Kinetics of protein binding in solid-phase immunoassays: Theory

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In a solid-phase immunoassay, binding between an antigen and its specific antibody takes place at the boundary of a liquid and a solid phase. One of the reactants (receptor) is immobilized on a surface. The other reactant (ligand) is initially free in solution. We present a theory describing the kinetics of immunochemical reaction in such a system. A single essential restriction of the theory is the assumption that the reaction conditions are uniform along the binding surface. In general, the reaction rate as a function of time can be obtained numerically as a solution of a nonlinear integral equation. For some special cases, analytical solutions are available. Various immunoassay geometries are considered, in particular, the case when the reaction is carried out on a microspot.
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I. INTRODUCTION

The detection of a given protein in a liquid sample is a routine problem in experimental biology. One of the most preferred and efficient tools developed for this purpose is the immunoassay technique, which makes use of the high affinity of antigen–antibody pairs.[1,2] In a solid-phase immunoassay, the liquid sample containing one of the reagents (say, antigen) is put in contact with a solid surface, where the other reagent (antibody) is immobilized. From the intensity of the immunochemical reaction in this system, one can estimate the initial concentration of the antigen in the sample. A new and very promising trend in this field is the microarray immunoassay, where the liquid sample is simultaneously in contact with a large number (up to several thousands) of antibodies arranged in an array of individual microspots. Thus, the protein profile of a sample can, in principle, be obtained very quickly. Unfortunately, this technique is far from well established, although many research efforts are currently being made in this direction. In many recent publications, the sensitivity of the microarray immunoassays appears considerably lower than expected (for review see, e.g., Refs. 3 and 4). The reason for this may lie in the fact that the experiments are designed without solid theoretical background.

The kinetics of solid-phase immunoassays and related problems have been a subject of many theoretical studies.[5–10] However, most theories deal with either the initial phase of the reaction or the steady-state regime. In the present paper, we suggest a more general (although sometimes less precise) theoretical approach, in the framework of which both the initial behavior and the steady-state kinetics are considered as particular cases.

II. GENERAL THEORETICAL CONSIDERATIONS

A. Basic equation

In general, we wish to describe a chemical reaction at the boundary of a liquid and a solid phase. One of the reactants, called receptor, is immobilized on a surface. The other reactant, called ligand, is initially free in solution. The ligand can bind to the receptor at 1:1 stoichiometry. The problem is to find the amount of the ligand bound to the receptor as a function of time t.

Let N be the total number of molecules of the receptor and $B(t)$ the number of bound molecules of the ligand. The reaction rate is

$$\dot{B}(t) = k_+[N - B(t)]c_L(t) - k_-B(t), \tag{1}$$

where k_+ and k_- are the intrinsic association and dissociation rate constants, respectively, and $c_L(t)$ is the local concentration of the ligand solution at the reaction surface. Here and further, a dot above a symbol denotes differentiation with respect to time: $\dot{B}(t)=dB/dt$. As implied by Eq. (1), we assume that the reaction conditions do not depend on the position on the surface.

In order to make use of Eq. (1), we should express the quantity $c_L(t)$ through the function $B(t)$. For this purpose, we introduce a "memory" function $G(t)$ in the following way. Imagine a deactivated molecule of ligand that cannot bind to the receptor, but retains all the original transport properties. Suppose that at time zero it is positioned somewhere at the

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reaction surface, the area of which we denote by σ. We define the quantity $G(t)\sigma dx$ as the probability to find such a molecule within an infinitesimally small distance dx from the reaction surface at the time t. In other words, the memory function $G(t)$ is the local concentration of a single deactivated molecule that starts from an arbitrary point of the reaction surface.

Let c be the initial ligand concentration. Imagine that, in addition to the usual course of the reaction, *deactivated* molecules of the ligand are created at the reaction surface with the rate $\dot{B}(t)$. Then the total local concentration of all ligand molecules (active and deactivated) will remain constant and equal to c. The local concentration of deactivated molecules can be expressed as a convolution integral $\int_0^t G(t-t')\dot{B}(t')dt'$, which we will further denote by $G(t)*\dot{B}(t)$. Hence, the local concentration of the active molecules is

$$c_L(t) = c - G(t) * \dot{B}(t). \tag{2}$$

Substituting this expression into Eq. (1) and dividing the latter by N, we obtain

$$\dot{\varphi}(t) = k_+[1-\varphi(t)][c - NG(t) * \dot{\varphi}(t)] - k_-\varphi(t), \tag{3}$$

where $\varphi(t)=B(t)/N$ is the fractional occupancy of the receptor binding sites. This is our basic kinetic equation. The transport processes are taken into account through the function $G(t)$, which will be explicitly found for some particular systems in Sec. III. In the present section we will concentrate on the general analysis of Eq. (3), assuming $G(t)$ to be known. Note that, for sufficiently large t, this function approaches $1/V$, where V is the volume of the ligand solution. Therefore it is convenient to introduce an auxiliary function $H(t)$ defined in the following way:

$$G(t) = 1/V + H(t)/V. \tag{4}$$

It is obvious that $H(t)\to 0$ as $t\to\infty$. In terms of $H(t)$, the basic kinetic equation can be rewritten as

$$\dot{\varphi}(t) = k_+[1-\varphi(t)][c - \nu\varphi(t) - \nu H(t) * \dot{\varphi}(t)] - k_-\varphi(t), \tag{5}$$

where $\nu=N/V$ is the would-be volume concentration of the receptor if it were detached from the reaction surface. In deriving Eq. (5) we used the fact that $1*\dot{\varphi}(t)=\varphi(t)$.

B. Limiting value of the fractional occupancy

In the limit $t\to\infty$, the system reaches dynamic equilibrium (φ=constant), and Eq. (5) reduces to

$$0 = k_+(1-\varphi)(c - \nu\varphi) - k_-\varphi. \tag{6}$$

This quadratic equation with respect to φ has two different positive real roots. We denote them as φ_1 and φ_2, assuming that $\varphi_1<\varphi_2$. The limiting value of the fractional occupancy $\varphi(t)$ is, of course, the lowest root, φ_1. (The other one, φ_2, is, in fact, always greater than unity.) Further in this paper, we will keep the notation φ_1 for the equilibrium value of $\varphi(t)$, although, in some approximations, it might not be necessarily found as a root of Eq. (6). For example, for a very large volume ($V\to\infty$) the term $\nu\varphi$ can be neglected in comparison with c, and Eq. (6) becomes

$$0 = k_+(1-\varphi)c - k_-\varphi. \tag{7}$$

In this case the limiting value of the fractional occupancy is given by the standard formula

$$\varphi_1 = k_+c/(k_+c + k_-). \tag{8}$$

C. Diffusion-controlled irreversible reaction

The kinetic equation has the simplest form when the reaction is diffusion-controlled and irreversible, i.e., every molecule of the ligand that reaches the reaction surface becomes permanently bound. Formally, this situation corresponds to an infinite association constant: $k_+\to\infty$. According to Eqs. (2) and (3), this means that the local concentration of the ligand solution should vanish:

$$c - NG(t) * p(t) = 0. \tag{9}$$

Here, for the future convenience, the notation $p(t)$ is used for $\dot{\varphi}(t)$. We assume that $c<\nu$ and, consequently, the limiting value of $\varphi(t)$ is $\varphi_1=c/\nu<1$.

