Steric Effects on Multivalent Ligand-Receptor Binding: Exclusion of Ligand Sites by Bound Cell Surface Receptors

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ABSTRACT Steric effects can influence the binding of a cell surface receptor to a multivalent ligand. To account for steric effects arising from the size of a receptor and from the spacing of binding sites on a ligand, we extend a standard mathematical model for ligand-receptor interactions by introducing a steric hindrance factor. This factor gives the fraction of unbound ligand sites that are accessible to receptors, and thus available for binding, as a function of ligand site occupancy. We derive expressions for the steric hindrance factor for various cases in which the receptor covers a compact region on the ligand surface and the ligand expresses sites that are distributed regularly or randomly in one or two dimensions. These expressions are relevant for ligands such as linear polymers, proteins, and viruses. We also present numerical algorithms that can be used to calculate steric hindrance factors for other cases. These theoretical results allow us to quantify the effects of steric hindrance on ligand-receptor kinetics and equilibria.

INTRODUCTION

For many cell surface receptors, interaction with a multivalent ligand is essential for signal transduction (Metzger, 1992). Multivalent ligand-receptor binding leads to aggregation of receptors, phosphorylation of intracellular receptor domains, and activation of cytoplasmic regulatory molecules. These receptors include multichain immune recognition receptors (Keegan and Paul, 1992), such as the B cell receptor, and receptor tyrosine kinases (Pazin and Williams, 1992; Fry et al., 1993), such as the epidermal growth factor receptor. Multivalent ligand-receptor binding is important not only in signal transduction, but also in a variety of other phenomena, such as antibody-mediated activation of the complement cascade (Burton and Woof, 1992) and viral attachment and entry into cells (Haywood, 1994).

Because of the importance of multivalent ligand-receptor binding, significant effort, involving both experimental and theoretical work, has been directed at understanding the interactions of multivalent ligands with cell surface receptors. Theoretical work on bivalent ligands (Dembo and Goldstein, 1978; Perelson and DeLisi, 1980) has been particularly influential. The theory for these ligands has been refined over a number of years (Posner et al., 1995b) and applied to a number of problems, particularly the analysis of FcϵRI aggregation on the surface of rat basophilic leukemia cells (Goldstein, 1988; Goldstein and Wofsy, 1994). Theoretical work on multivalent ligands (Gandolfi et al., 1978; DeLisi, 1980; Perelson, 1981) has also been applied to a number of problems in immunology and virology (Hlavacek et al., 1999; Sulzer and Perelson, 1997; Dee and Shuler, 1997; Goldstein and Wofsy, 1996; Wickham et al., 1990, 1995; Segal et al., 1983; Vogelstein et al., 1982; Dower and Segal, 1981; Dower et al., 1981). However, models for multivalent ligands have yet to be fully developed.

When the valence of a ligand is greater than two, models for ligand-receptor binding are complicated by a number of factors (Perelson, 1984; Macken and Perelson, 1985; Lauffenburger and Linderman, 1993). One complication arises when a bound receptor, because of its physical size, excludes receptor binding at neighboring ligand sites. Steric exclusion of ligand sites by bound receptors is illustrated in Fig. 1. Shown schematically is the surface of a multivalent ligand that is bound to two receptors. Each receptor binds a single site but physically covers an area that encompasses more than one site on the ligand. Sites on the ligand are bound or unbound, and unbound sites are covered, excluded, or available. A *covered* site is unavailable for receptor binding, because it is covered by a bound receptor. Likewise, an *excluded* site, although it is not covered, is unavailable for receptor binding because it lies near a bound receptor, and binding at this site would require an overlap of receptors. A covered or excluded site is distinguished from an *available* site, at which receptor binding is possible. Steric exclusion of ligand sites by bound receptors reduces the average reactivity of unbound ligand sites: available sites have the potential to bind receptors, but covered and excluded sites do not.

Steric exclusion of ligand sites is likely to play a role in many ligand-receptor interactions, as suggested by the interactions of antibodies with various types of antigens. Steric effects due to ligand site exclusion can be important in antibody binding to viruses. For example, the protein coat of tobacco mosaic virus (TMV), a nonenveloped virus, consists of 2130 repeating subunits, but subunit-specific, excess monoclonal antibody binds TMV with a stoichiometry of 800:1, which suggests that a bound antibody Fab arm

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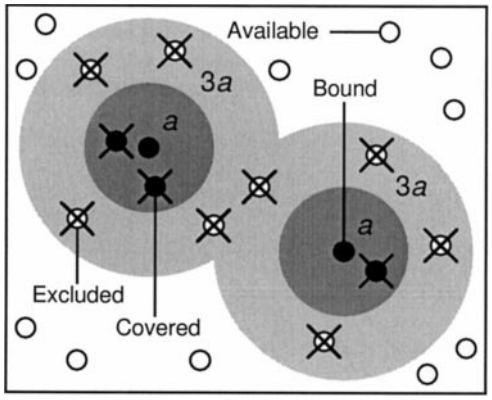

FIGURE 1 The surface of a ligand on which two receptors are bound. Ligand sites are bound, covered, excluded, or available, as indicated. A darkly shaded region represents the circular area a that is covered by each receptor. A lightly shaded region represents the "exclusion area" of each receptor, an annular region of area $3a$ in which sites cannot be bound without overlap of receptors. Note that sites combine with receptors at the center of the area covered by a bound receptor.

covers approximately three viral subunits (Pellequer and Van Regenmortel, 1993). Steric effects due to ligand site exclusion also can be significant in antibody binding to bacterial polysaccharides. Galactan from *Prototheca zopfii* is composed of ~1240 galactosyl residues. Galactan-specific antibodies, which can bind at sites along the entire polysaccharide chain, each cover at least 10 and as many as 30 sequential galactosyl residues when bound (Glaudemans et al., 1986). Steric effects can also be important in antibody binding to protein antigens, because the whole surface of a protein is potentially antigenic (Davies and Cohen, 1996) and domains important for protein-protein binding are smaller than protein-protein interfaces (Wells, 1996). Interactions of antibodies with densely haptenated carrier molecules are also likely to involve steric effects (Macken and Perelson, 1986; Hlavacek et al., 1999).

Here we develop a theoretical framework for modeling multivalent ligand-receptor binding when steric effects due to ligand site exclusion are important. In this framework, steric effects on cross-linking reactions are characterized by a steric hindrance factor. This factor indicates how the accessibility, and thus the average reactivity, of unbound ligand sites depends on ligand site occupancy. Steric hindrance factors are closely related to insertion probabilities (Widom, 1963). An insertion probability is the probability of inserting a particle onto a surface without overlap when the surface is partially covered with other particles. By adapting the method of Andrews (1975, 1976) for calculating insertion probabilities, we are able to derive expressions for steric hindrance factors for various types of ligands and receptors.

In our derivation of steric hindrance factors, we focus on receptors that cover a compact region on the ligand surface when bound, but we consider different types of ligands with binding sites distributed regularly or randomly in one or two dimensions. The one-dimensional results, which complement earlier work (Macken and Perelson, 1986), are relevant for linear polymers to which haptens have been conjugated at random positions or a polysaccharide with regularly spaced epitopes. The two-dimensional results are relevant for ligands such as multisubunit proteins, haptenated proteins, surface proteins on enveloped viruses, or whole nonenveloped viruses. Our expressions for steric hindrance factors are approximate, except for special one-dimensional cases. To determine the accuracy and usefulness of these approximations, we compare approximate results with those calculated via Monte Carlo or combinatoric methods.

THEORY

Here we develop a model for ligand-receptor binding that includes steric effects. We focus on a multivalent ligand that interacts with a monovalent receptor. The ligand is assumed to be symmetrical and much smaller than a cell, and binding sites on the ligand are assumed to be chemically identical. We allow for various arrangements of ligand sites. The receptor is mobile and disperse on the cell surface. We assume that receptor trafficking is inhibited such that the total amounts of ligand and receptor are each constant (Lauffenburger and Linderman, 1993). Steric effects arise when bound receptors hinder access to ligand sites, as illustrated in Fig. 1. In the absence of steric effects, the model reduces to the well-studied equivalent site model for multivalent ligand-receptor binding (Perelson, 1984; Macken and Perelson, 1985; Lauffenburger and Linderman, 1993).

