Semiflexible Oligomer−Polymer Binding: Combinatorial and Conditional Probability Analyses and Stochastic Simulation

Jiří Horský*

Institute of Macromolecular Chemistry, v.v.i., Academy of Sciences of the Czech Republic, Heyrovský Sq 2, 162 06 Prague 6, Czech Republic

Received November 9, 2007; Revised Manuscript Received April 9, 2008

ABSTRACT: Theoretical description of large ligand binding to a one-dimensional array of binding sites (lattice) is modified for oligomer−polymer binding and found to predict some experimentally observed phenomena. In the model, not only the lattice but also a ligand is defined as an array of binding sites. Additional assumptions made are the first-order intraligand cooperativity, the infinitely small lattice concentration, and semiflexible binding (i.e., ligand dangling ends are allowed, but not looping). The relations and procedures derived for equilibrium binding to a finite lattice, equilibrium binding to an infinite lattice, and for kinetics of binding to a finite lattice are mutually consistent and predict the existence of experimentally observed phenomena such as charge inversion in polyelectrolyte complexes, the effect of the rate or order in which the components are mixed on properties of formed polymer nanoparticles, and the salt effect on polycation−polyanion turbidimetric titration.

Introduction

Many important physicochemical and biological processes can be described as a binding of small ligands to a large molecule or to a molecular assembly; therefore, the problem has received continuous attention for over six decades.[1−16] A macromolecule or a surface is usually treated as a one- or two-dimensional array of binding sites (lattice). Theoretical analysis is a challenge even for arrays of identical binding sites because the binding properties of a site can be affected by the state (occupied/unoccupied) of other sites in the array. If other bound ligands have a stabilizing effect, the binding is cooperative, which means that the dependence of the occupation of the array on the concentration of the free ligand (a binding isotherm) is steeper than for the noncooperative case. For relatively small arrays such as hemoglobin, the observed stepwise binding constants can be related to microscopic constants of the underlying model.[8] For large arrays (including infinite ones), only the nearest-neighbor interactions are usually considered and treated theoretically using various approaches for obtaining the binding isotherm.

The cooperativity concept seems to be clear. Yet, the word conveys a different meaning in the realm of polymers where polymer−polymer complexes are widely studied,[13,17−19] and an array of binding sites thus describes not only the lattice but also the ligand. Consequently, the cooperativity is considered to manifest itself with the increasing length of an oligomeric/polymeric ligand, rather than with the increasing ligand concentration[20] or, in a qualitative way, by the stable structure of polymer−polymer complexes in comparison with low-molecular-weight analogues.[18] Kříž and Dautzenberg[20] were aware not only of the difference between classical (interligand) and polymer (intraligand) cooperativity but also of various sources of the latter. The first-order intraligand cooperativity is given by interactions between adjacent sites of the ligand and is always present because binding sites are chained in the ligand. The higher-order intraligand cooperativity occurs when the affinity of the binding site depends on the state (bound/free) of more remote binding sites in the ligand. Obviously, the first-order intraligand cooperativity cannot be avoided for polymeric ligands, but the interligand and higher-order cooperativity will be ignored in the present paper, which concentrates on the effect of the ligand length.

The length of a ligand, expressed as the number of binding sites required by the ligand, is the other most investigated aspect of binding to an array of binding sites besides cooperativity. If a ligand can cover several binding sites of the lattice, the "parking problem"[21] occurs. In this analogy, the lattice corresponds to a (one-dimensional) parking lot without parking spaces marked. Parking is easier in an empty unmarked parking lot than in a marked one. As the number of randomly parked cars increases, however, finding parking space becomes more difficult because some gaps between cars are too narrow. Thus, binding of a large ligand for a free lattice is promoted but inhibited for a highly occupied one. The problem was analyzed by various approaches, and binding isotherms were derived for noncooperative and cooperative binding of a large ligand to a one-dimensional lattice, both finite and infinite.[3,5,6,9]

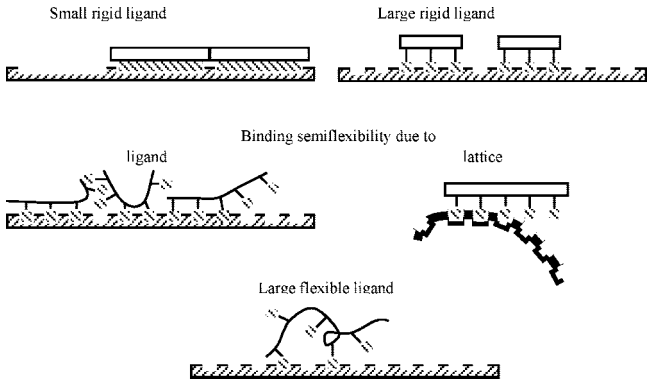
Thus, theoretical tools for analyzing the effect of increasing length of a ligand seem to be available. In all those theories, however, the value of the (micro)binding constant is postulated, and its relation to the size of a ligand is not investigated. This is understandable because the theories were derived with some purely biological processes in mind in which a ligand corresponds to a definite number of binding sites. The situation becomes different if a polymer or oligomer is used as a ligand because molecules of the same polymer but with a different number of monomeric units, and hence binding sites, can easily be prepared. In order to obtain the relationship for a binding constant of polymer ligand, the theoretical analysis of polymer ligand binding presented by Kříž and Dautzenberg[20] will be re-evaluated.

Polymer−polymer complexes received great attention, motivated by potential industrial or biomedical applications.[13,17,22] Mixing solutions of complex-forming polymers frequently leads to precipitation or, when done in a controlled manner, to formation of nanoparticles, envisaged as drug-delivery vehicles.[23,24] The formation of supramolecular assemblies of polymer−polymer complexes in water is not surprising as hydrogen bonding or Coulombic interactions are consumed (internalized) within complexes, which thus become more hydrophobic. The alternatively conceivable reason for aggregation/precipitation, compatible with the "scrambled eggs" model,[25,26] is cross-linking, occurring when a polymer chain

* E-mail: horsky@imc.cas.cz.

10.1021/ma702493w CCC: $40.75 © 2008 American Chemical Society
Published on Web 06/10/2008

Scheme 1. Various Modes of Binding to a One-Dimensional Lattice Relevant in the Present Context[a]



[a] A small ligand can bind to one lattice site only. All binding sites of a rigid ligand are bound simultaneously. A semiflexible ligand can have dangling ends; this can be due to the conformational flexibility of the ligand or of a lattice. A flexible ligand can make loops.

forms a complex with two or more chains of the complementary polymer. Cross-linking is a consequence of the fact that binding sites of a polymer ligand need not to be bound to a lattice as a rigid unit. Some binding sites can be bound to different complementary chains (cross-linking), some to other parts of the same complementary chain (looping), or some can be free (dangling). We ascribe this phenomenon to "flexibility" either of the ligand or of the lattice chains.

Even though the phenomenon seems to be plausible, direct experimental evidence is missing. Nevertheless, some supporting experimental evidence is available. The sign of zeta potential of polyelectrolyte complexes depends on the mixing ratio; complexes formed between polycations and DNA exhibit positive zeta potential at polycation excess, indicating the existence of partially dangling ligands.[27,28] Nagura et al.[29] observed the increase in Young's modulus of poly(vinyl alcohol) hydrogels upon incorporation of poly(sodium L-glutamate) and ascribe it to cross-linking in the polymer−polymer complex. This also shows that although the complexation of homopolymers, analyzed in this paper, leads to aggregation or precipitation, gelation due to interpolymer complexation can occur in more heterogeneous systems.