Equation (9) can be easily solved in terms of Laplace transform. The Laplace transform of an arbitrary function $F(t)$ is defined by

$$\hat{F}(s) = \int_0^t e^{-st}F(t)dt. \tag{10}$$

We apply this transformation to Eq. (9), keeping in mind that it converts convolution to ordinary multiplication

$$c/s - N\hat{G}(s)\hat{p}(s) = 0. \tag{11}$$

Note that $\hat{1}=1/s$. From Eq. (11) we have

$$\hat{p}(s) = c/Ns\hat{G}(s). \tag{12}$$

Since the fractional occupancy can be expressed as $\varphi(t)=1*p(t)$, its Laplace transform is

$$\hat{\varphi}(s) = \hat{p}(s)/s. \tag{13}$$

The Laplace transform inversion, required to find $p(t)$ and $\varphi(t)$, is, in general, a delicate matter. However, the mean reaction time τ can be easily found directly from $\hat{p}(s)$

$$\tau = \int_0^\infty t\,\frac{p(t)dt}{\varphi_1} = -\frac{1}{\varphi_1}\left[\frac{d\hat{p}(s)}{ds}\right]_{s=0}. \tag{14}$$

Note that $p(t)dt=d\varphi(t)$ is just an increase of the fractional occupancy in the time interval $(t,t+dt)$. Before differentiation of Eq. (12), it is more convenient to rewrite it in the form

$$\hat{p}(s)/\varphi_1 = 1/[1 + s\hat{H}(s)], \tag{15}$$

where $\hat{H}(s)$ is defined by Eq. (4). Substituting Eq. (15) into Eq. (14), we get

$$\tau = \hat{H}(0). \tag{16}$$

The quantity $\hat{H}(0)$ is, in fact, the time required for an average ligand molecule to find its way to the reaction surface. It is a very important parameter of the system. Furthermore, we will call it the (mean) first collision time and denote it by τ_F.

It should be mentioned that the solution $\varphi(t)$ defined by Eqs. (12) and (13) remains valid also in the case when $c > \nu$, so far as $\varphi(t) < 1$.

D. Linear approximation (case of a small fractional occupancy)

When the fractional occupancy $\varphi(t)$ is negligible in comparison with unity, Eq. (3) becomes linear

$$p(t) = k_+[c - NG(t) * p(t)] - k_-[1 * p(t)]. \tag{17}$$

The requirement $\varphi(t) \ll 1$ always holds for the initial phase of the reaction. If, in addition, the limiting value of $\varphi(t)$ is also small, it is defined by [cf. Eq. (6)]

$$0 = k_+(c - \nu\varphi) - k_-\varphi. \tag{18}$$

For self-consistency, the solution of this equation must satisfy the condition

$$\varphi_1 = k_+c/(k_+\nu + k_-) \ll 1. \tag{19}$$

As before, Eq. (17) can be treated in terms of Laplace transform to give

$$\hat{p}(s) = k_+c/[s + k_+N s\hat{G}(s) + k_-] \tag{20}$$

or, taking into account Eqs. (4) and (19),

$$\frac{\hat{p}(s)}{\varphi_1} = \frac{k_+\nu + k_-}{s[1 + k_+\nu\hat{H}(s)] + k_+\nu + k_-}. \tag{21}$$

Note that $\hat{p}(0) = \varphi_1$. The mean reaction time is, according to Eqs. (14) and (21),

$$\tau = \frac{1 + k_+\nu\tau_F}{k_+\nu + k_-}, \tag{22}$$

where $\tau_F = \hat{H}(0)$ is the first collision time.

E. Steady-state approximation

Under steady-state conditions, $\dot{\varphi}(t)$ is assumed to be constant. More precisely, the reaction rate $\dot{\varphi}(t)$ must decrease much more slowly than the memory function $G(t)$. In this case, one can neglect all the "memory" effects and approximate $H(t)$ by a delta-function,

$$H(t) = \tau_F\delta(t). \tag{23}$$

Equation (5) takes then the form

$$d\varphi/dt = k_+(1 - \varphi)[c - \nu\varphi - \nu\tau_F(d\varphi/dt)] - k_-\varphi. \tag{24}$$

Now, it is more convenient to consider time t as a function of φ. The solution of Eq. (24) [with initial condition $t(0) = 0$] is

$$t(\varphi) = \frac{1}{k_+\nu(\varphi_2 - \varphi_1)}\left\{[1 + k_+\nu\tau_F(1 - \varphi_2)]\ln\left(1 - \frac{\varphi}{\varphi_2}\right) - [1 + k_+\nu\tau_F(1 - \varphi_1)]\ln\left(1 - \frac{\varphi}{\varphi_1}\right)\right\}, \tag{25}$$

where φ_1 and φ_2 are, as before, the solutions of Eq. (6) ($\varphi_1 < \varphi_2$). The mean reaction time can be found by the formula

$$\tau = \frac{1}{\varphi_1}\int_0^{\varphi_1} t(\varphi)d\varphi, \tag{26}$$

which yields

$$\tau = \frac{1}{k_+\nu(\varphi_2 - \varphi_1)}\left\{[1 + k_+\nu\tau_F(1 - \varphi_1)] - [1 + k_+\nu\tau_F(1 - \varphi_2)]\left[1 + \frac{\varphi_2 - \varphi_1}{\varphi_1}\ln\left(1 - \frac{\varphi_1}{\varphi_2}\right)\right]\right\}. \tag{27}$$

When the amount of receptor, multiplied by the factor φ_1, is negligible in comparison with the total amount of ligand ($\varphi_1\nu \ll c$), Eq. (24) simplifies to

$$d\varphi/dt = k_+(1 - \varphi)[c - \nu\tau_F(d\varphi/dt)] - k_-\varphi. \tag{28}$$

The solution is

$$t(\varphi) = \frac{1}{k_+c + k_-} \times\left\{k_+\nu\tau_F\varphi - [1 + k_+\nu\tau_F(1 - \varphi_1)]\ln\left(1 - \frac{\varphi}{\varphi_1}\right)\right\}, \tag{29}$$

where the limiting value of fractional occupancy, φ_1, is given by Eq. (8). The mean reaction time is

$$\tau = [1 + k_+\nu\tau_F(1 - \varphi_1/2)]/(k_+c + k_-). \tag{30}$$

It should be noted that Eqs. (28)–(30) can also be obtained in the frame of the so-called two-compartment model.[10]

III. APPLICATION TO PARTICULAR SYSTEMS

A. One-dimensional diffusion to the bottom of a well

1. Memory function

Consider a well, of height a, filled with the ligand solution [Fig. 1(a)]. The receptor is immobilized at the bottom, of area σ. The only transport mechanism is supposed to be the diffusion characterized by a diffusion coefficient D. We start to analyze this system by finding the memory function $G(t)$. Since the motion of the ligand molecules in a horizontal direction has no influence on the reaction kinetics, the problem is essentially one-dimensional.