Valence

We define the following ligand valences (Fig. 2): v, f, n, and $\nu(i)$ for $i = 1, \ldots, f$. The valence v is the number of sites

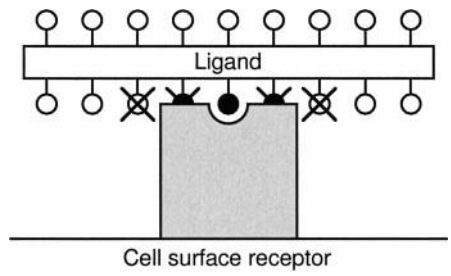

FIGURE 2 A ligand-receptor complex. Bound, covered, excluded, and available sites on the ligand are represented as in Fig. 1. Initially, the ligand can attach to the cell surface at any of 18 sites ($v = 18$). However, because of the geometry of this ligand, once the ligand is anchored to the cell surface, only nine of the 18 sites, those that are directed at the cell surface, are in position to interact with receptors. Thus $n = 9$. After a site is bound by a receptor, the number of sites available for further binding depends on the particular site at which the receptor is bound. The average number of sites that are available for receptor binding when the ligand is bound at a single site is $(6 + 5 + 4 + 4 + 4 + 4 + 4 + 5 + 6)/9$. Thus $\nu(1) = 14/3$. Likewise, we can determine that $\nu(2) = 62/45$ and $\nu(3) = 0$. At most, three sites on the ligand can be bound simultaneously by receptors ($f = 3$). A bound receptor can contact up to three ligand sites ($\eta = 3$).

on a ligand in solution at which a receptor can bind, and the *effective* valence f is the maximum number of sites on a ligand that can be bound simultaneously by receptors (Perelson, 1981, 1984). The *exposure* valence n is a new concept: we define it as the number of sites on a ligand that are exposed to receptors when the ligand is anchored to the cell surface. As illustrated in Fig. 2, a bound ligand may expose only a fraction of its sites to receptors. We also define for the first time the valence of the ith bound state $\nu(i)$ for $i = 1, \ldots, f$. Each $\nu(i)$ is the number of ways in which a ligand bound at i sites can be converted to a ligand bound at $i + 1$ sites, i.e., $\nu(i)$ is the number of sites that are available for receptor binding on a ligand that is bound at i sites averaged over all possible microscopic states of the ligand. The quantity $\nu(i)$ will also be called the number of available sites. Note that $\nu(f) = 0$, because no further binding can occur once f sites are bound, and that $\nu(i) \leq n - i$. If each exposed site on a ligand is always available for receptor binding, then $f = n$ and $\nu(i) = n - i$. However, if ligand sites can be covered or excluded by bound receptors, as illustrated in Figs. 1 and 2, then $f < n$ and $\nu(i) < n - i$.

Reaction scheme

Our model for ligand-receptor binding is based on the reaction scheme shown in Fig. 3. In this scheme, ligand-receptor binding proceeds through a series of reversible reactions. The initial reaction involves the binding of a solution-phase ligand to a receptor, and each subsequent reaction involves the addition of a receptor to a ligand-receptor complex on the cell surface. As indicated in Fig. 3, the model is developed in terms of ligand states. Variables in the model include the concentration of ligand in solution, which is denoted as L_0; the surface density of ligand that is bound at i sites, which is denoted as L_i; and the surface density of free receptors, which is denoted as R.

Equilibria

For the initial reaction in Fig. 3,

$$L_1 = \upsilon K R L_0 \tag{1}$$

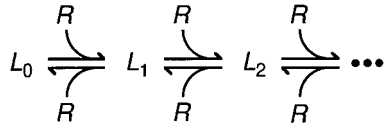

FIGURE 3 Reaction scheme. At each step in this scheme, the following reaction takes place: Free receptor + Free ligand site ⇌ Bound receptor. The first step involves the attachment of solution-phase ligand to a cell-surface receptor. Each subsequent step involves the addition of a receptor to a ligand-receptor complex on the cell surface. R indicates a receptor, L_0 indicates a ligand in solution, and L_1 and L_2 indicate ligands that are bound to one and two receptors, respectively.

where K is an equilibrium constant that represents the affinity of a receptor for a site on solution-phase ligand. For each subsequent reaction in Fig. 3,

$$(i + 1)L_{i+1} = \nu(i)K_x R L_i \quad i = 1, \ldots, f - 1 \tag{2}$$

where K_x is a two-dimensional cross-linking equilibrium constant that represents the affinity of a receptor site for an available site on surface-adsorbed ligand. The coefficient $(i + 1)$ in this equation represents the number of ways that a receptor can dissociate from a ligand bound at $i + 1$ sites.

Kinetics

The kinetics of ligand-receptor binding are difficult to model when steric effects influence binding, because to determine exact time courses of binding, we must write a kinetic balance equation for each microscopic ligand state (Epstein, 1979a,b; Munro et al., 1998). Nevertheless, exact tractable models can be developed for limiting cases (Epstein, 1979a,b; Schaaf and Talbot, 1989; Evans, 1993). Below, we present a model that is exact for the limiting case in which microscopic equilibrium is established instantaneously and approximate otherwise. Instantaneous establishment of microscopic equilibrium (IEME) occurs when ligands bound at i sites effectively redistribute themselves immediately among all possible microscopic states whenever a ligand enters or leaves the ith bound state. Microscopic equilibrium, which is a necessary condition for binding equilibrium, allows us to characterize the number of available sites on a ligand in a particular bound state at a particular time by the expected number of available sites at equilibrium. The IEME approximation improves as the rate of receptor dissociation increases, and this approximation is least accurate when receptor binding is irreversible. The usefulness of the IEME approximation can be determined by comparing approximate and exact results, which can be determined with numerical methods (Epstein, 1979b; Reiter and Epstein, 1990; Sild et al., 1996).

Given IEME, a kinetic description of ligand-receptor binding is provided by

$$\begin{aligned}
dL_0/dt &= -C(\upsilon k_f R L_0 - k_r L_1)\\
dL_1/dt &= \upsilon k_f R L_0 - k_r L_1 - \nu(1)k_x R L_1 + 2k_{-x} L_2\\
dL_i/dt &= \nu(i-1)k_x R L_{i-1} - i k_{-x} L_i\\
&\quad - \nu(i)k_x R L_i + (i+1)k_{-x} L_{i+1}\\
&\quad \text{for } i = 2, \ldots, f-1\\
dL_f/dt &= \nu(f-1)k_x R L_{f-1} - f k_{-x} L_f
\end{aligned} \tag{3}$$

where k_f is the forward rate constant for initial binding of a ligand to a receptor, k_r is the corresponding reverse rate constant, k_x is the forward cross-linking rate constant for the addition of a receptor to a ligand-receptor complex on the cell surface, and k_{-x} is the corresponding reverse rate constant. The constant C is a factor that converts surface

densities to concentrations, e.g., C is the cell concentration if surface densities are expressed on a per cell basis. Equation 3 is appropriate for reaction-limited binding. If diffusion of ligand to the cell surface were limiting, then the rate constants in Eq. 3 would have to be modified (Berg and Purcell, 1977; DeLisi and Wiegel, 1981; Shoup and Szabo, 1982; Goldstein, 1989; Goldstein et al., 1989; Lauffenburger and Linderman, 1993).

Conservation

Conservation of ligand can be expressed as

$$L_T = L_0 + C \sum_{i=1}^{f} L_i \tag{4}$$

where L_T is the total concentration of ligand. Similarly, conservation of receptor can be expressed as

$$R_T = R + \sum_{i=1}^{f} iL_i \tag{5}$$

where R_T is the total surface density of receptors.