In order to avoid cross-linking and to facilitate the analysis of polymer−polymer binding, the present paper deals with infinitely dilute solutions of the polymer taken as a one-dimensional lattice. As the length of the ligand increases, the distinction between the lattice and the ligand becomes disputable. Therefore, the finite concentration rather than size defines which polymer of the pair is considered as a ligand. A further assumption is that the ligand is bound in a zipper-like way by one contiguous block of binding sites (mers) to a contiguous sequence of lattice binding sites, which means that dangling ends are allowed but looping is prohibited. Such ligands (or binding) are denoted here as semiflexible as opposed to rigid ones, for which neither dangling ends nor looping are possible, and to flexible ones, for which both dangling ends and looping are allowed. Various possible modes of binding to a one-dimensional lattice are depicted in Scheme 1 to stress the specific meaning of the terms in the present context. The size of a ligand is taken with respect to the lattice binding sites, and thus the ligand that can bind to one lattice binding site is called small regardless of its actual size. (Semi)flexibility of ligand binding can be in fact incurred by the conformational flexibility of the lattice.

It is well-known that the properties of prepared polymer−polymer complexes or their assemblies can depend on such factors as the rate[30] or order[31] in which the components are mixed. This clearly indicates that such systems are not in equilibrium, and kinetics of the complex formation must be taken into account. Therefore, the kinetic aspects of binding of semiflexible ligands are also addressed in the present paper.

Although the derived equations and algorithm are valid regardless of the ligand size, their application to high polymer ligands becomes intractable due to the enormous number of available configurations. The problem becomes manageable if the number of binding sites in ligands is restricted; therefore, numerical examples are given for oligomeric ligands only. Shorter ligands are also more likely to be "semiflexible" as the probability of looping is decreased. Even such narrower analysis provides some insight into behavior of polymeric ligands and is of practical relevance by itself. Therapeutic efficacy of antisense oligonucleotides can be increased using polymer-based delivery systems.[32]

The aim of the present paper is to implement the concept of "semiflexibility", as defined above, into the theoretical schemes proposed for binding of large ligands to one-dimensional lattices.[3,5,6,9] Only the basic case of the first-order intraligand cooperativity and infinitely diluted lattices is considered. For ligands corresponding to a semiflexible array of binding sites, predictive procedures are developed for (i) equilibrium binding to a finite lattice, (ii) equilibrium binding to an infinite lattice, and (iii) kinetics of binding to a finite lattice. Numerical examples obtained with the procedures are given for oligomer−polymer binding mainly to illustrate (i) mutual consistency of the procedures, (ii) general and unexpected features of semiflexible binding, and (iii) qualitative agreement with some phenomena observed with oligomer−polymer and polymer−polymer complexes. In lieu of conclusions, the limitations and possibilities of further generalization are discussed.

Equilibrium Semiflexible Binding

Binding of an Array of Binding Sites to a Matching Lattice. Kříž and Dautzenberg partitioned the Gibbs energy of binding an isolated array of r binding sites to a lattice with coupling in a ladder-like manner, ΔG, as follows:[20]

$$\Delta G = \sum_{i=1}^{r} \Delta g_i + \sum_{i=1}^{r-1} \Delta g_{i,i+1} + \sum_{i=1}^{r-2} \Delta g_{i,i+1,i+2} + \ldots \quad (1)$$

The first right-hand-side term corresponds to the interaction of isolated binding sites; the second term is an additional contribution given by simultaneous binding of two neighboring binding sites; the third of three, etc. Kříž and Dautzenberg assumed that the first-order (apparent) cooperativity, in which no interaction between ligand binding sites exists except connectivity, is described by the first term only. The first term, however, describes binding of r independent sites, i.e., zero-order cooperativity (noncooperativity). For first-order intraligand cooperativity, the second term must be also taken into account because the remaining binding sites are no longer free after the first site becomes bound. For an array of identical binding sites $\Delta g_i = \Delta g$ and $\Delta g_{i,i+1} = \Delta g'$, and eq 1 can be rearranged for the first-order intraligand cooperativity as

$$\Delta G = \Delta g + (r-1)(\Delta g + \Delta g') \quad (2)$$

Taking into account the general thermodynamic relation between a binding constant, K_r, and the Gibbs energy of binding, $\Delta G = -RT \ln K_r$, eq 2 can be recast as

$$K_r = K_0 K_a^{\,r-1} \quad (3)$$

with $K_0 = \exp(-\Delta g/RT)$ and $K_a = \exp(-(\Delta g + \Delta g')/RT)$.

Binding to a Finite One-Dimensional Lattice. Equilibrium ligand binding between a lattice formed by M binding sites (M-

site lattice) and a ligand corresponding to n complementary sites (n-site ligand) is analyzed in this subsection. First, binding of a n-site ligand that always binds to n lattice sites (i.e., a rigid n-site ligand) is reviewed. As in the whole paper, interligand cooperativity is not considered.

For a rigid ligand, the binding state of a lattice is determined by a number of attached ligands. Elementary binding theory[4] gives the fraction of lattices with the number of ligands attached $k = m$, denoted as x_m:

$$x_m = \frac{P(m)(K_n L)^m}{\sum_{k=0}^{k_{max}} P(k)(K_n L)^k} \tag{4}$$

In eq 4, L is the free concentration of the n-site ligand in solution. K_n is the ligand binding constant, which can be determined from eq 3 with $r = n$ for oligomeric rigid ligands (i.e., one-dimensional arrays of identical complementary binding sites) with first-order intraligand cooperativity. The denominator is called the binding polynomial, and its summation index k goes from zero to the maximum number of n-site ligands which can be placed on the M-site lattice, k_{max}, that is to the greatest integer less than or equal to M/n for rigid n-site ligands. The combinatorial factor $P(k)$ corresponds to the number of ways in which k n-site ligands can be placed on M-site lattice. For a small ligand ($n = 1$), the combinatorial factor is equal to the binomial coefficient, $k_{max} = M$, and the binding polynomial simplifies to $(1 + K_1 L)^M$. Epstein showed[5] that the combinatorial factor for a rigid n-site ligand can be obtained by operationally collapsing bound ligands to small ones. Thus, $P(k)$ for both small and large rigid ligands is generally equal to the number of ways in which k ligands corresponding to a single binding site can be placed on a lattice comprising $M - nk + k$ sites:

$$P(k) = \frac{(M - nk + k)!}{(M - nk)!\, k!} \tag{5}$$

Alternatively, $P(k)$ can be identified with the number of ways in which $M - nk$ vacant lattice binding sites can be arranged along the $(M - nk + k)$-site lattice. With $P(k)$ known, the average number of n-site ligands bound per M-site lattice, k_{avg}, is calculated as

$$k_{avg} = \sum_{k=0}^{k_{max}} k x_k \tag{6}$$

The binding isotherm is obtained by combining eqs 4–6. In order to compare the lattices of different lengths, it is useful to express the extent of binding as an average number of ligands per binding site $v = k_{avg}/M$ or as an average occupancy of lattice binding sites w, which can be also defined as the fraction of occupied lattice binding sites. In a similar fashion, v can be alternatively defined as the fraction of lattice binding sites occupied by the first ligand sites. For completely attached ligands, $w = nv$ and two forms of binding isotherms given as the dependence of v or w on L are equivalent.

The relations for binding of rigid large ligands were derived disregarding the ligand internal structure.[3,5,6,9] The oligomeric nature of ligands becomes essential for semiflexible binding in which an n-site ligand is attached to the lattice in ladder-like fashion by one contiguous block formed by an arbitrary number of binding sites r, $0 < r \leq n$. For such ligands, w becomes an additional independent parameter characterizing binding, with values ranging from v to nv, and the binding isotherms $v(L)$ vs L and $w(L)$ vs L give different information. Similarly, the number of attached semiflexible ligands, k, does not determine fully the binding state of a lattice as the total number of binding sites through which k semiflexible n-site ligands can be attached to the lattice, h, can vary from k to nk. The binding constant for binding of a ligand by r binding sites is given by eq 3 for semiflexible binding with first-order intraligand and no interligand cooperativity. In this case, the values of k and h sufficiently define the binding state of a lattice because the product of k binding constants K_r with $\Sigma r = h$ is equal to $K_0{}^k K_a{}^{h-k}$ and thus does not depend on individual values of r. The binding polynomial becomes a double polynomial in $K_0 L/K_a$ and K_a, and the fraction of lattices with m semiflexible n-site ligands bound to the total of p binding sites, $x_{m,p}$, is given by