Suppose that a one-dimensional particle, of diffusion coefficient D, diffuses within the interval $(0, a)$ with reflecting boundaries. At time zero, the particle starts from the point $x = 0$. The quantity $\sigma G(t)dx$ coincides with the probability to find the particle in the interval $(0, dx)$ at the time instant t. Another view on this system is that the particle moves along

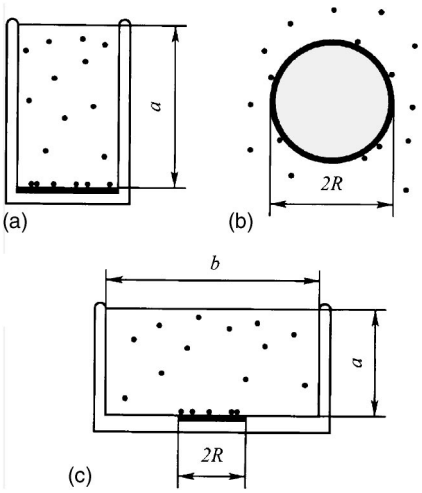

FIG. 1. Assay geometries: One-dimensional diffusion to the bottom of a well (a); three-dimensional diffusion to a spherical bead (b); and three-dimensional diffusion to a spot (c). The receptor on the surface is indicated by a fat line.

an infinite chain of straight segments of length a, but this chain has been folded like a folding rule: the direction of each segment is exactly opposite to its neighbors, so that the entire chain has been collapsed to within the size of a single segment. Now, the quantity $\sigma G(t)dx$ is equal to the probability to find the particle in one of the intervals $(2na-dx, 2na+dx)$ along the chain, where n is an arbitrary integer

$$\sigma G(t)dx = \sum_{k=-\infty}^{\infty} g_1(2na,t)2dx, \tag{31}$$

where

$$g_1(x,t) = (4\pi Dt)^{-1/2} \exp(-x^2/4Dt) \tag{32}$$

is the fundamental solution of the diffusion equation in one-dimensional space. The memory function can also be represented as

$$\sigma G(t) = (1/a) f(a/\sqrt{Dt}), \tag{33}$$

where $f(\,)$ is a universal function that does not depend on the parameters of the system,

$$f(y) = \frac{y}{\sqrt{\pi}}\left[1 + 2\sum_{n=1}^{\infty} \exp(-n^2y^2)\right]. \tag{34}$$

The Laplace transform of Eq. (33) is

$$\sigma\hat{G}(s) = (1/\sqrt{Ds})\coth(a\sqrt{s/D}). \tag{35}$$

2. Diffusion-controlled irreversible reaction

For a diffusion-controlled irreversible reaction, the Laplace transform of the reaction rate, according to Eqs. (12) and (35), is given by[11]

$$\hat{p}(s) = (c/\rho)\sqrt{D/s}\,\tanh(a\sqrt{s/D}), \tag{36}$$

where $\rho = N/\sigma$ is the surface density of the receptor. The inversion of Eq. (36) yields

$$p(t) = \frac{c}{\rho}\sqrt{\frac{D}{\pi t}}\left[1 + 2\sum_{n=1}^{\infty}(-1)^n \exp\left(-\frac{n^2a^2}{Dt}\right)\right], \tag{37a}$$

or, in an alternative representation,

$$p(t) = \frac{2cD}{a\rho}\sum_{n=1}^{\infty} \exp\left(-\frac{(2n-1)^2\pi^2}{4}\frac{D}{a^2}t\right). \tag{37b}$$

Equations (37a) and (37b) are equivalent. The series in Eq. (37a) converges faster for small t and the series in Eq. (37b) converges faster for large t. By integration of Eqs. (37a) and (37b), we get, respectively,

$$\varphi(t) = \frac{2c}{\rho}\sqrt{\frac{D}{\pi}}\left\{\sqrt{t} + 2\sum_{n=1}^{\infty}(-1)^n\left[\sqrt{t}\exp\left(-\frac{n^2a^2}{Dt}\right) - na\sqrt{\frac{\pi}{D}}\,\mathrm{erfc}\left(\frac{na}{\sqrt{Dt}}\right)\right]\right\}, \tag{38a}$$

$$\varphi(t) = \frac{8ac}{\pi^2\rho}\sum_{n=1}^{\infty}\frac{1}{(2n-1)^2}\left[1 - \exp\left(-\frac{(2n-1)^2\pi^2}{4}\frac{D}{a^2}t\right)\right]. \tag{38b}$$

Although Eq. (38b) is simpler, the convergence of Eq. (38a) for practically interesting values of t is better. The mean reaction time can be found as [cf. Eqs. (4) and (16)]

$$\tau = \tau_F = \hat{H}(0) = \lim_{s\to 0}(a\sigma\hat{G}(s) - 1/s) = a^2/3D. \tag{39}$$

3. Linear approximation

For the sake of simplicity, we will consider the solution of the linearized kinetic equation only for a very large height of the well: $a\to\infty$. The memory function in this case is reduced to

$$\sigma G_0(t) = 1/\sqrt{\pi Dt}. \tag{40}$$

Its Laplace transform is

$$\sigma\hat{G}_0(s) = 1/\sqrt{Ds}. \tag{41}$$

Substituting this expression into Eq. (20), we get

$$\hat{p}(s) = k_+c/(s + k_+\rho\sqrt{s/D} + k_-). \tag{42}$$

The denominator in Eq. (42) can be considered as a square polynomial in $\sqrt{s}$. If λ_1 and λ_2 are the roots of this polynomial, Eq. (42) can be rewritten as

$$\hat{p}(s) = \frac{k_+c}{\lambda_1 - \lambda_2}\left[\frac{1}{\sqrt{s} - \lambda_1} - \frac{1}{\sqrt{s} - \lambda_2}\right]. \tag{43}$$

In general, λ_1 and λ_2 are complex numbers with a negative real part. We assume that $\lambda_1 \neq \lambda_2$. The inverse Laplace transform of the function

$$\hat{F}_1(s) = 1/(\sqrt{s} - \lambda) \tag{44}$$

is known to be

$$F_1(t) = 1/\sqrt{\pi t} + \lambda \exp(\lambda^2 t)\mathrm{erfc}(-\lambda\sqrt{t}). \tag{45}$$

Thus, the expression in Eq. (43) can be easily inverted

$$p(t) = \frac{k_+ c}{\lambda_1 - \lambda_2}[\lambda_1 \exp(\lambda_1^2 t)\mathrm{erfc}(-\lambda_1\sqrt{t}) - \lambda_2 \exp(\lambda_2^2 t)\mathrm{erfc}(-\lambda_2\sqrt{t})]. \tag{46}$$

In order to obtain the fractional occupancy $\varphi(t)$, one can [keeping in mind Eq. (13)] make use of the following pair of the "image" and "original" functions:

$$\hat{F}_2(s) = 1/s(\sqrt{s} - \lambda), \tag{47}$$

$$F_2(t) = \frac{1}{\lambda}\exp(\lambda^2 t)\mathrm{erfc}(-\lambda\sqrt{t}) - \frac{1}{\lambda}. \tag{48}$$

The required expression for $\varphi(t)$ is

$$\varphi(t) = \frac{k_+ c}{\lambda_1 - \lambda_2}\left[\frac{1}{\lambda_1}\exp(\lambda_1^2 t)\mathrm{erfc}(-\lambda_1\sqrt{t}) - \frac{1}{\lambda_1} - \frac{1}{\lambda_2}\exp(\lambda_2^2 t)\mathrm{erfc}(-\lambda_2\sqrt{t}) + \frac{1}{\lambda_2}\right]. \tag{49}$$

The limiting value of the fractional occupancy is $\varphi_1 = k_+ c/k_-$ [cf. Eq. (19)]. The mean reaction time τ is not defined. The substitution of Eq. (42) into Eq. (14) leads to a divergence: $\tau = \infty$.

It should be noted that, in practice, it is inconvenient to evaluate expressions in Eqs. (46) and (49) directly as they are, because of the overflow/underflow errors caused by the functions exp( ) and erfc( ) at large t. This problem, however, can be easily avoided by using the formula

$$\exp(x^2)\mathrm{erfc}(x) = \frac{1}{x\sqrt{\pi}}\left[1 - \frac{1}{2x^2} + \frac{1\cdot 3}{2^2 x^4} - \frac{1\cdot 3\cdot 5}{2^3 x^6} + \cdots\right], \tag{50}$$

where $\mathrm{Re}(x)$ is assumed to be large and positive.