Steric effects

To account for steric effects, we must determine how the number of available sites $\nu(i)$ varies with ligand site occupancy i. The fraction of exposed ligand sites that are available for receptor binding is given by $\nu(i)/n$, which can be interpreted as the probability that an exposed site is available for receptor binding. This probability, which we denote as $P_i(\mathcal{A})$, is called the *insertion probability*, because if we were to attempt to add an additional receptor to a ligand-receptor complex at a randomly chosen site on the ligand, $P_i(\mathcal{A})$ is the probability that this insertion attempt is successful. Several approaches are available for calculating insertion probabilities. Here we follow an approach of Andrews (1975, 1976) to develop expressions for $P_i(\mathcal{A})$ for ligands with sites distributed randomly or ordered regularly in one or two dimensions.

Ligands with sites distributed randomly on a two-dimensional surface

Let us focus on the case illustrated in Fig. 1. A ligand is bound at i sites, and the ligand exposes n sites, which are distributed randomly over an area A, to receptors on the cell surface. A bound receptor covers a circular area a on the ligand surface. Later, we will consider cases in which a is noncircular. We assume that $a \ll A$, such that edge effects are negligible.

As illustrated in Fig. 1, an exposed ligand site is in one of four states: it is bound ($\mathcal{B}$), covered ($\mathcal{C}$), excluded ($\mathcal{E}$), or available ($\mathcal{A}$). The probability that a site is available can be expressed as the following product:

$$P_i(\mathcal{A}) = P_i(\bar{\mathcal{B}})P_i(\bar{\mathcal{C}}|\bar{\mathcal{B}})P_i(\bar{\mathcal{E}}|\bar{\mathcal{B}}\bar{\mathcal{C}}) \tag{6}$$

where $P_i(\bar{\mathcal{B}})$ is the probability that a site is not bound; $P_i(\bar{\mathcal{C}}|\bar{\mathcal{B}})$ is the conditional probability that a site is not covered, given that it is not bound; and $P_i(\bar{\mathcal{E}}|\bar{\mathcal{B}}\bar{\mathcal{C}})$ is the conditional probability that a site is not excluded, given that it is neither bound nor covered. We can determine $P_i(\bar{\mathcal{B}})$ and $P_i(\bar{\mathcal{C}}|\bar{\mathcal{B}})$ exactly, and we can determine $P_i(\bar{\mathcal{E}}|\bar{\mathcal{B}}\bar{\mathcal{C}})$ approximately.

The probability that a site is *not* bound, $P_i(\bar{\mathcal{B}})$, is related to the probability that a site *is* bound $P_i(\mathcal{B})$: $P_i(\bar{\mathcal{B}}) = 1 - P_i(\mathcal{B})$. Because $P_i(\mathcal{B})$ is equivalent to the fraction of exposed sites that are bound, i/n,

$$P_i(\bar{\mathcal{B}}) = 1 - i/n \tag{7}$$

The probability that a site is not covered given that it is not bound, $P_i(\bar{\mathcal{C}}|\bar{\mathcal{B}})$, is related to the probability that it is covered given that it is not bound $P_i(\mathcal{C}|\bar{\mathcal{B}})$: $P_i(\bar{\mathcal{C}}|\bar{\mathcal{B}}) = 1 - P_i(\mathcal{C}|\bar{\mathcal{B}})$. Because $P_i(\mathcal{C}|\bar{\mathcal{B}})$ is equivalent to the fraction of the exposed ligand surface that is covered by bound receptors ia/A,

$$P_i(\bar{\mathcal{C}}|\bar{\mathcal{B}}) = 1 - ia/A \tag{8}$$

The product $P_i(\bar{\mathcal{B}})P_i(\bar{\mathcal{C}}|\bar{\mathcal{B}}) = (1 - i/n)(1 - ia/A)$ is the probability that a site is neither bound nor covered.

If a site is neither bound nor covered, then it lies somewhere in an area of size $A - ia$. Sites in this area are either excluded or available. A site is excluded if it lies within the "exclusion area" of a bound receptor. As illustrated in Fig. 1, the exclusion area of a receptor that covers a circular area a is an annular region of area $3a$ that surrounds a. Thus the probability that a site is excluded by a given bound receptor is $3a/(A - ia)$, and the probability that the site is not excluded by this particular receptor is $1 - 3a/(A - ia)$. Following Andrews (1975, 1976), we estimate $P_i(\bar{\mathcal{E}}|\bar{\mathcal{B}}\bar{\mathcal{C}})$, the probability that a site is not excluded by any of the i receptors, as the ith power of the probability that a site is not excluded by a given receptor. Thus,

$$P_i(\bar{\mathcal{E}}|\bar{\mathcal{B}}\bar{\mathcal{C}}) = \left(1 - \frac{3a}{A - ia}\right)^i \tag{9}$$

This expression implies mutually independent events. In other words, the probability that a site is excluded by the first receptor in Fig. 1 is independent of whether the site is excluded by the second receptor. In general, this is an approximation, because the exclusion areas of two receptors can overlap, as depicted in Fig. 1.

By combining Eqs. 6–9, we obtain an expression for the insertion probability for the case illustrated in Fig. 1:

$$P_i(\mathcal{A}) = \left(1 - \frac{i}{n}\right)\left(1 - \frac{ia}{A}\right)\left(1 - \frac{3a}{A - ia}\right)^i \tag{10}$$

This expression also yields an expression for $\nu(i)$, which can be substituted into the model equations (Eqs. 1–5), because

$\nu(i) = nP_i(\mathcal{A})$. The general method that we have used to derive Eq. 10 is applied below to derive expressions for several other cases.

Equation 10 can be generalized for receptors that cover any convex area a by using the results of Boublík (1975). These results indicate that the exclusion area of a receptor that covers an area a is $(2\gamma + 1)a$ if a is convex and if receptors bind in random orientations. The shape factor $\gamma \geq 1$ is defined as $s^2/(4\pi a)$, where s is the perimeter of a. This factor has a value of $\sim$1 for many shapes. If a is disk shaped, then $\gamma = 1$, and the exclusion area is $3a$, as expected (Fig. 1). If a is equilateral triangular, $\gamma = 3\sqrt{3}/\pi \approx 1.6$. If a is square, $\gamma = 4/\pi \approx 1.3$. If a is hexagonal, $\gamma = 2\sqrt{3}/\pi \approx 1.1$. In general, if a is a regular polygon with k sides, $\gamma = (k/\pi)\tan(\pi/k)$. If a is rectangular with aspect ratio $\rho = 2$ (the length of the longer side is twice that of the shorter side), $\gamma = 9/(2\pi) \approx 1.4$. In general, if a is rectangular with aspect ratio $\rho \geq 1$, $\gamma = (\rho + 1)^2/(\rho\pi)$. These results indicate that insertion probabilities are somewhat insensitive to the shape of a as long as a is compact. The generalized form of Eq. 10 is given in Table 1 (Case 1).

Ligands with sites ordered regularly on a two-dimensional surface

If ligand sites are ordered regularly on a lattice instead of distributed randomly, then we can adapt the results of Stankowski (1983) to calculate the insertion probability $P_i(\mathcal{A})$. These results were derived originally for adsorption reactions, also by using the approach of Andrews (1975, 1976). Earlier, we characterized the ligand-receptor interface with an area a, but here we characterize this interface with the contact number η, which represents the number of adjacent sites that are bound or covered by a bound receptor. The pattern of ligand sites contacted by a receptor must be symmetrical; a hexagonal contact pattern is illustrated in Fig. 4. Exact expressions for $P_i(\bar{\mathcal{B}})$ and $P_i(\bar{\mathcal{C}}|\bar{\mathcal{B}})$ and an approximate expression for $P_i(\bar{\mathcal{E}}|\bar{\mathcal{B}}\bar{\mathcal{C}})$ are given in Table 1 (Case 2). This latter expression involves an excluded-area parameter α, which depends on the contact pattern of the receptor and the lattice of ligand sites. A recipe for calculating the excluded-area parameter is given by Stankowski (1983). For a ligand with sites arranged on a square lattice and a square contact pattern, $\alpha = 3 - (4\sqrt{\eta} - 1)/\eta$, where $\eta \in [1, 4, 9, \ldots]$. For a ligand with sites arranged on a hexagonal lattice and a hexagonal contact pattern (Fig. 4), $\alpha = 3 - \sqrt{12\eta - 3}/\eta$, where $\eta \in [1, 7, 19, \ldots]$.

