Architectural and Structural Optimization of the Protective Polymer Layer for Enhanced Targeting

Chun-Chung Chen and Elena E. Dormidontova*

Department of Macromolecular Science and Engineering, Case Western Reserve University, Cleveland, Ohio 44106

Received November 24, 2004. In Final Form: March 15, 2005

Using Monte Carlo simulations we study the influence of ligand architecture (valence, branching length) and structure (polydispersity) of a flat protective polymer layer on the accessibility of its functional groups and efficiency of receptor targeting. Two types of receptor surfaces were considered: the surface homogeneously covered with receptors and the surface containing a finite number of receptor sites. We found that multivalent ligands provide a larger density of targeting groups on the periphery of the layer compared to monovalent ligands for the same overall number of targeting groups per polymer layer. Because of their cooperativity in binding, multivalent ligands were also considerably more efficient in binding to both types of receptor surfaces. With an increase of ligand valence the number of functional groups attached to receptors noticeably increases. Short-branched divalent ligands show an especially high cooperativity in binding to closely packed receptors. However, in the case of immobile receptors separated by a finite distance from each other, the average distance between the functional groups belonging to the same short divalent ligand is too small to reach different receptors simultaneously and the receptor binding is less efficient than in the monovalent ligand case. Using a bidisperse protective polymer layer formed by short nonfunctional polymers and long functionalized polymers considerably increases the fraction of functional groups on the periphery of the layer. Simulations of receptor binding confirm the high efficiency of receptor targeting by bidisperse polymer layers, which is achieved by means of larger compressibility and higher capability of the ligands to reach out compared to the corresponding monodisperse layers. The concepts of multivalent ligands and a bidisperse protective polymer layer each have their own advantages which can be combined for an enhanced targeting effect.

1. Introduction

Targeted gene and drug deliveries hold a great potential for the successful treatment of many deadly diseases. In the case of both hydrophobic drugs or polymer/DNA complexes with a high surface charge the use of a protective polymer layer is desirable to ensure solubility or prevent nonspecific binding.[1−3] This can be achieved by using a biocompatible hydrophilic polymer such as poly(ethylene oxide) (PEO). The favorable PEO−water interactions inhibit protein adsorption, and such shielded gene/drug delivery systems offer the advantage of lower toxicities and prolonged circulation times in vivo.[1,2,4] Additionally, neutralization of the surface charges of polymer/DNA complexes results in the lower level of nonspecific interactions demonstrated by these systems.[2,5,6] Besides protection of the drug/gene from unfavorable external interactions, another requirement for a drug delivery carrier is the possibility to "navigate" the delivery to the required area. This targeting effect can be achieved by terminating the hydrophilic polymer by ligands having a strong binding affinity to the receptors overexpressed on the cell surface of interest.[7,8] A variety of targeting moieties (such as galactose, transferrin, folate, RGD−peptides, different types of saccharides, antibodies, or antibody fragments) have been used for active gene/drug delivery.[5,6,8−11] Whereas many studies provide rather positive results of such targeting, especially in the cases of single synthetic vectors conjugated with DNA/drugs,[5,6,10,11] there is also a range of problems encountered in specific targeting. To improve the targeting efficiency a more careful consideration of the factors influencing the distribution and binding capability of ligands attached to hydrophilic chains is required. In this paper we study the influence of ligand architecture and structure of the protective polymer layer on the targeting efficiency (Figure 1).

One of the factors diminishing the success of specific targeting is shielding of the targeting functional groups by the protective polymer layer. There are a number of reports of a reduction of transfection efficiency of large PEO-covered nanoparticles carrying targeting groups.[12−14] Also recent experimental studies of phospholipid, liposome, and polymersome adhesion show low or no adhesion achieved when functional groups were attached either directly to the surface or to the polymer spacer of shorter or comparable to stabilizing polymer length.[15−17] Similar

* Corresponding author. E-mail: eed@case.edu.

(1) Allen, C.; Maysinger, D.; Eisenberg, A. *Colloids Surf., B* **1999**, *16*, 3−27.
(2) Ogris, M.; Brunner, S.; Schüller, S.; Kircheis, R.; Wagner, E. *Gene Ther.* **1999**, *6*, 595−605.
(3) Torchilin, V. P.; Omelyanenko, V. G.; Papisov, M. I.; Bogdanov, A. A.; Trubetskoy, V. S.; Herron, J. N.; Gentry, C. A. *Biochim. Biophys. Acta* **1994**, *1195*, 11−20.
(4) Lee, J. H.; Lee, H. B.; Andrade, J. D. *Prog. Polym. Sci.* **1995**, *20,* 1043−1079.
(5) Leamon, C. P.; Weigl, D.; Hendren, R. W. *Bioconjugate Chem.* **1999**, *10*, 947−957.
(6) Merdan, T.; Callahan, J.; Peterson, H.; Bakowsky, U.; Kopeckova, P.; Kissel, T.; Kopecek, J. *Bioconjugate Chem.* **2003**, *14*, 989−996.
(7) Nobs, L.; Buchegger, F.; Gurny, R.; Allemann, E. *J. Pharm. Sci.* **2004**, *93*, 1980−1992.
(8) Kristiansen, K. *Pharmacol. Ther.* **2004**, *103*, 21−80.
(9) Lundquist, J.; Toone, E. *Chem. Rev.* **2002**, *102*, 555−578.
(10) Blessing, T.; Kursa, M.; Holzhauser, R.; Kircheis, R.; Wagner, E. *Bioconjugate Chem.* **2001**, *12*, 529−537.
(11) David, A.; Kopeckova, P.; Minko, T.; Rubinstein, A.; Kopecek, J. *Eur. J. Cancer* **2004**, *40*, 148−157.
(12) Kunath, K.; Merdan, T.; Hegener, O.; Haberlein, H.; Kissel, T. *J. Gene Med.* **2003**, *5*, 588−599.
(13) Erbacher, P.; Bettinger, T.; Belguise-Valladier, P.; Zou, S.; Coll, J.; Behr, J.; Remy, J. *J. Gene Med.* **1999**, *1*, 210−222.
(14) Dauty, E.; Remy, J.; Zuber, G.; Behr, J. *Bioconjugate Chem.* **2002**, *13*, 831−839.
(15) Kim, D.; Klibanov, A.; Needham, D. *Langmuir* **2000**, *16*, 2808−2817.

10.1021/la047109v CCC: $30.25
Published on Web 05/03/2005

observations were made by Torchilin et al. in studying TAT-targeted liposome internalization by several cell lines: when targeting groups were attached directly to liposome surface or to a spacer shorter than stabilizing PEG chains, no cell uptake was registered.[18] In all the above-mentioned cases shielding of targeting groups by the protective polymer layers is thought to be the reason for the targeting failure. This explanation is consistent with the classical result that the maximum of the end-group distribution of a flat polymer layer occurs at approximately 2/3 of the layer thickness[19−22] and it is even closer to the surface for spherical layers.[23−25] To overcome the effect of shielding of targeting functional groups, a bidisperse structure of the protective polymer layer can be employed. As is known from the results of experimental and theoretical studies of classical polymer brushes, an increase of polymer concentration near the surface results in depletion of this area by end groups and therefore enhancement of end-group distribution in the periphery of the layer.[19,21,26−28] In this case the short polymer acts as a concentration barrier preventing end groups of the longer chains from bending back close to the surface and therefore improving accessibility of functional groups. As a result, when the length of the polymer end-functionalized by the targeting group was larger than the length of a stabilizing polymer, the adhesion of phospholipids and polymersomes[15,16] as well as liposome internalization by cells[18] was very high. The enhancement of targeting achieved by using such a bidisperse polymer layer will depend on the degree of functionalization,[16] chain length ratio, density of grafting, and so on. Computer modeling proved to be an effective tool in studying various factors influencing complex behavior of polymers tethered to surfaces[20−22,27,29] and receptor−ligand interactions.[8] Below we will present the results of Monte Carlo simulations regarding ligand distribution in the planar protective polymer layer and receptor binding capability of ligands in structurally different polymer layers. We note that in so far most of the simulations were concerned either with modeling of nonfunctional polymer layers[20,21,27] or deal with specific ligand−receptor pair interactions[8] or address the kinetics of ligand−receptor binding.[30] Some simulation methods were also applied to aid in numerical solutions of equations involved in analytical considerations of ligand−receptor binding.[31−33] We will model a bidisperse protective polymer layer consisting of short (nonfunctional) and long (functionalized with ligands) chains as shown in Figure 1b. On the basis of the results presented below, we will make predictions concerning the optimal composition (density of short vs long chains) and structure (length ratio of short to long chains) of the protective polymer layer to ensure the highest targeting efficiency.

