad (6)$$

Thus, the overall free energy of a system with wall separation $z = s$ and binding energy E_{bind} between the functional groups and receptors with respect to the reference state with the wall separation distance $z = \infty$ (ensuring no interaction between the polymer layer and cell surface) was the sum of the compression free energy and binding free energy, i.e.

$$F(s, E_{\text{bind}}) = F_{\text{compr}}(s) + F_{\text{bind}}(s, E_{\text{bind}}) \quad (7)$$

For all simulations, the configuration of interest was equilibrated through a series of MC steps before any analysis was performed. In compression simulations, 2^{20} (≈ 1 million) equilibration steps were performed, and binding simulations were equilibrated for 2^{22} (≈ 4 million) MC steps. We note that the absolute number of equilibration steps was considerably larger, as for the initial configuration for each separation distance s and each binding energy E_{bind} we used the equilibrated configuration obtained for the separation distance $s + a$ and binding energy $E_{\text{bind}} - \Delta E_{\text{bind}}$. The number of binding and unbinding events at equilibrium were found to be equal to each other, as expected. The analysis of the simulation results was performed on at least an additional 3 million MC steps in the following way. For each simulation we accumulated configuration averages over three consecutive periods, each of at least one million MC steps. This gave us three samples for each system as the results were considered to be uncorrelated due to the large simulation times. Thereby each pairwise free energy difference calculation could be performed nine different ways, which allowed us to calculate the mean value and an error estimate (from the variance σ of the distribution of free-energy-differences) $\delta F_{\text{error}} = \sqrt{\sigma^2/9}$. In the summing of free energy differences this error was then propagated assuming the variance was of a normal distribution (i.e. for $A+B: \sigma_{A+B}^2 = \sigma_A^2 + \sigma_B^2$). A similar method, based on three data points obtained from different time intervals, was used to determine the average value and the error in the single ensemble results, such as the number of bound receptors.

Results

Distance-Dependent Interaction Profiles

In our simulations we studied four main design parameters for the polymer layer: degree of functionalization x , chain degree of polymerization N , density of grafting σ and binding energy E_{bind} . To some extent E_{bind} can be controlled experimentally via chemical modification of the ligand used.³⁴ From our simulation data for each separation distance s between the polymer-tethered surface and cell wall we obtained the average number of bound ligands $\langle n_{\text{bind}} \rangle$ and the total free energy of polymer layer-cell surface interaction F . These values are shown in Figures 2, 3-4 for $E_{\text{bind}} = -15kT$ and the relatively high receptor density $\rho = 8.1 \times 10^{-6} (a^{-2})$ as a function of the separation distance s for different degrees of functionalization x , chain degree of polymerization N and density of grafting σ , correspondingly. Besides the free energy of

interaction $F(s)$, we also calculated the interaction force F between the targeting groups and cell surface:²⁵

$$f = - \frac{\partial F(s)}{\partial s} \quad (8)$$

The free energy profile was fitted to an empirical function to facilitate the calculation of the force.

Effect of degree of functionalization and binding energy—Figure 2 shows the average number of bound ligands $\langle n_{bind} \rangle$, the free energy F and force f of polymer layer-cell surface interaction for $\sigma = 0.0061 \text{ (}\text{\AA}^{-2}\text{)}$ and $N = 16$ as a function of separation distance s for different degrees of functionalization x . It is seen that a decrease in the separation distance s leads to an increase in polymer layer-receptor binding and in an attractive interaction force and corresponding decrease in the free energy of interaction for all degrees of functionalization x . Qualitatively similar behavior, i.e. decrease in the minimum free energy and increase in number of bindings with an increase in the degree of functionalization was predicted analytically in ref. 18. The maximum number of bound ligands $n_{bind}^{\max}$ achieved at small separation distances increases linearly with the degree of functionalization for smaller x when $n_{bind}^{\max}$ is determined by the number of functional groups. For large x , $n_{bind}^{\max}$ reaches the saturation level defined by the maximum number of receptors which can fit in the $64\text{\AA} \times 64\text{\AA}$ cell surface area. In general, $\langle n_{bind} \rangle$ is strongly influenced by the degree of functionalization - a larger degree of functionalization leads to a larger number of bound ligands.

