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Kinetics of ligand binding to a cluster of membrane-associated receptors

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Abstract. The process of ligand binding to a cluster of membrane-associated receptors is examined theoretically. The theoretical model proposed involves the diffusion of ligands from the solution to the disc-like cluster of receptors on the surface of the spherical cell. When the ligand hits the internal part of the disc-like cluster, it begins to move laterally until it leaves the disc through its outer surface or is bound by one of the receptors inside the disc. If the ligand leaves the cluster, it returns to the solution and hits the disc again after a certain period, etc. According to our model the transition from a diffusion-limited to a reaction-limited process of binding is determined by the dimensionless parameter $\lambda \equiv \tilde{D}t_c/a^2$, where $\tilde{D}$ is the lateral diffusion coefficient, t_c is the characteristic time of reaction, and a is the radius of the disc-like cluster. The forward rate constant k_f turns out to be a function of λ. Comparing the results of our calculations of k_f with some experimental data we found that agreement is achieved at high λ, i.e. the process of ligand binding by clustered receptors is predominantly reaction-limited.

Key words: Chemoreception – Diffusion of ligands – Receptors' cluster

1. Introduction

The kinetic and equilibrium parameters of a biomolecular reaction (e.g., between an individual substrate A and an enzyme molecule B, or between ligand A and receptor B) are often used to characterize biochemical systems. The general scheme of biomolecular reactions is as follows (Berg and Purcell 1977; DeLisi 1981; Abbott and Nelsestuen 1989):

$$A + B \underset{k_-}{\overset{k_+}{\rightleftharpoons}} A \ldots B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} A - B. \qquad (1.1)$$

separated reactants — encounter complex — product

where k_+, k_- are the effective forward and reverse diffusive rate constants, and k_1, k_{-1} are the intrinsic forward and reverse rate constants.

The kinetics of the binding of the ligands to spherical cells of radius R that have N uniformly distributed receptors per cell has received broad theoretical attention (Berg and Purcell 1977; Goldstein and Wiegel 1983; Goldstein 1989; Wiegel 1991). If the cell is modeled as a sphere of radius R, k_+ is identified with the well known Smoluchowski diffusion-limited rate constant (Berg and Purcell 1977; Shoup and Szabo 1982):

$$k_+ = 4\pi RD. \qquad (1.2)$$

If the receptor is modeled as a perfectly adsorbing disc of radius $a \ll R$, while the rest of the cell surface is assumed to be perfectly reflecting (flux through it is zero), k_1 can be calculated as (Hill 1975):

$$k_1 = 4Da. \qquad (1.3)$$

In this case the theory predicts that the ligand-receptor forward rate constant per receptor, k_f, will depend on N according to the following equation (Berg and Purcell 1977):

$$k_f = \frac{k_1}{1 + Nk_1/k_1} = \frac{4Da}{1 + Na/\pi R}. \qquad (1.4)$$

The same approach also applies to system in which receptors cluster on the membrane surface and form coated pits. In the simplest case one can treat clusters as perfect sinks. Then, Eq. (1.4) is still valid, provided one interprets N and a as number and radius of clusters rather than individual receptors. The forward rate constant for the binding to the entire cell, $k_{f\text{cell}}$, is $Nk_{f\text{rec}}$, is k_f/N_{rec}, where N_{rec} is the number of receptors per cluster.

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In the present work we carry out the kinetic analysis of the process of ligand binding to a cluster of membrane-associated receptors within the framework of a more general model, conceiving clusters of receptors as non-perfect sinks. Our approach makes it possible to describe the transition from a diffusion-limited to a reaction-limited process of binding, the latter being characterized by the finite t_c. In part 2 we present a mathematical solution of the problem of diffusion of ligand molecules to the disc-like cluster of receptors which takes into account the lateral diffusion of ligands over the surface of the cluster before they are bound by one of the receptors of the cluster. This solution is used to determine the general representation of the constant k_1 involved in Eq. (1.4) as a function of the lateral diffusion coefficient $\tilde{D}$, t_c and a, i.e. to generalize Eq. (1.3). In part 3 the results of Sect. 2 will be utilized to describe the ligand-receptor interactions in the systems with clustered receptors on a cell surface. We will show that our theory predicts k_f to be a non-monotonous function of a (as distinct from the simplified Eq. (1.4) according to which k_f monotonously decreased as a increases). The theoretical results will be compared with the experimental data. Our theory can also be used to estimate cluster radius a from the data on rate constants. We will see that the original model of Berg and Purcell (1977) (see also Shoup and Szabo 1982) overestimates k_f and a, while the predictions of our model are in better agreement with the experiments for reasonable values of the model parameters. Thus we confirm the fact that most ligand-receptor interactions are not diffusion limited (Varfolomeyev and Zaitsev 1982).