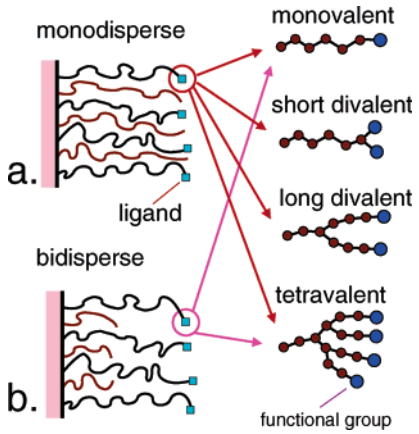

Figure 1. Schematic representation of monodisperse polymer layer (a) functionalized with ligands of different valence (monovalent, short divalent, long divalent, and tetravalent) and bidisperse polymer layer (b) functionalized by either monovalent or tetravalent ligands. The bidisperse layer is formed by (chemically identical) nonfunctional short chains and functionalized long chains. The depicted length of the branches in multivalent ligand cases reflects the exact simulation setup.

One of the recent developments in the field of targeting is the use of multivalent ligands. So far multivalent ligands were mainly used to target single chains with covalently attached drugs.[11,34] However, a few recent publications also report on use of mono- and multivalent ligands attached to dendrimers.[9,35] Extensive experimental[9,11,34,36] and theoretical[31−33] studies of ligand−receptor interactions show that multivalent ligands have an evident advantage compared to monovalent ones. Multivalent ligands can interact with receptors via many possible mechanisms, such as cooperative binding, statistical rebinding, chelate effects, and subside binding, which are not available for monovalent receptors.[9,32,35−37] Furthermore, multivalent ligand binding often results in receptor clustering (in the case of highly mobile receptors), which may stimulate signaling pathways.[9,35,36,38] The degree of complexation between multivalent ligands and receptors depends on many factors such as the density of ligands and receptors, size of receptors, architecture of the ligand, and so on.[9,33,36,37] It is also not evident whether the hindrance effect taking place in the monovalent ligand case will also be a problem for multivalent ligands. In this paper we will attempt to answer this question. An additional factor to consider in the application of multivalent ligands for drug/gene delivery purposes is steric exclusion of some of the functional groups belonging to the same (multivalent) ligand.[31,33,35] To address this effect we will model ligands of different valence and branching length as shown in

(16) Lin, J.; Silas, J.; Bermudez, H.; Milam, V.; Bates, F.; Hammer, D. *Langmuir* **2004**, *20*, 5493−5500.
(17) Blume, G.; Cevc, G.; Crommelin, M. D. J. A.; Bakkerwoudenberg, I. A. J. M.; Kluft, C.; Storm, G. *Biochim. Biophys. Acta* **1993**, *1149*, 180−184.
(18) Torchilin, V. P.; Rammohan, R.; Weissig, V.; Levchenko, T. S. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *98*, 8786−8791.
(19) Fleer, G. J.; Cohen Stuart, M. A.; Scheutjens, J. M. H. M.; Cosgrove, T.; Vincent, B., Eds.; *Polymers at Interfaces*; Chapman and Hall: London, 1993.
(20) Murat, M.; Grest, G. S. *Macromolecules* **1989**, *22*, 4054−4059.
(21) Chakrabarti, A.; Toral, R. *Macromolecules* **1990**, *23*, 2016−2021.
(22) Lai, P.-Y.; Binder, K. *J. Chem. Phys.* **1991**, *95*, 9288−9299.
(23) Lindberg, E.; Elvingson, C. *J. Chem. Phys.* **2001**, *114*, 6343−6352.
(24) Wijmans, C. M.; Zhulina, E. B. *Macromolecules* **1993**, *26*, 7214−7224.
(25) Dan, N.; Tirrell, M. *Macromolecules* **1992**, *25*, 2890−2895.
(26) Milner, S. T.; Witten, T. A.; Cates, M. E. *Macromolecules* **1989**, *22*, 853−861.
(27) Lai, P. Y.; Zhulina, E. B. *Macromolecules* **1992**, *25*, 5201−5207.
(28) Dan, N.; Tirrell, M. *Macromolecules* **1993**, *26*, 6467−6473.
(29) Sides, S.; Grest, G.; Stevens, M. *Macromolecules* **2002**, *35*, 566−573.
(30) Jeppesen, C.; Wong, J.; Kuhl, T.; Israelachvili, J.; Mullah, N.; Zalipsky, S.; Marques, C. *Science* **2001**, *293*, 465−468.
(31) Huskens, J.; Mulder, A.; Auletta, T.; Nijhuis, C.; Ludden, M.; Reinhoudt, D. *J. Am. Chem. Soc.* **2004**, *126*, 6784−6797.
(32) Hubble, J. *Mol. Immunol.* **1999**, *36*, 13−18.
(33) Hlavacek, W.; Posner, R.; Perelson, A. *Biophys. J.* **1999**, *76*, 3031−3043.
(34) Kudryashov, V.; Glunz, P.; Williams, L.; Hintermann, S.; Danishefsky, S.; Lloyd, K. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3264−3269.
(35) Woller, E.; Cloninger, M. *Org. Lett.* **2002**, *4*, 7−10.
(36) Gestwicki, J.; Cairo, C.; Strong, L.; Oetjen, K.; Kiessling, L. *J. Am. Chem. Soc.* **2002**, *124*, 14922−14933.
(37) Kramer, R. H.; Karpen, J. W. *Nature* **1998**, *395*, 710−713.
(38) Lauffenburger, D. A.; Linderman, J. J. *Receptors: models for binding, trafficking, and signaling*; Oxford University Press: New York, 1993.

Figure 1a (while keeping the same overall density of functional groups in the polymer layer). We will consider the functional groups distribution inside the planar monodisperse polymer layer and study the binding efficiency of ligands upon interaction with two types of the receptor surfaces: the surface homogeneously covered with receptors and the surface containing a finite number of receptor sites. We will also consider bidisperse layers carrying multivalent ligands and compare the results with that obtained for monovalent bidisperse layers or multivalent monodisperse layers. On the basis of these results, we will make predictions concerning the optimal structure of polymer layers and the use of multivalent ligand in targeting.

2. Simulation Details

The aim of this paper is to make general predictions concerning the influence of ligand architecture and polymer layer structure on targeting efficiency. Therefore, the natural choice of the computational technique is Monte Carlo (MC) simulations, which allow the study of multiple chains without specifying their atomistic details. A polymer chain was represented by a sequence of monomers connected by means of chemical bonds. We note that the number of monomers does not have to be equal to the number of actual atoms on a real chain but can represent a number of repeat units in the polymers. We have applied the bond-fluctuation model (BFM)[39] on a cubic lattice. To account for the excluded volume effects the positions of neighboring monomers (bond length) should satisfy BFM rules. Besides these interactions we do not consider any attraction/repulsion between monomers or enforce any chain rigidity, such that our simulations correspond to flexible chains in good solvent conditions. This computational setup was chosen taking into account that the functional groups are normally attached to a flexible biocompatible hydrophilic polymer, for which the physiological media (water) represents a good solvent.

Since the interaction between the functional targeting groups and cell receptors occurs in the immediate vicinity of the functional groups, our main subject of study will be the protective polymer layer. As we will discuss below, only the outer 20% of such polymer layer will influence the targeting efficiency, making immaterial the shape of the surface as long as its curvature is large enough compared to the polymer layer thickness.[23−25] Taking this into account below, we will consider a simple planar surface to which stabilizing polymers terminated by the targeting groups will be grafted (Figure 1). Polymers were grafted uniformly on a square surface (x−y plane) with the density $\sigma = (8a)^{-2}$ (where a is a cell size of the lattice). We note that the chosen density is about 16 times smaller than the maximum possible in our simulations. We have applied the periodic boundary conditions in the x and y directions ($L_x = L_y = 64a$ for the targeting group distribution and $L_x = L_y = 32a$ for binding studies), while the size of the simulation box in z-direction (perpendicular to the surface) was fixed at $L_z = 256a$. In all cases discussed below, the polymer length was well within the range of experimentally relevant values: $N = 64$ for monodisperse layers and variable for bidisperse layers. To compare the efficiency of ligands of different valence we kept the overall density of targeting groups the same. In most of the cases considered below the number of targeting groups was twice smaller than the overall number of chains, i.e., 50% functionalization. For multivalent ligand cases, the corresponding number of targeting end groups were attached to the polymer as shown in Figure 1. We note that we modeled tetravalent ligands using three branching points which is more chemically relevant[9,36] and computationally less constrained (i.e. having a larger acceptance rate). Chains end-functionalized with ligands were uniformly distributed among those which do not carry end groups. The influence of the extent of functionalization on the targeting capability will be discussed below.