The free energy of the polymer layer-cell surface interaction firstly decreases with a decrease in the separation distance s following the increase in the number of bound ligands and reaches a minimum at some intermediate separation distance. The absolute value of the free energy at its minimum ($F_{\min}$) noticeably increases with an increase in the degree of functionalization and the position of the minimum shifts to smaller separation distances (especially for low x). At even smaller s the free energy F increases due to the conformational penalty for compression of the polymer layer. The interaction force follows a similar pattern as the free energy but exhibits a maximum in polymer layer-cell surface attraction at larger separation distances than the corresponding minimum of the free energy. The attractive force increases and the separation distance at which the maximum force is achieved decreases with an increase of the degree of functionalization (following the free energy behavior).

As is seen from Figure 2 an increase in the degree of functionalization results in larger number of bound ligands, lower free energy achieved over the larger range of separation distances and a correspondingly larger attractive force. A qualitatively similar trend is observed in our simulations for a constant degree of functionalization with increasing absolute value of the energy of binding E_{bind} (results not shown). We note that the results obtained for spherical polymer micelles (which will be a subject of a separate publication) follow a qualitatively similar trend. Experimental observations for functionalized by targeting groups polymersomes,⁹ dendrimers¹¹ and block copolymer micelles^{7,8} also show noticeable increase in binding (or association constant) with the increase in degree of functionalization.

Effect of chain length—The average number of bound ligands $\langle n_{bind} \rangle$, the free energy F and force f of polymer layer-cell surface interaction for $\sigma = 0.0061 \text{ (}\text{\AA}^{-2}\text{)}$ and $x = 1$ are shown in Figure 3 as a function of separation distance s for different degrees of polymerization N . With increasing chain length the distance at which ligand-receptor binding is first observed increases, in agreement with experimental observations,¹⁴⁻¹⁶ while the maximum $\langle n_{bind} \rangle$ at small separation distances is independent of N . Correspondingly, the free energy of the polymer

layer-cell surface interaction is lower for larger N at larger separation distances s . However, longer polymer chains start to lose conformational entropy (i.e. feel the presence of the cell surface) at larger separation distances compared to the shorter chains. As a result, the minimum of the free energy is more shallow and occurs at larger separation distances for longer chains. The attractive force follows the same pattern as the free energy with its maximum value being smaller, which qualitatively agrees with the predictions of ref. 18, and is achieved at larger distances for the longer chains (Figure 3). We found that scaling of the separation distance with the radius of gyration of polymer chains while bringing the free energy and binding curves somewhat closer together does not collapse all curves for different chain lengths into one, as was anticipated in ref. 18. It is possible that the chains considered here are not long enough for the prediction to hold or the distribution of chain ends in polymer layer is in practice wider and plays a more substantial role in receptor binding than anticipated in ref. 18. The latter argument is consistent with the finding of several experimental studies employing surface force apparatus¹⁴⁻¹⁶ that the maximum binding distance is larger than (but not proportional to) the chain radius of gyration or even brush height but smaller than a fully extended chain length. We also found that for all chain lengths considered the maximum binding distance increases with an increase in the absolute value of binding energy (see supporting information), which qualitatively agrees with theoretical predictions.^{15, 17, 18, 35} At the same time the equilibrium binding distance corresponding to the free energy minimum is found to be a decreasing function of $|E_{bind}|$ (see supporting information) also in qualitative agreement with recent theoretical predictions.¹⁸

Based on these profiles (Figure 3) it can be concluded that polymer layers composed of shorter chains show a larger attractive force to a cell surface, a deeper and more narrow free energy minimum and a slightly larger ligand-receptor binding $\langle n_{bind} \rangle$ (at least for $x = 1$) at the free energy minimum compared to the layers containing longer chains. At the same time, longer polymer chains can “detect” the cell surface at a larger separation distance and the free energy profile is broader (i.e. binding to a cell surface can occur in a larger range of separation distances) even though less deep than for shorter chains. We note that these results are valid for long planar polymer layers, where chains cannot escape compression by changing their orientation. For any finite-size layer, where edge-effects can be substantial, these results can be altered in the way that can give preference to longer chains, as chains close to the edge of the layer can escape compression by re-orientation and they also can reach additional receptors if the receptor surface is larger than polymer layer surface. The latter was observed experimentally for a comb-like functionalized layer interacting with cells capable of migration³⁶ and was predicted theoretically for polymer layers interacting with free-floating proteins.²⁰

For the case of spherical polymer micelles, which will be a subject of our future publication, an increase in tether chain length leads to an increase in both the degree of ligand-receptor binding and attractive force of interaction with the receptor surface. In this case the longer tether chains can reach further along the cell surface from the micelle center and interact with a larger number of receptors. Using longer polymer chains may also have some advantages in stabilizing and protecting of nanoparticles.