Ligands with sites distributed randomly along a one-dimensional array

For a ligand with sites distributed randomly along a one-dimensional array, expressions for $P_i(\bar{\mathcal{B}})$, $P_i(\bar{\mathcal{C}}|\bar{\mathcal{B}})$, and $P_i(\bar{\mathcal{E}}|\bar{\mathcal{B}}\bar{\mathcal{C}})$ are given in Table 1 (Cases 3 and 4). The derivation of these expressions is analogous to the derivation of Eqs. 7–9. In the expressions of Table 1, the parameter L, which is analogous to A, represents the total length of the array of sites exposed to receptors, and the parameter l, which is analogous to a, represents the length of the array that is covered by a bound receptor. The expressions for

TABLE 1 Theoretical expressions for calculating insertion probabilities and steric hindrance factors

Case	Arrangement of sites on the ligand	$P_i(\bar{\mathcal{C}}\|\bar{\mathcal{B}})$	$P_i(\bar{\mathcal{E}}\|\bar{\mathcal{B}}\bar{\mathcal{C}})$
1*	Randomly distributed on 2-D surface	$1 - ia/A$	$\left[1 - (2\gamma + 1)\left(\frac{a}{A - ia}\right)\right]^i$
2#	Ordered on regular 2-D lattice	$1 - i\left(\frac{\eta - 1}{n - i}\right)$	$\left[1 - \alpha\left(\frac{\eta}{n - i\eta}\right)\right]^i$
3§	Randomly distributed on 1-D ring	$1 - il/L$	$\left(1 - \frac{l}{L - il}\right)^i$
4	Randomly distributed on 1-D chain	$1 - il/L$	above $\times \left(1 - \frac{l}{L - il}\right)$
5¶	Ordered on regular 1-D lattice ring	$1 - i\left(\frac{\eta - 1}{n - i}\right)$	$\prod_{j=1}^{\eta-1}\left(1 - \frac{i}{n - i(\eta - 1) - j}\right)$
6‖	Ordered on regular 1-D lattice chain	$1 - i\left(\frac{\eta - 1}{n - i}\right)$	above $\times \left(1 - \frac{\eta - 1}{n - i(\eta - 1)}\right)$

On a ligand bound at i sites, the expected number of sites available for receptor binding, $\nu(i)$, is given by $nP_i(\mathcal{A})$ or $(n - i)H(i)$, where n is the number of sites exposed to receptors, $P_i(\mathcal{A}) = P_i(\bar{\mathcal{B}})P_i(\bar{\mathcal{C}}|\bar{\mathcal{B}})P_i(\bar{\mathcal{E}}|\bar{\mathcal{B}}\bar{\mathcal{C}})$ is the insertion probability, and $H(i) = P_i(\bar{\mathcal{C}}|\bar{\mathcal{B}})P_i(\bar{\mathcal{E}}|\bar{\mathcal{B}}\bar{\mathcal{C}})$ is the steric hindrance factor. Expressions for $P_i(\bar{\mathcal{C}}|\bar{\mathcal{B}})$ and $P_i(\bar{\mathcal{E}}|\bar{\mathcal{B}}\bar{\mathcal{C}})$ are given above for cases discussed in the text. In all six cases, $P_i(\bar{\mathcal{B}}) = 1 - i/n$.

*Sites exposed to receptors are distributed over an area A. A receptor binds with random orientation and covers an area a, which is convex. The shape of a determines the shape factor $\gamma = s^2/(4\pi a)$, where s is the perimeter of a.

#A bound receptor contacts η adjacent symmetrically arrayed sites (Fig. 4). The excluded-area parameter α is determined according to the recipe of Stankowski (1983).

§Sites exposed to receptors are arrayed along a length L. A bound receptor covers a length l.

¶A bound receptor contacts η sequential sites.

‖If receptors can bind at edge sites, n in these expressions is replaced by $n + \eta - 1$ and $\nu(i) = (n + \eta - 1 - i)H(i)$ (cf. Eq. 11).

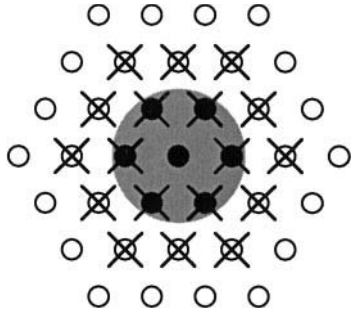

FIGURE 4 A hexagonal pattern of ligand-receptor contacts on a ligand with a hexagonal lattice of sites. As shown, a bound receptor contacts seven sites ($\eta = 7$ and $\alpha = 12/7$). The area covered by a bound receptor is indicated by the shaded region. Bound, covered, excluded, and available sites on the ligand are represented as in Fig. 1.

$P_i(\bar{\mathcal{B}})$ and $P_i(\bar{\mathcal{C}}|\bar{\mathcal{B}})$ are exact. The expression for $P_i(\bar{\mathcal{E}}|\bar{\mathcal{B}}\bar{\mathcal{C}})$ also is exact when $L/l \ll n$, but it is approximate otherwise. The expressions given for Case 3 in Table 1 are for a ligand with sites distributed along a ring, i.e., a closed one-dimensional array. The expressions given for Case 4 in Table 1 are for a ligand with sites distributed along a chain, i.e., an open one-dimensional array. The expressions for the two cases are essentially the same, except that $P_i(\bar{\mathcal{E}}|\bar{\mathcal{B}}\bar{\mathcal{C}})$ is multiplied by a factor to account for edge effects when sites are ordered along a chain instead of a ring. This factor is $1 - l/(L - il)$, where $l/(L - il)$ is the probability that a site is excluded because it is close to an edge. We assume that a site is excluded because of edge effects if a receptor is unable to bind at that site entirely within the length L, as might be the case if the receptor, in addition to binding the ligand site, requires nonspecific interactions over a larger contact area. If receptor binding is possible at such a site, then the expressions for Case 4 in Table 1 can be used to determine a lower bound on the insertion probability.

Ligands with sites ordered regularly along a one-dimensional array

For a ligand with sites ordered along a ring and a receptor that contacts η sequential sites, we can calculate $P_i(\bar{\mathcal{B}})$, $P_i(\bar{\mathcal{C}}|\bar{\mathcal{B}})$, and $P_i(\bar{\mathcal{E}}|\bar{\mathcal{B}}\bar{\mathcal{C}})$ exactly. The results are summarized in Table 1 (Case 5). When $\eta \ll n$, the expression for $P_i(\bar{\mathcal{E}}|\bar{\mathcal{B}}\bar{\mathcal{C}})$ reduces to $(1 - i/(n - i(\eta - 1)))^{\eta-1}$, which corresponds to the result of McGhee and von Hippel (1974) for an infinite lattice. If sites on a ligand are ordered along a chain, as depicted in Fig. 2, rather than along a ring, then the expressions for $P_i(\bar{\mathcal{B}})$, $P_i(\bar{\mathcal{C}}|\bar{\mathcal{B}})$, and $P_i(\bar{\mathcal{E}}|\bar{\mathcal{B}}\bar{\mathcal{C}})$ are essentially the same, except that $P_i(\bar{\mathcal{E}}|\bar{\mathcal{B}}\bar{\mathcal{C}})$ is multiplied by a factor to account for edge effects (Table 1, Case 6). When a receptor is unable to bind at "edge" sites (i.e., sites at which a bound receptor would contact fewer than η sites), the correction factor is $1 - (\eta - 1)/(n - i(\eta - 1))$, where $(\eta - 1)/(n - i(\eta - 1))$ is the probability that a site is an edge site. As before, the expressions for $P_i(\bar{\mathcal{B}})$, $P_i(\bar{\mathcal{C}}|\bar{\mathcal{B}})$, and $P_i(\bar{\mathcal{E}}|\bar{\mathcal{B}}\bar{\mathcal{C}})$ are exact. If receptors are able to bind at edge sites, the expressions for Case 6 in Table 1 can be used to obtain exact results. We simply replace n with $n + \eta - 1$, i.e., we introduce $\eta - 1$ virtual edge sites.