B. Three-dimensional diffusion to a sphere

1. Diffusion-controlled irreversible reaction

Consider a spherical bead, covered with the immobilized receptor, in a large volume of ligand solution [Fig. 1(b)]. Imagine for a while that the ligand molecules can freely move through the space occupied by the bead. The memory function for this simplified case, $G'(t)$, can be easily found. Suppose that at time zero a ligand molecule is positioned at the topmost point of the bead. Its time-dependent concentration at any observation point (in the absence of reaction) is given by the fundamental solution of the diffusion equation in three-dimensional space

$$g_3(x,t) = (4\pi D t)^{-3/2}\exp(-x^2/4Dt), \tag{51}$$

where x is the distance from the starting point. The "simplified" memory function $G'(t)$ is just the average of $g_3(x,t)$ over all observation points belonging to the bead surface,

$$G'(t) = \frac{1}{4\pi}\int_{\phi=0}^{2\pi} d\phi \int_{\cos\theta=-1}^{1} d(\cos\theta) g_3[R\sqrt{2(1-\cos\theta)}, t] = \frac{1 - \exp(-R^2/Dt)}{8\pi R^2\sqrt{\pi D t}}. \tag{52}$$

Here we used a spherical coordinate system with the origin at the center of the bead; R is the bead radius. The simplified memory function, although not suited for the kinetic equation [Eq. (3)], is quite useful in the case of a diffusion-controlled irreversible reaction. In such a reaction, the ligand molecules cannot penetrate inside the bead anyway, because they are all captured at the surface. It is, however, important to take into account explicitly that the space occupied by the bead is free from the ligand molecules at time zero. Let $c'(r,t)$ be the time-dependent ligand concentration in the absence of a reaction as a function of the distance r to the center of the bead, which is assumed to be permeable. Then the initial conditions are

$$c'(r,0) = 0 \quad \text{if } r \leqslant R, \qquad = c \quad \text{if } r > R. \tag{53}$$

For $t > 0$, the function $c'(r,t)$ at the bead surface can be found as

$$c'(R,t) = c\int_{\phi=0}^{2\pi} d\phi \int_{\cos\theta=-1}^{1} d(\cos\theta)\int_{r=R}^{\infty} r^2\, dr \times g_3(\sqrt{R^2 + r^2 - 2Rr\cos\theta}, t) = \frac{c}{R}\sqrt{\frac{Dt}{\pi}}\left[1 - \exp\left(-\frac{R^2}{Dt}\right)\right] + \frac{c}{2}\left[1 + \mathrm{erfc}\left(\frac{R}{\sqrt{Dt}}\right)\right]. \tag{54}$$

Since in the presence of a reaction the local concentration at the bead surface should vanish, we can write [cf. Eq. (9)]

$$c'(R,t) - NG'(t) * p(t) = 0. \tag{55}$$

Hence, in terms of Laplace transforms,

$$\hat{p}(s) = \hat{c}'(R,s)/N\hat{G}'(s). \tag{56}$$

Substituting Eqs. (52) and (54) into Eq. (56), we get

$$\hat{p}(s) = (4\pi DRc/N)(1/s + R/\sqrt{Ds}). \tag{57}$$

The inversion of Eq. (57) yields

$$p(t) = (4\pi DRc/N)(1 + R/\sqrt{\pi D t}), \tag{58}$$

$$\varphi(t) = (4\pi DRc/N)(t + 2R\sqrt{t/\pi D}). \tag{59}$$

Equation (59) is the well-known Smoluchowski formula.[12] It should be recalled that in our case it holds only for $\varphi(t) < 1$.

2. Linear approximation

The correct memory function, for an impermeable bead, can now be easily found by comparison of Eqs. (12) and (57)

$$\hat{G}_0(s) = \frac{1}{4\pi R^2\sqrt{D}(\sqrt{s} + \sqrt{D}/R)}, \tag{60}$$

$$G_0(t) = \frac{1}{4\pi R^2\sqrt{D}}\left[\frac{1}{\sqrt{\pi t}} - \frac{\sqrt{D}}{R}\exp\left(\frac{Dt}{R^2}\right)\mathrm{erfc}\left(\frac{\sqrt{Dt}}{R}\right)\right]. \tag{61}$$

Substituting Eq. (60) into Eq. (20), we get the Laplace transform of the reaction rate in the linear approximation

$$\hat{p}(s) = \frac{k_+c(\sqrt{D}/R + \sqrt{s})}{s\sqrt{s} + (k_+\rho/\sqrt{D} + \sqrt{D}/R)s + k_-\sqrt{s} + k_-\sqrt{D}/R}, \tag{62}$$

where $\rho = N/4\pi R^2$ has, again, the meaning of the surface density of the receptor. This expression can be inverted by the method already used [cf. Eq. (42) and the text thereafter]. Let λ_1, λ_2, and λ_3 be the roots of the denominator in Eq. (62) considered as a polynomial in $\sqrt{s}$. Then

$$\hat{p}(s) = k_+c\left[\frac{\sqrt{D}/R + \lambda_1}{(\lambda_2 - \lambda_1)(\lambda_3 - \lambda_1)(\sqrt{s} - \lambda_1)} + \text{cyc. perm.}\right], \tag{63}$$

$$p(t) = k_+c\left[\frac{(\sqrt{D}/R + \lambda_1)\lambda_1\exp(\lambda_1^2 t)\mathrm{erfc}(-\lambda_1\sqrt{t})}{(\lambda_2 - \lambda_1)(\lambda_3 - \lambda_1)} + \text{cyc. perm.}\right], \tag{64}$$

$$\varphi(t) = k_+c\left\{\frac{\sqrt{D}/R + \lambda_1}{(\lambda_2 - \lambda_1)(\lambda_3 - \lambda_1)\lambda_1}[\exp(\lambda_1^2 t)\mathrm{erfc}(-\lambda_1\sqrt{t}) - 1] + \text{cyc. perm.}\right\}. \tag{65}$$

Here, by "cyc. perm." we denote the terms obtained from the first one by cyclic permutations of the indices of λ.

The mean reaction time τ can, in principle, be found by the standard formula [Eq. (22)]. However, this formula should be adapted for a very large volume of ligand solution: $V \to \infty$. In this case $G_0(t) = H_0(t)/V$, so that

$$\hat{G}_0(0) = \hat{H}_0(0)/V = \tau_F/V. \tag{66}$$

Taking into account Eq. (66) and neglecting the term $k_+\nu$ in comparison with k_-, we can rewrite Eq. (22) as

$$\tau = [1 + k_+N\hat{G}_0(0)]/k_-. \tag{67}$$

This equation can, of course, be derived directly from Eqs. (14) and (20) with $\varphi_1 = k_+c/k_-$. For the memory function under consideration [Eq. (60)], we have

$$\hat{G}_0(0) = 1/4\pi DR. \tag{68}$$

Thus, the mean reaction time is, according to Eqs. (67) and (68),

$$\tau = (1 + k_+\rho R/D)/k_-. \tag{69}$$

C. Three-dimensional diffusion to a spot

1. Memory function

Consider a circular spot of receptor molecules on a flat reflecting boundary between a solid phase and a large volume of ligand solution. From a practical point of view, this is the most interesting case relevant for the microarray technique. However, in the frame of our formalism it can be treated only approximately, because the reaction conditions on the spot depend on the distance to its center. The memory function is, according to its formal definition, the function $2g_3(x,t)$ [Eq. (51)] averaged over all pairs of points belonging to the spot, with the argument x being the distance between the points in each pair

$$G_0(t) = \frac{1}{(\pi R^2)^2}\int_{\phi_1=0}^{2\pi} d\phi_1 \int_{r_1=0}^{R} r_1 dr_1 \int_{\phi_2=0}^{2\pi} d\phi_2 \times \int_{r_0=0}^{R} r_2 dr_2 \cdot 2g_3(\sqrt{r_1^2 + r_2^2 - 2r_1r_2\cos\phi_2}, t). \tag{70}$$

Here, we use a polar coordinate system with the origin at the center of the spot. R is the spot radius. The factor 2 preceding $g_3(\,)$ accounts for the fact that only half of the space is available for diffusion. Although a closed form of Eq. (70) is not known, this equation can be used to obtain a very important parameter of the system, namely, the first collision time τ_F divided by the volume V of ligand solution

$$\tau_F/V = \hat{G}_0(0) = \int_0^\infty G_0(t)dt = \frac{8}{3\pi^2 DR}. \tag{71}$$

It should be recalled that Eq. (71) was obtained in the approximation of uniform reaction conditions over the spot. The exact value of τ_F/V is known to be[5]

$$\tau_F/V = 1/4DR. \tag{72}$$

The difference between the two values of τ_F/V is $\approx 8\%$, which can serve as an estimate of accuracy of our approach for this system.