Effect of polymer grafting density—The average number of bound ligands $\langle n_{bind} \rangle$, the free energy F and force f of the polymer layer-cell surface interaction for $N = 16$ and the same number of targeting groups (25) are shown in Figure 4 as a function of the separation distance s for three polymer grafting densities. The lowest polymer grafting density, $\sigma = 0.0061(a^{-2})$, corresponds to the mushroom regime, when the conformation of individual chains is not affected by their neighbors. While for the highest grafting density considered, $\sigma = 0.0488(a^{-2})$, chains overlap each other in the polymer layer leading to an increase in their radius of gyration (see supporting information), which we will refer as the brush regime. As is seen from

Figure 4, an increase in grafting density leads to an increase in the largest separation distance at which binding starts. This is the result of chain stretching in the polymer layer at high grafting densities that increases the concentration of functional groups on the outer periphery of the polymer layer and thus allows binding at larger distances. For the larger grafting density the maximum number of bound ligands is also achieved at the larger distance compared to that for smaller σ . Similar to the chain length effect, a larger grafting density results in a lower free energy of polymer layer-receptor surface interaction at larger separation distances s . However a higher chain density in the polymer layer also implies smaller tolerance for compression, which starts to play a role at larger separations as well. As a result, the free energy minimum is not as deep for the highest grafting density and occurs at a larger separation distance compared to that for the lower grafting density cases. However, in contrast to the chain length effect, a larger grafting density produces a more narrow free energy profile and a correspondingly larger attractive force for the polymer layer-receptor surface interactions compared to that for smaller σ .

The results presented in Figure 4 imply that while a smaller polymer grafting density (mushroom regime) leads to a lower free energy minimum and a somewhat larger ligand-receptor binding, an increase of σ can increase ligand availability for binding and thus, the attraction force and distance at which polymer layer is capable of binding to a cell surface. In the case of a nanoparticle a higher polymer density can also be beneficial for better protection of the core and incorporated drugs. We find that for the spherical micelle the effect of polymer density is not as dramatic as for layers but follows somewhat similar qualitative behavior.

Binding Affinity

Of particular interest in this study is the affinity of the functionalized polymer layers for the targeted cell surface. To compare the effect of different design parameters of the polymer layer one can compare the corresponding minima of the free energy of polymer-layer-cell surface interactions, $F_{\min}$. We note that $F_{\min}$ which was estimated from fitting the free energy profiles $F(s)$ is achieved at different separation distances, as is seen in Figures 2-4. The free energy minimum $F_{\min}$ is related to the maximum equilibrium binding constant for the polymer layer as

$$K_{bind}^{eq} = \exp\left(-\frac{F_{\min}}{kT}\right) \quad (9)$$

Using the free energy minimum, $F_{\min}$ as a measure of affinity, one can study the influence of polymer layer design parameters. Figure 5 shows the effect of the degree of functionalization x , the energy of binding E_{bind} and chain degree of polymerization N on the affinity of polymer layers to receptor surface for $\sigma = 0.0061(a^{-2})$ and $\rho = 8.1 \times 10^{-6}(a^{-2})$. As one could anticipate from the distance-dependent free energy profiles (Figure 2) an increase in degree of functionalization x or the absolute value of the free energy $|E_{\text{bind}}|$ leads to a higher affinity (lower free energy $F_{\min}$). These results are consistent with theoretical predictions,^{17,18} simulation results²³ and experimental observations.^{7-9,11,37} From Figure 5 it can also be seen that polymer layers composed of shorter chains show somewhat higher affinity than longer chains. We can attribute this to the larger entropic penalty for polymer layer compression in the case of longer chains which reduces the absolute value of free energy. As we discussed above, for a polymer micelles we expect to have somewhat different dependence: longer polymer chains allow recruitment of receptors from a larger area of the cell surface as well as give greater freedom of binding to mobile receptors. As one can anticipate from Figure 4 an increase in the polymer grafting density decreases the affinity (not shown) with overall dependence of $F_{\min}$ on $|E_{\text{bind}}|$ and x being rather similar to what is shown in Figure 5.