$$x_{m,p} = \frac{P(m,p)\left(\frac{K_0 L}{K_a}\right)^m K_a{}^p}{\sum_{h=0}^{M}\sum_{k=k_{min}}^{h} P(k,h)\left(\frac{K_0 L}{K_a}\right)^k K_a{}^h} \tag{7}$$

where k_{min} is the smallest number of n-site ligands which can bind to h lattice binding sites, that is, the smallest integer equal to or greater than h/n. The combinatorial factor $P(k,h)$ is the number of ways in which k n-site ligands can be attached to the total of h sites from a lattice M sites long. Overall extent of binding is then given by

$$v = \frac{1}{M}\sum_{h=0}^{M}\sum_{k=k_{min}}^{h} k x_{k,h} \tag{8}$$

and

$$w = \frac{1}{M}\sum_{h=0}^{M}\sum_{k=k_{min}}^{h} h x_{k,h} \tag{9}$$

To complete derivation of the binding isotherms for semiflexible n-site ligands, the relation for the combinatorial factor $P(k,h)$ is to be derived. We take advantage of the fact that the number of ways in which $M - h$ vacant lattice sites can be arranged between and around k ligands, $P_f(k,h)$, is independent of the distribution of h occupied lattice sites to k ligands and that, conversely, the number of ways how h occupied sites can be divided into k blocks of n sites at most, $P_b(k,h)$, is independent of arrangement of vacancies. Therefore, $P(k,h)$ can be written as

$$P(k,h) = P_f(k,h)\, P_b(k,h) \tag{10}$$

The alternative Epstein approach introduced in eq 5 gives $P_f(k,h)$ as the number of arrangements of $M - h$ vacancies on $(M - h + k)$-site lattice

$$P_f(k,h) = \frac{(M - h + k)!}{(M - h)!\, k!} \tag{11}$$

Determination of $P_b(k,h)$ is more complicated. We start with a special case of oligomeric ligand for which the block of bound sites always starts at the first ligand binding site. This can be achieved by making the first site very strongly binding. The violation of the assumption of identical ligand binding sites does not matter as eq 3 is still valid. Such a special ligand serves not only as an introduction to a general semiflexible ligand, which can have two dangling ends, but may be of interest by itself.

Even in this case, a direct combinatorial approach to $P_b(k,h)$ can be used only for $n > h - k$, when $P_b(k,h)$ can be identified with the number of ways in which $k - 1$ boundaries between k blocks can be drawn at $h - 1$ potential places between h sites. Otherwise, the fact that the block cannot be longer than n sites must be taken into account. A recursive procedure how to calculate $P_b(k,h)$ in this case is describe in Appendix A.

Oligomers without any special binding group are modeled as a linear array of identical binding sites, and the bound block

is allowed to start at any binding site. We denote that as sliding binding. Such an approach is more appropriate for typical oligomeric ligands. The computation of $P_b(k,h)$ in this case must account for the fact that there is $n - r + 1$ ways in which the n-site ligand can be attached through a contiguous block of r binding sites. A direct enumeration procedure for computing $P_b(k,h)$ is given in Appendix B. Once calculated, the values of $P_b(k,h)$ can be collected into a table for further use.

Binding to an Infinite Lattice. The combinatorial approach cannot be used for long lattices as its computational requirements steeply increase with the length of lattice; however, it is possible to derive the relations for binding to a one-dimensional infinite lattice. Binding of large rigid ligands to such a lattice was theoretically studied by McGhee and von Hippel,[3] who used the conditional probability method to get the expression for the extent of binding. They started with the mass-action equation describing binding between a rigid n-site ligand and a sequence of n vacant lattice binding sites:

$$[\text{bound ligand}] = K_n L[n \text{ vacant site sequence}] \qquad (12)$$

where square brackets mean molar concentration. Dividing both sides of eq 12 by the molar concentration of lattice binding sites and by L, we obtain

$$\frac{v}{L} = K_n f_n \qquad (13)$$

where f_n is the fraction of vacant lattice binding sites followed by at least $n - 1$ vacant sites. The determination of f_n is not trivial because a ligand cannot bind in gaps between bound ligands comprising less than n vacant lattice sites (see the "parking problem" above). There are $q - n + 1$ positions in which an n-site ligand can bind in a gap comprising $q \geq n$ sites. Thus, a vacant lattice binding site contributes to f_n only if followed by $n - 1$ vacant binding sites. For rigid ligands, the fraction of occupied lattice binding sites is nv and the fraction of vacant sites is $1 - nv$. Denoting the probability that a vacant site is immediately followed by another vacant site p_{v-v}, a general relation for f_n can be written

$$f_n = (1 - nv)p_{v-v}^{\,n-1} \qquad (14)$$

Even though $1 - nv$ gives the probability that a lattice binding sites is vacant $1 - nv \neq p_{v-v}$ because the randomness of vacant binding sites is distorted by the connectivity of the ligand. A vacant binding site can be followed only by the *first* binding site of a ligand. As in calculation of the combinatorial factor for a rigid n-site ligand on a finite lattice, the correct value of p_{v-v} can be obtained by collapsing the ligand length to one:

$$p_{v-v} = \frac{1 - nv}{1 - nv + v} \qquad (15)$$

Thus, eq 13 becomes

$$\frac{v}{L} = K_n(1 - nv)\left(\frac{1 - nv}{1 - v(n-1)}\right)^{n-1} \qquad (16)$$

The equation describing concomitant binding of i different ligands can be derived from eq 12 in the same way:

$$\frac{v_i}{L_i} = K_i\left(1 - \sum_i n_i v_i\right)\left(\frac{1 - \sum_i n_i v_i}{1 - \sum_i (n_i - 1)v_i}\right)^{n_i - 1} \qquad (17)$$

The subscript i denotes quantities describing the ith ligand.

McGhee and von Hippel[3] were aware of the fact that their approach can be extended to partially bound ligands (i.e., partially dangling ligands) but did not pursue this direction (nor to our knowledge anybody else) even though they derived eq 17 which represents the starting point for the development of the relation for semiflexible binding.

In analysis of the binding of semiflexible oligomers, we are dealing not with a set of different ligands but with one n-site ligand in different binding states, that is, bound by a different number of sites to the lattice. Thus, the index i in eq 17 is no longer an arbitrary assigned identification but identifies the ligand state by the number of bound ligand sites and runs from 1 to n. In this notation, $n_i = i$ and $\sum n_i v_i = w$. The subscript i at L can be dropped because oligomer ligands in different binding states are in equilibrium with the free oligomer ligand. Taking into account that $\sum v_i = v$, eq 17 becomes

$$\frac{v}{L} = \sum_{i=1}^{n} K_i(1 - w)\left(\frac{1 - w}{1 - w + v}\right)^{i-1} \qquad (18)$$

or taking into account that $\sum n_i v_i = w$

$$\frac{w}{L} = \sum_{i=1}^{n} iK_i(1 - w)\left(\frac{1 - w}{1 - w + v}\right)^{i-1} \qquad (19)$$

For the binding starting at the first binding site of the oligomer ligand we can use K_i given by eq 3. For sliding binding, the existence of different ways in which an oligomer can be attached must be accounted for:

$$K_i = (n - i + 1)K_0 K_a^{\,i-1} \qquad (20)$$

After substituting eq 20 into eqs 18 and 19, all the summations are found to correspond to the summation of several first terms of a geometrical series or of its first or second derivatives, and eqs 18 and 19 can be rewritten as

$$v = K_0 L(1 - w)\frac{(n+1)(1-q) - (1 - q^{n+1})}{(1-q)^2} \qquad (21)$$

$$w = K_0 L(1 - w)\frac{n(1 - q^{n+2}) - (n+2)q(1 - q^n)}{(1-q)^3} \qquad (22)$$

with

$$q = \frac{K_a(1 - w)}{1 - w + v} \qquad (23)$$

Thus, the values of v and w can be found for given L, n, K_0, and K_a by simultaneously solving eqs 21–23.