The steric hindrance factor

To connect our model equations with the equivalent site model (Perelson, 1984; Lauffenburger and Linderman, 1993), we introduce the following formalism. We define $H(i)$ for $i = 1, \ldots, f - 1$ as $\nu(i)/(n - i)$, which is the fraction of exposed unbound ligand sites that are available for receptor binding. Thus,

$$\nu(i) = (n - i)H(i) \tag{11}$$

and

$$H(i) = \frac{\nu(i)/n}{1 - i/n} = \frac{P_i(\mathcal{A})}{P_i(\bar{\mathcal{B}})} = P_i(\bar{\mathcal{C}}|\bar{\mathcal{B}})P_i(\bar{\mathcal{E}}|\bar{\mathcal{B}}\bar{\mathcal{C}}) \tag{12}$$

We can interpret $H(i) \leq 1$ as a factor that corrects for steric hindrance. In the absence of steric effects, $H(i) = 1$, because $\nu(i) = n - i$, as discussed earlier. When $H(i) = 1$ for all i, the model equations (Eqs. 1–5) reduce to the equivalent site model. In the presence of steric effects, $H(i) < 1$, because $\nu(i) < n - i$, as discussed earlier. The smaller the value of $H(i)$, the larger the effect of steric hindrance on ligand-receptor binding.

METHODS

Algorithms

The insertion probability $P_i(\mathcal{A})$ is equivalent to $\nu(i)/n$, and the steric hindrance factor $H(i)$ is equivalent to $\nu(i)/(n - i)$. Thus, $P_i(\mathcal{A})$ and $H(i)$ can be calculated from $\nu(i)$. To determine $\nu(i)$ directly, we specify the geometry of a ligand-receptor interface and the spacing of exposed ligand sites. Then we count the number of sites that are available on average for the different microscopic bound states of the ligand. Here, we present two algorithms for calculating insertion probabilities that are based on this approach: a combinatoric algorithm, which is efficient when n is small, and a Monte Carlo algorithm, which is efficient when n is large. The Monte Carlo algorithm is similar to that used by Siepmann et al. (1992) to calculate insertion probabilities for fluids of hard rods and disks.

Combinatoric algorithm

A configuration of n ligand sites is generated. A recursive procedure then is used to generate every possible configuration of i bound receptors (Taylor et al., 1991). If a configuration of receptors is acceptable, meaning that none of the i receptors overlap, then the number of available sites is counted. A site is available if a receptor can be placed at that site without overlapping other receptors. We compute the number of available sites averaged over all acceptable con-

figurations of receptors. For ligands with randomly distributed sites, multiple configurations of sites are generated, and the above process is repeated for each configuration. The efficiency of the algorithm varies inversely with $n!/[(n-i)!i!]$, which is the number of ways that i receptors can be distributed among n ligand sites. A similar but more efficient algorithm has been described (Badcoe, 1992) that can be used with one-dimensional ligands that have regularly ordered sites.

Monte Carlo algorithm

Initialization. A configuration of n ligand sites is generated. We then attempt to distribute i receptors among these sites such that none of the receptors overlap. The receptors are placed at i randomly chosen but distinct sites. Each receptor, except the first, is then checked for overlap in sequential order. If a receptor overlaps another receptor, we attempt to move it to an unbound site. If all attempts to move a receptor to an unbound site result in overlap, we randomly redistribute the i receptors among the n sites and attempt to eliminate overlap as before. If an acceptable distribution of receptors cannot be obtained after a fixed number of attempts, we abandon the configuration of sites, i.e., we assume that this configuration of n ligand sites does not permit the binding of i receptors. Because this procedure may abandon configurations of sites that do indeed permit the binding of i receptors, extreme caution must be exercised when calculating small insertion probabilities, for which the algorithm is inefficient in any case.

Execution of a Monte Carlo cycle. After an initial configuration of i nonoverlapping receptors is generated, we then generate a new configuration of receptors by executing a Monte Carlo cycle. In a Monte Carlo cycle, we sequentially attempt to move each of the i receptors once from its present ligand site s_j to a neighboring ligand site s_k. A move is rejected if it results in an overlap of receptors. If the move results in no overlap, it is accepted with probability $\min(N_j/N_k, 1)$, where N_j is the number of sites that neighbor site s_j and N_k is the number of sites that neighbor site s_k. This acceptance criterion is necessary to ensure that a move from site s_j to s_k is as likely as a move from site s_k to s_j. The neighborhood of a site is defined as the collection of sites within a fixed distance of the site. This distance is chosen so that a site's expected number of neighbors is well above 1. After each Monte Carlo cycle, the number of available sites on the ligand is determined. We perform a fixed number of Monte Carlo cycles and compute the average number of available sites. For ligands with randomly distributed sites, multiple configurations of ligand sites are generated, and the above process, including the initialization procedure, is repeated for each configuration.

Calculating insertion probabilities

For ligands with $n \leq 20$, we use the combinatoric algorithm, whereas for ligands with $n > 20$, we use the Monte Carlo algorithm. We use periodic boundary conditions in all calculations. For ligands with randomly distributed sites, each reported insertion probability is the mean of 100 computational runs. To obtain reproducible results, we adjust algorithmic parameters so that the standard deviation divided by the mean is less than 0.1.

Calculating equilibrium states

Calculation of equilibrium states is aided by combining Eqs. 1, 2, 4, and 5, which yields

$$1 = R/R_T + \frac{vKL_T \sum_{i=1}^{f} i\pi(i)}{n + vKCR_T \sum_{i=1}^{f} \pi(i)}, \tag{13}$$

where

$$\pi(i) = (K_x R_T)^{i-1}(R/R_T)^i \prod_{j=1}^{i} \left[\left(\frac{n-j+1}{j}\right) H(j-1)\right] \tag{14}$$

We adopt the convention that $H(0) = 1$. If $H(j-1) = 1$ for $j = 1, \ldots, i$, then the product in Eq. 14 reduces to the statistical factor $n!/[(n-i)!i!]$.

When values for the parameters (v, n, C, L_T, R_T, K, and K_x) are specified and a value for the steric hindrance factor $H(i)$ is specified for $i = 1, \ldots, f-1$, Eq. 13 is a nonlinear equation involving a single unknown: the fraction of free receptors R/R_T. To determine the fraction of free receptors at equilibrium, we solve this equation by using the method of bisection (Press et al., 1992). Once R/R_T is known, other states at equilibrium can be determined by using the relations $L_0/L_T = n/[n + vKCR_T \sum_{i=1}^{f} \pi(i)]$ and $L_i/R_T = (L_0/L_T)(vKL_T/n)\pi(i)$, which are derived from Eqs. 1, 2, and 4.

Calculating time courses

To calculate time courses of ligand-receptor binding, we solve an initial value problem that involves f differential equations and two auxiliary algebraic equations. These equations are derived from Eqs. 3–5 by using $K = k_f/k_r$ and $K_x = k_x/k_{-x}$ and by introducing dimensionless variables: $\tau = k_{-x}t$, $r = R/R_T$, $l = L_0/L_T$, and $x_i = L_i/R_T$ for $i = 1, \ldots, f$. From Eq. 3, we obtain

$$dx_i/d\tau = u_{i-1} - u_i, \quad \text{for } i = 1, \ldots, f \tag{15}$$

where

$$u_0 = rx_i(k_r/k_{-x})[(vKL_T)rl - x_1],$$

$$u_i = (n-i)H(i)(K_x R_T) - (i+1)x_{i+1}$$

for $i = 1, \ldots, f-1$, and $u_f = 0$. From Eqs. 4 and 5, we obtain

$$l = 1 - (CR_T/L_T)\sum_{i=1}^{f} x_i \tag{16}$$

and

$$r = 1 - \sum_{i=1}^{f} i x_i \tag{17}$$

If we consider a system in which all ligand molecules are initially in solution, then $r = 1$, $l = 1$, and $x_i = 0$ for $i = 1, \ldots, f$ at $\tau = 0$. These initial conditions and Eqs. 15–17 define an initial value problem, which we solve numerically by using the FORTRAN subroutine LSODE (http://www.netlib.org/odepack; Hindmarsh, 1983).

