Diffusion-induced competitive two-site binding

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ABSTRACT

The influence of diffusion on the kinetics of ligand binding to a macromolecule with two sites is considered for a simple model where, in the reaction-controlled limit, there is no cooperativity and hence the sites are independent. By applying our recently developed formalism to describe a network of coupled diffusion-influenced reactions, we show that the rate constants of chemical kinetics cannot just be renormalized. Rather a new reaction channel, which connects the two singly occupied states, must be introduced. The rate constants of this new channel depend on the committor or capture probability that a ligand that just dissociated from one site rebinds to the other. This result is rederived in an elementary way using the encounter complex model. Illustrative calculations are presented where the kinetics of the fractional saturation of one site is compared with that of a macromolecule that has only this site. If all sites are initially empty, then the second site slows down binding to the first due to competition between the sites. On the other hand, if the second site is initially occupied, the binding of the first site speeds up because of the direct diffusion-induced transitions between the two singly bound states.

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I. INTRODUCTION

Consider a macromolecule with two different binding sites for a ligand. Let us assume that, in the framework of chemical kinetics, binding to one site does not depend on the occupancy of the other. We are interested in how the diffusive motion of the ligands influences the kinetics. There are two distinct physical effects that any theory must deal with. First, the sites compete for the ligands. A ligand that has diffused close to the macromolecule can bind to either site. Thus, the rate of binding to one site in the presence of the other should be slower. This occurs even when the binding is irreversible and was first discussed in the context of cell-bound receptors involved in chemotaxis by Berg and Purcell.¹

When the binding is reversible, another process must be considered. Namely, a ligand that has just dissociated from one site can geminately rebound to the other site. Since this ligand is already close to the macromolecule, rebinding will occur faster than binding a ligand from the bulk. In this paper, we will bring our general theory of coupled diffusion-influenced reactions² to bear on this problem. We will see that even

the simplest version of our formalism leads to the modification of the standard chemical kinetic scheme by introducing a new reaction channel that directly connects the two states that are singly occupied. However, when all binding sites are equivalent, we will show that it is sufficient to replace the intrinsic association and dissociation rate constants by their diffusion-influenced counterparts.³

We first found that diffusion can modify the connectivity of a chemical kinetic scheme in the context of multisite phosphorylation where an enzyme can modify a substrate at several sites.⁴ Specifically, the chemical kinetic scheme for double phosphorylation is $S_0 + E \rightleftharpoons S_0E \rightarrow S_1 + E \rightleftharpoons S_1E \rightarrow S_2 + E$, where S_i represents the substrate with i sites phosphorylated and S_0E and S_1E are the enzyme-substrate complexes. We showed that diffusion modifies this kinetic scheme by inducing a direct transition between S_0E and S_1E .^{4,5} When the catalytic rate is very fast, the above scheme reduces to $S_0 + E \rightarrow S_1 + E \rightarrow S_2 + E$. We were able to solve exactly the corresponding many-particle time-dependent problem when the diffusing enzymes are in excess and not interacting and the substrates are static.⁴ By taking the Markovian limit, we showed that diffusion induces a direct transition between

$S_0 + E$ and $S_2 + E$. Prior to our work, Zhou studied a similar problem in the context of “substrate channeling.”⁶ Specifically, he considered an enzyme with two active sites that modifies a substrate via its first site forming an intermediate and then again via its second site forming a product. When the catalytic rates are very fast, he found analytical expressions for the time dependence of the substrate concentrations. If one identifies S_1 as the intermediate and S_2 as the product, his kinetic scheme is identical to our irreversible one. It can be shown that the results in Ref. 6 can be obtained from our diffusion-modified scheme with the extra transition $S_0 + E \rightarrow S_2 + E$ (see Fig. 2B in Ref. 4).

The scheme that describes two-site binding in the framework of chemical kinetics is shown in Fig. 1. A macromolecule can be in four states: unliganded (M_1), with one ligand bound (M_2 and M_3) and with two ligands bound (M_4). We assume that the binding rate constants do not depend on the state of the macromolecule; therefore, there is no kinetic and thermodynamic cooperativity in the system. All effects discussed in this paper are solely due to correlations that arise as a result of the diffusive motion of the reactants.

The probability of the first site to be bound is

$$B_1(t) = \frac{[M_2] + [M_4]}{[M_1] + [M_2] + [M_3] + [M_4]}, \quad (1)$$

where $[M_i]$ is the concentration of the macromolecule in state M_i . We will ascertain the influence of the second site by comparing this with the binding probability of another macromolecule M that only has the first site, described by the kinetic scheme

FIG. 1. Chemical kinetic scheme for two-site binding. A macromolecule with two sites binds a ligand L and forms four states: with both sites empty (M_1), with the first (M_2) or second (M_3) site bound, and with both sites occupied (M_4). κ_{ai} and κ_{di} are the association and dissociation intrinsic rate constants for site i , $i = 1, 2$. v_i , $i = 1, 2, 3, 4$, indicate the net reaction rates. The sites are assumed to be thermodynamically and kinetically independent. The ligands are in excess, so the ligand concentration, $[L]$, does not change.

The corresponding binding probability is

$$B_1^s(t) = \frac{[ML]}{[ML] + [M]}. \quad (3)$$

Here, the superscript “s” (i.e., single) indicates that the molecule has only the first site. By calculating $B_1(t)$ for the kinetic scheme in Fig. 1 and $B_1^s(t)$ for the scheme in Eq. (2), it can be shown that $B_1(t) = B_1^s(t)$, and thus in this case the first site is not affected by the presence of the second site. However, as will be shown in this paper, when diffusion of reactants is sufficiently slow, this is no longer true because the two sites become coupled.