The function given by Eq. (70) is very inconvenient to use in the kinetic equation. Instead of using it, we wish to construct an approximate memory function that (i) is sufficiently simple, (ii) similar in form to Eqs. (60) and (61), (iii) provides the same value of τ_F/V as the true memory function [Eq. (71)], and (iv) has the correct asymptotic behavior at small and large times t:

$$\sigma G_0(t) = 2g_1(0,t) \quad \text{when } t \to 0, \tag{73}$$

$$G_0(t) = 2g_3(0,t) \quad \text{when } t \to \infty. \tag{74}$$

Here $\sigma = \pi R^2$ is the area of the spot and $g_1(\,)$ is defined by Eq. (32). A function satisfying these conditions is

$$\hat{G}_0(s) = \frac{1}{2\pi R^2\sqrt{D}}\left(\frac{1}{\sqrt{s} + \xi_1} + \frac{1}{\sqrt{s} + \xi_2}\right), \tag{75}$$

$$G_0(t) = \frac{1}{\pi R^2\sqrt{D}}\left\{\frac{1}{\sqrt{\pi t}} - \mathrm{Re}[\xi_1 \exp(\xi_1^2 t)\mathrm{erfc}(\xi_1\sqrt{t})]\right\}, \tag{76}$$

where

$$\xi_1 = \alpha + i\beta, \quad \xi_2 = \alpha - i\beta, \tag{77}$$

$$\alpha = [48\pi/(256 - 9\pi^2)]\sqrt{D}/R, \tag{78}$$

$$\beta = (1 - 9\pi^2/128)^{1/2}\alpha, \tag{79}$$

with i being the imaginary unit.

The corrections due to a finite volume of the ligand solutions can be found as follows. Suppose that the receptor spot is positioned exactly in the center of the bottom of an incubation chamber that has the form of a parallelepiped with the dimensions $length \times width \times height = b \times b \times a$ [Fig. 1(c)]. We assume that $R \ll a$ and $R \ll b$, so that at the times of the order a^2/D and b^2/D the function $G_0(t)$ is already close to $2g_3(0,t)$. Using the three-dimensional analogue of Eq. (33), we can write

$$G(t) = G_0(t) - 2g_3(0,t) + (1/ab^2) f(a/\sqrt{Dt}) f^2(b/2\sqrt{Dt}), \tag{80}$$

where the function $f(\,)$ is defined by Eq. (34).

2. Diffusion-controlled irreversible reaction

Assuming that $V \to \infty$ and substituting the function $\hat{G}_0(s)$ [Eq. (75)] into the general solution of the kinetic equation for the diffusion-controlled irreversible reaction [Eq. (12)], we get

$$\hat{p}(s) = \frac{c}{N}\left[\frac{3\pi^2 DR}{8s} + \frac{9\pi^3 R^2\sqrt{D}}{128\sqrt{s}} + \frac{(128 - 9\pi^2)\pi R^2\sqrt{D}}{128}\frac{1}{\alpha + \sqrt{s}}\right], \tag{81}$$

$$p(t) = (c/N)[(3\pi^2/8)DR + R^2\sqrt{\pi D/t} - (1 - 9\pi^2/128)\pi R^2\sqrt{D}\alpha \exp(\alpha^2 t)\mathrm{erfc}(\alpha\sqrt{t})], \tag{82}$$

$$\varphi(t) = (c/N)\{(3\pi^2/8)DRt + (9\pi^2/64)R^2\sqrt{\pi Dt} + (1 - 9\pi^2/128)(\pi R^2\sqrt{D}/\alpha)[1 - \exp(\alpha^2 t)\mathrm{erfc}(\alpha\sqrt{t})]\}$$
$$\varphi(t) < 1. \tag{83}$$

The inaccuracy of Eq. (82) does not exceed 8% as compared with a more rigorous solution that can be found in Refs. 9 and 13.

3. Linear approximation

In the linear approximation for $V \to \infty$, we have, according to Eqs. (20) and (75),

$$\hat{p}(s) = \frac{k_+ c[(\sqrt{s} + \alpha)^2 + \beta^2]}{[(\sqrt{s} + \alpha)^2 + \beta^2](s + k_-) + (k_+\rho/\sqrt{D})(\sqrt{s} + \alpha)s}, \tag{84}$$

$$p(t) = k_+ c\left\{\frac{[(\lambda_1 + \alpha)^2 + \beta^2]\lambda_1 \exp(\lambda_1^2 t)\mathrm{erfc}(-\lambda_1\sqrt{t})}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)(\lambda_1 - \lambda_4)} + \text{cyc. perm.}\right\}, \tag{85}$$

$$\varphi(t) = k_+ c\left\{\frac{[(\lambda_1 + \alpha)^2 + \beta^2][\exp(\lambda_1^2 t)\mathrm{erfc}(-\lambda_1\sqrt{t}) - 1]}{\lambda_1(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)(\lambda_1 - \lambda_4)} + \text{cyc. perm.}\right\}. \tag{86}$$

Here $\rho = N/\pi R^2$ and λ_1, λ_2, λ_3, and λ_4 are the roots of the denominator in Eq. (84) considered as a polynomial in $\sqrt{s}$ [cf. Eqs. (63)–(65)]. The mean reaction time can be found by means of Eqs. (67) and (71),

$$\tau = (1 + 8k_+\rho R/3\pi D)/k_-. \tag{87}$$

IV. NUMERICAL SOLUTION

A. Computational scheme

In general, the full (nonlinear) kinetic equation can be solved only numerically. In terms of the reaction rate $p(t) = \dot{\varphi}(t)$, it has the form [cf. Eq. (3)]

$$p(t) = k_+\left(1 - \int_0^t p(t')dt'\right)\left(c - N\int_0^t G(t - t')p(t')dt'\right) - k_-\int_0^t p(t')dt'. \tag{88}$$

The numerical solution of this equation was obtained as follows. Let $t_0, t_1, t_2, \ldots$ denote the control points along the time axis: $t_0 = 0$, $t_n < t_{n+1}$, $(n = 0, 1, 2, \ldots)$. Within each interval (t_n, t_{n+1}), the function $p(t)$ was approximated as $p(t) = p_n \exp[-\gamma_n(t - t_n)]$, where p_n and γ_n are constants. Note that $p_0 = k_+ c$ and $p_{n+1} = p_n \exp[-\gamma_n(t_{n+1} - t_n)]$. Thus, the required solution is defined by a sequence of the γ_n values. When the first m terms of this sequence $(\gamma_0, \gamma_1, \ldots, \gamma_{m-1})$ were known, the next one, γ_m, was found by numerical solution of the equation

$$p_m \exp[-\gamma_m(t_{m+1} - t_m)] = k_+\left(1 - \int_0^{t_{m+1}} p(t)dt\right) \times \left(c - N\int_0^{t_{m+1}} G(t_{m+1} - t)p(t)dt\right) - k_-\int_0^{t_{m+1}} p(t)dt, \tag{89}$$

where the integrals containing $p(t)$ were considered as functions of γ_m. The convolution integral was evaluated numeri-

cally. The step size (i.e., the interval between the control points) was controlled adaptively by carrying out two steps of an identical size Δt and comparing the result with that of a single step of the size $2\Delta t$. This computational scheme proved to be stable, in the sense that the solution was not significantly affected by the choice of the three parameters that defined (i) the accuracy of numerical integration, (ii) the accuracy of finding the root of Eq. (89), and (iii) the step size—provided that these parameters were sufficiently small.