Quantifying receptor aggregation

A ligand bound at i sites is bound to i receptors. Thus the fraction of receptors in ligand-induced aggregates of i or more receptors is given by

$$\alpha(i) = \sum_{j=i}^{f} j L_j / R_T \tag{18}$$

The fraction of receptors in aggregates of all sizes, which has been proposed as a measure of the extent of receptor cross-linking (Gandolfi et al., 1978; Perelson, 1981), is given by $\alpha(2)$. Receptor aggregates of size 10 or more, termed *immunons*, have been suggested to be the minimum signaling unit for B cells (Dintzis et al., 1976, 1983). The fraction of receptors in immunons is given by $\alpha(10)$.

RESULTS

We have developed a model for ligand-receptor binding (Eqs. 1–5) in which $\nu(i)$ represents the expected number of ligand sites that are available for receptor binding when a ligand is bound at i sites. We have related $\nu(i)$, which is sensitive to steric effects (Figs. 1 and 2), to a steric hindrance factor $H(i) \leq 1$ (Eq. 11). When $H(i) = 1$ for all i, Eqs. 1–5 reduce to an equivalent site model (Perelson, 1984; Lauffenburger and Linderman, 1993). The steric hindrance factor $H(i)$ is related to the insertion probability $P_i(\mathcal{A})$ (Eq. 12), the probability that a site on a ligand is available for receptor binding when the ligand is bound at i sites. By following the approach of Andrews (1975, 1976), we have derived exact or approximate expressions for $P_i(\mathcal{A})$ for different types of ligands and receptors (Table 1). Below, we examine the accuracy of these expressions. We also examine steric effects on ligand-receptor binding at equilibrium and steric effects on time courses of ligand-receptor binding.

Accuracy of theoretical expressions

Theoretical expressions for the insertion probability $P_i(\mathcal{A})$ and the steric hindrance factor $H(i)$ are given for six cases in Table 1. Expressions for the first four cases are approximate, whereas expressions for the last two cases are exact. The accuracy of these expressions is illustrated in Figs. 5 and 6, in which insertion probabilities calculated using the expressions in Table 1 are compared with those calculated using the Monte Carlo algorithm.

In Fig. 5, results are shown for ligands with regularly ordered sites. In each panel, we consider three ligands, which interact with the same receptor. The ligands have different numbers of sites but are otherwise identical. The solid and broken lines in Fig. 5 *A* are based on the expression for $P_i(\mathcal{A})$ for Case 5 in Table 1, which is exact. Thus comparison of these results with the corresponding numerical results, which are represented by points, provides a test of our Monte Carlo algorithm for calculating insertion probabilities. As expected, the theoretical and numerical results are indistinguishable. The solid and broken lines in Fig. 5 *B* are based on the expression for $P_i(\mathcal{A})$ for Case 2 in Table 1, which is approximate. Despite the approximate nature of these results, they agree closely with the corresponding numerical results. The potential for this level of accuracy is consistent with earlier observations (Stankowski, 1984).

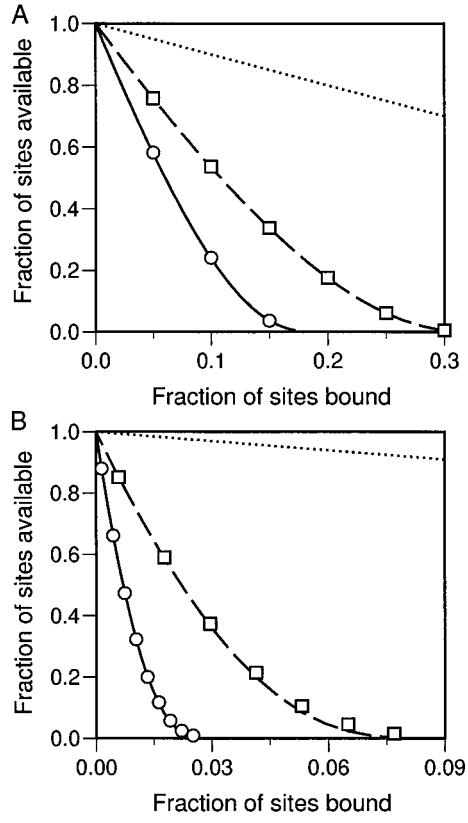

FIGURE 5 Insertion probabilities for ligands with regularly ordered sites. The fraction of sites available for receptor binding, $P_i(\mathcal{A}) = \nu(i)/n$, is plotted as a function of the fraction of sites bound, i/n. (*A*) The lattice of ligand sites is linear (Case 5 in Table 1). The solid line corresponds to a ligand with $n = 400$ and $\eta = 5$. The broken line corresponds to a ligand with $n = 200$ and $\eta = 3$. (*B*) The lattice of ligand sites is square (Case 2 in Table 1); $\alpha = 3 - (4\sqrt{\eta} - 1)/\eta$. The solid line corresponds to a ligand with $n = 676$ and $\eta = 25$. The broken line corresponds to a ligand with $n = 169$ and $\eta = 9$. In each panel, the dotted line corresponds to a ligand with $H(i) = 1$. Numerical results are represented by points.

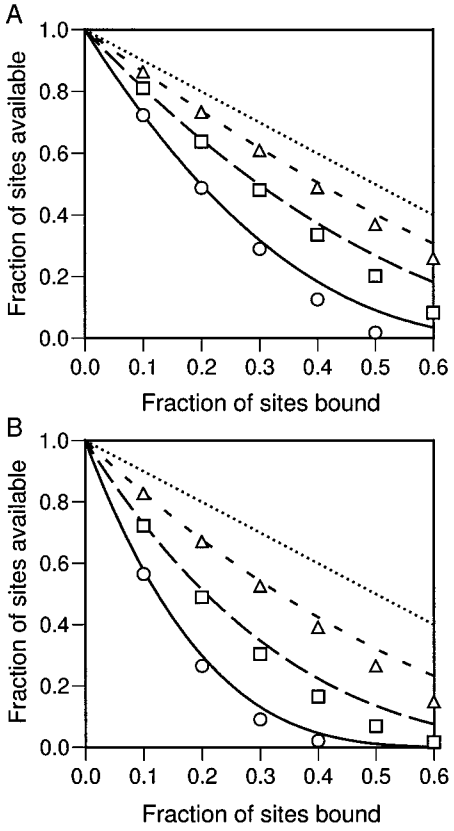

FIGURE 6 Insertion probabilities for ligands with randomly distributed sites. The fraction of sites available for receptor binding, $P_i(\mathcal{A}) = \nu(i)/n$, is plotted as a function of the fraction of sites bound, i/n. (*A*) Ligand sites are distributed along a one-dimensional ring (Case 3 in Table 1); $l/L = 0.01$. (*B*) Ligand sites are distributed over a two-dimensional surface (Case 1 in Table 1); $a/A = 0.01$ and $\gamma = 1$. In each panel, the solid line corresponds to a ligand with $n = 100$, the broken line corresponds to a ligand with $n = 50$, and the dashed line corresponds to a ligand with $n = 20$. The dotted line corresponds to a ligand with sites ordered such that $H(i) = 1$. Numerical results are represented by points.

In Fig. 6, results are shown for ligands with randomly distributed sites. In each panel, we consider four ligands, three with different numbers of sites and one with ordered sites, all of which can be bound simultaneously by receptors. The theoretical results, which are based on expressions in Table 1, can be compared with the corresponding numerical results. As can be seen, the theoretical expressions are capable of predicting how insertion probabilities, and therefore steric effects, vary with the number of binding sites on a ligand.