Another interesting observation, which can be made from Figure 5, is that the minimal absolute energy of binding $E_{\text{bind}}^{\text{min}}$ necessary to achieve at least some attraction between the polymer layer and cell surface, i.e. $K_{\text{bind}}^{\text{max}} \geq 1$ (or $F_{\text{min}} \leq 0$) strongly depends on the degree of functionalization. Thus for $N = 32$ with 100% functionalization $E_{\text{bind}}^{\text{min}} \simeq -9.2kT$, for 50% of functionalization $E_{\text{bind}}^{\text{min}} \simeq -10kT$, while for 4% of functionalization $E_{\text{bind}}^{\text{min}}$ is close to $-13.3kT$. The corresponding values for $E_{\text{bind}}^{\text{min}}$ are only slightly lower for $N = 8$. Naturally, $E_{\text{bind}}^{\text{min}}$ will also be dependent on the density of polymers σ and receptors ρ : an increase in polymer density from $\sigma = 0.0061(a^{-2})$ to $\sigma = 0.0488(a^{-2})$ decreases $E_{\text{bind}}^{\text{min}}$ from $-8.26kT$ to $-9.45kT$ (for $N = 16$, $n_{\text{ligand}} = 25$), whereas a nearly 100 times decrease in receptor density (for $N = 16$, $x = 1$) from $\rho = 8.1 \times 10^{-6}(a^{-2})$ to $\rho = 9.1 \times 10^{-8}(a^{-2})$ reduces the minimum free energy of binding from $-8.26kT$ to $-13.7kT$ (results not shown).

While the free energy minimum, F_{min} is a natural choice for comparison of the affinities of polymer layers of different architecture, it is not the only possible measure of affinity. For instance, one can compare also the average number of bound ligands $\langle n_{\text{bind}} \rangle_{\text{eq}}$ corresponding to the free energy minimum F_{min} . Even though the interaction between the polymer layer and receptor surface can occur over the whole range of distances (Figures 2-4) where the free energy is negative, our analysis shows that the interaction at the minimum of the free energy will be strongly dominating (unless the free energy profile is very shallow) and hence it can be taken as the equilibrium value. Figure 6 shows the equilibrium number of bound ligands $\langle n_{\text{bind}} \rangle_{\text{eq}}$ as a function of the total number of ligands n_{ligand} for $\sigma = 0.0061(a^{-2})$, $\rho = 8.1 \times 10^{-6}(a^{-2})$ at different values of binding energies obtained for two different chain lengths, $N = 16$ and $N = 32$. As is seen for the highest binding energy considered $E_{\text{bind}} = -15kT$, $\langle n_{\text{bind}} \rangle_{\text{eq}}$ is practically independent of chain length (for the range considered, $8 \leq N \leq 32$, data for $N = 8$ and 24 not shown) and increases linearly $\langle n_{\text{bind}} \rangle_{\text{eq}} \simeq 0.97n_{\text{ligand}}$ with the number of ligands n_{ligand} in the range $0 < n_{\text{ligand}} \lesssim 17$ (where the maximum binding is determined by the number of ligands). For the larger degree of functionalization $\langle n_{\text{bind}} \rangle_{\text{eq}}$ reaches saturation based on the limited number of receptors which can be placed on the corresponding cell surface. A similar type of saturation behavior was observed in experimental studies on functionalized dendrimer binding.^{11,37} This saturation level is weakly dependent on N with somewhat larger $\langle n_{\text{bind}} \rangle_{\text{eq}}$ achieved for shorter chains because of the smaller entropic loss upon compression. For lower absolute values of binding energies $|E_{\text{bind}}|$ the overall dependence of $\langle n_{\text{bind}} \rangle_{\text{eq}}$ on n_{ligand} although remaining qualitatively similar, results in a smaller equilibrium number of bound ligands with the slope for the linear portion of the dependence decreasing and the difference between $\langle n_{\text{bind}} \rangle_{\text{eq}}$ achieved for shorter and longer chains increasing. Evidently, polymer layers with longer tethers cannot achieve as high number of bound ligands as the layers with shorter chains due to the larger conformational entropy loss. Only when the binding energy becomes so high that the maximum possible level of binding is achieved (defined either by number of ligands or number of receptors which can cluster on cell surface), as in the case $E_{\text{bind}} = -15kT$, the difference in conformational properties for the layers of different chain length becomes insignificant.