The outline of this paper is as follows. We start with the rate equations of chemical kinetics written in terms of reaction rates and stoichiometric coefficients in Sec. II A. By applying the theory of diffusion-influenced reaction networks,² we obtain a set of the diffusion-modified rate equations, in which the chemical reaction rates become coupled (Sec. II B). These rate equations correspond to a diffusion-modified kinetic scheme, where the two states of the macromolecule with one bound site are directly connected (Sec. II C). This kinetic scheme is rederived in the framework of the phenomenological encounter complex model in Sec. III. Illustrative calculations are presented in Sec. IV. We first consider capture and escape probabilities, which modify the intrinsic rate constants, and their connection to the Smoluchowski diffusion-controlled rate constant (Sec. IV A). The special case of irreversible binding to the first site (but still reversible binding to the second) is considered in Sec. IV B. The binding probability for arbitrary reactivities is considered in Sec. IV C. In Sec. V, we present some concluding remarks.

II. THEORY

To describe the binding of diffusing ligands to a macromolecule with two sites, we use the general theory developed recently² for a network of coupled association-dissociation reactions. The theory is formulated in terms of stoichiometric matrices and reaction rates of conventional chemical kinetics and the matrix of diffusion-influenced irreversible association fluxes, obtained by solving pair problems. We assume here that the ligands are in excess so that their concentration $[L]$ does not change.

A. Chemical kinetics

The rate equations of conventional chemical kinetics for the system of four species involved in four bimolecular association-dissociation reactions (see Fig. 1) can be written as

$$\frac{d\mathbf{x}}{dt} = \mathbf{S}\mathbf{v}, \quad (4)$$

where $\mathbf{x}$ is the vector with elements $x_i = [M_i]$ equal to the concentration of species M_i , $i = 1, \dots, 4$, $\mathbf{v}$ is the vector with

elements v_α , $\alpha = 1, \dots, 4$, of the bimolecular net reaction rates defined as

$$\begin{aligned} v_1 &= \kappa_{a1}[L][M_1] - \kappa_{d1}[M_2], \\ v_2 &= \kappa_{a2}[L][M_1] - \kappa_{d2}[M_3], \\ v_3 &= \kappa_{a2}[L][M_2] - \kappa_{d2}[M_4], \\ v_4 &= \kappa_{a1}[L][M_3] - \kappa_{d1}[M_4], \end{aligned} \quad (5)$$

where κ_{ai} and κ_{di} are the association and dissociation intrinsic rate constants of site i ($i=1, 2$). The stoichiometric matrix $\mathbf{S}$ is a 4×4 matrix of stoichiometric coefficients

$$\mathbf{S} = \begin{pmatrix} -1 & -1 & 0 & 0 \\ 1 & 0 & -1 & 0 \\ 0 & 1 & 0 & -1 \\ 0 & 0 & 1 & 1 \end{pmatrix}. \quad (6)$$

Here, the i th column of $\mathbf{S}$ corresponds to the i th reaction channel. For example, the first column of $\mathbf{S}$ shows that M_1 and M_2 are involved in the first reaction with net rate v_1 .

The rate equations [Eq. (4)] can be reformulated as

$$\frac{d\mathbf{x}}{dt} = \mathbf{K}\mathbf{x}, \quad (7)$$

where $\mathbf{K}$ is the rate matrix corresponding to the kinetic scheme in Fig. 1. This rate matrix is the product of the stoichiometric matrix $\mathbf{S}$ and the matrix $\mathbf{F}$ with the elements equal to the derivatives of the net reaction rates with respect to the species concentrations, $F_{ij} = \partial v_i / \partial [M_j]$

$$\mathbf{K} = \mathbf{S}\mathbf{F}, \quad (8)$$

where

$$\mathbf{F} = \begin{pmatrix} \kappa_{a1}[L] & -\kappa_{d1} & 0 & 0 \\ \kappa_{a2}[L] & 0 & -\kappa_{d2} & 0 \\ 0 & \kappa_{a2}[L] & 0 & -\kappa_{d2} \\ 0 & 0 & \kappa_{a1}[L] & -\kappa_{d1} \end{pmatrix}. \quad (9)$$

Here, the i th row of $\mathbf{F}$ corresponds to the i th reaction channel. For example, the first row of $\mathbf{F}$ contains the association and dissociation rate constants for the first site in the first reaction channel.

The solution of the rate equation [Eq. (7)] is $\mathbf{x}(t) = \exp(\mathbf{K}t)\mathbf{x}(0)$. Using this, it can be shown that, if all sites are initially empty ($[M_2] = [M_3] = [M_4] = 0$ at $t = 0$), the probability that site 1 is occupied [Eq. (1)] is

$$B_1(t) = \frac{\kappa_{a1}[L]}{\kappa_{a1}[L] + \kappa_{d1}} (1 - e^{-(\kappa_{a1}[L] + \kappa_{d1})t}). \quad (10)$$

This kinetics is identical to that where site 2 does not exist [Eqs. (2) and (3)]. Thus, in this model, the kinetics of binding to the first site is unaffected by the presence of the second site. We shall see that when the influence of diffusion is taken into account, this will no longer be the case.

In the special case when the two sites are indistinguishable (i.e., when $\kappa_{a1} = \kappa_{a2} = \kappa_a$ and $\kappa_{d1} = \kappa_{d2} = \kappa_d$), the kinetic scheme in Fig. 1 simplifies. By adding the rate equations for $[M_2]$ and $[M_3]$ and defining $[ML] = [M_2] + [M_3]$, one

obtains rate equations that correspond to the following kinetic scheme:

The statistical factor 2 in the rate constants accounts for the fact that a ligand can bind to two sites of M_1 and that a ligand can dissociate from either site of M_4 .