B. One-dimensional diffusion

The numerical computations for the one-dimensional diffusion to the bottom of a well [Fig. 1(a)] were performed for the following set of parameters. The rate constants were $k_+=5.3\times10^5$ 1/M s and $k_-=3.2\times10^{-4}$ 1/s, the diffusion coefficient of the ligand molecules $D=1\times10^{-7}$ cm^2/s, the receptor surface density $\rho=1.5\times10^{-11}$ mole/cm^2, and the height of the incubation chamber $a=0.3$ cm. These parameters correspond approximately to the microtiter well assay with the human interferon-γ as a ligand and its monoclonal antibody as a receptor.[14] The initial ligand concentration c was varied over a large range. The receptor was assumed to cover the entire bottom of the incubation chamber. The memory function was defined by Eqs. (33) and (34).

The examples of numerical solution of Eq. (88), for $c=0.1\nu$, $c=\nu$, and $c=10\nu$, are displayed in Fig. 2 (thick solid line also denoted as line 1). The quantity ν is equal to $N/V=\rho/a=5.0\times10^{-8}$ M. In the same figure, the following analytical curves are shown for comparison. Line 2 (dotted line) corresponds to a diffusion-controlled irreversible reaction [Eqs. (37a) and (37b)]. Line 3 (thin solid line) is the linear approximation in the limit $a\to\infty$ [Eq. (46)]. Line 4 (dashed line) represents the steady-state approximation. In the latter case, the dependence of $p(t)=d\varphi/dt$ on t is given parametrically by Eqs. (24) and (25), with $\tau_F=a^2/3D=0.3\times10^6$ s [see Eq. (39)]. The quantity φ plays the role of the parameter.

The corresponding curves for the fractional occupancy φ are shown in Fig. 3. The numerical solution is represented by line 1 (thick solid line), the solution for a diffusion-controlled irreversible reaction [Eqs. (38a) and (38b)] by line 2 (dotted line), the linear approximation in the limit $a\to\infty$ [Eq. (49)] by line 3 (thin solid line), and the steady-state approximation [Eq. (25)] by line 4 (dashed line).

It should be recalled that the functions represented by curves 2 and 3 are proportional to the initial ligand concentration c. Thus, in logarithmic scale (Fig. 2), an increase of c does not change the form of these curves; it only shifts them upwards. By derivation of curve 2, the backward reaction was completely ignored. However, it was taken into consideration that the number of ligand molecules is finite (as the height of the incubation chamber, a, is finite). On the contrary, curve 3 was obtained under the assumption that $a\to\infty$; but the backward reaction was accounted for. Since curve 2 lies lower than curve 3 (for $t>\tau_F$), the role of the backward reaction for the given set of parameters is negligible. Therefore, the diffusion-controlled irreversible reaction (curve 2) serves as a good approximation at low concentrations c (i.e., $c<\nu$). Although the initial behavior of curve 2 is not correct (Fig. 2), this can hardly be observed experimentally (Fig. 3).

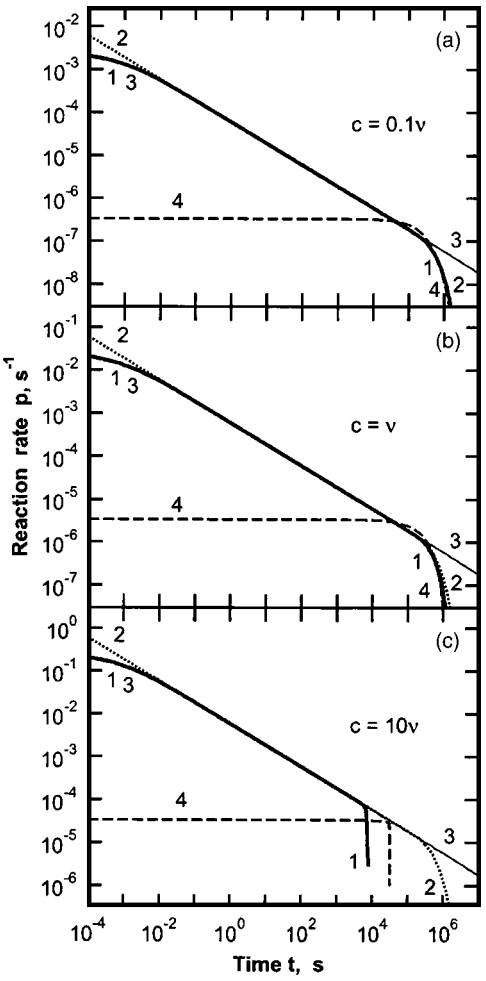

FIG. 2. Reaction rate p as a function of time t for various initial ligand concentrations c. The receptor is immobilized at the bottom of the incubation chamber of height $a=0.3$ cm. The other parameters are the rate constants $k_+=5.3\times10^5$ 1/M s and $k_-=3.2\times10^{-4}$ 1/s, the diffusion coefficient $D=1\times10^{-7}$ cm^2/s, and the receptor surface density $\rho=1.5\times10^{-11}$ mole/cm^2. The initial ligand concentration c is indicated in the units of $\nu=\rho/a=5.0\times10^{-8}$ M. Line 1 (thick solid line) is the numerical solution of Eq. (88) with $G(t)$ defined by Eq. (33). Line 2 (dotted line) is given by Eqs. (37a) and (37b) (diffusion-controlled irreversible reaction: $k_+\to\infty$). Line 3 (thin solid line) is given by Eq. (46) (linear approximation in the limit $a\to\infty$). Line 4 (dashed line) is given parametrically by Eqs. (24) and (25) with $\tau_F=a^2/3D$ (steady-state approximation).

In Sec. III A we did not consider the linearized kinetic equation [Eqs. (17) and (20)] with the memory function for a well of a finite height a [Eqs. (33)–(35)] because of mathematical complexity. It is clear, however, that the solution would be close to curve 3 at small t, and to curve 2 at large t (for the given set of parameters).

When the concentration c is greater that ν, the limiting fractional occupancy φ_1 is approximately equal to unity. In this case, curves 2 and 3, though completely wrong at large times t, can serve as useful estimates of the initial course of the reaction. They are valid almost in the whole region $\varphi<\varphi_1$, if $c\gg\nu$ [Fig. 3(c)].