2. Binding of ligands to the isolated disc-like cluster of receptors associated with the plane membrane

In this section we consider the flux of Brownian particles (ligand molecules) to the isolated disc-like sink (cluster of receptors). Our analysis should be considered as a generalization of the one of Hill (1975) to the case of non-perfect sink, i.e. ligands are allowed to leave the disc-like sink. We will calculate the number of ligands, I, which are bound by the receptors of the given cluster per unit time. Provided I is calculated, the rate constant, k_1, is defined as I/n_0, where n_0 is the number concentration of ligands at infinite distance from the cluster.

To avoid misunderstanding we will use the term captured ligands to describe those which are moving along the surface of the cluster and are attracted by the receptors as distinct from free ligands (which stay in the solution) and bound ligands (which are taken away from the analysis).

The scheme of the process is shown in Fig. 1.

Since we assume that $a \ll R$, the cell membrane can be described as locally planar. In other words, the disc is considered in this section as a part of the plane such that on the external part of the plane (outside the disc) the condition of ideal reflection is satisfied (the flux through the plane is zero) while on the internal part of the plane (inside the disc) ligands can be bound by receptors. When a free ligand hits the internal part of the disc, it is assumed

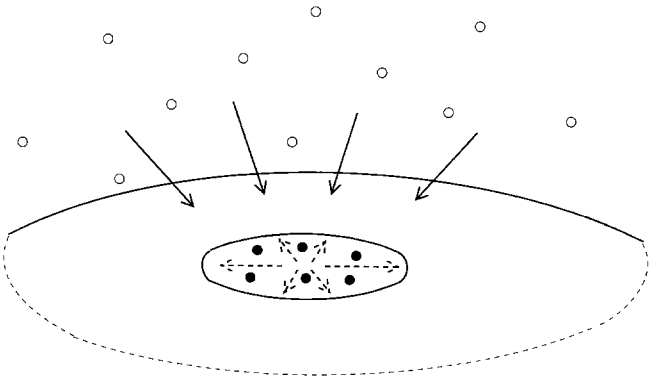

Fig. 1. Scheme of the process of ligand capture. Disk-like cluster of receptors is shown on the surface of the spherical cell. Empty circles designate free ligands, arrows show their flux to the cluster. Filled circles designate captured ligands, dotted arrows shown their flux to the edge of the disk

to be captured. In this case it begins to move laterally until it leaves the disc. If the ligand leaves the disc, it returns to the solution and hits the disc again after a certain period, etc.

Thus, our model is based on the following *principal assumptions*:

(i) Capture of ligands by the receptor-free part of the cell surface is neglected. To justify this assumption let us recall that the forces which are responsible for the capture of ligands by the cell surface are mostly of electrostatic origin (Bell 1978). These forces are higher if the ligand contacts the part of the surface which contains recptors, than at the receptor free surface. However, we admit that a more general theory should also take into account the capture of ligands by the receptor-free part of the surface.
(ii) Captured ligand can leave the cluster of receptors only from the cluster edge. This assumption is valid of the captured ligand is attracted by any receptors, i.e. if the distance at which the receptors attract the ligand is larger than the size of the receptor. In this case the attraction of the captured ligand to the internal part of the cluster is higher than its attraction to the edge of the cluster, so that the desorption of the ligand from the internal surface of the cluster is less probable than the desorption from the edge of the cluster. However, in a more general model the desorption from the internal part of the cluster should also be taken into account in calculating I.

Despite these simplifying assumptions we will see that our model turns out to be in rather good agreement with the experimental data (see Sect. 3).

In addition to these two principal assumptions, we employ the following simplification:
(iii) Those ligands which leave the disc from its surface are immediately removed to infinity. In other words, the problem which we consider in this section corresponds to the situation where the possibility of a ligand being captured more than one time by the same receptor is neglected.

It is worthwhile noting that, as distinct from assumption (i) and (ii), assumption (iii) is not necessary for the formulation of our model, but it simplifies the calculation

considerably. As shown in Appendix 1, removal of assumption (iii) results only in some alteration of a numerical coefficient in the final equation for the flux. In the end of this section we will discuss a possible way to account for this alteration.