We have examined the accuracy of Eq. 10 in more detail (unpublished results). We find that accuracy decreases as either the fraction of sites bound i/n or the fraction of ligand surface covered by receptors ia/A increases. In other words, Eq. 10 is less accurate when the surface of the ligand is tightly packed with receptors, as can be expected. Thus, under conditions that favor close packing of receptors on the ligand surface, such as a receptor concentration in excess of ligand concentration or a large cross-linking constant, the usefulness of Eq. 10 should be checked. We expect that the results of this analysis are typical for the approximate expressions in Table 1, because all of these expressions were derived by the same method.

Steric effects on ligand-receptor equilibria

Equilibrium cross-linking curves are shown in Fig. 7 for cases where steric effects do and do not influence binding. Cross-linking, as measured by $\alpha(2)$ or $\alpha(10)$ (Eq. 18), is plotted as a function of ligand concentration for two ligands. One ligand has ordered sites, which all can be bound simultaneously, and the other ligand has randomly distributed sites, not all of which can be bound simultaneously, because of potential for steric exclusion of ligand sites by bound receptors. To ensure a controlled comparison, the two ligands are otherwise identical.

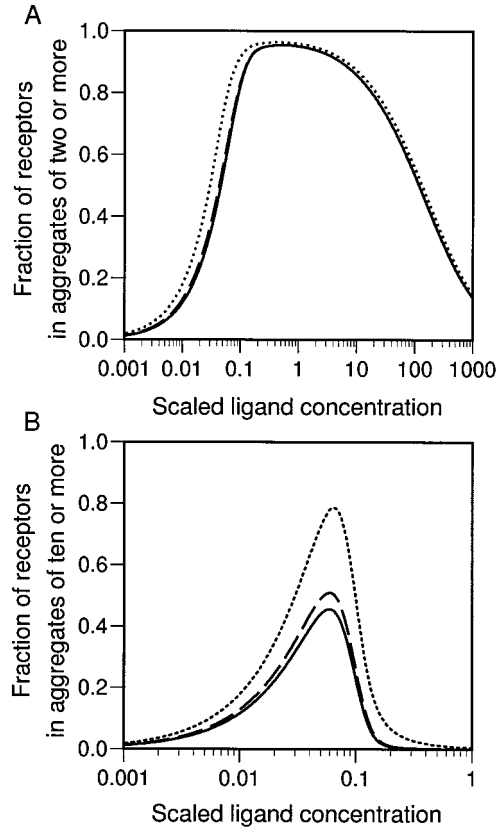

FIGURE 7 Equilibrium cross-linking curves. (*A*) $\alpha(2)$ and (*B*) $\alpha(10)$ are plotted as a function of υKL_T. The binding curves are determined by solving Eq. 13 with the following parameter values: $n = 20$, $K_xR_T = 10$, and $\upsilon KCR_T = 1$. The dotted line is for a ligand with ordered sites and $H(i) = 1$. The broken and solid lines are for a ligand with sites distributed randomly in two dimensions and $H(i) < 1$. To plot the broken line, we calculate $H(i)$ by using Eq. 10 with $a/A = 0.01$. To plot the solid line, we calculate $H(i)$ by using Eq. 12 and the following values for $P_i(\mathcal{A})$ for $i = 1, \ldots, 19$: 0.9120, 0.8282, 0.7482, 0.6715, 0.598, 0.527, 0.458, 0.392, 0.328, 0.265, 0.205, 0.149, 0.100, 0.062, 0.037, 0.0216, 0.0119, 0.0060, and 0.0024. These values are determined with the combinatoric algorithm for the case where a is circular, A is square with periodic boundary conditions, and $a/A = 0.01$.

In the comparison of Fig. 7 *A*, we see that steric effects on equilibrium cross-linking, as measured by $\alpha(2)$, are minor. Essentially the same fraction of receptors are aggregated in the presence or absence of steric effects. This result is typical for other cases that we have examined. However, as illustrated in Fig. 7 *B*, steric effects can significantly influence the distribution of receptor aggregates. Steric effects inhibit the formation of higher-order complexes, such as immunons. As can be seen, the peak fraction of receptors in immunons, which is given by $\alpha(10)$, is reduced by approximately twofold because of steric effects. This result suggests that steric effects on ligand-receptor binding can have different consequences for cellular responses, depending on how the cell senses receptor aggregation. One can expect signals that are triggered by dimeric and larger aggregates to be less sensitive to steric effects than signals that are triggered only by oligomeric aggregates.

By comparing the broken and solid lines in Fig. 7, we can see that Eq. 10, an approximate expression, is capable of accurately modeling steric effects on equilibrium cross-linking.

Steric effects on ligand-receptor kinetics

Time courses of ligand-receptor binding are shown in Fig. 8 for cases where steric effects do and do not influence binding. As in Fig. 7, we consider a ligand with ordered sites, which all can be bound simultaneously, and a ligand with randomly distributed sites, only a fraction of which can be bound simultaneously because of the potential for steric exclusion of ligand sites by bound receptors.

For the time courses of Fig. 8, ligand is initially in solution. As time progresses, ligand-receptor binding leads to the formation of receptor aggregates on the cell surface. In the upper panel, we follow the fraction of receptors in aggregates of all sizes, which is given by $\alpha(2)$, and in the lower panel, we follow the fraction of receptors in aggregates of 10 or more receptors, which is given by $\alpha(10)$. As can be seen, steric effects limit the fraction of receptors that are ultimately aggregated, as we observed earlier (Fig. 7). Steric effects also slow the temporal formation of aggregates. For example, in Fig. 8 *B*, the value of $\alpha(10)$ reaches a value of 0.2 faster for the ligand with ordered sites than for the ligand with randomly distributed sites. As we noted earlier, steric hindrance has a greater effect on higher order ligand-receptor complexes than on lower-order complexes. The fraction of receptors in aggregates of all sizes is comparable with or without steric effects (Fig. 8 *A*), but the fraction of receptors in large aggregates is significantly different when steric effects are present or absent (Fig. 8 *B*).

By comparing the broken and solid lines in Fig. 8, we can see that Eq. 10 is capable of accurately modeling steric effects on ligand-receptor binding kinetics.

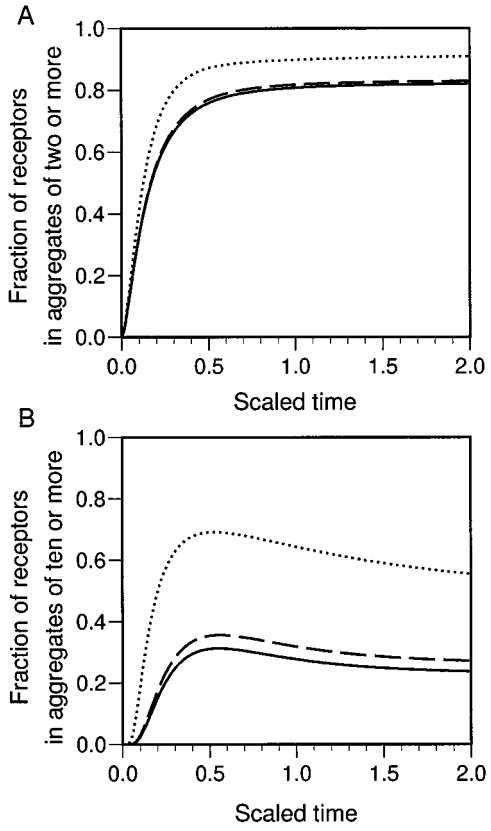

FIGURE 8 Time courses of ligand-receptor binding. The fraction of receptors in (*A*) aggregates of two or more receptors $\alpha(2)$ and in (*B*) aggregates of 10 or more receptors $\alpha(10)$ is plotted as a function of the scaled time $k_{-x}t$. Initially, ligand is in solution. Time courses are determined by solving Eqs. 15–17 with the following parameter values: $vKL_T = 1$, $K_xR_T = 10$, $n = 20$, $CR_T/L_T = 10$, and $k_r/k_{-x} = 1$. The dotted line is for a ligand with ordered sites and $H(i) = 1$. The broken and solid lines are for a ligand with sites distributed randomly in two dimensions and $H(i) < 1$. To plot the broken line, we calculate $H(i)$ by using Eq. 10 with $a/A = 0.01$. To plot the solid line, we calculate $H(i)$ by using Eq. 12 and the numerical values for $P_i(\mathcal{A})$ given in Fig. 7.