It is interesting to note that the steady-state approximation (curve 4) proved to be reasonably good at low concen-

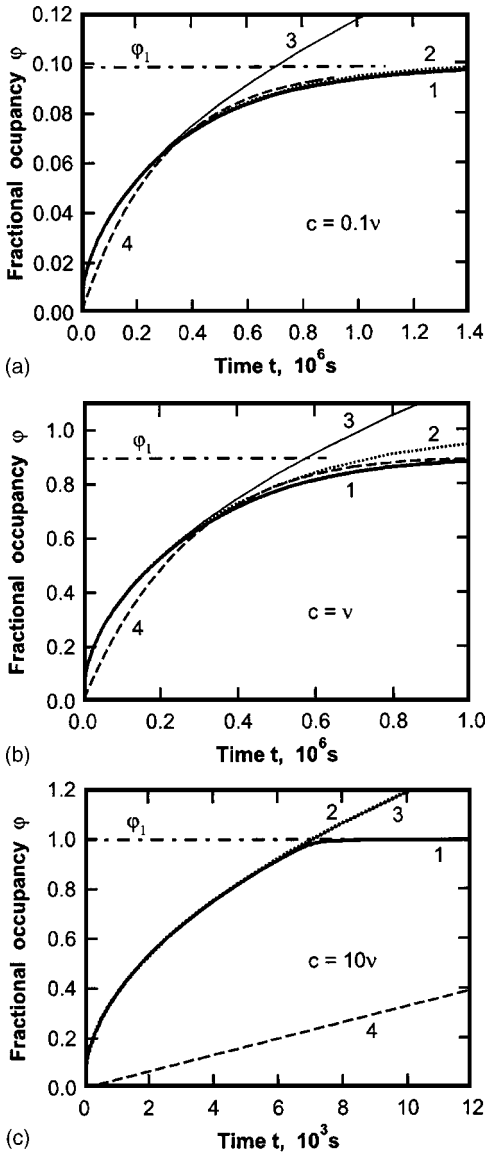

FIG. 3. Fractional occupancy φ as a function of time t for various initial ligand concentration c. The reaction conditions are the same as in Fig. 2. Line 1 (thick solid line) corresponds to the numerical solution of Eq. (88). Line 2 (dotted line) is given by Eqs. (38a) and (38b) (diffusion-controlled irreversible reaction). Line 3 (thin solid line) is given by Eq. (49) (linear approximation). Line 4 (dashed line) is given by Eq. (25) with $\tau_F = a^2/3D$ (steady-state approximation).

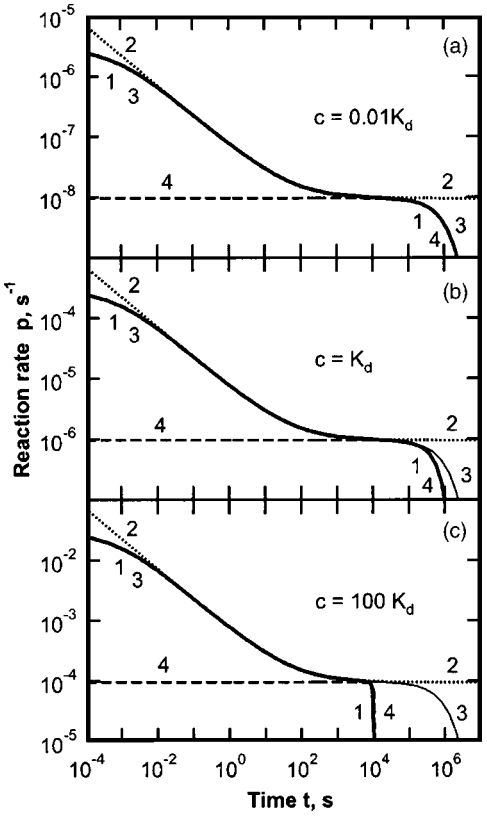

FIG. 4. Reaction rate p as a function of time t for various initial ligand concentrations c. The receptor is immobilized within a circular spot of radius $R=0.005$ cm at the center of the bottom of an incubation chamber. The dimensions of the incubation chamber are $length \times width \times height = b \times b \times a$, where $a=0.3$ cm and $b=0.5$ cm. The other parameters are the rate constants $k_+ = 5.3\times10^5$ 1/M s and $k_- = 3.2\times10^{-4}$ 1/s, the diffusion coefficient $D = 1\times10^{-7}$ cm^2/s, and the receptor surface density $\rho = 1.5\times10^{-11}$ mole/cm^2. The initial ligand concentration c is indicated in the units of $K_d = k_-/k_+ = 6.0\times10^{-10}$ M. Line 1 (thick solid line) is the numerical solution of Eq. (88) with $G(t)$ defined by Eq. (80). Line 2 (dotted line) is given by Eq. (82) (diffusion-controlled irreversible reaction: $k_+ \to \infty$). Line 3 (thin solid line) is given by Eq. (85) (linear approximation in the limit $a, b \to \infty$). Line 4 (dashed line) is given parametrically by the Eqs. (28) and (29) with $\tau_F = ab^2\hat{G}(0)$ (steady-state approximation).

trations c [Fig. 3(a)]. Only when the concentration becomes high ($c \gg \nu$), the steady-state approximation fails completely [Fig. 3(c)].

C. Three-dimensional diffusion to a spot

The numerical computations for the three-dimensional diffusion to a spot [Fig. 1(c)] were performed for the same set of parameters as in Sec. IV B. In addition, two other parameters were used that follow. The bottom of the incubation chamber was assumed to be a square with the side $b=0.5$ cm. The spot of radius $R=0.005$ cm was positioned in the center. The memory function was defined by Eq. (80).

The numerical solutions in terms of the reaction rate $p(t)$ are displayed in Fig. 4 (thick solid line or line 1) for three values of the initial ligand concentration: $c=0.01K_d$, $c=K_d$, and $c=100K_d$, where $K_d = k_-/k_+ = 6.0\times10^{-10}$ M is the equilibrium dissociation constant. The following analytical results are also shown: the diffusion-controlled irreversible reaction [Eq. (82)] is represented by line 2 (dotted line), the linear approximation [Eq. (85)] by line 3 (thin solid line), and the steady-state approximation by line 4 (dashed line). Line 4 is given parametrically by the Eqs. (28) and (29), which were derived under the condition that $\nu \ll c/\varphi_1$. The use of these equations in the present case is justified, because $\nu = N/V = \rho\pi R^2/ab^2 = 0.026K_d$, and the quantity c/φ_1 is always greater than K_d. For the first collision time we used the value $\tau_F = 3.99\times10^7$ s, which was obtained numerically as $ab^2\hat{G}(0)$, with $G(t)$ defined by Eq. (80). For comparison, the analytical expression [Eq. (71)] gives $\tau_F = 4.05\times10^7$ s. (The difference between the two values of τ_F is not principal, but we used the more exact one, in order not to mix up the inaccuracy of the formula with the inaccuracy of the parameter value.)