For each chain architecture, the distributions of monomers and targeting end groups inside the polymer layer are measured and averaged over 1×10^7 MC time steps after equilibration. In the binding study these measurements (plus additional calculations for free energy and force) were repeated for each distance between the surface grafted with polymers and a receptor surface. In our simulations we considered two types of target surfaces: plane surfaces homogeneously covered with receptors and the surfaces carrying distinct receptor sites. In the latter case, receptor sites were placed uniformly on the surface with the separation distance $8a$. The density of receptor sites on the surface was the same as the polymer grafting density so for most of the cases a 1:2 ratio between the targeting groups and receptors was obeyed. It was assumed that each receptor can form a complex with only one targeting group. (We note that, similar to most of experimental cases, we consider no interactions between the functional groups and between the functional groups and the surface to which the polymer spacers are attached. The influence of these factors will be addressed in our future work.)

3. Results and Discussion

3.1. Targeting Group Distribution. Since one of the important factors in successful targeting is the accessibility of the targeting groups, we first will study the functional group distribution inside the protective polymer layer for the ligands of different functionality (as shown in Figure 1a). In all cases the total number of functional groups was the same: twice smaller than the number of polymer chains. To achieve the same number of functional groups for ligands of different valence the degree of ligand substitution was chosen appropriately (i.e. 1/8 was the degree of substitution for tetravalent ligands, 1/4 for divalent ligands, and 1/2 for monovalent ligands). For the considered polymer length ($N = 64$) and grafting density ($\sigma = (8a)^{-2}$), the polymer layer is in so-called brush regime[19−21] with a characteristic parabolic monomer density profile (Figure 2a). In the case of monovalent ligands the maximum of the functional-group distribution is positioned as expected at about 2/3 of the polymer layer height having a relatively small fraction of end groups located on the periphery of the layer. This implies that some of the functional groups can indeed be hidden inside the layer and become inaccessible for targeting. We note that the distribution of functional groups and end groups of nonfunctional chains is the same only in this case. If one uses divalent ligands instead (with the same overall number of functional groups as in monovalent ligand case) the distribution of functional groups becomes slightly more narrow and, more importantly, the position of its maximum shifts toward larger distances from the surface resulting in a larger fraction of functional groups on the periphery of the layer (Figure 2a) compared to monovalent case. Among divalent ligands, those with a longer branch length produced a more narrow distribution and is more efficient in enhancing functional group concentration on the periphery of the polymer layer. Tetravalent ligands, having the same number of functional groups as in divalent and monovalent cases, produced the highest level of functional groups on the periphery of the polymer layer (Figure 2a). In a comparison of ligands of different functionality, it is evident that the higher the valence the stronger is the tendency for the functional groups to be located on the periphery of the layer. This is likely the result of branching: having a larger area per ligand makes it less probable to be buried inside the polymer layer.

We have also studied the functional group distribution inside bidisperse polymer layers containing nonfunctional short chains, $N_S = 48$, and long end-functionalized chains, $N_L = 64$. As is expected, bidispersity of the layer helps to prevent penetration of the functional groups inside the layer (Figure 2b). Due to the presence of short chains, the relative monomer density concentration near the surface

(39) (a) Carmesin, I.; Kremer, K. *Macromolecules* **1988**, *21*, 2819−2823. (b) Deutsch, H. P.; Binder, K. *J. Chem. Phys.* **1991**, *94*, 2294−2304.

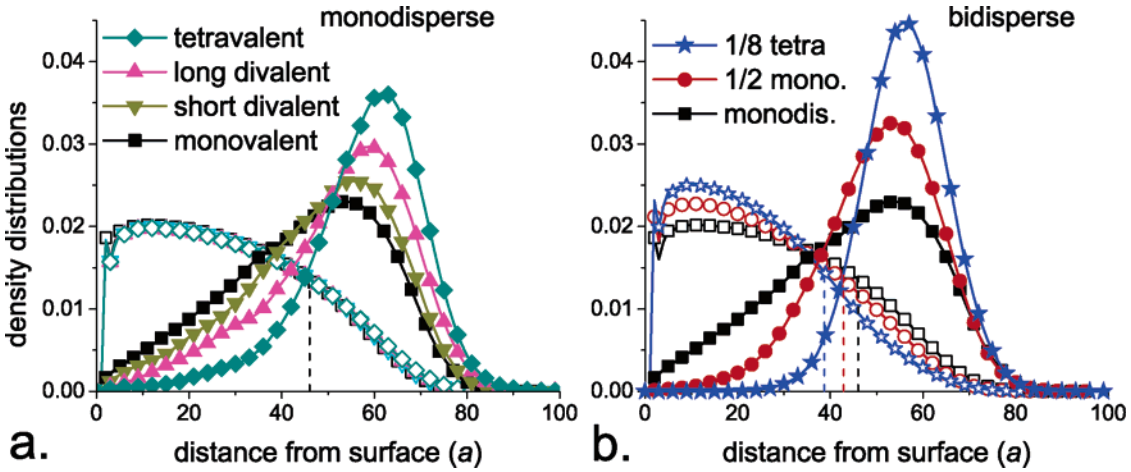

Figure 2. Density distribution for monomers (open symbols) and functional end groups (solid symbols) for monodisperse polymer layers ($N = 64$) modified by ligands of different valence (a) and bidisperse polymer layers (b) in comparison with monovalent monodisperse layer. In all cases the number of functional groups was the same (twice smaller than the number of chains). Bidisperse layers consist of short nonfunctional chains of $N_S = 48$ monomers and long functionalized chains of $N_L = 64$. Bidisperse layers modified by monovalent ligands contained an equal fraction of short and long chains, whereas the bidisperse layers functionalized by tetravalent ligands contained 7/8 of short chains and 1/8 of long chains (to maintain the same overall number of functional groups). Vertical dashed lines mark the exterior part of the layer containing 20% of the polymer mass.

is higher and in the exterior of the layer is lower than for the monodisperse case.[19,21,27,28,40] As a result, the functional group distribution in the bidisperse layer is also noticeably different compared to a monodisperse layer: there are practically no functional groups close to the surface resulting in an enhancement of end-group density on the periphery of the layer.[19,21,27,28] For a bidisperse layer having an equal number of (nonfunctional) short and long chains modified by monovalent ligands, the maximum of the end-group distribution was similar to the case of monodisperse layer of long divalent ligands (compare to Figure 2a) having the same overall number of functional groups. When the protective bidisperse layer was modified by tetravalent ligands (i.e. contained 7/8 short (nonfunctional) chains and 1/8 long tetravalent chains) (Figure 2b), the highest level of functional groups on the periphery of the polymer layer has been achieved among all considered cases (having the same overall number of functional groups) (Figure 2a,b). Combining the multivalency of ligands with bidispersity of the polymer layer allows one to increase the fraction of functional groups on the periphery of the layer due to more bulky nature of the ligand and because of the surface concentration barrier created by the shorter chains of the bidisperse layer.

Degree of Functionalization. The results presented in Figure 2 all correspond to 50% of functionalization; i.e., the overall number of functional groups was twice smaller than the number of chains. The degree of functionalization is an important factor influencing the end-group distribution inside the polymer layer and ultimately the binding capability. We have varied the overall degree of functionalization of monodisperse polymer layers modified by ligands of different valence and calculated the fraction of functional groups in periphery area of the layer containing up to 20% of the overall polymer mass (Figure 3). For the cases considered in Figure 2a, this corresponds to the area beyond the vertical dashed line. As is seen, the majority of the functional groups is located in this area. Also, as will be discussed below (binding study section), only the functional groups located in this area have a strong influence on the interactions with the surface carrying

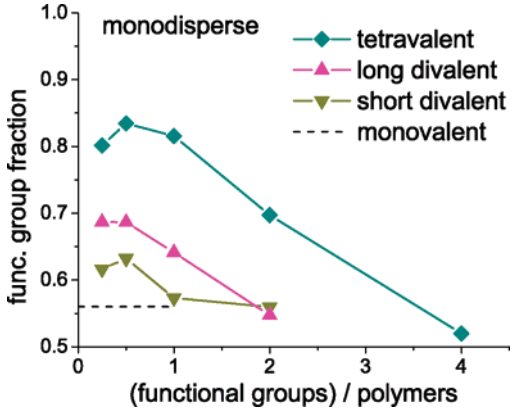

Figure 3. Fraction of functional groups in the exterior part of the monodisperse polymer layers ($N = 64$) containing 20% of polymer mass vs the ratio of the number of functional groups to the number of polymer chains. The overall number of functional groups was given by the product of ligand valence and the degree of ligand substitution.

receptor sites. As is seen from Figure 3, for monovalent ligands the degree of functionalization does not have any effect on the fraction of functional groups in the exterior of the layer. In contrast, for multivalent ligands (short- and long-branched divalent and tetravalent ligands, all with the same overall degree of functionalization), the fraction of functional groups in the periphery of the layer first increases and then decreases with the degree of functionalization. In all cases 50% functionalization (i.e. the number of functional groups is half the number of chains) was the most efficient in producing the largest fraction of functional groups on the periphery of the polymer layer. As we discussed above, the difference in the behavior of mono- and multivalent ligands is due to the branching effect for the latter resulting in an enhanced tendency for the functional groups to stay in the exterior of the layer.