Comparing the average number of bound ligands $\langle n_{\text{bind}} \rangle_{\text{eq}}$ corresponding to the free energy minimum F_{min} for different grafting densities as a function of the number of ligands n_{ligand} for different binding energies (see supporting materials) one can observe rather similar behavior to what is presented in Figure 6: for high binding energy $\langle n_{\text{bind}} \rangle_{\text{eq}}$ is independent of the grafting density, whereas at lower absolute values of binding energies the average number of bound ligands decreases with lower values achieved for higher grafting densities. Thus, for high absolute binding energies considering the equilibrium number of bound ligands gives a somewhat different perspective on the dependence of polymer chain length and grafting density on the affinity of the polymer layer for the cell surface compared to the free energy minimum consideration: in such a case the maximum possible level of binding is achieved with no

noticeable dependence on chain length or polymer grafting density. At the same time for lower absolute values of binding energies, both criteria of affinity provide a qualitatively similar result: shorter chains with smaller grafting densities achieve higher affinity. We note that based on the free energy vs distance profiles shown in Figures 2-4, the increase in the degree of functionalization x (or the absolute energy of binding E_{bind}) leads to both a deeper free energy minimum and a larger average number of bound ligands. Therefore comparison of the equilibrium number of bindings $\langle n_{bind} \rangle_{eq}$ results in a qualitatively similar dependence (not shown) as presented in Figure 5.

Binding to Surfaces with Different Receptor Densities

So far we discussed the influence of different design parameters of a polymer layer on its affinity to a receptor surface with a relatively high density $\rho = 8.1 \times 10^{-6}(a^{-2})$ (corresponding to nearly 80% of the surface covered by receptors for high degrees of functionalization and absolute binding energies). The average receptor densities can vary considerably between different cells¹³ with higher receptor densities typical for diseased cells (such as cancerous cells) and 100 times lower densities for normal cells. Thus, it would be beneficial to investigate the affinity of a targeted polymer layer for cells with different receptor densities.

Figure 7 shows the affinity of binding (i.e. the free energy minimum of polymer layer-cell surface interactions) as a function of the degree of functionalization for cell surfaces with different receptor densities (ranged from $9.1 \times 10^{-8}(a^{-2})$ to $8.1 \times 10^{-6}(a^{-2})$). As is seen, the increase in the receptor density results in a substantial enhancement of polymer layer-receptor surface interactions and the free energy minimum decreases from values close to zero for the cells with the lowest receptor densities to $-70kT$ for the largest receptor density considered. The decrease in the free energy with an increase in the density of mobile receptors is qualitatively consistent with theoretical predictions.^{17,18} The difference in the free energy minimum for the interactions with high and low receptor density cells is related to the ratio of the corresponding equilibrium binding constants as

$$\frac{K_{bind}^{high}}{K_{bind}^{low}} = \exp\left(-\frac{F_{min}^{high} - F_{min}^{low}}{kT}\right) \quad (10)$$

Comparing different curves in Figure 7 one can conclude that the ratio of the binding constants (eq 10) will increase with an increase in the difference in receptor densities and with an increase in the degree of functionalization x . As is seen from Figure 7 the free energy minimum F_{min} decreases nearly linearly with the degree of functionalization x for lower x and/or lower receptor densities. At the higher degree of functionalization (and high receptor densities) this decrease slows down as the receptor concentration reaches saturation in the area exposed to the polymer layer. Nevertheless for all receptor concentrations considered an increase in x results in lower values of F_{min} (i.e. higher affinity) and hence a larger difference in the free energy minimum values for cells with high and low receptor densities $|F_{min}^{high} - F_{min}^{low}|$.

Binding Specificity

Besides achieving high affinity to the cells with a high density of receptors, the successful targeting system should also be highly selective, i.e. exhibit no or very low affinity to normal cells (with a low density of receptors). To analyze the influence of different design parameters on the specificity of binding, it is useful to plot the number of bound ligand as a function of receptor density. Theoretically the amount of binding is expected to increase with an increase of receptor density until it reaches saturation level,^{17,18,38} which is supported by experimental observations.³⁹ Figure 8 shows the effect of the energy of binding E_{bind} on the binding

specificity. As is seen for the highest (absolute) binding energy considered, $E_{\text{bind}} = -15kT$, the increase in receptor density results in a systematic increase in the fraction of bound groups (a nearly linear increase in the linear-log scale of the Figure). This implies low selectivity to the cells with different receptor density - simply the higher the receptor density the higher is the affinity. For lower absolute binding energies the increase in the bound fraction starts at somewhat higher receptor density and for $|E_{\text{bind}}| \lesssim 13kT$ there is no noticeable binding up to receptor densities of about $\rho = 3 \times 10^{-7}(a^{-2})$, above which binding enhances considerably. Thus a lower absolute binding energy provides higher selectivity of binding to high receptor density cells.