B. Diffusion-modified rate equations

Now consider how diffusion can alter the kinetics of binding. In general, the influence of diffusion on time dependence of concentrations is described by non-Markovian rate equations²

$$\frac{d\mathbf{x}}{dt} = \mathbf{S} \left(\mathbf{v} - \int_0^t \mathbf{J}(t-t') \mathbf{v}(t') dt' \right), \quad (12)$$

where $\mathbf{J}(t)$ is the 4×4 matrix of the irreversible pair association fluxes. Each element of this matrix is the probability density that, in an isolated ligand-macromolecule pair, the ligand irreversibly binds to one reaction site at time t having just dissociated from the same or another site at time $t = 0$. In the limit of fast diffusion, the molecules formed after dissociation immediately diffuse apart, so all elements of $\mathbf{J}$ are zero, and we recover the rate equations of ordinary chemical kinetics [Eq. (4)]. The above rate equation involves the convolution of the pair association fluxes with the reaction rates, and hence the rate at time t depends on the rates at previous times. When the pair association fluxes change much faster than the bulk reaction rates (the Markovian limit), the memory effects can be neglected.

The diffusion-modified rate equation in the Markovian limit (which is the main focus in this paper) is

$$\frac{d\mathbf{x}}{dt} = \mathbf{S}(\mathbf{I} - \mathbf{Q})\mathbf{v}, \quad (13)$$

where $\mathbf{I}$ is the 4×4 identity matrix and $\mathbf{Q}$ is the 4×4 committor matrix,² $\mathbf{Q} = \int_0^\infty \mathbf{J}(t) dt$. The elements of $\mathbf{Q}$ are the probabilities that the ligand that has just dissociated from one site of the macromolecule irreversibly rebinds to the same or another unoccupied site rather than diffusing away. These committors or capture probabilities are defined for an isolated pair of a ligand and a macromolecule (in any state) that can only bind irreversibly and thus cannot dissociate. The matrix $\mathbf{Q}$ has the following structure in the space of net reaction rates:

$$\mathbf{Q} = \begin{pmatrix} Q_{11} & Q_{12} & 0 & 0 \\ Q_{21} & Q_{22} & 0 & 0 \\ 0 & 0 & Q_2^s & 0 \\ 0 & 0 & 0 & Q_1^s \end{pmatrix}. \quad (14)$$

The off-diagonal elements are not independent, but are related² by the detailed balance condition, $Q_{12}\kappa_{a2} = Q_{21}\kappa_{a1}$. The physical interpretation of the elements of $\mathbf{Q}$ is shown schematically in Fig. 2. Consider the first reaction channel with rate v_1 , where a ligand binds to site 1 of the unliganded macromolecule. Q_{11} is the probability that a ligand that has just

FIG. 2. Schematic definition of the committers (capture and escape probabilities). (a) Q_{11} is the probability that a ligand that has just dissociated from the first site (i.e., from M_2) rebinds to the first site (to form M_2) rather than binding to the second site (to form M_3) or diffusing away. Q_{21} is the probability that a ligand that has just dissociated from the first site rebinds to the second site to form M_3 . $\varepsilon_1 = 1 - Q_{11} - Q_{21}$ is the probability that a ligand that has just dissociated from the first site does not rebound and diffuses away (i.e., escapes to infinity). The plus sign “+” indicates that the ligand and the macromolecule are infinitely far apart. Q_{12} , Q_{22} , and $\varepsilon_2 = 1 - Q_{22} - Q_{12}$ are defined analogously for a ligand that has just dissociated from the second site (from M_3). (b) Q_1^s is the probability that a ligand that has just dissociated from the first site of M_4 rebinds to that site. $\varepsilon_1^s = 1 - Q_1^s$ is the probability to diffuse away (escape). (c) Q_2^s ($\varepsilon_2^s = 1 - Q_2^s$) is the probability that a ligand that has just dissociated from the second site of M_4 rebinds to that site (diffuses away).

dissociated from site 1 will eventually rebound to site 1 (i.e., will be captured by site 1) rather than bind to site 2 or diffuse away. Q_{21} is the probability that a ligand initially near site 1 rebinds to site 2. $\varepsilon_1 = 1 - Q_{11} - Q_{21}$ is the probability that this ligand does not rebound but diffuses away (escapes). The meaning of Q_{22} , Q_{12} , and ε_2 is analogous.

Now consider the reaction channel with rate v_4 , where a ligand binds to site 1 of a macromolecule that already has a ligand bound at site 2. A ligand that has just dissociated from site 1 can only rebound to site 1 (with probability Q_1^s) or diffuse away (with probability $\varepsilon_1^s = 1 - Q_1^s$) because the other site is already occupied. These probabilities are the same as those for a macromolecule that has only site 1 and hence the superscript “s” (single site) on the capture and escape probabilities. The interpretation of Q_2^s and $\varepsilon_2^s = 1 - Q_2^s$ is analogous.