3D diffusion of free ligands in cylindrical coordinates with the origin in the center of the disc and the z axis perpendicular to the disc is governed by the following equation

$$D\nabla^2 n(r,z) \equiv D\left[\frac{1}{r}\frac{\partial}{\partial r} r \frac{\partial}{\partial r} n(r,z) + \frac{\partial^2}{\partial z^2} n(r,z)\right] = 0, \quad (2.1)$$

where D is the coefficient of 3D diffusion and $n(r,z)$ is the number concentration of free ligands. Boundary conditions are given by

$$n(r<a,0) = 0,\ \nabla n(r>a,0) = 0,\ n(\infty,\infty) = n_0, \quad (2.2)$$

where a is the radius of the disc.

2D diffusion of captured ligands in polar coordinates are governed by the following equation

$$\tilde{D}\frac{1}{r}\frac{\mathrm{d}}{\mathrm{d}r} r \frac{\mathrm{d}}{\mathrm{d}r}\tilde{n}(r) - \frac{1}{t_c}\tilde{n}(r) = -j(r), \quad (2.3)$$

where $\tilde{D}$ is the coefficient of lateral (2D) diffusion (in the general case $D \geq \tilde{D}$, because the mobility of ligands near the surface can not be higher than the one in the bulk of the solution), $\tilde{n}(r)$ is the number of ligands per unit area, t_c^{-1} is the probability for the reaction to occur per unit time, $j(r)$ is the number of free ligands captured by the disc per unit time per unit area. Boundary conditions are given by

$$\tilde{n}(a) = 0,\ \mathrm{d}\tilde{n}(0)/\mathrm{d}r = 0. \quad (2.4)$$

Equations (2.1) and (2.3) are not independent. Indeed, the flux in the right hand side of Eq. (2.3) takes the form

$$j(r) = D\,\partial n(r<a,0)/\partial z. \quad (2.5)$$

Hence one has to combine Eqs. (2.1–2.5) to calculate the distribution of captured ligands over the disc, $\tilde{n}(r)$. The total number of ligands bound by all the receptors of the disc-like cluster per unit time is given by

$$I = 2\pi \int_0^a J(r)\, r\, \mathrm{d}r, \quad (2.6)$$

where the number of ligands bound by receptor per unit time per unit area, $J(r)$, is identified with $t_c^{-1}\tilde{n}(r)$.

Equation (2.1) is reduced to the Laplace equation, i.e. the calcualation of $n(r,z)$ is mathematically equivalent to the calculation of the electrostatic potential of the conducting disc. The solution of the latter problem (see e.g. Morse and Feschbach 1953) is given by

$$n(r,z) = n_0 - \frac{2}{\pi} n_0 \operatorname{arctg}\frac{1}{\xi}, \quad (2.7)$$

where the oblate ellipsoidal coordinates (ξ, v) are used, which are related to the cylindrical coordinates by the following equations:

$$z = a\,\xi v,\ r = a[(\xi^2+1)(1-v^2)]^{1/2}. \quad (2.8)$$

In these coordinates the surface of the disc corresponds to the axis $\xi = 0$. When one considers 2D diffusion on a disc, it is sometimes convenient to introduce the coordinate $\tilde{v} \equiv [1-(r/a)^2]^{1/2}$, which is identical to v in the limit $\xi \to 0$. We will use $\tilde{v}$ and r interchangeably. We substitute (2.7) into (2.5) to calculate the flux of ligands to the surface of the disc as

$$j(r) = \frac{2n_0 D}{\pi a[1-(r/a)^2]^{1/2}},\ j(\tilde{v}) = \frac{2n_0 D}{\pi a \tilde{v}}. \quad (2.9)$$

The general form of the solution is determined by the value of the dimensionless parameter $\lambda \equiv \tilde{D}t_c/a^2$. At $\lambda \ll 1$ the ligands, after hitting the surface of the disc, are immediately bound to it, while at $\lambda \gg 1$ the ligands most likely leave the disc after reaching its edge. These two cases can be described as diffusion-limited and reaction-limited respectively. Below we will consider both limiting cases and obtain the general solution valid at arbitrary λ.

In the diffusion-limited case ($\lambda \ll 1$) the solution of Eq. (2.3) is obtained by substituting (2.9) into (2.3), the first term in the left-hand side of (2.3) being neglected:

$$\tilde{n}(r) = \alpha/[1-(r/a)^2]^{1/2},\ \tilde{n}(\tilde{v}) = \alpha/\tilde{v}, \quad (2.10)$$

where $\alpha \equiv \dfrac{2n_0 D t_c}{\pi a}$. Since Eq. (2.10) is obtained for a perfect sink with the lateral diffusing being totally neglected, it is not applicable in the vicinity of the edge, i.e. at distances $\leq (\tilde{D}t_0)^{1/2}$ from the edge. However, in the limit $\lambda \to 0$ (i.e. at $t_c \to 0$) the area in which Eq. (2.10) is not applicable is infinitesimally small. Evidently, in this case $J(r)$ is identical to $j(r)$. Integrating (2.6) gives the well known result (Hill 1975):

$$I = 4Dan_0. \quad (2.11)$$

At $\lambda \geq 1$ one has to take into account the lateral diffusion of captured ligands and their leaving the cluster after reaching its edge. In other words, in this case I and the rate constants of the process depend on t_c, i.e. it can not be described as diffusion limited.