Kinetics of Semiflexible Oligomer−Polymer Binding: Stochastic Simulation

Binding kinetics of a rigid n-site ligand (oligomer) to a finite lattice in the absence of interligand cooperativity is reviewed before dealing with a semiflexible ligand. The number of bound rigid large ligands, k, does not define fully the lattice binding state from a kinetic point of view; the arrangement of ligands on the lattice (microstates) must be taken into account. Transition between microstates occurs in two elementary processes: (i) separation of a bound n-site ligand from the lattice and (ii) attachment of an n-site ligand to a sequence of n vacant lattice sites. The number of the processes (i) that can occur on a lattice with k bound ligands is of course k. The number of the processes (ii) depends on the mutual position of the bound ligands. As discussed in the previous subsection, an additional ligand cannot bind in gaps comprising less than n vacant lattice sites. A ligand can bind in $q - n + 1$ ways in a gap comprising $q \geq n$ sites. Thus, for example, the 10-site lattice with two 3-site rigid ligands at the lattice ends has two different 3-site sequences of vacancies but none with ligands located in the lattice middle. The elementary processes can be described by standard deterministic rate equations with the rate constant k_f for the process (i) and k_b for the process (ii) because the ligand consists of identical

binding sites and the same holds for the lattice. Thus, the set of ordinary differential equations describing the rate of transition between microstates can be, in principle, written down for the complete set of possible microstates. As the number of the microstates increases steeply with the number of lattice sites, the deterministic formulation of binding kinetics becomes intractable even for relatively short lattices. An essentially exact solution, however, can be obtained by stochastic simulation.[6,33,34]

In stochastic formulation of binding kinetics,[6,33,34] the state of the system is defined not by concentrations of the lattice in N permissible microstates but as the N-dimensional vector of microstate populations; that is, the state of the system is defined as a point in the N-dimensional space. The temporal evolution of the system then takes the form of a Markovian random walk in this N-dimensional space, and reaction rates become probabilities of the processes per unit time. For binding of a rigid large ligand to a one-dimensional array of binding sites, the random walk is realized in elementary processes (i) and (ii), and the stochastic form of basic kinetic equations becomes

$$p_+ = k_b L \tag{24}$$

$$p_- = k_f \tag{25}$$

where $p_+ \, dt$ gives the probability that an n-site ligand binds to a certain sequence of n vacant lattice binding sites within the time interval t; $t + dt$, and p_- gives the analogous probability that the certain bound ligand separates from the lattice. Monte Carlo techniques are employed to simulate Markovian random walk. In the straightforward implementation, the state of the system at the time $t + \Delta t$ is obtained from the state at time t as follows. All elementary processes possible at time t are identified. A random number R from the unit-interval uniform distribution is generated for each process and compared with the probability p calculated from eq 24 or 25. If $R < p\Delta t$, the state of the system is adjusted according to the process. The state of the system at $t + \Delta t$ obtained after going through all possible elementary processes is an adequate approximation of the solution of deterministic rate equations for sufficiently high number of lattices in the system, S. Setting $t = t + \Delta t$, the state in the time $t + 2\Delta t$ can be obtained by repeating the described procedure (a simulation step), and so on. The selection of Δt is critical for the successful implementation of the algorithm. If too short Δt is chosen, no change may occur for many consecutive simulation steps and the simulation becomes inefficient. On the other hand, several processes may occur during long Δt. This is not a problem for processes occurring on different lattices because behavior of the lattice does not depend on the state of other lattices in infinitely dilute solution considered here. That is also the reason why the stochastic simulation does not need to be done for all S lattices in the system at once, but evolution of the population statistic can be obtained by repeating the simulation for a single lattice for S times. However, several elementary processes can occur even on a single lattice during Δt, and some of them can be mutually exclusive. The probability of the co-occurrence of the elementary processes can be decreased by shortening Δt, at the expense of simulation efficiency, but never avoided because the interval between two consecutive elementary processes is a random variable by itself.

The elegant way out of the quandary is to recognize randomness of the interval between two consecutive elementary processes and use it to our advantage. The question is no longer which elementary processes occur during a predetermined time interval, but a Monte Carlo approach is used twice in each simulation step—first to find at what time the next elementary process occurs and then which of the possible elementary processes it is. In this way not only co-occurrence of elementary processes is avoided but also simulation efficiency is increased as unfruitful steps are absent, and even though the inventory of possible elementary processes must be updated in each step, only two random numbers are drawn. The fast simulation algorithm was introduced to chemical kinetics by Gillespie[33,34] and used for the binding of large rigid ligands to a one-dimensional lattice by Reiter and Epstein.[9]

The simulation step of the fast algorithm starts by the inventory in which numbers of each type of the possible elementary processes are determined. For the sake of generality, let us say that there are ζ types of the number of elementary processes; for the binding of rigid large ligands $\zeta = 2$. Then the overall probability $p \, dt$ that any of the possible processes occurs within the time interval dt is given with p from eq 26

$$p = \sum_{i=1}^{\zeta} \eta_i p_i \tag{26}$$

where η_i is the number of the elementary processes of the type i. The product $p_i \, dt$ gives the probability that the process of type i occurs during dt. For binding a rigid large ligand, p_i is given by one of eqs 24 and 25. The random time t_p at which the next elementary process occurs is generated using a random number R_1 from the unit-interval uniform distribution

$$t_p = t - \frac{\ln R_1}{p} \tag{27}$$

where t is the initial time of the simulation step.

During the inventory, possible elementary process are numbered from 1 to $\Sigma\eta$. The number of the elementary process that is to occur, z, is determined so that the relation 27 holds

$$\sum_{i=0}^{z-1} p_i \le R_2 p < \sum_{i=1}^{z} p_i \tag{28}$$

where R_2 is the second random number drawn in the simulation step, $p_i \, dt$ gives the probability that the ith elementary process occurs during dt, and $p_0 = 0$. The simulation step ends after the state of the system is adjusted by the elementary process z. The simulation steps are repeated until some criterion is met, for example $t_p > t_{final}$. If the simulation run is done for a single lattice, it should be repeated many times and the results averaged in order to get a reliable estimate of the binding kinetics. The consecutive simulation runs are possible for infinitely dilute lattice solutions not only because the lattices are independent but also because the binding of a ligand to the lattice does not change free ligand concentration, L, used in eq 24. In practice, the change in the free-ligand concentration usually occurs and can be included in the simulation; however, parallel simulations for all lattices have to be done and free ligand concentration adjusted after each simulation step according to the elementary process chosen.[9]

The fast simulation algorithm for semiflexible binding is the same as for the rigid one, but a different set of elementary processes must be considered. Any vacant binding site can become occupied regardless of the gap size, and a free ligand can attach through any of its binding sites. Thus, for semiflexible binding, eq 24 describes a process in which a certain free lattice binding site becomes occupied by an arbitrary binding site of some free ligand. Binding of an attached ligand can proceed in a zipper-like way—vacant ligand and lattice binding sites adjacent to occupied ones and facing each other can couple. The probability that a certain possible zipper-like bindings occurs during dt is $p_+' \, dt$ and is related to the rate constant of the process k_z

$$p_+' = k_z \tag{29}$$

The zipper-like mechanism applies also to ligand detachment—only outer-bound ligand sites can become free. We assume the single

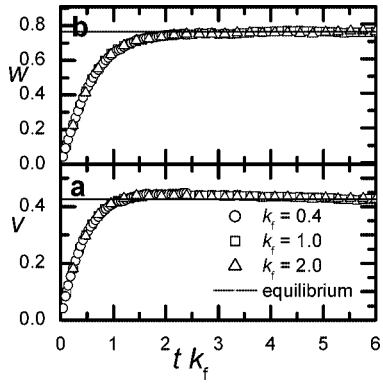


Figure 1. Comparison of the kinetics of binding with the equilibrium results for a semiflexible ligand comprising 10 binding sites and a lattice comprising 30 binding sites with $K_0L = 0.1$ and $K_a = 1.5$. The stochastic simulation was carried out for three different rates of binding as given by the values of k_f; 500 runs were accumulated in each simulation. The equilibrium values obtained by the combinatorial method are indicated by full straight lines. (a) Time evolution of the average number of ligands per binding site, v. (b) Time evolution of the average occupancy of lattice binding sites, w.

detachment probability given by eq 25 for unzipping including the final detachment of the ligand.