C. Diffusion-modified kinetic scheme

When the matrix $\mathbf{Q}$ in Eq. (14) is substituted into Eq. (13), the rates v_1 and v_2 become coupled. The resulting equation

[Eq. (13)] can be recast in a form that involves a diffusion-modified rate matrix, $\mathbf{K}_{\text{DM}}$, i.e.,

$$\frac{d\mathbf{x}}{dt} = \mathbf{K}_{\text{DM}}\mathbf{x}, \quad (15)$$

where $\mathbf{K}_{\text{DM}} = \mathbf{S}(\mathbf{I} - \mathbf{Q})\mathbf{F}$ [cf. Eq. (8)]. The diffusion-modified kinetic scheme that corresponds to this rate matrix is shown in Fig. 3. The rate constants that appear in the chemical kinetic scheme shown in Fig. 1 are scaled by the escape probabilities (in such a way that the equilibrium constants are unchanged). However, not only are the rate constants scaled but also a new reaction channel appears, which directly connects the two singly occupied states. The physical reason for this is that a ligand that has just dissociated from one site can rebound to the other site rather than diffusing away into the bulk. When diffusion is very fast (the reaction-controlled limit), the probability of this occurring approaches zero, and hence is not considered in ordinary chemical kinetics. The rate constants for these new transitions have a transparent physical interpretation. They are equal to the product of the dissociation rates and the probabilities that, after dissociation from one site, the ligand rebinds to the other site.

As before, we are interested in comparing the probability $B_1(t)$ that site 1 is occupied [Eq. (1)], calculated for the diffusion-modified scheme in Fig. 3, with the result that would be obtained if site 2 did not exist. The corresponding diffusion-modified kinetic scheme is

FIG. 3. Diffusion-modified kinetic scheme for two-site binding. The new reaction channel not present in the conventional kinetic scheme in Fig. 1 is shown in red. The intrinsic rate constants of the reaction channels of chemical kinetics (in Fig. 1) are scaled by the two-site, ε_1 and ε_2 , and single-site, ε_1^s and ε_2^s , escape probabilities. The rate constants of the new reaction channels are equal to the product of the dissociation rate constants, κ_{dj} , and the probabilities that the ligand that has just dissociated from one site rebinds to the other, Q_{12} and Q_{21} . Here, we have taken artistic license and put the binding sites on the opposite poles of the macromolecule. However, the role of diffusion is greater when the sites are located close to each other.

where the intrinsic rate constants κ_{a1} and κ_{d1} are scaled by the single-site escape probability, ε_1^s . We shall see that the probability that site 1 is occupied for two-site and single-site binding is no longer the same, i.e., $B_1(t) \neq B_1^s(t)$.

In the special case when the singly bound states are indistinguishable and thus the intrinsic association and dissociation rate constants of the two sites are the same ($\kappa_{a1} = \kappa_{a2} = \kappa_a$, $\kappa_{d1} = \kappa_{d2} = \kappa_d$, and consequently $\varepsilon_1 = \varepsilon_2 = \varepsilon$ and $\varepsilon_1^s = \varepsilon_2^s = \varepsilon^s$), the kinetic scheme in Fig. 3 simplifies. By adding the diffusion-modified rate equations for $[M_2(t)]$ and $[M_3(t)]$ and defining $[ML(t)] = [M_2(t)] + [M_3(t)]$, one can show that the scheme in Fig. 3 reduces to

This kinetic scheme for indistinguishable sites is the same as that in ordinary chemical kinetics [see Eq. (11)], but with the intrinsic rate constants scaled with the corresponding escape probabilities. Thus, the new reaction channel only appears when the sites are inequivalent.

III. ENCOUNTER COMPLEX MODEL

We have obtained the diffusion-modified kinetic scheme in Fig. 3 by using our general formalism as a “black box.” Although the diffusion-influenced rate constants for the old and new reaction channels turned out to have a physically appealing interpretation, it would be nice if they could be obtained in an elementary self-contained way. Here, we will do this within the framework of the encounter-complex model originally introduced by Eigen⁷ and then used⁸ to obtain the diffusion-influenced rate constants for $M + L \rightleftharpoons ML$. In this model, the diffusion-influenced binding consists of two steps. First, a macromolecule and a ligand diffuse together and form an encounter complex (with the bimolecular rate constant k_{Di}^+ for site $i = 1, 2$) and then they either diffuse apart (with the rate constant k_{Di}^-) or bind (with the rate constant a_i). A bound state can dissociate with the rate constant d_i to form an encounter complex (see Fig. 4). The two encounter complexes of the unbound macromolecule M_1 with a ligand close to the first site, $M_1 \dots L(1)$, and to the second site, $M_1 \dots L(2)$, can interconvert because the ligand can diffuse from one site to the other. This is described by phenomenological rate constants b_1 and b_2 . These rates are not arbitrary, but restricted by the detailed balance condition, $b_1 k_{D1}^+ k_{D2}^- = b_2 k_{D2}^+ k_{D1}^-$. Note that all rate constants in Fig. 4 are the unimolecular ones, except k_{Di}^+ , which are bimolecular rate constants with dimensionality volume/time.

Starting with the rate equations corresponding to the extended chemical kinetic scheme in Fig. 4, invoking the steady-state approximation for the concentrations of the encounter complexes ($d[M_1 \dots L(i)]/dt = 0$, $i = 1, 2$, and $d[M_1 \dots L]/dt = 0$, $i = 2, 3$), one can eliminate the concentrations of the encounter complexes. The resulting rate equations for $[M_1]$, $[M_2]$, $[M_3]$, and $[M_4]$ contain rate constants that are

FIG. 4. Kinetic scheme for the encounter complex model. The scheme in Fig. 1 is augmented by four encounter complexes, where the ligand L is close to the first or to the second unbound site of the macromolecule. The macromolecule with two empty sites, M_1 , forms two such complexes with a ligand near the first, $M_1 \dots L(1)$, and the second, $M_1 \dots L(2)$, site, respectively.

rather complicated combinations of the original phenomenological rate constants, k_{Di}^+ , k_{Di}^- , a_i , d_i , b_1 , and b_2 . However, if one calculates the capture and escape probabilities within the framework of the encounter complex model, these rate constants can be simply expressed in terms of these probabilities, as we have shown before⁵ in the context of two-site phosphorylation.