Let us first consider the limiting case $\lambda \gg 1$, which corresponds to the reaction-limited process of binding. In this case the second term in the left hand side of Eq. (2.3) can be neglected and we find

$$\tilde{n}(x) = \frac{\alpha}{\lambda}\left\{\left[1-\left(\frac{x}{x_1}\right)^2\right]^{1/2} - \ln\left[1+\left[1-\left(\frac{x}{x_1}\right)^2\right]^{1/2}\right]\right\}.$$
$$\tilde{n}(\tilde{v}) = \frac{\alpha}{\lambda}[\tilde{v} - \ln(1+\tilde{v})], \quad (2.12)$$

where $x \equiv r/(Dt_c)^{1/2}$ is a dimensionless variable, $x_1 \equiv a/(\tilde{D}t_c)^{1/2} \equiv \lambda^{-1/2}$. Integration according to Eq. (2.6) yields

$$I = \pi t_c^{-1}\int_0^{a^2}\tilde{n}(r)\,\mathrm{d}r^2 = \pi a^2 t_c^{-1}\int_0^1 \tilde{n}(\tilde{v})\,\mathrm{d}\tilde{v} = \frac{aDn_0}{3\lambda}, \quad (2.13)$$

Comparing (2.11) and (2.13) we can write the asymptotic representations for the ratio $I_r \equiv I/(4Dan_0)$:

$$I_r(\lambda) = \begin{cases} 1, & \lambda \ll 1, \quad (2.14.1) \\ 1/(\beta\lambda), & \lambda \gg 1, \quad (2.14.2) \end{cases}$$

where the numerical coefficient, β, is found equal to 12 in within the framework of our above analysis.

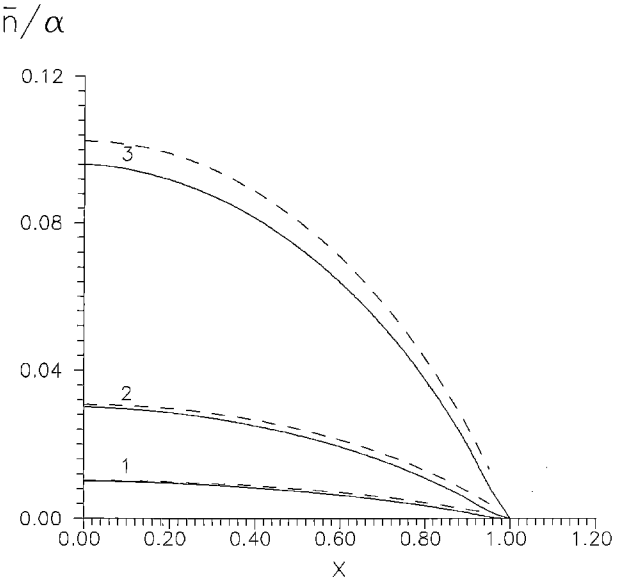

Fig. 2. Dimensionless concentration of ligands on the disk-like cluster of receptors is plotted vs. radial coordinate at $\lambda = 30$ (1), 10 (2), 3 (3). *Solid curves* are calculated according to Eq. (2.16); *dashed curves* – according to (2.12)

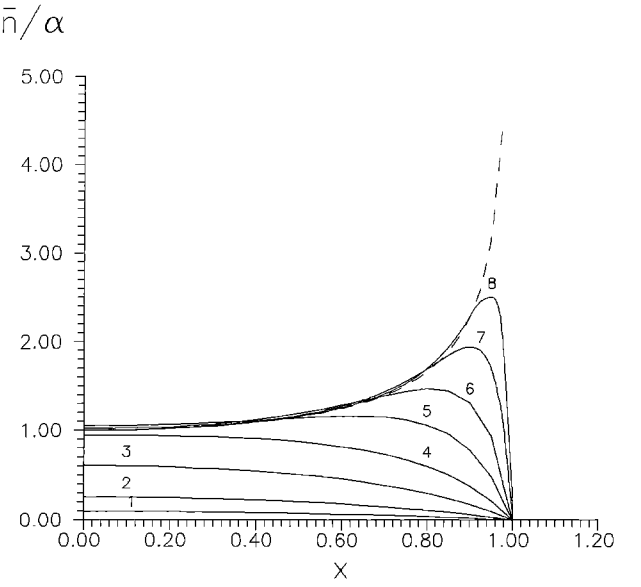

Fig. 3. The same as in Fig. 1 at $\lambda = 3$ (1), 1 (2), 0.3 (3), 0.1 (4), 0.03 (5), 0.01 (6), 0.003 (7), 0.001 (8). *Dashed curve* is calculated according to Eq. (2.10)