Another factor having considerable influence on the selectivity of binding to cells with different receptor densities is the number of functional groups on a polymer layer n_{ligand} (Figure 9). As is seen from Figure 9, polymer layers with a large number of functional groups (13-25) for a given polymer grafting density and box size do not exhibit strong selectivity: the corresponding curves follow a nearly linear dependence (in the linear-log scale of the Figure). Polymer layers with a lower number of functional groups (< 13) show practically no affinity for cells with low density of receptors and enhanced binding to the cells with higher receptor density. We note that a very low number of functional groups (1 - 2) cannot provide high selectivity of binding since the maximum possible number of bound ligands is also low. Thus some intermediate number of functional groups (e.g. 4 - 12) may be the best choice to ensure the specificity of binding. The tether chain length and density of polymer grafting do not have a significant impact on the selectivity of binding, at least in the range of the values considered (data not shown).

To identify the design parameters of a polymer layer which allow us to achieve a low affinity for cells with a low receptor density and at the same time show strong binding to the cells with a high receptor density, we plotted in Figure 10 $F_{\text{min}}^{\text{high}} - F_{\text{min}}^{\text{low}}$ vs $F_{\text{min}}^{\text{low}}$ for different degrees of functionalization, energies of binding, polymer lengths and grafting densities. For the low receptor density we considered the case with $\rho = 1.5 \times 10^{-7}(a^{-2})$ and for high receptor density $\rho = 8.1 \times 10^{-6}(a^{-2})$, about 50 times difference. As is seen from Figure 10 most of the simulation data follow rather similar behavior, which can be described by the following phenomenological power-law dependence:

$$F_{\text{min}}^{\text{high}} - F_{\text{min}}^{\text{low}} = -40.7 |F_{\text{min}}^{\text{low}}|^{0.234} \quad (11)$$

Some of the observed deviations from the fitting curve correspond to either a lower degree of functionalization or higher polymer grafting density. While this phenomenological dependence is not likely to be unique, it emphasizes the importance of not only the ratio of equilibrium binding constants for high and low receptor density cells, but also the binding constant for normal cells when both high affinity and selectivity is considered. It seems that the main trend is primarily defined by the binding energy E_{bind} with lower binding energy resulting in low $F_{\text{min}}^{\text{low}}$, but also low absolute value of $|F_{\text{min}}^{\text{high}} - F_{\text{min}}^{\text{low}}|$ (upper right corner of the Figure). At the same time the highest binding energies provide large $|F_{\text{min}}^{\text{high}} - F_{\text{min}}^{\text{low}}|$ value but also a relatively large $F_{\text{min}}^{\text{low}}$ (lower left corner of the Figure). To achieve both low or no affinity to cells with low receptor density and high affinity to cells with high receptor density one has to be in a lower right corner of the Figure where the curve bends from the vertical asymptote at $F_{\text{min}}^{\text{low}} \rightarrow 0$ to a linear slope at more negative values of $F_{\text{min}}^{\text{low}}$. The precise requirements for binding selectivity depend on the application. As an illustration we selected the following criteria

$$F_{\text{min}}^{\text{high}} - F_{\text{min}}^{\text{low}} < -30kT \quad \text{and} \quad F_{\text{min}}^{\text{low}} > -1.5kT, \text{ shown as a shaded area in Figure 10. One can}$$

achieve a high selectivity and high affinity for cells with high receptor density by using several different strategies (as shown in Table 1). For example, one can use a relatively low polymer grafting density, high degree of functionalization and intermediate range of binding energies or one can use a high binding energy for low polymer grafting density with a lower degree of functionalization or high polymer grafting density with a higher degree of functionalization. Thus it seems that several different design parameters of a polymer layer result in a relatively similar behavior as concerns the achievement of high selectivity of binding.