To show how this works, let us start with the fragment of the scheme in Fig. 4 that involves $M_3 \dots L$

By invoking the steady-state approximation for $[M_3 \dots L]$, this becomes

To find the escape (ε_1^s) and capture ($Q_1^s = 1 - \varepsilon_1^s$) probabilities within the framework of the encounter complex model, we need to consider the scheme that is an irreversible analogue of Eq. (18):

Here, the ligand in the encounter complex $M_3 \dots L$ can diffuse away (escape) to form M_3 or irreversibly bind to form M_4 . The probability that it escapes is $\varepsilon_1^s = k_{D1}^- / (k_{D1}^- + a_1)$. The probability to bind is $Q_1^s = a_1 / (k_{D1}^- + a_1)$. Thus, if we define the intrinsic association and dissociation constants as $\kappa_{a1} = a_1 k_{D1}^+ / k_{D1}^-$ and $\kappa_{d1} = d_1$, then the kinetic scheme in Eq. (19) can be rewritten as

This scheme is identical to the fragment of the diffusion-modified kinetic scheme in Fig. 3 that connects M_3 and M_4 . The rate constants that connect M_2 and M_4 in Fig. 3 are obtained in a similar way.

Finally, consider the fragment of the scheme in Fig. 4 that involves complexes $M_1 \dots L(1)$ and $M_1 \dots L(2)$. By using the steady-state approximation for these encounter complexes, the kinetic scheme in Fig. 4 can be reduced to one that has the same connectivity as the diffusion-modified one in Fig. 3, but with the rate constants that at first sight look hopelessly complicated. For example, the rate constant for the transition $M_2 \rightarrow M_3$ is

$$\frac{d_1 b_1 a_2}{(k_{D1}^- + a_1 + b_1)(k_{D2}^- + a_2 + b_2) - b_1 b_2}. \quad (22)$$

However, we will now show that all these rate constants become identical to those in Fig. 3 when expressed in terms of the capture and escape probabilities calculated within the framework of the encounter complex model.

To do this, we consider the irreversible kinetic scheme involving $M_1 \dots L(1)$ and $M_1 \dots L(2)$ shown in Fig. 5(a). The probability p_i of finding the system in state $M_1 \dots L(i)$ at time t satisfies

$$\frac{d\mathbf{p}}{dt} = -\mathbf{R}\mathbf{p}, \quad (23)$$

where $\mathbf{p}$ is a column vector with elements p_1 and p_2 and $\mathbf{R}$ is the 2×2 matrix

$$\mathbf{R} = \begin{pmatrix} k_{D1}^- + a_1 + b_1 & -b_2 \\ -b_1 & k_{D2}^- + a_2 + b_2 \end{pmatrix}. \quad (24)$$

The capture and escape probabilities are defined as follows [see Fig. 5(b)]: Q_{ij} is the probability that the system initially in state $M_1 \dots L(j)$ decays through the binding channel with rate constant a_i . ε_j is the probability that the system initially in state $M_1 \dots L(j)$ decays through either diffusion channel with rate constants k_{D1}^- and k_{D2}^- . These can be expressed in terms of the probability that the system is in state $M_1 \dots L(i)$ at time t having been in state $M_1 \dots L(j)$ initially, $p(i, t|j, 0)$, as

$$Q_{ij} = a_i \int_0^\infty p(i, t|j, 0) dt, \quad (25)$$

$$\varepsilon_j = \sum_{i=1}^2 k_{Di}^- \int_0^\infty p(i, t|j, 0) dt.$$

Now $p(i, t|j, 0) = [\exp(-\mathbf{R}t)]_{ij}$, and since $\int_0^\infty \exp(-\mathbf{R}t) dt = \mathbf{R}^{-1}$, the above capture and escape probabilities can be rewritten as

$$Q_{ij} = a_i [\mathbf{R}^{-1}]_{ij},$$

$$\varepsilon_j = \sum_{i=1}^2 k_{Di}^- [\mathbf{R}^{-1}]_{ij}. \quad (26)$$

These probabilities can be obtained analytically by inverting $\mathbf{R}$.

Using these results and defining $\kappa_{ai} = a_i k_{Di}^+ / k_{Di}^-$ and $\kappa_{di} = d_i$, $i = 1, 2$, one can show that the kinetic scheme and rate constants obtained by invoking the steady-state approximation for the concentrations of the encounter complexes

FIG. 5. (a) The irreversible kinetic scheme used to calculate the committors in the encounter complex model. (b) Schematic definitions of the committors (capture/escape probabilities) within the framework of the encounter complex model. This is similar to Fig. 2(a) with the pairs formed immediately after dissociation being replaced by the corresponding encounter complexes. For example, Q_{21} is the probability that, starting with $M_1 \dots L(1)$, the system decays via the channel with rate constant a_2 , whereas ε_1 is the probability that this system decays via the channel with rate constant k_{D2}^- or the one with rate constant k_{D1}^- .

are identical to the diffusion-modified scheme shown in Fig. 3. Thus, in this case, the encounter complex model reproduces the diffusion-modified kinetic scheme obtained for a more general microscopic model.

IV. ILLUSTRATIVE CALCULATIONS

To calculate the time dependence of the concentrations, we need to know the magnitude of the elements of the committor matrix $\mathbf{Q}$. These depend on the behavior of an isolated pair of reactants that can irreversibly associate. For realistic microscopic models of the reaction, they can be obtained from the Brownian dynamics simulations. For certain simple models, the elements of $\mathbf{Q}$ can be found analytically.