For bidisperse polymer layers one can also choose different lengths for the long (functionalized) and short (nonfunctional) chains. We have kept the length of long (functionalized) chains constant, $N_L = 64$, and varied the length of nonfunctional chains and the overall degree of functionalization. The fraction of functional groups in the

(40) Levicky, R.; Koneripalli, N.; Tirrell, M.; Satija, S. *Macromolecules* **1998**, *31*, 2616−2621.

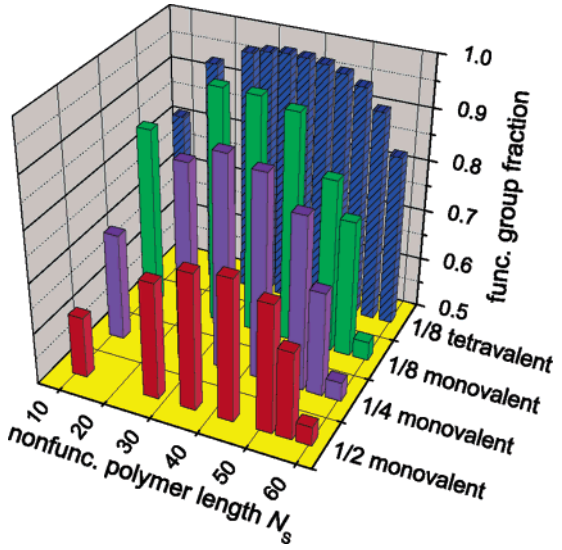

Figure 4. Fraction of functional groups in the exterior part of the bidisperse polymer layers ($N_L = 64$) containing 20% of polymer mass vs the length of short (nonfunctional) polymer, N_S, and the degree of ligand substitution (fraction of long chains).

Table 1. Optimal Composition for Bidisperse Polymer Layers ($N_L = 64$) Shown in Figure 4 Ensuring the Maximal Fraction of Functional Groups in the Periphery of the Layers

ligand type	substitution[a]	functionalization,[b] %	functional group fraction in periphery[c]	optimal N_S[d]
monovalent	1/2	50	0.77	48
monovalent	1/4	25	0.92	40
monovalent	1/8	12.5	0.96	32
tetravalent	1/8	50	0.98	40

[a] The fraction of long (functionalized by ligands) chains in the layer. [b] The percentage of the total number of functional groups in the layer to the overall number of polymer chains. [c] The maximal fraction of functional groups in the exterior part of the layers containing 20% of polymer mass. [d] The length of short chains at which the maximal fraction of functional groups in the exterior part of the layers is achieved.

exterior part of the polymer layer (containing 20% of overall polymer mass) was calculated and shown in Figure 4 for monovalent ligands with three different degrees of functionalization and for tetravalent ligands with 50% functionalization. As is seen in all cases, there is a strong dependence on the short chain length: if the difference in chain length of functionalized and nonfunctional chains is too large or too small, the advantage of having bidisperse polymer layer compared to the monodisperse layer vanishes. With an increase of the degree of functionalization for monovalent ligands the fraction of functional groups on the periphery of bidisperse layers decreases (but the absolute number of functional groups may increase). The highest fraction of functional groups on the periphery of the layer is reached when 1/8 of the chains modified by the monovalent ligands and the length for the remaining 7/8 of short chains was $N_S = 32$ (Table 1). When the degree of substitution for monovalent ligands was higher (1/4 or 1/2), the corresponding fraction of functional groups on the periphery of the layer was lower even at their maximal values (reached at $N_S = 40$ and $N_S = 48$, respectively). It is interesting to note that with a decreasing degree of functionalization the largest fraction of functional groups was achieved at smaller N_S: $N_S/N_L \simeq 3/4$ for 1/2-substitution, $N_S/N_L \simeq 5/8$ for 1/4-substitution, and $N_S/N_L \simeq 1/2$ for 1/8-substitution. Bidisperse polymer layers modified by 1/8 of tetravalent ligands (having 50% functionalization) demonstrate a rather large fraction of functional groups on the periphery of the layer (Figure 4). Its level is comparable only with the best of the monovalent ligands case (1/8 functionalized chains) but shows a somewhat weaker dependence on the nonfunctional chain length. For the tetravalent ligands any chain length ratio in the range $0.5 \le N_S/N_L \le 0.8$ produces nearly the maximal fraction of functional groups on the periphery of the layer. Comparing mono- and bidisperse polymer layers (Figures 3 and 4), one can see that the latter produces a noticeably larger fraction of functional groups in the exterior part of the polymer layer.

In this section and throughout the paper we discuss mainly the efficiency of targeting, i.e., how to achieve the maximal percentage of targeting groups bound to receptors at equilibrium. The maximal efficiency of the use of the functional groups does not automatically guarantee the highest absolute number of functional groups bound to receptors. The latter depends on the ratio of ligands to receptors, nature of ligand−receptor interactions, accessibility of receptors, method of delivery, types of cell, and so on. The range of the factors influencing targeting is rather broad, and we plan to continue studying their importance in our future work. In this paper our main interest is to elucidate the design of the functionalized protective polymer layer to ensure a high accessibility of functional groups and to achieve a high degree of receptor targeting. On the basis of the functional-group distribution discussed above, a high degree of accessibility of functional groups can be achieved by using high-valence ligands and/or by applying bidisperse protective polymer layers.

3.2. Binding Study (Interaction of Functionalized Polymer Layer with the Target Surface). While the distribution of the functional groups in a polymer layer can be a good indicator of how accessible they are to the receptors, the real test of targeting efficiency can be performed in receptor binding simulations. Upon interaction with receptors, the polymer layer can be stretched or compressed depending on the distance to the target surface and the strength of the binding. As a result, the number of functional groups available to participate in binding may change as well. In our simulations we considered two types of target surfaces: plane surfaces homogeneously covered with receptors (Figure 5a) and the surfaces carrying distinct receptor sites (Figure 5b). In the latter case immobile receptors (which occupy one cell sticking out of the surface) were homogeneously distributed on the target surface with an average separation distance $8a$. The surface between receptor sites was neutral and impenetrable for polymers. The number of receptor sites on the targeted surface was the same as the number of polymers in the polymer layer (so for most of the cases there was a 1:2 ratio between the targeting groups and receptors). In general an increase of the degree of functionalization results in an increase of the absolute number of the bound functional groups which in turn would lead to stronger adhesion to the receptor surface, as observed in ref 16, until the maximum saturation level is reached. For both types of receptor surfaces the interaction energy between a functional group and a receptor was $\epsilon = 8kT$ (which is somewhat smaller than the typical energy of ligand−receptor interaction[37,38]). This energy was chosen to ensure good convergence of our simulation results for the statistical analysis (e.g. free energy and force calculations) and to be able to monitor the details of the binding mechanism for chains of different architecture. The influence of the higher binding energy results mainly in the increase of interactions between the

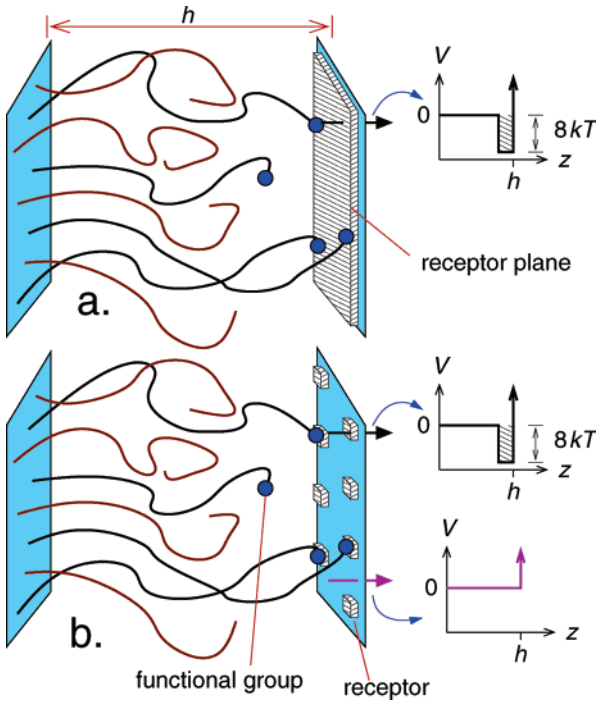

Figure 5. Schematic representation of the binding simulations between the bidisperse polymer layer functionalized by monovalent ligands and a target surface homogeneously covered with receptors (a) or containing a finite number of distinct receptor sites (b). In this latter case the surface between receptor sites was neutral and impenetrable for polymers. Receptors were immobile and stick out of the surface by one cell size. In both cases the interaction energy between a functional group and a receptor was $\epsilon = 8kT$. The distance between the polymer layer and the surface, h, was varied.

polymer layer and receptor surface leading to the saturation predicted in refs 41 and 42 when all functional groups are attached to the receptor surface.