The kinetic parameters of eqs 24, 25, and 29 are related to equilibrium binding constants because the rates of attachment and detachment processes must equal at equilibrium:

$$\begin{aligned} K_0 &= k_b/k_f \\ K_a &= k_z/k_f \end{aligned} \tag{30}$$

All sets of k_b, k_f, and k_z complying with the same values of K_0 and K_a give the identical dependence of v or w on $k_f t$ because arbitrary time units can be used in the description of the binding kinetics.

Proper "bookkeeping" is crucial for successful implementation of the stochastic simulation to kinetics of large-ligand binding.[9] Description of the semiflexible ligand binding brings own challenges in this respect. Some details on the procedures used for calculating the numerical examples given below are presented in Appendix C.

Numerical Examples and Discussion

Numerical examples obtained by application of the procedures outlined above are presented to illustrate three main points: (1) the agreement between results of these procedures; (2) general and unexpected features of semiflexible binding; (3) qualitative agreement with experimental findings.

It is seen in Figure 1 that the stochastic simulation of binding of a semiflexible oligomer ligand to a longer lattice agrees with the results of equilibrium binding at long times both for the average number of ligands per binding site, v, and the average occupancy of lattice binding sites, w. The stochastic simulation was carried out for three different rates, and the results are equivalent if plotted against the scaled time tk_f, as predicted. An interesting feature of the plot is that while the average occupancy increases smoothly toward the equilibrium value, the number of bound ligands grows above the equilibrium value at intermediate times before decreasing to the equilibrium value again. Such behavior might be expected for high equilibrium occupancy as the lattice site freed after a ligand detachment from the lattice can be occupied by an adjacent ligand before a free ligand is attached again. While for small ligands ($n = 1$) higher-than-equilibrium occupancy would be a purely statistical phenomenon, disappearing with the increasing number of simulations runs, the overshooting displayed for large ligands is inherent to the process.

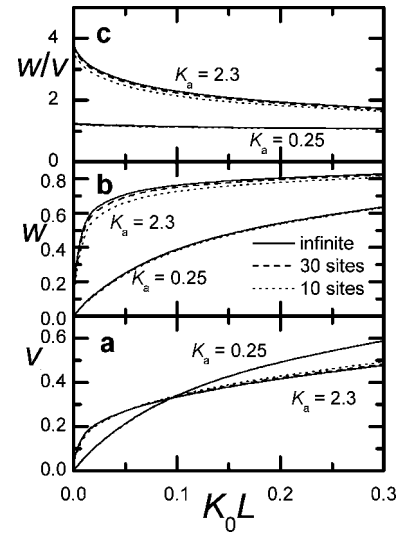


Figure 2. Effect of lattice length on the average number of ligands per lattice binding site, v (panel a), on the average occupancy of a lattice site, w (panel b), and the average number of bound sites per ligand, w/v (panel c). The ligand consists of five identical binding sites with $K_a = 2.33$ and $K_a = 0.25$. Binding isotherms are drawn for the infinite lattice and for the lattices comprising 30 or 10 binding sites.

Figure 2 presents the binding isotherms v vs K_0L (Figure 2a) and w vs K_0L (Figure 2b) for two semiflexible ligands and lattices of various lengths. The ligands comprise five binding sites each but have different binding flexibility, that is, the different values of K_a: 2.3 the more rigid one and 0.25 the more flexible one. The results for finite lattices obtained by modified combinatorial methods are consistent with those for the infinite lattice obtained by the modified conditional probability method. The effect of the lattice length is minor for the more rigid ligand and almost negligible for the more flexible one. Generally, the effect of the lattice length is the manifestation of the "parking problem", which for the semiflexible binding is given not by the ligand length but by the average number of binding sites through which the ligand is attached to the lattice, r_{avg}. The insignificant "parking problem" may be expected for a ligand with small K_a and r_{avg} only slightly higher than 1. On the other hand, r_{avg} can approach n for a ligand with high K_a at low lattice saturation (low w); as the fraction of occupied binding sites increases, however, the "parking problem" sets in. The semiflexible ligand can cope with the situation by decreasing r_{avg}. These features of semiflexible binding are ilustrated in Figure 3c where the binding isotherms are combined to provide the dependence of r_{avg} on K_0L ($r_{avg} = w/v$) for both ligands. The value of r_{avg} decreases from 1.25 to 1.07 for the more flexible ligand and from about 4 to 1.7 for the more rigid one as K_0L increases from 0 to 0.3. The distinct effect of the lattice length is observed in the latter case. Figure 2c can explain some features found with the binding isotherms. The average lattice-site occupancy, w, given by effective strength of binding, is always higher for the more rigid ligand with a higher value of K_a. The average number of ligands per binding site, v, is more sensitive to the spatial arrangement of ligands on the lattice. The value of v is higher for the more rigid ligand at low K_0L only. The effect of lattice length is minor even for the more rigid ligand nevertheless interesting. While w is always higher for a longer lattice, the same applies to v only at low K_0L and higher v is found for a shorter lattice at high K_0L.

The effect of the ligand length is explored in the next set of results. Since the ligands consist of a various number of identical binding sites, it is more appropriate to make the comparison on the mass rather than molar scale and to express the free ligand concentration as the concentration of binding sites, nL. As in

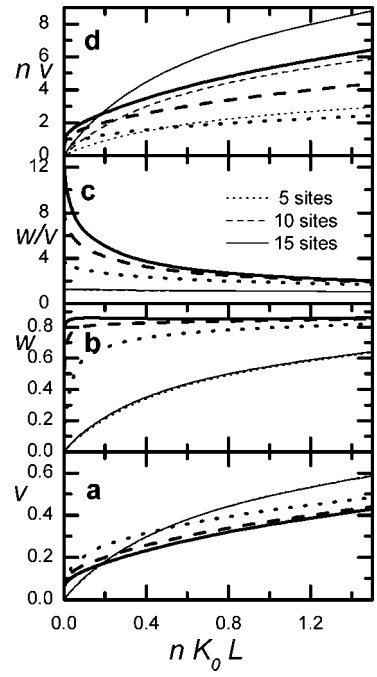


Figure 3. Effect of the ligand length on the average number of ligands per a lattice site v (panel a), the average occupancy of lattice binding sites w (panel b), the average number of bound sites per ligand w/v (panel c), and the total number of ligand binding sites (both bound and dangling) per lattice binding site nv (panel d). nK_0L is the binding-site-based dimensionless concentration of a free ligand. The ligand lengths $n = 5$, 10, and 15 binding sites; the lattice comprises 30 binding sites. Results are given for two ligands of different binding flexibility $K_a = 0.25$ (thin lines) and $K_a = 2.33$ (thick lines).

the case of the lattice length, the ligand length has a negligible effect on the binding isotherms v vs nK_0L (Figure 3a) and w vs nK_0L (Figure 3b) for more flexible ligand ($K_a = 0.25$). The data for $n = 5$ were already presented in Figure 2 and showed that the more flexible ligand ($K_a = 0.25$) is attached to the lattice predominantly through one binding site only. That applies also to ligands with $n = 10$ and 15 (Figure 3c). After substituting $i = 1$ into eq 20, it is seen that the effective overall binding constant can be approximated as nK_0, and the coincidence of the binding isotherms v vs nK_0L or w vs nK_0L for the more flexible ligand is understandable. On the other hand, it is equally understandable that the effect of the ligand length for the more flexible ligand becomes very strong if the extent of binding is expressed on mass scale as nv, that is, as the total number of ligand binding sites, both bound and dangling, attached to a lattice binding site (Figure 3d). For the more rigid ligands ($K_a = 2.3$), the effect of the ligand length is observed not only with the binding isotherm nv vs nK_0L but also with both v vs nK_0L and w vs nK_0L. However, the effect of the ligand length is opposite in the last two cases. While the increased ligand length promotes the average lattice site occupancy w, the number of attached ligands per a lattice binding site v is higher for shorter lattices as the "parking problem" is less severe.