A. Analytic results for the committors

Consider the following microscopic model of association of a macromolecule and a ligand and the dissociation of the resulting complex. Assume for the sake of simplicity that there are no interaction potentials between the reactants other than short-range repulsive ones. Let $\mathbf{r}$ denote the distance between reactants and their orientations. The formation and breakage of stereospecific interactions are described by sink functions. An M_1 - L pair with relative coordinate $\mathbf{r}$ can form M_2 with

the rate constant $\kappa_{a1}\sigma_1(\mathbf{r})$, where the normalized sink function $\sigma_1(\mathbf{r})$ ($\int \sigma_1(\mathbf{r}) d\mathbf{r} = 1$) is nonzero only in the region where M_1 and L have the appropriate separation and orientation to form M_2 . M_2 can dissociate with unimolecular rate constant κ_{d1} to form M_1 and L with relative coordinate $\mathbf{r}$ chosen from the probability distribution $\sigma_1(\mathbf{r})$. The dependence of $\sigma_1(\mathbf{r})$ on $\mathbf{r}$ for association and dissociation is the same because of detailed balance. An M_3 - L pair associates to form M_4 with the same rate constant $\kappa_{a1}\sigma_1(\mathbf{r})$. Thus, the association and dissociation via the fourth reaction channel are described by the same sink function $\sigma_1(\mathbf{r})$. The association and dissociation via the second and the third reaction channels are described by the sink function $\sigma_2(\mathbf{r})$.

To find the elements of the committor matrix $\mathbf{Q}$, one has to solve an irreversible pair problem with the appropriate boundary conditions.² For an arbitrary position-dependent reactivity, this can be done only numerically. However, using the Wilemski-Fixmann closure approximation, the capture probabilities can be expressed in terms of sink-sink correlation functions calculated in the absence of reactions.² It was shown that the diffusion-influenced association rate constants for a macromolecule with one or two reactive spots on the surface found within this approximation differ from the exact ones by no more than 9%.⁹⁻¹²

Within the framework of the Wilemski-Fixmann closure approximation, the committor matrix $\mathbf{Q}$ is given by²

$$\mathbf{Q} = (\mathbf{I} + \mathbf{Q}^0)^{-1} \mathbf{Q}^0, \quad (27)$$

where $\mathbf{Q}^0$ has the same structure as $\mathbf{Q}$ [Eq. (14)]. The elements of $\mathbf{Q}^0$ can be expressed in terms of the time integrals of the sink-sink correlation functions, C_{ij} , as

$$\mathbf{Q}^0 = \begin{pmatrix} \kappa_{a1}C_{11} & \kappa_{a1}C_{12} & 0 & 0 \\ \kappa_{a2}C_{21} & \kappa_{a2}C_{22} & 0 & 0 \\ 0 & 0 & \kappa_{a2}C_{22} & 0 \\ 0 & 0 & 0 & \kappa_{a1}C_{11} \end{pmatrix}, \quad (28)$$

where C_{ij} are defined as

$$\begin{aligned} C_{11} &= \int_0^\infty dt \int d\mathbf{r} d\mathbf{r}' \sigma_1(\mathbf{r}) g(\mathbf{r}, t | \mathbf{r}', 0) \sigma_1(\mathbf{r}'), \\ C_{22} &= \int_0^\infty dt \int d\mathbf{r} d\mathbf{r}' \sigma_2(\mathbf{r}) g(\mathbf{r}, t | \mathbf{r}', 0) \sigma_2(\mathbf{r}'), \\ C_{12} &= C_{21} = \int_0^\infty dt \int d\mathbf{r} d\mathbf{r}' \sigma_1(\mathbf{r}) g(\mathbf{r}, t | \mathbf{r}', 0) \sigma_2(\mathbf{r}'). \end{aligned} \quad (29)$$

Here, $g(\mathbf{r}, t | \mathbf{r}', 0)$ is the Green's function for free translational and rotational diffusion with the reflecting boundary condition at contact.

Using Eq. (28) in Eq. (27), we can find the matrix elements of $\mathbf{Q}$ [Eq. (14)]. The single-site capture and escape probabilities in terms of C_{ii} are ($i = 1, 2$)

$$Q_i^s = 1 - \varepsilon_i^s = \frac{\kappa_{ai}C_{ii}}{1 + \kappa_{ai}C_{ii}}, \quad (30)$$

and the two-site probabilities are

$$\begin{aligned} Q_{ii} &= 1 - \frac{\varepsilon_i^s}{1 - \phi_1\phi_2Q_1^sQ_2^s}, \quad i = 1, 2, \\ Q_{ij} &= \frac{\phi_iQ_i^s\varepsilon_j^s}{1 - \phi_1\phi_2Q_1^sQ_2^s}, \quad ij = 12, 21, \\ \varepsilon_i &= \frac{\varepsilon_i^s(1 - \phi_jQ_j^s)}{1 - \phi_1\phi_2Q_1^sQ_2^s}, \end{aligned} \quad (31)$$

where $\phi_i = C_{12}/C_{ii}$ is the ratio of the time integrals of the cross- and auto-correlation functions. This ratio determines the degree of competition between the two sites. When $C_{12} = 0$ (and hence $\phi_i = 0$), the sites are independent, and the committor matrix $\mathbf{Q}$ is diagonal with the elements equal to the single-site capture probabilities. The magnitude of ϕ_i depends on the proximity of the two sites. The closer the sites, the larger the influence of the second site on the first.