Conclusions

We have applied Monte Carlo simulations to study the reversible binding between a functionalized planar polymer layer and a planar cell surface containing different densities of mobile receptors. The distance-dependent curves for the average number of ligands bound to receptors, the total free energy for the polymer layer-cell surface interaction and the interaction force were obtained (Figures 2-4) using Bennett's *acceptance-ratio method*.²⁹ Four main design parameters for the polymer layer were considered: the degree of functionalization, chain degree of polymerization, polymer density of grafting and the binding energy for the ligand-receptor interaction. By analyzing the distance dependent interactions between the polymer layer and cell surface we found that an increase in the degree of functionalization or in the absolute energy of ligand-receptor binding results in a larger number of ligands bound to the receptors, lower free energy and stronger attractive force, both of which are achieved over a larger range of separation distances (Figure 2). The maximum binding distance increases with an increase in the (absolute) energy of binding $|E_{\text{bind}}|$ in agreement with theoretical predictions,^{15, 17, 18, 35} while the equilibrium separation distance (corresponding to the free energy minimum) decreases with an increase in $|E_{\text{bind}}|$ (cf. ref. 18]) and the degree of functionalization.

We also found that polymer layers composed of shorter chains exhibit a deeper and narrower free energy profile and a larger attractive force (Figure 3). However, a polymer layer with longer chains can interact with the cell surface at a larger and broader range of separation distances, as is seen experimentally.¹⁴⁻¹⁶ The increase in polymer grafting density from the mushroom to brush regime is found to increase the ligand availability and the maximum binding distance (Figure 4). This results in a larger attractive force, but at the same time produces a higher free energy minimum due to the smaller tolerance for compression for a polymer layer of high grafting density.

We used two measures of the binding affinity for the polymer layer to the cell surface: the free energy minimum, which is related to the equilibrium binding constant and the equilibrium fraction of bound ligands. As discussed above, based on the free energy minimum criterion the polymer layers with a smaller grafting density, shorter chain length, larger degree of functionalization and larger absolute binding energy possess a larger affinity to the cell surface (Figure 5). The equilibrium free energy F_{min} is also found to decrease with an increase in receptor density (Figure 7), in qualitative agreement with theoretical predictions.^{17, 18} The application of the other criterion of affinity, the equilibrium fraction of binding, leads to a similar conclusion, except for high absolute values of binding energy when the maximum possible number of bound ligands is achieved regardless of chain length or polymer grafting density (Figure 6). As a result, for high absolute values of binding energy polymer layers of different chain lengths and grafting density possess similar affinity. We also found that the minimum absolute energy of binding $|E_{\text{bind}}^{\text{min}}|$ required to achieve any attraction between the polymer layer and receptor surface decreases with an increase in degree of functionalization, receptor density and a decrease in the polymer grafting density.

Achieving binding specificity to cells with a high concentration of receptors compared to normal cells (with low receptor density) is another important factor for designing a targeted

polymer layer.^{10, 11} We showed that high binding specificity can be achieved by the polymer layers with intermediate ligand-receptor binding energies, as a larger binding energy ensures a high binding affinity but lacks specificity while a smaller binding energy provides inadequate affinity (Figure 8). The degree of functionalization has a similar effect on the binding specificity, i.e. an intermediate number of functional groups is the best choice to ensure the specificity of binding (Figure 9). The polymer chain length and the density of polymer grafting have shown little impact on the binding specificity.

Clearly, the optimal design of the polymer layers requires synergistic confluence of the different design parameters to satisfy both criteria: achieving high binding affinity to cells with high receptor density and at the same time having high binding specificity, i.e. low or no affinity to cells with low receptor density. As is shown, this can be achieved by using several different strategies as illustrated in Figure 10 and Table 1. For example, one can use a relatively low polymer grafting density, high degree of functionalization and intermediate range of binding energies or one can use a high binding energy for low polymer grafting density with a lower degree of functionalization or high polymer grafting density with a higher degree of functionalization. Those strategies conform to one general rule: the polymer layers with intermediate degrees of affinity to the cells with high receptor density are more likely to possess high specificity of interaction with cells of different receptor density.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Schematic representation of the end-functionalized polymer layer interacting with the cell surface containing mobile receptors.

Figure 2.

The average number of bound ligands $\langle n_{bind} \rangle$, the free energy F and force f of polymer layer-cell surface interaction for $E_{bind} = -15kT$, $\sigma = 0.0061(a^{-2})$, $\rho = 8.1 \times 10^{-6}(a^{-2})$ and $N = 16$ as a function of separation distance s for different degrees of functionalization x .

Figure 3.

The average number of bound ligands $\langle n_{bind} \rangle$, the free energy F and force f of polymer layer-cell surface interaction for $E_{bind} = -15kT$, $\sigma = 0.0061(a^{-2})$, $\rho = 8.1 \times 10^{-6}(a^{-2})$ and $x = 1$ as a function of separation distance s for different degree of polymerization N .