The curves for the fractional occupancy $\varphi(t)$ are shown in Fig. 5. Line 1 (thick solid line) corresponds to the numeri-

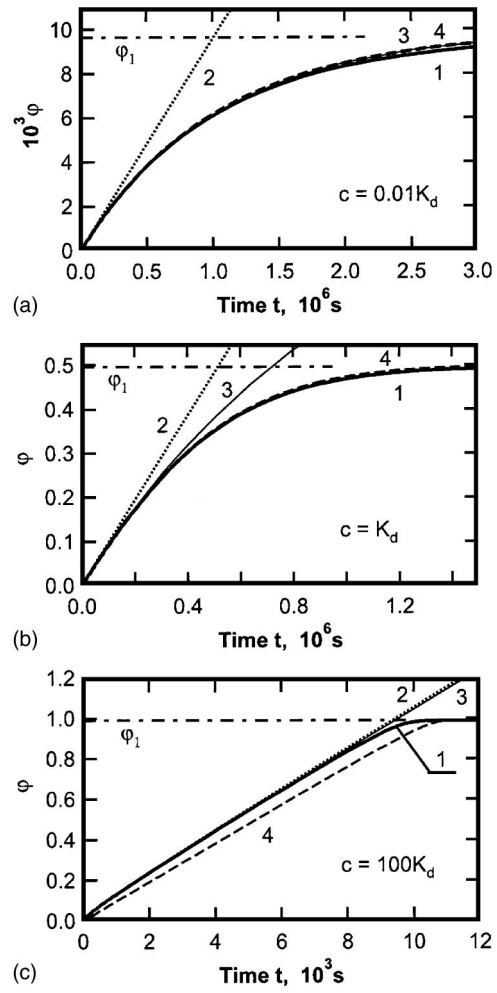

FIG. 5. Fractional occupancy φ as a function of time t for various initial ligand concentration c. The reaction conditions are the same as in Fig. 4. Line 1 (thick solid line) corresponds to the numerical solution of Eq. (88). Line 2 (dotted line) is given by Eq. (83) (diffusion-controlled irreversible reaction). Line 3 (thin solid line) is given by Eq. (86) (linear approximation). Line 4 (dashed line) is given by Eq. (29) with $\tau_F = ab^2\hat{G}(0)$ (steady-state approximation).

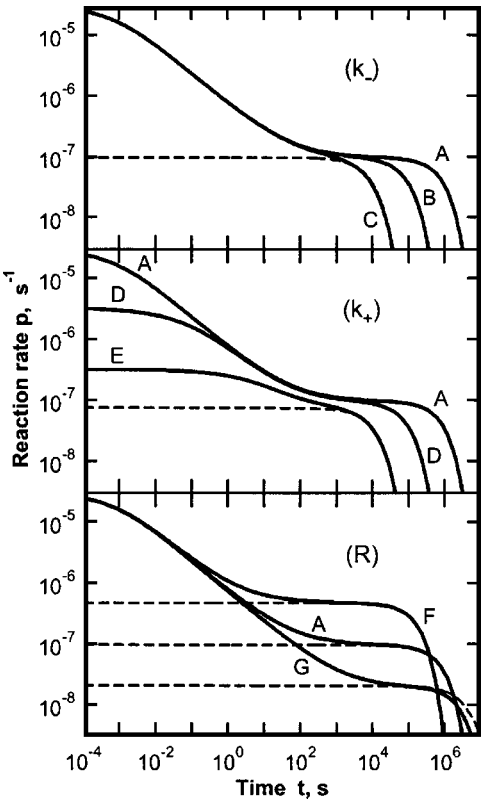

FIG. 6. Dependence of the function $p(t)$ on the dissociation rate constant k_- (upper frame), on the association rate constant k_+ (middle frame), and on the spot radius R (lower frame). The numerical solutions are shown with the thick solid lines. Line A, presented in all frames, corresponds to the default set of parameters that are given in the legend of Fig. 4. The ligand concentration is $c = 6.0 \times 10^{-11}$ M. For each of the other curves, one parameter has a nondefault value. Upper frame: Line A, $k_- = 3.2 \times 10^{-4}$ 1/s; line B, $k_- = 3.2 \times 10^{-3}$ 1/s; line C, $k_- = 3.2 \times 10^{-2}$ 1/s. Middle frame: Line A, $k_+ = 5.3 \times 10^5$ 1/M s; line D, $k_+ = 5.3 \times 10^4$ 1/M s; line E, $k_+ = 5.3 \times 10^3$ 1/M s. Lower frame: Line F, $R = 0.001$ cm; line A, $R = 0.005$ cm; line G, $R = 0.025$ cm. For some curves, the corresponding steady-state solution is given by the dashed line.

cal solution of the full kinetic equation, line 2 (dotted line) to the diffusion-controlled irreversible reaction [Eq. (83)], line 3 (thin solid line) to the linear approximation [Eq. (86)], and line 4 (dashed line) to the steady-state approximation [Eq. (29)]. It should be mentioned that Eq. (25) yields a curve that would be indistinguishable from line 1 in Figs. 5(a) and 5(b) and from line 4 in Fig. 5(c).

Note that curves 2 and 3 were obtained in the assumption that $a, b \to \infty$ or, in other words, that the supply of ligand molecules is inexhaustible. If the limited number of ligand molecule were taken into account, curve 2 would lie lower at the times exceeding $\tau_F \sim 10^7$ s. However, the backward reaction, which is accounted for by curve 3, comes to play an important role already at the times $\sim 10^4$ s. Hence, at low ligand concentrations ($c \ll K_d$), the reaction reaches the dynamic equilibrium due to the high rate of the backward reaction, while the supply of ligand molecules is still far from being exhausted. For that reason, curve 2 fails to predict the course of reaction correctly (if $t > 10^4$ s), whereas curve 3 is quite a good approximation [Fig. 5(a)].

At high ligand concentrations ($c > K_d$), saturation of binding sites ($\varphi_1 \approx 1$) becomes the main reason for slowing down the reaction. In this case, only the initial part of curves 2 and 3 is relevant. However, this relevant part can be as large as almost the whole region $\varphi < \varphi_1$ [Fig. 5(c)].

For the given set of parameters, the steady-state regime develops after $\sim 10^4$ s. At this point, curve 4 merges with curve 1 (Fig. 4). The steady-state approximation describes the further course of the reaction very well. If the mean reaction time exceeds 10^4 s (which is the case at low and intermediate concentrations c), this approximation leads to sufficiently precise results [Figs. 5(a) and 5(b)].

Figure 6 illustrates the dependencies of the numerical solution on the dissociation rate constant k_-, on the association rate constant k_+, and on the spot radius R (thick solid lines). For most of the curves, the corresponding steady-state solution [Eqs. (28) and (29)] is also shown (dashed lines). The steady-state approximation proved to be very good at the time scales of practical interest. However, the simplified formulas [Eqs. (28) and (29)] do not hold for the increased spot radius $R = 0.025$ cm (cf. curve G), because the ratio $\varphi_1 \nu / c = 0.38$ is not sufficiently small. In this case, one should rather

use Eqs. (24) and (25), which are free from the assumption $\varphi_1 \nu \ll c$. All the data presented in Fig. 6 correspond to quite a low concentration $c = 6.0 \times 10^{-11}$ M, so that the inequality $c \ll K_d$ is always satisfied. Under these conditions, the analytical linear approximation should hold. Indeed, one can easily verify that Eq. (85) practically exactly reproduces the thick solid curves in Fig. 6, except for line G corresponding to the large spot radius. In the latter case, the solution given by Eq. (85) follows the dashed line at large t.

V. CONCLUDING REMARKS

We believe that the theoretical approach presented in this paper will be useful for the design and interpretation of immunoassays, in particular, those involving an array of antibody microspots. From a practical point of view, the most interesting situation is when the ligand concentration is low. We have considered here three theoretical approaches that are appropriate for treating this case: (i) the steady-state approximation, (ii) the analytical solution of the linearized kinetic equation, and (iii) the numerical solution of the full nonlinearized kinetic equation. However, at least one essential point still remains to be clarified. It is common practice to stir the ligand solution in order to accelerate the reaction. Taking into account the stirring will be the next step in developing the theory. Although a thorough treatment of this problem is beyond the scope of the present study, we would like to suggest some preliminary considerations.

The stirring does not change the form of the basic kinetic equation [Eq. (3)]. The problem is to find an appropriate memory function. It is clear that such a function will decay faster than in the case of pure diffusion. Hence, the steady-state approximation [Eq. (23)] will be even more justified. The effect of stirring results essentially in a decrease of the first collision time τ_F, which can be treated as a new experimental parameter (substituting in this role the diffusion coefficient D).

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