Thus, all elements of the committor matrix $\mathbf{Q}$ (and hence the escape probabilities) can be expressed in terms of the intrinsic rate constants and the time integrals of sink-sink correlation functions. The latter are related to the steady-state diffusion-controlled Smoluchowski rate constants, which can be found from the literature for several models.⁹⁻²¹ Specifically,

$$C_{ii} = 1/k_{DC}(i), \quad (32)$$

where $k_{DC}(i)$ is the diffusion-controlled rate constant that describes irreversible binding to a macromolecule with single site i ($i = 1, 2$). This follows from the fact that the diffusion-influenced binding rate constant for a single site, $\varepsilon_i^s\kappa_{ai}$, becomes $1/C_{ii}$ in the diffusion-controlled limit, $\kappa_{ai} \rightarrow \infty$ [see Eq. (30)]. When the whole surface of a macromolecule is absorbing, $k_{DC}(i) = 4\pi DR$, where R is the radius of the molecule, so that $C_{ii} = 1/4\pi DR$.

The cross-correlation function C_{12} can be found if the diffusion-controlled rate constant for irreversible binding to two sites, $k_{DC}(1 + 2)$, is also known. Specifically, $k_{DC}(1 + 2)$ is related to C_{12} as

$$k_{DC}(1 + 2) = \frac{C_{11} + C_{22} - 2C_{12}}{C_{11}C_{22} - C_{12}^2}. \quad (33)$$

This follows from the diffusion-influenced binding rate constant for two sites, $\varepsilon_1\kappa_{a1} + \varepsilon_2\kappa_{a2}$, with ε_i given in Eq. (31). This rate constant reduces to Eq. (33) in the diffusion-controlled limit, $\kappa_{ai} \rightarrow \infty$.

The rate constants $k_{DC}(i)$ have been obtained for a spherical macromolecule with an absorbing "cap."^{9,13-15} The rate constant $k_{DC}(1 + 2)$ for two sites has been calculated for a spherical macromolecule with two absorbing caps with arbitrary locations.^{12,18} These analytic expressions are rather complicated, and so the results calculated below were obtained for specific values of the dimensionless parameter $\kappa_{ai}C_{ii}$, which is the ratio of the reaction-controlled and diffusion-controlled rate constants. We assume that $C_{11} = C_{22} = C$, but $\kappa_{a1} \neq \kappa_{a2}$, so that the sites are inequivalent. Then, for illustrative purposes, we set $C_{12} = C/2$ so that $\phi_1 = \phi_2 = 1/2$.

B. Irreversible binding in the presence of a second reversible site

In the special case when the ligand cannot dissociate from the first site ($\kappa_{d1} = 0$), the binding kinetics can be obtained analytically. Let assume that initially the first site is empty and the second site is bound with probability p . Then, the probability for the first site to be bound is

$$B_1(t) = 1 - (1 + f(t))e^{-\varepsilon_1^s \kappa_{a1}[L]t},$$

$$f(t) = \xi_1 \frac{(1-p)\kappa_{a2}[L](1-\xi_1) - p\kappa_{d2}}{\kappa_{a2}[L](1-\xi_1)^2 + \kappa_{d2}} \times \left(1 - \exp\left(-t \varepsilon_2^s \frac{\kappa_{a2}[L](1-\xi_1)^2 + \kappa_{d2}}{1 - \xi_1 \xi_2}\right)\right), \quad (34)$$

where $\xi_i = \phi_i Q_i^s = C_{12}\kappa_{ai}/(1 + C_{ii}\kappa_{ai})$, $i = 1, 2$. In the absence of the second site, the binding probability $B_1^s(t)$ is given by the above equation with $f(t) = 0$. Function $f(t)$ describes the effect of the second site on binding to the first site. This function is either positive or negative, depending on the initial population of the second site. When all sites are initially unoccupied ($p = 0$), $f(t)$ is positive, so the kinetics of binding to the first site is always slower than when the second site does not exist. This is because binding to the second site depletes the concentration of ligands around the first site. When the second site is occupied initially ($p = 1$), $f(t)$ is negative, so the binding of the first site speeds up. This is because a ligand that just dissociated from the second site is already relatively close to the first site and can bind faster than a ligand in the bulk, which must diffuse close to the macromolecule.

Thus, diffusion influences the kinetics of two-site binding in two different ways. First, the sites compete for the ligand during association; therefore, the association rates for $M_1 \rightarrow M_2$ and $M_1 \rightarrow M_3$, $\kappa_{a1}\varepsilon_1$ and $\kappa_{a2}\varepsilon_2$, are smaller than those for independent sites, $\kappa_{a1}\varepsilon_1^s$ and $\kappa_{a2}\varepsilon_2^s$. Second, there is an additional reaction channel $M_2 \rightleftharpoons M_3$, which speeds up binding because a ligand that has dissociated from one site can directly rebound to the other.

C. Numerical results

Here, we compare the fractional saturation of site 1, $B_1(t)$, obtained from the diffusion-modified kinetic scheme in Fig. 3, with the result for diffusion-influenced binding to a macromolecule that has only site 1 [Eq. (16)] which is

$$B_1^s(t) = \frac{\kappa_{a1}[L]}{\kappa_{a1}[L] + \kappa_{d1}} \left(1 - e^{-\varepsilon_1^s(\kappa_{a1}[L] + \kappa_{d1})t}\right). \quad (35)$$

We calculate $B_1(t)$ [using Eq. (15) with Q_{ij} in Eq. (31)] for two initial conditions: (1) both sites are empty and (2) site 1 is empty, but site 2 is occupied. Recall that in the framework of chemical kinetics $B_1(t) = B_1^s(t)$ for both initial conditions since we assumed that there is no cooperativity in the reaction-controlled limit (see Fig. 1).