The results presented so far have demonstrated that the extent of binding has to be explicitly defined for the semiflexible oligomer−polymer binding. Not only are there two basic binding isotherms v vs K_0L and w vs K_0L but these can be combined and/or modified to provide some specific information. The combinations/modifications which can be compared with experiment will be used in the following examples.

For the ligand and lattice binding sites carrying opposite but unit charges, the net number of unit charges of the complex per lattice binding site is $1 - nv$. For the rigid binding, the quantity $1 - nv$ is proportional to the dimensionless linear

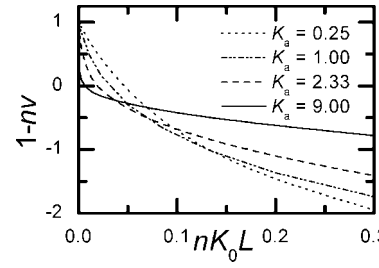


Figure 4. Net charge of an oligomer−polymer complex per lattice site $1 - nv$. Oligomeric ligand comprises five negatively charged binding sites, and polymeric lattice comprises 30 positively charged binding sites. The oligomer flexibility is given by values of $K_a = 0.25$, 1, 2.33, and 9.

charge density ξ used in the theory of polyelectrolytes and defined as the ratio of the Bjerrum length and the average axial spacing of charge sites.[35] Although the relation between ξ and $1 - nv$ may be formally applied also for the semiflexible binding, it is not physically justified. Because of the ligand dangling ends, the complex resembles a graft copolymer, and the overall charge is given by the compensation of opposite charges on the spatially separated groups.

The model used makes no assumptions about the nature of forces responsible for binding between ligand and lattice sites. The only restriction is given by eq 2, which allows only for the one-to-one contact interactions between ligand and lattice binding sites and the nearest-neighbor interactions between the lattice binding sites occupied by the same ligand. Thus, the model can be applied to polyelectrolytes at the suitable concentration of the supporting low-molecular-weight electrolyte which makes the electrostatic interactions adequately screened and effects such as counterion release or interligand negative cooperativity due to dangling charged ligand ends negligible. An example of the dependence of the net charge $1 - nv$ on the concentration of the free anionic ligand is given in Figure 4 for several values of K_a. It is seen that the overall charge of the complex changes the sign as the concentration of the free ligand increases. Thus, the model predicts the existence of the experimentally observed[27,28] charge inversion of a polymer complex upon addition of a charged ligand to a solution of an oppositely charged lattice. Such qualitative agreement with experiment is significant because the charge inversion is not predicted for rigid ladder-like binding for which $1 - nv \geq 0$. One of the reasons why the quantitative agreement between the theory and experiment cannot be expected is aggregation or cross-linking of lattice−ligand complexes. However, even those not fully compensated charged ligands on the surface of formed nanoparticles appear to be bound by Coulombic interactions, thus complying with the present concept of flexible bindings.[36] The idea of partially bound polyelectrolytic ligands has been developed to the theory explaining the counterintuitive phenomenon of charge inversion.[37] The theory is based on electrostatic consideration; here, we show that the analogous results can be obtained for semiflexible large ligand binding in general by combinatorial considerations.

The properties of the complex can be significantly influenced by its net charge. For example, if nanoparticles are to be prepared, the charge can prevent aggregation[36] but also disintegrate the particle if is too high.[27] Figure 4 indicates two ways of manipulating the net charge of the oligomer−polymer complex: by changing the ligand concentration or the amount of the added low-molecular-weight salt. The propensity of a free ligand to attach through one binding site to the lattice, given by K_0L, is affected in both cases; the ligand flexibility, given by K_a, only in the latter.

The assumption that the concentration of the lattice is infinitely small has two consequences that make the theory and

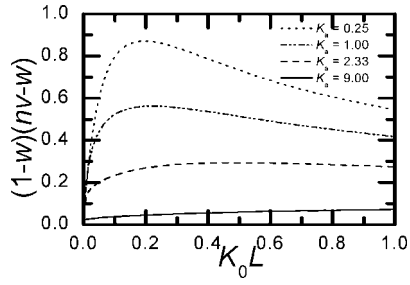


Figure 5. Aggregation propensity of the oligomer−polymer complexes given as the product of the content of dangling ligand binding sites and the content of unoccupied lattice binding sites. The ligands comprise five binding sites and have $K_a = 0.25$, 1, 2.33, and 9. The lattice comprises 25 binding sites.

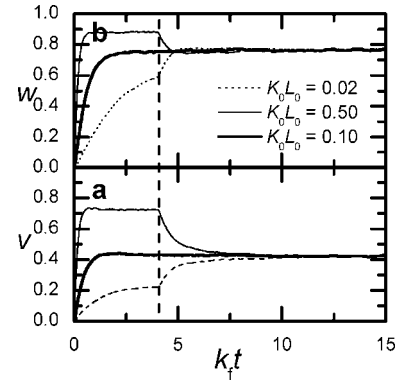


Figure 6. Results of the stochastic simulation of reorganization of the complex after jump in the ligand concentration. A dashed vertical line indicates the time of the change in the values of K_0L to 0.1. The initial values of K_0L_0 were 0.02, 0.1, and 0.5. $K_a = 1.5$ in all simulations, consisting of 500 runs each. The ligand comprises 10 binding sites and the lattice 30. Panel a gives the average number of ligands per lattice binding site; panel b gives the average occupancy of a lattice site.

simulations more manageable. First, the free ligand concentration is not affected by binding to the lattice. This is not essential but makes computation easier; moreover, constant free ligand concentration can be maintained experimentally. The other consequence is the absence of cross-linking and aggregation. These phenomena are much more difficult to be treated theoretically and avoided experimentally; however, some indirect information can be obtained even with the infinitely dilute-lattice approach.

At fixed lattice concentration, the proportionality may be assumed between the cross-linking propensity and the product of the number of free lattice binding sites and of the number of dangling ligand binding sites $(1 - w)(nv - w)$. An example of the dependence of the product on the ligand concentration is given in Figure 5; the dependence goes through a maximum if the ligand is flexible enough. The product generally decreases as the value of K_a increases and displays no maximum for a high value of K_a.

Because the value of K_a for polyelectrolyte complexes can be modified by addition of low-molecular-weight salt, Figure 5 can be confronted with experiment. Chen et al.[31] measured turbidity while titrating poly(diallyldimethylammonium chloride) with poly(potassium vinyl sulfate) and vice versa in the presence of various amounts of a low-molecular-weight salt. The turbidity titration curves measured at high ionic strengths displayed a maximum. At low ionic strengths, the turbidity either leveled off or decreased only slightly after passing through the maximum. In pure water the increase in turbidity was negligible. Taking into account that the strength of Coulombic interactions decreases with increasing ionic strength, the observation of Chen et al. is in agreement with Figure 5, which shows more and more pronounced maxima for decreasing values of K_a. Chen et al.[31] tentatively attributed a dip in the turbidity at highest levels of titrant addition to weakening of complexation interactions at high salt levels. The present results substantiate this notion. As suggested above, the values of K_0L and K_a for polyelectrolytes can be manipulated by addition of low-molecular-weight electrolyte; the procedure can be generalized for uncharged polymers as an addition of suitable cosolvent and used for controlling precipitation in solutions of complexing polymers. The presented theory may then serve as a guide.

The data obtained by the combinatorial and conditional probability methods describe equilibrium binding; nevertheless, they can also shed some light on nonequilibrium phenomena such as the effect of the addition rate or mixing on properties of oligomer−polymer complexes. After addition of a highly concentrated droplet of a ligand to a large volume of lattice (or vice versa), the concentrations at which complexation occurs are different from the overall ones. The local concentration of the added component is higher whereas the bulk component is temporarily depleted. If these temporal local concentrations are favorable for cross-linking (cf. Figure 5), aggregates can be formed, which—if kinetically stable—can survive equilibration of the ligand concentration.