We have systematically varied the distance h between the polymer-grafted surface and the target surface and measured the probability P_n to have n functional groups bound to receptors. Knowing the distribution P_n as a function of h, we estimate the average fraction of functional groups bound to receptors as

$$\langle x(h)\rangle = \frac{\sum_n (nP_n(h))}{N_f} \qquad (1)$$

where N_f is the total number of functional groups in a polymer layer.

Multivalent Ligands Binding a Target Surface Homogeneously Covered with Receptors. Figure 6a shows the average fraction of functional groups bound to the receptors, $\langle x(h)\rangle$, as a function of the distance to the receptor surface (homogeneously covered with receptors) h for monodisperse polymer layers modified by ligands of different functionality. In all cases $\langle x(h)\rangle$ abruptly increases with a decrease of the distance to the target surface, reaching a plateau corresponding to nearly 100% binding of the functional groups. The separation distance h_b at which the abrupt increase of $\langle x(h)\rangle$ occurs can be considered as the binding distance. As is seen, this distance is maximal for the tetravalent ligands, somewhat smaller for divalent, and even smaller for monovalent ligands. As we have discussed in the previous section, the overall density profile of monodisperse layer functionalized with ligands of different functionality is essentially the same (Figure 2a).

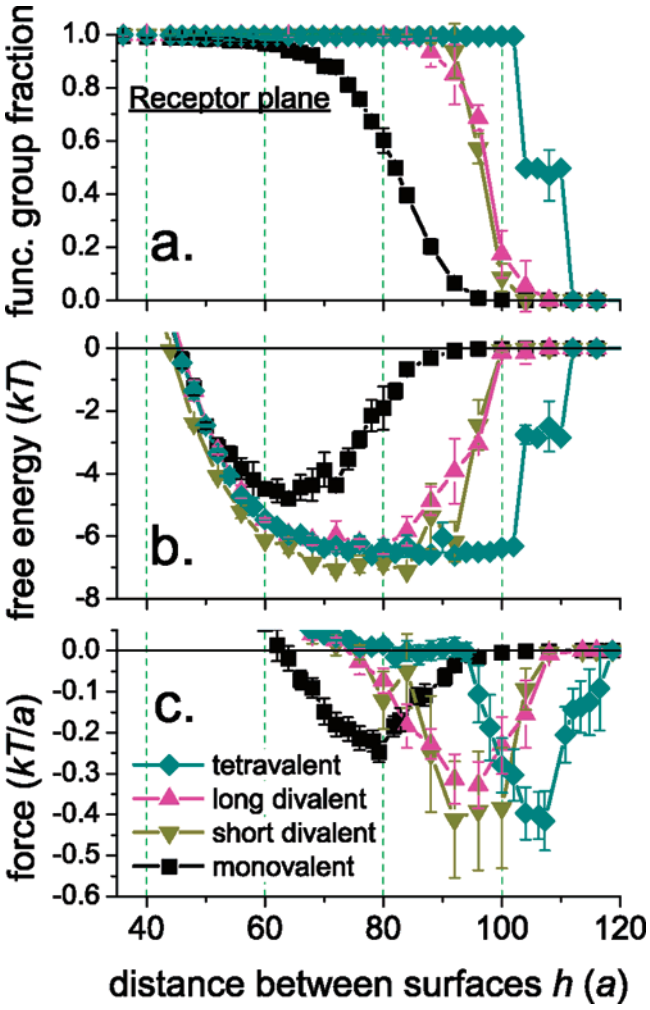

Figure 6. Binding simulation results for interactions between the homogeneous receptor surface and the monodisperse layers modified by ligands of different functionality: fraction of bound functional groups (a), free energy per functional group (b), and attractive force (between the polymer layer and receptor surface) per functional group (c) as a function of the distance to surface containing receptors. In all cases the polymer chain length was $N = 64$.

This implies that tetravalent ligands have greater capability to reach out compared to divalent or monovalent ligands. Similarly, the level of nearly 100% involvement of functional groups in receptor binding is reached at larger separation distances for tetravalent ligands compared to divalent and monovalent ones. Such a strong advantage of ligands of higher functionality is due to cooperativity of binding: as soon as any of functional groups get bound to a receptor, it effectively brings all other functional groups belonging to the same ligand in the close vicinity of receptor surface and increases their probability to be bound.[31] Also, the probability to stay bound is higher for multivalent ligands compared to monovalent ones (the statistical effect).[31,36]

We have also evaluated the free energy of interactions between the polymer layer and receptor surface as a function of the distance h to the target surface. To this end we have calculated the overall number of configurations of the system $C_{total}(h) = \Sigma_n C_n(h)$ (where n is the number of functional groups bonded to receptors) and applied the following equation:

$$F(h) \equiv -kT \ln Z(h) \qquad (2)$$

where $Z(h)$ is the partition function given by

$$Z(h) \equiv \sum_n C_n(h)\, e^{-\epsilon n/(kT)} = \frac{\Sigma_n\, C_n(h)}{\Sigma_n P_n(h)\, e^{\epsilon n/(kT)}} = \frac{C_{total}(h)}{\langle e^{\epsilon n/(kT)} \rangle} \quad (3)$$

Figure 6b shows the free energy (per functional group) of interactions between the monodisperse polymer layers modified by ligands of different valence and receptor surface. (The error bars of the free energy calculations were estimated from the statistical fluctuations in the Monte Carlo time sequence.[43]) After the initial abrupt decrease, the free energy reaches shallow minimum and then starts to increase with decreasing distance to the target surface. The depth of the minimum characterizes how strongly the polymer layer is bound to the target surface, whereas the position of the minimum of the free energy indicates the equilibrium distance between the polymer layer and the target surface.[42] For tetravalent ligands the initial free energy decrease occurs at larger separation distances compared to ligands of lower functionality following the tendency observed for $\langle x(h) \rangle$. While for divalent and tetravalent ligands the free energy minimum is approximately the same, it is interesting to note that the short (-branched) divalent ligands seem to have a slightly lower free energy compared to long (-branched) divalent or tetravalent ligands. This is because of the high cooperativity of short divalent ligands: having the shortest separation distance (branch length) functional groups belonging to the same short divalent ligand will be more likely bound to receptors.

At shorter separation distances deformation of the polymer layer occurs, resulting in the free energy increase. It is worthwhile to note that for all types of ligands the zero values of the free energy is reached at practically the same separation distance. This result is connected with the fact that the free energy increase is governed by the deformation of the polymer layer and therefore depends on the overall density distribution. As we discussed above, the density distribution is very similar for all types of ligands for monodisperse brushes (Figure 2a); thus, it comes as no surprise that the free energies also follow the same pattern in the deformation-controlled region.

Another value which can be obtained on the basis of the simulations is the interaction force f between the polymer layer and the target surface, which is a derivative of the free energy

$$f(h) = -\partial F(h)/\partial h \quad (4)$$

The interaction force characterizes how strong is the pulling of the polymer layer to the surface created by interactions between the functional groups and receptor surface. In principle, the value of the force can be compared with that obtained in AFM measurements using a tip modified by receptors (or polymer layer) in contact with a functionalized polymer layer (or receptor surface).[30,44,45] Figure 6c compares the interaction forces between a functional group and the homogeneous receptor surface for monodisperse layers functionalized with ligands of different valence. Numerically, the force at a given distance h between the two surfaces (and its error bar) was estimated from the slope of linear fit (and its standard deviation) of the free energy data points in the range $h \pm 8a$. The interaction force behavior follows qualitatively the same pattern as the free energy except the position of its extremum is shifted to larger distances between the polymer layer and a target surface. As before, the largest separation distance corresponding to the extremum of the force is achieved by the tetravalent ligands. The maximum absolute value of the force is relatively similar for all but monovalent ligands (Table 2).