Figure 6 shows the ligand-binding curves of the first site in the presence of the second binding site, $B_1(t)$, together with

FIG. 6. The fractional saturation, B_1 , of the first site in the presence of the second site as a function of the dimensionless time, $\tau = t \varepsilon_1^s (\kappa_{a1}[L] + \kappa_{d1})$. The curve in the middle (black dashed) corresponds to the single-site binding, B_1^s [Eq. (35)]. The lower (green) curves correspond to the initial state where both sites are unbound (M_1). The upper (red) curves correspond to the initial state where the first site is empty, but the second site is occupied (M_3). (a) Irreversible binding to the first site ($\kappa_{d1} = 0$) in the presence of the second reversible site [Eq. (34)] $K_{1eq}[L] = \infty$, $K_{2eq}[L] = 1$; (b)–(d) reversible binding to the sites with various binding affinities: (b) $K_{1eq}[L] = K_{2eq}[L] = 1$; (c) $K_{1eq}[L] = 0.2$, $K_{2eq}[L] = 0.1$; and (d) $K_{1eq}[L] = 1$, $K_{2eq}[L] = 5$. $K_{ieq} = \kappa_{ai}/\kappa_{di}$, $i = 1, 2$. Other dimensionless parameters are $\kappa_{a1}C_{11} = 10$, $\kappa_{a2}C_{22} = 5$, $C_{11} = C_{22}$, and $C_{12}/C_{11} = 0.5$.

that for a single site, $B_1^s(t)$ (the black dashed curves in the middle). All curves are plotted as a function of the time scaled to the relaxation time, $\tau = t \varepsilon_1^s (\kappa_{a1}[L] + \kappa_{d1})$. Then $B_1^s(\tau)$ depends only on $K_{1eq}[L] = \kappa_{a1}[L]/\kappa_{d1}$, which specifies the equilibrium population of the first site. For two-site binding, the additional dimensionless parameters are $K_{2eq}[L] = \kappa_{a2}[L]/\kappa_{d2}$, $\kappa_{ai}C_{ii}$, and $\phi_i = C_{12}/C_{ii}$, $i = 1, 2$. Because of diffusion, $B_1(t)$ and $B_1^s(t)$ are no longer identical and $B_1(t)$ depends on the initial conditions. Figure 6(a) shows the results for irreversible binding to the first site calculated from Eq. (34). The initially unbound second site leads to the slower ligand binding to the first site (lower green curves), whereas the initially bound second site leads to the faster ligand binding (upper red curves). This is true for arbitrary parameters of the model. The deviation from the single-site binding curve is larger when the binding affinity of the sites is low and the second site is initially occupied. In this case the effect of the new transitions in the diffusion-modified kinetic scheme in Fig. 3 is most noticeable and can lead to a non-monotonic ligand-binding curve [the upper red curve in Fig. 6(c)]. The curves in Fig. 6(d) correspond to the second site having larger binding affinity compared to the first site, in which case the deviation from the single-site binding is larger if the second site is initially unbound (the lower green curve).

Thus, depending on its initial occupancy, the second site can either accelerate or slow down the binding to the first site. If the second site is initially empty, it binds

ligands that would otherwise bind to the first site, so the binding to the first site is slower because of competition. If the second site is initially occupied, the ligand can dissociate from this site and bind to the first site much faster than a ligand from the bulk that has to diffuse close to the macromolecule.

V. CONCLUDING REMARKS

Diffusion-induced correlations alter the kinetics of ligand binding of a macromolecule with two sites. In this paper, we studied the effect of diffusion on reversible ligand binding when the sites are independent in the reaction-controlled (i.e., fast diffusion) limit, and thus, in the framework of conventional chemical kinetics, the kinetics of binding to the first site does not depend on the presence of the second site. We used the Markovian version of our general theory of diffusion-influenced reaction networks² to obtain rate equations that involve capture (also called splitting or commitment) probabilities, i.e., the probabilities that a ligand just dissociated from one site of the macromolecule irreversibly binds to the other site or rebinds to the same site or diffuses away (escapes). We found that the influence of diffusion cannot be described by simply modifying the rate constants of ordinary chemical kinetics. Diffusion actually changes the chemical kinetic scheme by inducing a direct transition between the two singly bound states, as shown in Fig. 3. The physical reason for this is that a ligand that has dissociated from one site can diffuse to the other site and then bind. This would never occur if diffusion were very fast, hence it is not considered in ordinary chemical kinetics.

Diffusion influences the kinetics in two opposing ways. The additional reaction channel accelerates binding because a ligand just dissociated from one site can quickly bind to the other. On the other hand, the two sites compete for the ligands during association. Therefore, the binding kinetics to the first site slows down when the macromolecule is initially unliganded and speeds up when the second site is initially occupied. The magnitude of these effects increases with the proximity of the binding sites and with any process that increases the probability that the dissociated ligand stays near the macromolecule, such as a short range attractive force or the cage effect due to crowding.

In this paper, we used the simplest version of the theory, i.e., Markovian diffusion-modified equations in the limit of small ligand concentrations. The non-Markovian theory with memory [Eq. (12)] leads to non-exponential kinetics and

power-law asymptotics at long times.^{22–25} To get the correct amplitude of the asymptotics, one must consider the reaction of a pair with bulk ligands when calculating the irreversible association fluxes. We have done this for single-site binding $A + B \rightleftharpoons C$ ^{2,26} and more recently for stochastically gated binding, $A + B \rightleftharpoons C$, $A \rightleftharpoons D$.²⁷ Such an extended version of theory would be needed here to obtain results that are accurate for all times and all ligand concentrations.

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