The kinetic stability of aggregates may reflect the stability of individual ligand−lattice complexes or the complexes may be stabilized by mutual cross-linking. The stochastic model developed here clearly shows (Figure 6) that the reorganization of individual oligomer−polymer complexes after a jump in the ligand concentration is comparably fast as the complex formation from scratch, for both the concentration increase and decrease. Thus, if the effect of addition rate resulted from a difference in local and overall concentrations, the stabilization of formed structures would be caused by aggregation, i.e., by cross-linking in the present context.

Limitations and Prospects

The proposed extension of the theory of large ligand binding is based on some assumptions probably not fully met in real oligomer−polymer systems. Some of them such as infinite lattice concentration have been already discussed. The basic assumption of the theory is equality of all binding sites in the oligomer as well as equality of all binding sites in the lattice. However, some differences between terminal and inner sites might be expected in both binding enthalpy (due to the presence of end groups) and binding entropy (due to different conformational entropies of a chain attached through a terminal site and through an inner one). Another assumption that can be challenged is that of noncooperativity. For example, slight interligand anticooperativity may be expected due to the crowding of dangling ends of adjacent ligands, which would be much more pronounced with charged ligands. In stochastic simulations, each possible process is treated independently; therefore, the variability of binding or kinetic constants and of anticooperativity might be implemented, at least in principle. The experimental data discussed deal predominantly with polyelectrolytes for which the theory is applicable but only in the presence of adequate amount of a low-molecular-weight salt because long-range forces are not considered.

Long-range forces, full ligand flexibility (i.e., loops), or the finite lattice concentration (i.e., aggregation) makes the interactions between individual ligand and lattice binding sites a function of their coordinates and overall configuration of the complex/aggregate. Incorporation of more structural and functional details into the model would mean not just another

extension but a fundamental change, ultimately leading to coarse-grain or even atomistic simulation techniques of polymer science.

Why, then, analyze oligomer−polymer binding by the extension of the combinatorial and conditional probability methods and related stochastic simulation rather than, for example, by direct application of molecular dynamics? The main reason is that the current computational resources do not allow for the problem to be simulated in full atomistic representation. Therefore, some reduction in the level of detail is inevitable. The model developed here is in fact at the opposite end of the scale than the atomistic simulations. The reduction, not only spatial but also functional, is absolute, and only the features defining the oligomer−polymer binding are included. Thus, the phenomena reproduced or predicted by the model are essential for the process. In spite of the conceptual simplicity, additional simplifying assumptions were necessary in order to obtain numerical results. Thus, further development of the model can continue in two intertwining ways: by relaxation of the assumptions and by incorporation of the results of general polymer science.

Acknowledgment. The support by the Grant Agency of the Academy of Sciences of the Czech Republic (project IAA100500501) is acknowledged.

Appendix A

The problem is to determine the number of ways in which h elements can be arranged into k numbered groups of which none can contain more than n elements. We will call such groups containers of size n and designate the number $P(k,h,n)$. Since at least one element must be in each container, the sought number is equal to the number of arrangements for placing $h - k$ elements to k or less containers having size reduced to $n - 1$. If the number of arrangements with containers allowed to be empty is denoted $P'(k,h,n)$, then $P(k,h,n) = P'(k,h-k,n-1)$. Note that from now on h, k, and v mean parameters of the function P' rather than their initial values.

In some cases, $P'(k,h,n)$ can be computed directly in a single step. If $h \le n$, then the container size is irrelevant and

$$P'(k,h,n) = \frac{(h+k-1)!}{h!\,(k-1)!} \tag{A1}$$

Similarly, we can directly compute P' if $n(k-1) \le h$ because in that case we distribute vacancies rather than elements, and the container size limit is again irrelevant as it is used only to determined the number of vacancies

$$P'(k,h,n) = \frac{(nk+k-h-1)!}{(nk-h)!\,(k-1)!} \tag{A2}$$

And finally, for $n = 1$ for which $h \le k$ must hold

$$P'(k,h,1) = \frac{k!}{h!\,(k-h)!} \tag{A3}$$

In all other cases, we proceed recursively. The number of arrangements with i completely filled containers is equal to the number of ways how to select i from k containers multiplied by the number of arrangements for remaining elements and remaining containers, reduced in size by 1 to keep the number of completely filled containers equal to i. To get P', the products for all permissible i are summed.

$$P'(k,h,n) = \sum_{i=s}^{f} \frac{k!}{(k-i)!\,i!} P'(h-in, k-i, n-1) \tag{A4}$$

where the index i corresponds to the number of filled containers and runs from

$$\begin{aligned} s &= 0 && \text{for} \quad h \le k(n-1) \\ s &= h - k(n-1) && \text{for} \quad h > k(n-1) \end{aligned}$$

to

$$f = \text{floor}(h/n)$$

where floor(a) means the greatest integer smaller than a.

Recursion is stopped whenever a condition for direct calculation is met.

Appendix B

For sliding binding of semiflexible oligomers, the number of ways how to fully cover a linear lattice composed of h binding sites by k partially attached n-site ligands, $P(k,h,v)$, is higher than that for binding starting always at the first binding site (see Appendix A) because there is $n - r + 1$ ways in which the n-mer can be attached through a contiguous block of r binding sites. Unfortunately, such an increase is not identical for all configurations complying with a given set of h, k, and n. For example for $h = 6$, $k = 2$, and $n = 5$, the number of configurations is $3 \times 3 = 9$ for $r_1 = 3$ and $r_2 = 3$, whereas it is $1 \times 5 = 5$ for $r_1 = 5$ and $r_2 = 1$. Thus, it is not possible to use combinatorial identities, and all possible configurations must be run through as we sequentially place the oligomers starting from one side of the lattice. The recursive relation has the form

$$P(k,h,n) = \sum_{i=s}^{f} (n-i+1) P(h-i, k-1, n) \tag{B1}$$

The index i corresponds to the number of sites occupied by the currently placed oligomer and generally can run from 1 to n but in order to cover completely the lattice by k oligomers $h - i \ge k - 1$ and $h - i \le (k-1)n$. Thus

$$\begin{aligned} s &= 1 && \text{for} \quad h - 1 \le (k-1)n \\ s &= h - (k-1)n && \text{for} \quad h - 1 > (k-1)n \end{aligned}$$

and

$$\begin{aligned} f &= n && \text{for} \quad h - n \ge k - 1 \\ f &= h - k + 1 && \text{for} \quad h - n < k - 1 \end{aligned}$$

The scheme is completed by the initial value $P = 1$ for $k = 0$.

Appendix C

Stochastic simulation of the binding of an n-site-ligand to an M-site lattice requires a precise knowledge of the lattice state at every simulation step. The method for binding of a semiflexible ligand used in calculation of the numerical examples given above is described here. The state of a lattice is recorded using an array of $M + 2$ integers. The first integer (with the index $i = 0$) and the last one ($i = M + 1$) are always set to zero. Integers $i = 1$ to $i = M$ record the state of the ith lattice binding site. Zero means that the corresponding binding site is vacant; any other value says which one of the ligand binding sites, numbered from left to right, is attached. To store just the position of the binding site on the ligand is not sufficient since two or more ligands can be bound to the lattice in such a way that a continuous sequence of numbers is created, mimicking the attachment of one ligand. Therefore, the integer sign provides additional information. The sequence of integers corresponding to the same ligand is assigned the opposite sign than the sequence of the immediately preceding ligand. If conflict occurs after gap closing, the signs of integers to the right of the original gap are reversed as necessary, to keep the rule.