Multivalent Ligands Binding a Target Surface Carrying Distinct Receptor Sites. We have also repeated the set of binding simulations for the case of a target surface carrying receptor sites (homogeneously distributed with an average separation distance $8a$). In this case the number of receptors was only twice larger than the number of functional groups in the polymer brush. The results of binding simulations for monodisperse polymer layers terminated by the same types of ligands as before are shown in Figure 7 as a function of the distance to the target surface carrying receptors and summarized in Table 3. As is seen, the results obtained for receptor sites (Figure 7) are noticeably different from that obtained for the homogeneous receptor surface (Figure 6). First the maximal fraction of bound functional groups reached at low separation distances is considerably smaller in the case of receptors sites. As a result the absolute values of the free energy at minimum are more than 10 times smaller than for a homogeneous receptor surface. These effects are caused by the decrease in the number of available receptor sites. Second, the increase in the fraction of bound functional groups and correspondingly the decrease in the free energy occurs much more smoothly when the functionalized polymer brush interacts with surface carrying receptor sites. Also the binding distance at which the first stable bond between the functional group and a receptor forms is considerably smaller than in the case of the homogeneous receptor surface. Evidently the capability of ligands to reach out is the same in both cases since we deal with the same polymer layers. However, formation of a stable bond between the target surface and a functional group is much easier to achieve when there is a considerable excess of binding sites. Another interesting observation is that the separation distances at which the minimum of the free energy (and the extremum of the force) are reached for different polymer layers are practically the same in the case of interaction with distinct receptor sites. Since the density profile is essentially the same for all polymer layers (Figure 2 and Table 3), the separation distance at which the entropic penalty for brush deformation starts to dominate is also the same for all cases resulting in a rather similar position of the free energy minimum (and consequently the force). The increase of entropic penalty for deformation is the reason the free energy has a sharp minimum and never reaches saturation level for the case of receptor sites. The common feature for both the receptor surface and receptor sites cases is the evident preference of ligands of higher functionality in the receptor targeting. Indeed, in all cases tetravalent ligands show the largest binding distance, the lowest (or one of the lowest) free energy, and the largest pulling force, whereas monovalent ligands show the

(41) (a) Johner, A.; Joanny, J. F. *J. Chem. Phys.* **1992**, *96*, 6257−6273. (b) Johner, A.; Joanny, J. F. *Europhys. Lett.* **1991**, *15*, 265−270.

(42) Wijmans, C. *Macromolecules* **1996**, *29*, 789−791.

(43) A small error bar implies that simulations over a large number of MC steps produce a rather small variation of the value, which is likely to be the equilibrium value. However, this method of error estimation is not valid in the case when the system is trapped in a deep local free energy minimum (as in some cases of tetravalent ligands). In such case, a representative sampling through the entire configuration space is required, which can be done using methods described in the following: Berg, B. A.; Neuhaus, T. *Phys. Rev. Lett.* **1992**, *68*, 9−12. Marinari, E.; Parisi, G. *Europhys. Lett.* **1992**, *19*, 451−458.

(44) Wong, J.; Kuhl, T.; Israelachvili, J.; Mullah, N.; Zalipsky, S. *Science* **1997**, *275*, 820−822.

(45) Courvoisier, A.; Isel, F.; Francois, J.; Maaloum, M. *Langmuir* **1998**, *14*, 3727−3729.

Table 2. Binding Simulation Results for the Homogeneous Receptor Surface: Absolute Value of the Free Energy at Its Minimum ($-F_{min}$), Force at Its Extremum ($-f_{extr}$), and the Bound Fraction of Functional Groups x_{eq} at the Free Energy Minimum[a]

brush type	ligand type	$\langle h \rangle^b(a)$	$x_{20\%}{}^c$	x_{eq}	$-F_{min}(kT)$	$-f_{extr}(kT/a)$
monodisperse	monovalent	28.9	0.56	0.913	4.00	0.300
bidisperse	monovalent	26.5	0.77	0.957	4.76	0.240
monodisperse	short divalent	29.0	0.63	0.999	6.62	0.415
monodisperse	long divalent	29.3	0.69	0.993	6.19	0.328
monodisperse	tetravalent	29.6	0.83	0.994	6.44	0.398
bidisperse	tetravalent	24.4	0.98	0.994	6.53	0.519

[a] Both free energy and force are calculated per functional group. For all monodisperse brushes $N = 64$ and for all bidisperse brushes $N_L = 64$ and $N_S = 48$. In all cases considered the percentage of functional groups to the overall number of polymer chains was 50%, which was achieved by 1/2 ligand substitution for monovalent ligands, 1/4 for divalent ligands, and 1/8 for tetravalent ligands. For comparison we also show some characteristics of the polymer brushes in the absence of target surface [b] Average height of the free standing brush. [c] Fraction of functional groups in the exterior 20% the free standing brush (based on molecular weight).

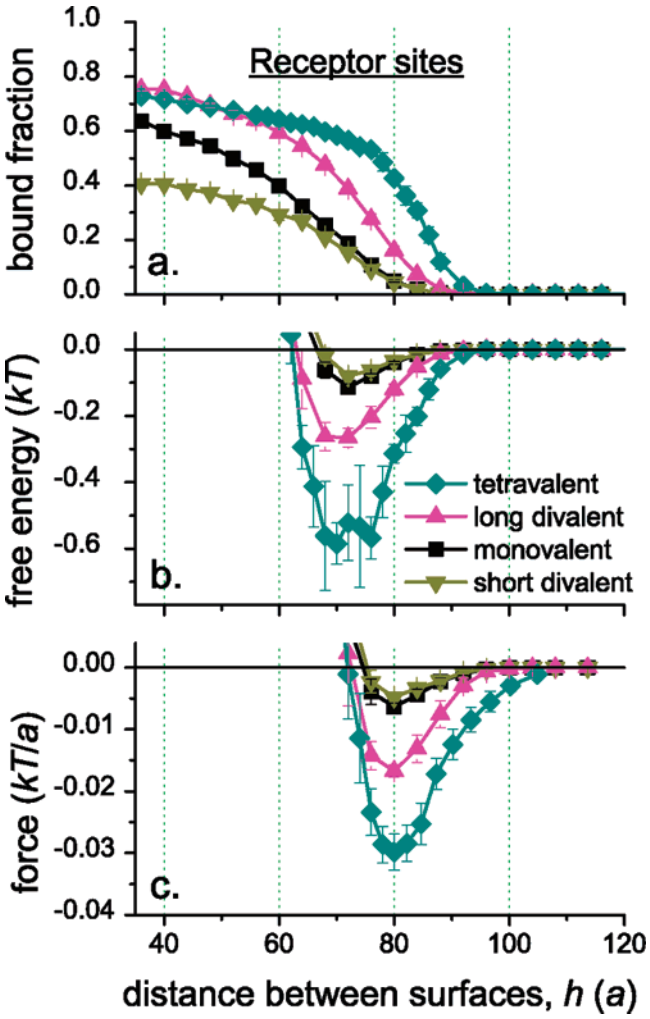

Figure 7. Binding simulation results for interactions between receptor sites and the monodisperse layers modified by ligands of different functionality: fraction of bound functional groups (a), free energy per functional group (b), and attractive force (between the polymer layer and receptor surface) per functional group (c) as a function of the distance to surface containing receptors. In all cases the ratio of functional groups to receptors was 1:2 and the polymer chain length was $N = 64$.

poorest (or one of the poorest) results. This tendency becomes especially evident when the number of available receptors is limited. The only exception from this trend was the short divalent ligands.

Influence of Ligand Branching Length on the Efficiency of Receptor Binding. On the basis of the results presented in Tables 2 and 3 and Figures 2, 6, and 7, it is evident that there is an advantage in using of multivalent ligands for enhancing receptor targeting. In part the reason for this is that the more bulky multivalent ligands tend to be localized on the periphery of the polymer layer. However, as we can see from the example of divalent ligands, this factor alone is not sufficient to ensure success in targeting. To study the effect of branch length in targeting, we compared divalent ligands with a short and long branch length (Figure 1). As is seen from Figure 2 and Table 2 the fraction of functional groups of divalent short ligands on the periphery of the layer is somewhat lower compared to divalent long ligands and is somewhat higher compared to the monovalent ligands. At the same time binding simulations show that upon interaction with the target surface homogeneously covered with receptors the fraction of functional groups bound to receptors was very similar for both long-branched and short-branched divalent ligands with a slight preference in the free energy for the latter. Among the factors ensuring success of targeting by multivalent ligands is the statistical effect and cooperativity of binding.[31,32,36] The latter would depend on the branch length for multivalent ligands and on the size and distribution (or mobility) of receptors.[33,37] When binding to the homogeneous receptor surface short-branched divalent ligands will possess a higher cooperativity since the smaller distance between the functional groups increases the probability of receptor binding (or rebinding) for the second functional group if the first is already bound. Due to this, short divalent ligands have shown one of the best results (comparable with that tetravalent ligands in the depth of the free energy minimum and the pulling force at its extremum) in binding to the receptor surface.