Figure 4.

The average number of bound ligands $\langle n_{bind} \rangle$, the free energy F and force f of the polymer layer-cell surface interaction for $E_{bind} = -15kT$, $N = 16$, $\rho = 8.1 \times 10^{-6}(a^{-2})$ as a function of separation distance s for three polymer grafting densities. In all cases the polymer layers contained the same number of targeting groups (25).

Figure 5.

Free energy minimum, $F_{\min}$ as a function of the degree of functionalization x and the absolute energy of binding $|E_{\text{bind}}|$ for polymer layers of the same grafting density $\sigma = 0.0061(a^{-2})$ and different degree of polymerization: $N=32$ (upper surface) and $N=8$ (lower surface), interacting with a cell surface with receptor density $\rho = 8.1 \times 10^{-6}(a^{-2})$. The minimum binding energy $E_{\text{bind}}^{\min}$ necessary to achieve at least some attraction between the polymer layer and cell surface is shown by symbols (circles) for the $N=32$ case.

Figure 6.

Average number of bound ligands $\langle n_{bind} \rangle_{eq}$ for polymer layers with two different degrees of polymerization $N = 16$ (solid symbols) and $N = 32$ (open symbols) at the same grafting density $\sigma = 0.0061(a^{-2})$ interacting with a cell surface ($\rho = 8.1 \times 10^{-6}(a^{-2})$) with different binding energies E_{bind} (shown next to the curves) as a function of the number of ligands n_{ligand} . The linear slope $0.97n_{ligand}$ for the low degree of functionalization is shown next to the data.

Figure 7.

Free energy minimum for the polymer layers with degree of polymerization $N = 16$ and grafting density $\sigma = 0.0061(a^{-2})$ interacting with cells with different density of receptors (shown next to the curves) with binding energy $E_{bind} = -15kT$ as a function of the degree of functionalization x .

Figure 8.

Average number of bound ligands for 100% functionalized polymer layers with degree of polymerization $N = 16$ and grafting density $\sigma = 0.0061(\text{\AA}^{-2})$ interacting with a cell surface with different binding energies (shown next to the curves) as a function of receptor density of cell surface ρ .

Figure 9. Average number of bound ligands for polymer layers with degree of polymerization $N = 16$ grafting density $\sigma = 0.0061(\text{\AA}^{-2})$ and different number of functional groups (shown next to the curves) interacting with a cell surface with binding energy $E_{\text{bind}} = -15kT$ as a function of receptor density of cell surface ρ .

Figure 10.

The difference in the free energy minima for the polymer layer interacting with cells with high ($\rho = 8.1 \times 10^{-6} a^{-2}$) and low ($\rho = 1.5 \times 10^{-7} a^{-2}$) density of receptors vs the free energy minimum for polymer layer-cell surface interaction for a low receptor density ($\rho = 1.5 \times 10^{-7} a^{-2}$). Different symbols correspond to different design parameters for the polymer layer (and variable binding energy) (see Table 1). The curve corresponds to the best fit to the data (see text). The shaded lower right corner ($F_{\min}^{\text{high}} - F_{\min}^{\text{low}} < -30kT$ and $F_{\min}^{\text{low}} > -1.5kT$) is taken as the optimal area to achieve strong binding to cells with high receptor density and weak binding to cells with low receptor density. The details of the design parameters for the corresponding polymer layers are listed in Table 1.

Table 1

The design parameters for the polymer layer corresponding to the shaded area in Figure 10

Symbols from Figure 10	Grafting Density, $\sigma \times 10^3$ ($\text{\AA}^{-2}$)	Tether Degree of Polymerization, N	Number of Ligands, n_{ligands} (degree of functionalization, x)	Optimal $E_{\text{bind}}(kT)$ [range] [*]
▼	6.1	16	9 (0.36)	-15 [-15 : -14.7]
●	6.1	16	13 (0.52)	-14 [-14.4 : -13.8]
■	6.1	16	25 (1.0)	-13 [-13.8 : -12.7]
◆	6.1	32	25 (1.0)	-14 [-14.5 : -13.5]
⊞	12	16	25 (1.0)	-15 [-13.9 : -13.0]
○	48	16	13 (0.007)	-15 [-15 : -14.5]
□	48	16	25 (0.125)	-15,-14 [-15.0 : -13.5]

^{*} Supplemental materials Section S4 contains an explanation for the optimal binding energy range and calculation details