The state of the lattice is transformed into possible elementary processes by deciding which processes are allowed at each lattice site. The possible elementary processes at site i with the value of the corresponding integer $h(i)$ are (i) occupation by a new n-site ligand if $h(i) = 0$; (ii) occupation by the left neighbor if

$h(i) = 0$ and $0 < |h(i-1)| < n$; (iii) occupation by the right neighbor if $h(i) = 0$ and $|h(i+1)| > 1$; (iv) dangling end extension (unzipping) if $h(i) \neq 0$ and $\mathrm{sgn}(h(i-1)) = \mathrm{sgn}(h(i)) \neq \mathrm{sgn}(h(i+1))$ or $\mathrm{sgn}(h(i-1)) \neq \mathrm{sgn}(h(i)) = \mathrm{sgn}(h(i+1))$; (v) ligand detachment if $h(i) \neq 0$ and $\mathrm{sgn}(h(i-1)) \neq \mathrm{sgn}(h(i)) \neq \mathrm{sgn}(h(i+1))$.

On the first pass through integers from 1 to M, probabilities of possible elementary processes given by eq 19 for (i), eq 20 for (ii) and (iii), and eq 24 for (iv) and (v) are summed to give overall probability p, which is used with a random number R_1 in eq 22 to determine the time of the next occurring process, t_p. After drawing the second random number R_2, the second pass commences and continues until the condition given by eq 23 is met and thus the realized elementary process is found. The elementary process changes only the state of the lattice site i at which it occurs; therefore, only the value of the ith integer is changed in order to record the new state of the lattice. The assigned value depends on the elementary-process type and is for the process (i) $-u$ if $h(i-1) > 0$ and u otherwise, u being the index number of the attached ligand site; (ii) $h(i-1) + \mathrm{sgn}(h(i-1))$; (iii) $h(i+1) - \mathrm{sgn}(h(i-1))$; (iv, v) 0. If $\mathrm{sgn}(h(i+1)) = \mathrm{sgn}(h(i))$ for the process (i) or (ii), then the integers from $(i+1)$th up are multiplied by -1 until zero value is found. A similar procedure but starting at ith is necessary for the process (iii) if $\mathrm{sgn}(h(i-1)) = \mathrm{sgn}(h(i))$.

The number of bound ligands, V, is increased by one by the process (i) and decreased by one by the process (v). The number of bound ligand sites attached, W, is increased by one by the processes (i)−(iii) and decreased by one by processes (iv) and (v). If the simulation is completed in one run, as if the state of all Q lattices in the system is considered and adjusted at each simulation step, the values of t_p, V, and W are stored after each simulation step to form a record of binding evolution. If the simulation is done in Q runs, each for a single lattice, such recording is not possible since an different sequence of t_p is obtained in each run. Therefore, the binding state of the system is recorded at predetermined sampling times, identical for all Q runs. The simple way is to record the actual state at the sampling time, t_s. For example, if the next elementary process is found at t_p at the step with the starting time t, then the individual run values of V and W at t are added to the simulation overall values of V and W at all t_s such that $t \leq t_s \leq t_p$. In this way, however, the results of many simulations steps are ignored if $\Delta t_s \gg t_p - t$; therefore, in order to decrease the noise the average run values of V and W in the time interval from $t_s - \Delta t_s$ to $t_s + \Delta t_s$ are added to the simulation overall values of V and W at t_s. After the simulation is completed, the final overall values of V and W are converted to v and w for each t_s, using the relations $v = V/QM$ and $w = W/QM$.

The simultaneous simulation can be used for a finite molar concentration of the lattice, c_{LT}, low enough to neglect the lattice cross-linking. In this case, also the free ligand molar concentration, L, is a function of time and must be adjusted in each simulation step. An elementary process (v) decreases and an elementary process (i) increases L by c_{LT}/Q.

References and Notes

(1) Klotz, I. M. *Arch. Biochem.* **1946**, *9*, 109–117.
(2) Scatchard, G. *Ann. N.Y. Acad. Sci.* **1949**, *51*, 660–672.
(3) McGhee, J. D.; von Hippel, P. H. *J. Mol. Biol.* **1974**, *86*, 469–489.
(4) Schellman, J. A. *Biopolymers* **1975**, *14*, 999–1018.
(5) Epstein, I. R. *Biophys. Chem.* **1978**, *8*, 327–339.
(6) Epstein, I. R. *Biopolymers* **1979**, *18*, 2037–2050.
(7) Hill, T. L.; Eisenberg, E.; Greene, L. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3186–3190.
(8) Smith, R. F.; Ackers, G. A. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 5347–5351.
(9) Reiter, J.; Epstein, I. R. *Biopolymers* **1990**, *29*, 543–547.
(10) Nykas, Y. J.; Blankchstein, D. *Langmuir* **1994**, *10*, 3512–3528.
(11) Wang, L.; DiCerra, E. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 12953–12958.
(12) DiCerra, E.; Kong, Y. *Biophys. Chem.* **1996**, *61*, 107–124.
(13) Bekturov, E. A.; Bimendina, L. A. *J. Macromol. Sci., Part C: Polym. Rev.* **1997**, *37*, 501–518.
(14) Nishio, T.; Shimizu, T.; Kwak, J. C. T.; Minakata, A. *Biophys. Chem.* **2003**, *104*, 501–508.
(15) Liu, J.; Li, Q. *Chem. Phys. Lett.* **2006**, *422*, 67–71.
(16) Teif, V. B. *Nucleic Acids Res.* **2007**, *35*, e80.
(17) Tsuchida, E. *Macromolecular Complexes, Dynamic Interactions and Electronic Processes*; VCH Publishers: New York, 1991.
(18) Nurkeeva, Z. S.; Mun, G. A.; Khutoryanskiy, V. V. *Macromol. Biosci.* **2003**, *3*, 283–295.
(19) Jiang, M.; Li, M.; Xiang, M.; Zhou, H. *Adv. Polym. Sci.* **1999**, *146*, 121–195.
(20) Kříž, J.; Dautzenberg, H. *J. Phys. Chem. A* **2001**, *105*, 3846–3854.
(21) Texter, J. *J. Chem. Phys.* **1989**, *91*, 6295–6301.
(22) Thunemann, A. F.; Muller, M.; Dauzentberg, H.; Joanny, J.-F.; Hartmut, L. *Adv. Polym. Sci.* **2004**, *166*, 113–171.
(23) Kabanov, A. V.; Kabanov, V. A. *Bioconjugate Chem.* **1995**, *6*, 7–20.
(24) Wagner, E.; Kloeckner, J. *Adv. Polym. Sci.* **2006**, *192*, 135–173.
(25) Michaels, A. S. *Ind. Eng. Chem.* **1965**, *57*, 32–40.
(26) Thunemann, A. F.; Muller, M.; Dautzenberg, H.; Joany, J.-F.; Lowen, H. *Adv. Polym. Sci.* **2004**, *166*, 113–171.
(27) Veron, L.; Ganee, A.; Ladaviere, C.; Delair, T. *Macromol. Biosci.* **2006**, *6*, 540–554.
(28) Gonzáles Ferreiro, M.; Tillman, L.; Hardee, G.; Bodmeier, R. *J. Controlled Release* **2001**, *73*, 381–390.
(29) Nagura, M.; Saitoh, H.; Gotoh, Y.; Ohkoshi, Y. *Polymer* **1996**, *37*, 5649–5652.
(30) Dragan, E. S.; Schwarz, S. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 5244–5252.
(31) Chen, J.; Heitmann, J. A.; Hubbe, M. A. *Colloids Surf., A* **2003**, *223*, 215–230.
(32) Merdan, T.; Kopeček, J.; Kissel, T. *Adv. Drug Delivery Rev.* **2002**, *54*, 715–758.
(33) Gillespie, D. T. *J. Phys. Chem.* **1977**, *81*, 2340–2361.
(34) Gillespie, D. T. *J. Comput. Phys.* **1976**, *22*, 403–434.
(35) Manning, G. S. *J. Phys. Chem.* **1984**, *88*, 6654–6661.
(36) Junghans, M.; Kreuter, J.; Zimmer, A. *Biochim. Biophys. Acta* **2001**, *1544*, 177–188.
(37) Grosberg, A. Y.; Nguyen, T. T.; Shklovskii, B. I. *Rev. Mod. Phys.* **2002**, *74*, 329–345.

MA702493W