At the same time binding to distinct receptor sites reveals quite a different story. The fraction of bound functional groups of short divalent ligands (as well as pulling force) was noticeably smaller and free energy was considerably less negative than that for not only long divalent ligands but also the monovalent ligands (Table 3, Figure 7). This, on first sight a surprising result, has its origin in the ligand architecture. The length of the branches carrying functional groups of short divalent ligands turned out to be insufficient to reach different receptor sites (which were separated by a twice larger distance than the functional groups, Figure 8). At the same time functional groups of the long divalent ligand were on average separated by a distance 1.17 times larger than the average distance between (immobile) receptors. As a result, functional groups of long divalent or monovalent ligands could reach different receptors without much trouble, while half of the functional groups of the short divalent ligands were wasted. We note that the functional groups of short divalent ligands could still replace each other in binding receptors (a statistical effect), but this

Table 3. Binding Simulation Results for the Surface with Distinct Receptor Sites: Absolute Value of the Free Energy at Its Minimum ($-F_{min}$), Force at Its Extremum ($-f_{extr}$), and the Bound Fraction of Functional Groups x_{eq} at the Free Energy Minimum[a]

brush type	ligand type	$\langle h \rangle^b(a)$	$x_{20\%}{}^c$	x_{eq}	$-F_{min}(kT)$	$-f_{extr}(kT/a)$
monodisperse	monovalent	28.9	0.56	0.14	0.073	0.0064
bidisperse	monovalent	26.5	0.77	0.27	0.214	0.0146
monodisperse	short divalent	29.0	0.63	0.11	0.051	0.0048
monodisperse	long divalent	29.3	0.69	0.36	0.267	0.0167
monodisperse	tetravalent	29.6	0.83	0.55	0.518	0.0298
bidisperse	tetravalent	24.4	0.98	0.56	0.626	0.0353

[a] Both free energy and force are calculated per functional group. For all monodisperse brushes $N = 64$ and for all bidisperse brushes $N_L = 64$ and $N_S = 48$. In all cases considered the percentage of functional groups to the overall number of polymer chains was 50%, which was achieved by 1/2 ligand substitution for monovalent ligands, 1/4 for divalent ligands, and 1/8 for tetravalent ligands. For comparison we also show some characteristics of the polymer brushes in the absence of target surface. [b] Average height of the free standing brush. [c] Fraction of functional groups in the exterior 20% the free standing brush (based on molecular weight).

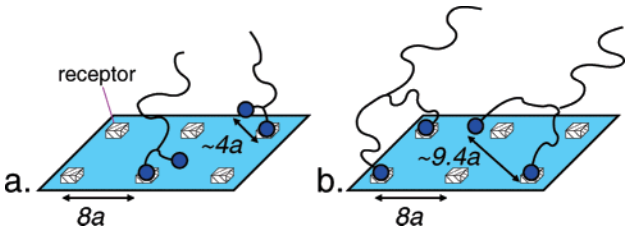

Figure 8. Schematic presentation of receptor site binding mechanism for the divalent ligands with short (a) and long (b) branches carrying functional groups. Density and nature of receptor sites are the same in both cases. The average distance between receptors and between functional groups belonging to the same ligand is indicated.

does not compensate for the overall loss in the absolute number of bound function groups, resulting in the poor targeting performance.

In general to take advantage of the cooperativity of binding the branch length of multivalent ligands should be small enough to increase the local concentration of functional groups resulting in larger probability of receptor binding (or rebinding) for the second functional group if the first is already bound. At the same time to be efficient in targeting, the branch length should be sufficiently large to reach different receptors, i.e., to be larger than at least the physical size of a receptor for mobile receptors or larger than the average distance between immobile receptors.[33,35,37] Therefore, to ensure a high efficiency of receptor binding by multivalent ligands, the architecture (branch length) of the ligand should be chosen on the basis of the receptor properties (size, mobility, number of binding sites per receptor, and so on).

Bidisperse Layer Binding. As we discussed above, using a bidisperse polymer layer (with short nonfunctional chains and long chains functionalized with ligands), one can achieve a considerable increase in the fraction of functional groups on the periphery of the layer (Figure 2b). In Figure 9 we show the simulation results of binding between bidisperse polymer layer ($N_L = 64$, $N_S = 48$) containing either tetra- and monofunctional ligands and homogeneous receptor surface. For comparison we also show the results for the corresponding monodisperse layers (open symbols). Since the monomer density profile for the bidisperse polymer layers varies with the fraction of short chains in the layer and is noticeably more compact (due to presence of short chains) than the monodisperse layer (Figure 2b, Table 2), the undisturbed monomer density distribution was also included as an inset to the Figure 9 for comparison. Despite having the smallest thickness of the free standing polymer layer (due to the largest fraction of short chains, 7/8), tetravalent ligands show one of the largest bonding distances (Figure 9a) exceeded only by monodisperse tetravalent ligands. With the

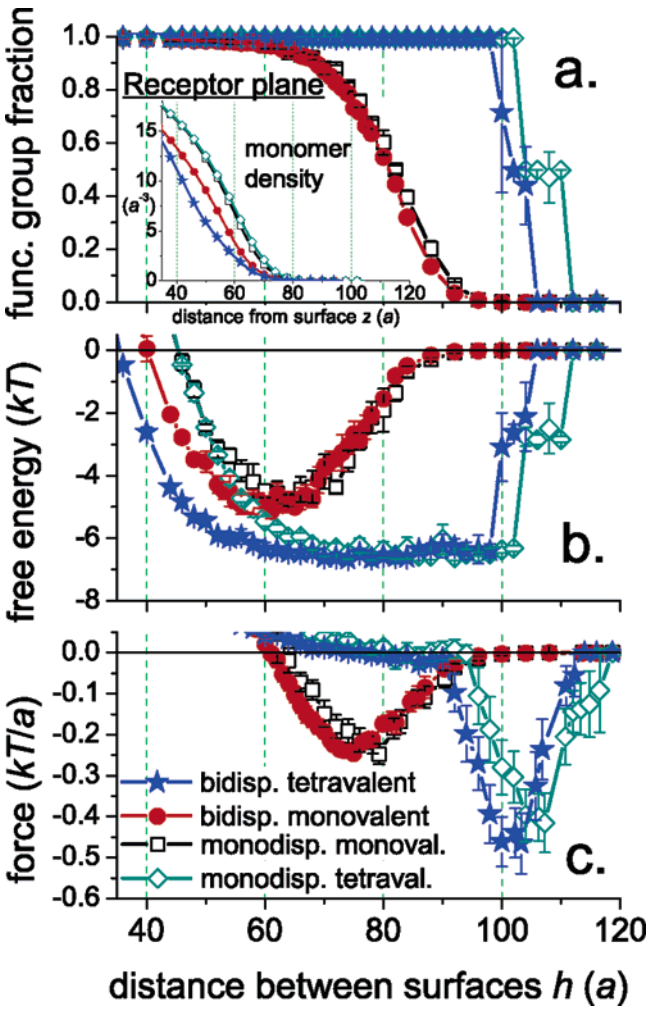

Figure 9. Binding simulation results for interactions between the homogeneous receptor surface and the bidisperse polymer layers ($N_S = 48$, $N_L = 64$) functionalized by monovalent or tetravalent ligands in comparison with the results for the corresponding monodisperse layers (open symbols): fraction of bound functional groups (a), free energy per functional group (b), and attractive force per functional group (c) as a function of the distance to surface containing receptors. The corresponding part of the undisturbed density profile (as in Figure 2b) is shown as an inset.

decrease of separation distance h, the fraction of bound functional groups increases more abruptly for bidisperse compared to monodisperse tetravalent layer reaching the same saturation level at nearly 100% involvement of functional groups in receptor binding. Similarly the plateaulike minimum of the free energy is the same in its absolute value for bidisperse and monodisperse tetravalent

layers. Since the free energy decreases more abruptly for bidisperse compared to monodisperse layers containing tetravalent ligands, the pulling force is somewhat stronger for the bidisperse layer. The fraction of bound functional groups in bidisperse and monodisperse layers of monovalent ligands increases with decrease of h in a very similar manner (but more slowly compared to the tetravalent ligands). If one takes into account that the undisturbed thickness of the bidisperse layer is smaller (having 50% short chains) than that of monodisperse layer (having no short chains), this implies that the monovalent ligands that are part of a bidisperse layer effectively reach out considerably further than those in a monodisperse layer. For monovalent ligands the free energy of interaction (and the force) between the bidisperse surface and receptor layer is only slightly deeper and shifted to somewhat smaller separation distances compared to that for the monodisperse layer. It is worthwhile to note that the separation distance at which the zero free energy level is reached in the deformation-controlled region (small h) follows the same pattern as the monomer density profile: it is the largest for monodisperse layers, somewhat smaller for bidisperse monovalent, and the smallest for the bidisperse tetravalent layer. The main benefit of bidisperse polymer layers in interactions with a homogeneous receptor surface is that they allow ligands to reach further out (due to smaller monomer concentration on the periphery) compared to monodisperse layers.