DISCUSSION

The present work was motivated by our interest in immunoreceptors. When immunoreceptors are aggregated through interactions with an antigen, signals are generated that lead to cellular activation (Metzger, 1992). This process is best characterized for FcεRI, the high-affinity receptor for IgE, and the immunoglobulin receptor on B cells. Signals induced by aggregation of these receptors depend on various properties of the aggregates that are formed on the cell surface, such as the size of aggregates (Fewtrell and Metzger, 1980; Dintzis et al., 1976) and the time that individual receptors spend in aggregates (Torigoe et al., 1998). These properties in turn depend on the properties of the ligand that mediates receptor aggregation.

Different types of ligands have been used in quantitative studies of receptor aggregation, such as symmetrical bivalent haptens and haptenated proteins and polysaccharides. The binding of bivalent haptens to immunoglobulin receptors has been well studied both theoretically and experimentally (Dembo and Goldstein, 1978; Perelson and DeLisi, 1980; Posner et al., 1995b; Holowka and Baird, 1996). However, bivalent ligands typically stimulate only weak

cellular responses (Kane et al., 1986; Posner et al., 1995a), which limits their usefulness for studying events that follow receptor aggregation. Multivalent ligands can be used to generate strong cellular responses, but the kinetics and thermodynamics of their interactions with receptors have been more difficult to study, and a complete theoretical understanding is not yet at hand. Here we have focused on methods for including steric hindrance effects in both kinetic and equilibrium binding calculations.

When a single site on a multivalent ligand binds to a cell surface receptor, nearby sites on the ligand may not be available for binding because receptors have a finite size and can "cover" sites and "exclude" others by preventing receptors from getting close enough to bind them. Here we have developed a theoretical framework for modeling steric exclusion of potential ligand sites by bound cell surface receptors. In this framework, steric effects on ligand-receptor binding are characterized by a steric hindrance factor, $H(i)$ (Eq. 11), which gives the fraction of unbound ligand sites that are available for receptor binding as a function of i, the number of ligand sites already occupied by receptors. The functional form of $H(i)$ depends on the size of the receptor and the spacing of ligand binding sites. We derived analytical expressions for $H(i)$ that apply under different circumstances (Table 1). These expressions are relevant for receptors that cover a compact region on the ligand surface and for ligands such as linear polymers, proteins, and viruses. In addition, we have presented numerical algorithms that can be used to calculate steric hindrance factors for other types of ligands and receptors.

We derived both exact and approximate expressions for steric hindrance factors (Table 1). To test the accuracy and usefulness of the approximate expressions, we used them to calculate insertion probabilities (Figs. 5 and 6). We then compared these insertion probabilities with those calculated using either a combinatoric or Monte Carlo algorithm. The approximate and numerical results are in close agreement for the cases that we examined. Because all of the expressions in Table 1 were derived by the same method, we focused on one approximate expression (Eq. 10), the equation for a ligand with sites randomly distributed in two dimensions. This type of ligand corresponds to a haptenated protein. We found, for the cases examined in Figs. 7 and 8, that this approximate expression is adequate for quantifying steric hindrance effects on both the kinetic and equilibrium behaviors of ligand-receptor binding.

Figs. 7 and 8 indicate that the fraction of receptors in large aggregates is more sensitive to steric effects than the fraction of receptors in aggregates of all sizes. Thus transmembrane signals that are generated in response to large aggregates may be sensitive not only to the number of binding sites per ligand, but also to the arrangement and spacing of ligand sites, which in turn are related to the overall size of the ligand.

In this paper we have focused on steric effects that influence receptor binding due to the exclusion of ligand sites by bound receptors. However, steric effects also can influence ligand adsorption to a cell if a bound ligand can sterically hinder the binding of other ligands to unbound receptors. This might occur if the ligand were large, as in the case of a bacterium binding to a cell, and in circumstances where receptor mobility is impeded. Stankowski (1984) discusses steric effects on ligand adsorption and suggests an approach for modeling these effects.

A number of theories and methods are available for modeling steric effects on adsorption reactions. For example, McGhee and von Hippel (1974) and others (Schwarz, 1977; Epstein, 1978; Reiter and Epstein, 1990; Badcoe, 1992; Di Cera and Kong, 1996) have developed theories and methods for modeling the binding of "large" ligands, which cover more than one site, to one-dimensional lattices of binding sites. The equations of McGhee and von Hippel have been widely used to analyze the binding of proteins to DNA. Similarly, Stankowski (1983, 1984) and others (Tamm and Bartoldus, 1988; Cowan and Underwood, 1988; Schaaf and Talbot, 1989; Chatelier and Minton, 1996; Sild et al., 1996) have developed methods for modeling the binding of large ligands to two-dimensional lattices of binding sites and potential surfaces. The theory developed here is distinguished from earlier work for several reasons: 1) We have considered steric effects on adsorption reactions in the specific context of a multivalent ligand binding to cell surface receptors. Steric effects on equilibrium and kinetic binding are characterized by a steric hindrance factor, which we have introduced into a previously developed model for ligand-receptor binding (Perelson, 1984; Lauffenburger and Linderman, 1993). 2) We have considered adsorption at randomly distributed sites, which is important for analyzing the binding of haptenated carrier molecules to cell surface antibodies. Most earlier work has focused on adsorption at regularly ordered sites or adsorption to a surface on which all points can potentially serve as a binding site. An exception is the study of Macken and Perelson (1986), in which equilibrium binding to a one-dimensional random distribution of sites was considered. 3) We have accounted for a wide array of cases (Table 1) with a single general approach. We also have presented numerical algorithms that can be used to calculate steric hindrance factors for any conceivable case.

The theory that we have developed for quantifying steric effects on ligand-receptor binding is closely related to statistical thermodynamic theories for one- and two-dimensional fluids of hard-core particles. A bound receptor that covers a circular area on the surface of a ligand is analogous to a hard disk in a two-dimensional fluid of hard disks. In thermodynamics, the insertion probability, the probability of adding a particle to a fluid without overlap, is related to the excess chemical potential of the fluid (Widom, 1963). A number of methods are available for calculating insertion probabilities, both numerically and in closed form. Here we have adapted some of these methods (Andrews, 1975, 1976; Boublík, 1975; Siepmann et al., 1992) to calculate insertion probabilities that are relevant for ligand-receptor binding. These methods required modification, because on the sur-

face of a ligand, a bound receptor is constrained to particular ligand sites, whereas in a thermodynamic fluid, a particle can be located potentially anywhere. Our expressions for insertion probabilities (Table 1) reduce to expressions for hard-core particle fluids in the limit $n \to \infty$.

The model that we have developed is for a monovalent receptor or for the case in which the sites on a multivalent receptor are assumed to act independently, as is assumed in equivalent site models. The model also applies to experimental systems, such as the basophil and related cell lines that express FcϵRI, in which bispecific chimeric antibodies can be used as receptors. These antibodies are effectively monovalent, because the specificity of each Fab arm is different. To extend our theory to multivalent receptors in which the structure of the receptor is explicitly taken into consideration is beyond the scope of this paper. Macken and Perelson (1986) have modeled the size and range of possible motions of the two Fab arms of immunoglobulin receptors in their study of the binding of a bivalent antibody to a linear antigen with randomly distributed binding sites. Wiegel and Goldstein (1987) have considered the binding of a bivalent antibody to a linear antigen with regularly ordered binding sites. Tamm and Bartoldus (1988) have considered the binding of a bivalent antibody to lipid membranes.

The main result reported here is the development of new methods for increasing the realism of models for multivalent ligand-receptor binding by taking steric hindrance effects into consideration. We believe these improvements will have importance in a variety of studies examining cellular responses to complex antigens, such as haptenated proteins. Theoretical and experimental efforts to understand such systems are of current and increasing interest (Sulzer and Perelson, 1997; Xu et al., 1998; Hlavacek et al., 1999).

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