The advantage of using bidisperse polymer layers becomes more evident when there is a limited number of receptors. The simulation results for the binding interactions between the surface containing a finite number of receptor sites and the same bidisperse layers as before (terminated by monovalent or tetravalent ligands) is shown in Figure 10 in comparison with that for the corresponding monodisperse layers. The overall number of functional groups was the same: twice smaller than the number of receptors. Similar to the receptor surface case (Figure 9) the position of the free energy minimum and the extremum of the force are shifted to smaller separation distances since the height of the undisturbed bidisperse brush is smaller and it allows larger deformation (due to low monomer concentration on the periphery). The new feature of the receptor binding is that the free energy minimum (and pulling force extremum) is noticeably deeper for both bidisperse layers compared to the corresponding monodisperse layers. This is the result of both higher accessibility of functional groups (Figure 2b) resulting in larger fraction of bound functional groups (especially for monovalent ligands, Table 3) and larger compressibility of bidisperse layers. The accessibility of functional groups manifests itself in capability to reach out, which is important for both kinds of receptor surfaces. The tolerance to larger compressibility of bidisperse brushes becomes especially importance for binding to a finite number of receptor sites since now the limit of nearly 100% involvement of functional groups in receptor binding cannot be reached without considerable deformation of the brush. Thus, the brush allowing larger deformation will achieve a higher fraction of bound receptor sites and lower free energy leading to observed preference of bidisperse layers over the monodisperse ones.

Comparing the binding simulation results shown in Figures 6, 7, 9, and 10, one can see that the ligands of highest functionality (tetravalent) were always the most efficient in receptor binding. This observation supports the idea that multivalent ligands have the advantage of cooperativity and statistical exchange upon binding.[31,32,36] On the other hand, bidisperse polymer

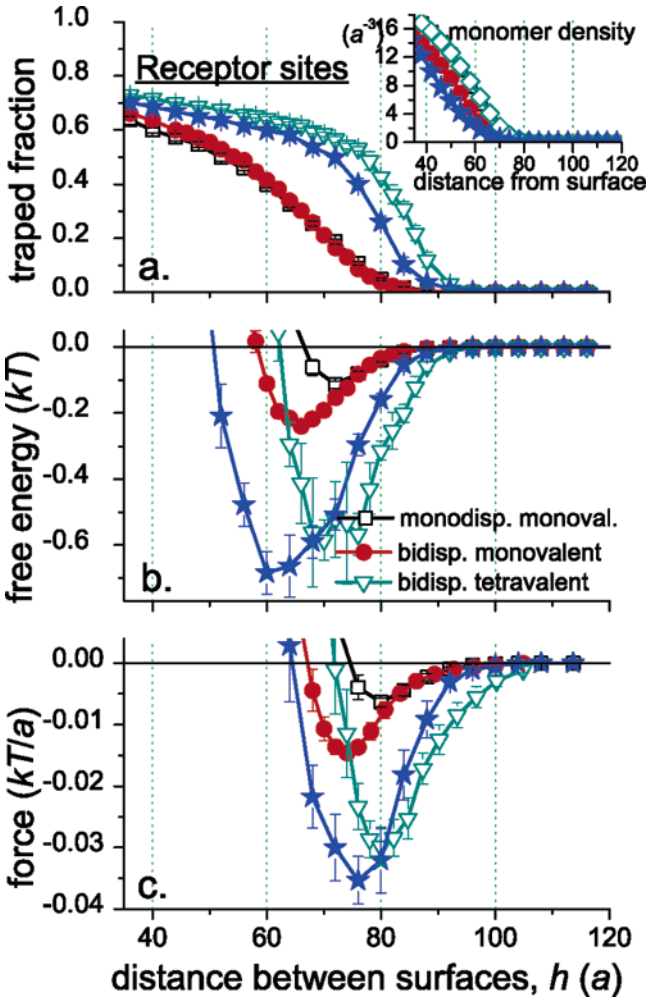

Figure 10. Binding simulation results for interactions between the receptor sites and the bidisperse polymer layers ($N_S = 48$, $N_L = 64$) functionalized by monovalent or tetravalent ligands in comparison with the results for the corresponding monodisperse layers (open symbols): fraction of bound functional groups (a), free energy per functional group (b), and attractive force per functional group (c) as a function of the distance to surface containing receptors. In all cases the ratio of functional groups to receptors was 1:2. The corresponding part of undisturbed density profile (as in Figure 2b) is shown as an inset.

layers allow closer contact with receptors due to their capability to reach out and high compressibility. As a result, more functional groups are exposed to the receptors and the interaction with the receptor surface is stronger. In general the binding simulation results (Figures 6, 7, 9, and 10) closely follow our predictions on the basis of the density of functional groups on the periphery of the layers (Figures 2−4). This implies that the accessibility of functional groups is one of the important factors in targeting. The case of short divalent ligands provides an interesting exception to this statement. If the functional groups belonging to the same ligand cannot simultaneously reach different receptors, then all the functional groups but one are "wasted" with respect to receptor binding.

4. Conclusions

The accessibility of ligands inside the protective polymer layer and their capability to reach out are among the important factors ensuring success of receptor targeting. In this paper we have considered the influence of both

ligand architecture (valence, branching length) and composition (polydispersity) of the protective polymer layer on the efficiency of receptor surface targeting. Two types of receptor surfaces were considered: a surface homogeneously covered with receptors and a surface containing a finite number of receptor sites. In accordance with experimental observations[9,11,34,36] and some of the theoretical results,[31−33] we found that the use of multivalent ligands has significant advantages in improving the targeting efficiency. First, having a physically more bulky ligand helps to increase the functional group concentration on the periphery of the layer. As a result, one can expect that hindrance of multivalent ligands inside the protective polymer layer will be less of a factor compared to the monovalent ligand case. Second, multivalent binding can occur in a cooperative or statistical manner: having one group bound to a receptor increases the probability that other functional groups of the same ligand will be close to the target surface and bind another receptor.

Despite these evident advantages the application of multivalent ligands to targeting should be taken with some care. Comparison of short- and long-branched divalent ligands shows that when the receptors are closely packed (homogeneous receptor surface), the former have the advantage of a higher degree of cooperativity: the shorter the average distance between functional groups belonging to the same ligand, the larger probability of the second receptor to be bound if the first one is already bound to a receptor. On the other hand, when the receptors are more sparsely distributed, the average distance between the functional groups of the same ligand should be comparable or larger than the average distance between receptors. If this condition is not satisfied, as it was in the case of short-branched divalent ligands, a considerable fraction of functional groups will be wasted and such multivalent ligands will be less efficient in targeting than monovalent ligands. Therefore, if one has in mind a particular receptor or receptor surface, the branch length for multivalent ligands should be chosen appropriately; at the very least it should be sufficiently long to bind different receptors. In the case of mobile receptors, which we plan to consider in our future work, we expect that the binding results will have some common features of both receptor sites and receptor planes cases considered here. The physical size of receptors will define the minimal separation distance between them and hence the minimal branch length for multivalent ligands.

Considerable enhancement of targeting efficiency can be also achieved by altering the composition of the protective polymer layer. In this paper we considered a bidisperse polymer layer composed of short, nonfunctional and long functionalized chains. Short chains acting as a concentration barrier prevent the functional groups from penetrating deeply into the layer. As a result, by using a bidisperse layer (which is easy to make experimentally[15,16]), one can achieve as high a fraction of functional groups on the periphery of the layer as when using multivalent ligands. Binding simulations confirm that bidisperse layers offer the advantages of larger compressibility and a higher capability for the ligands to reach out compared to the monodisperse layers. This results either in the deeper free energy minimum for bidisperse layers compared to the corresponding monodisperse layers (for the case of a finite number of receptor sites) or in the broader free energy plateau region (for the case of homogeneous receptor surface). Therefore, the bidisperse structure of the protective polymer layers can considerably contribute to the enhancement of targeting.[15,16] Both motifs discussed in this paper, using multivalent ligands and using bidisperse polymer layers, can be applied simultaneously for further improvement of targeting.

We found that the distribution of functional groups inside the protective polymer layer can provide a good measure of targeting efficiency. Therefore, using fluorescence or other measurement techniques that characterize the functional groups distribution inside the polymer layer, one can get some quantitative measure of what fraction of functional groups will be easily accessible for receptor binding. There are many other factors such as interactions between ligands or between ligands and polymers, curvature of the surface, mobility and size of receptors, and so on which can influence the efficiency of targeting. Systematic study of these factors will allow considerable improvement in the design and efficiency of targeted gene/drug delivery.

Acknowledgment. We appreciate helpful discussions with Dr. Jinming Gao (CWRU). This work was supported by the Biomimetic and Bioactive Polymers Program of Case Western Reserve University (OBR Challenge, CSE RC 00-01).

LA047109V