To find the solution valid at arbitrary λ, one has to solve Eq. (2.3) which is an inhomogeneous linear differential equation. Corresponding fundamental systems of solutions of homogeneous equations consist of Bessel functions of imaginary argument $I_0(..)$ and $K_0(..)$. Hence the solution of (2.3) takes the form

$$\tilde{n}(x) = C_1 I_0(x) + C_2 K_0(x) - I_0(x) \int \frac{x j_{tot}(x)}{\Delta(I_0, K_0)} K_0(x)\,dx + K_0(x) \int \frac{x j_{tot}(x)}{\Delta(I_0, K_0)} I_0(x)\,dx, \tag{2.15}$$

where

$$\Delta(I_0, K_0) \equiv I_0(x) \frac{dK_0(x)}{dx} - \frac{dI_0(x)}{dx} K_0(x) = -[I_0(x) K_1(x) + I_1(x) K_0(x)] = -1/x.$$

The constants C_1 and C_2 and the limits of integration are defined by taking into account the boundary conditions. The final result can be written as

$$\tilde{n}(x) = \alpha \left[I_0(x) \int_x^{x_1} \frac{K_0(x)\,x\,dx}{(1-(x/x_1)^2)^{1/2}} - \frac{K_0(x_1)}{I_0(x_1)} I_0(x) \int_0^{x_1} \frac{I_0(x)\,x\,dx}{(1-(x/x_1)^2)^{1/2}} + K_0(x) \int_0^{x} \frac{I_0(x)\,x\,dx}{(1-(x/x_1)^2)^{1/2}} \right]. \tag{2.16}$$

The integrals in (2.16) are easily calculated numerically. Results are given in Figs. 2 and 3 where it is shown that in the limits of low and high λ the dependence $\tilde{n}(x)$ approaches the approximate solutions (2.10) and (2.12) respectively.

Substituting the results into (2.6) we calculate I numerically as a function of λ. The results of these calculations are presented in Fig. 4.

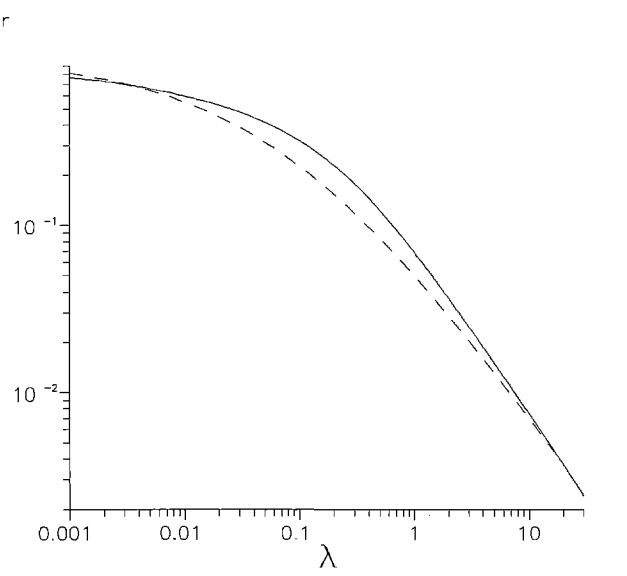

Fig. 4. $I_r \equiv I/(4Dan_0)$ is plotted vs. λ. *Solid lines* – numerical calculations, *dashed lines* are calculated according to the interpolation formula (2.17) with $\beta = 12$

In some cases it is desirable to have an analytical representation of $I(\lambda)$. To find such a representation we make use of the asymptotic equations (2.14) and search the analytical approximation for $I(\lambda)$ as an interpolation formula valid in both limiting cases. One of the simplest equations of this type is given by:

$$I_r(\lambda) \equiv I(\lambda)/(4Dan_0) = \frac{1}{[(\beta\lambda)^{1/2} + 1]^2}, \tag{2.17}$$

Calculation according to Eq. (2.17) are compared with the numerical calculation in Fig. 4. One can see that Eq.

(2.17) turns out to be in rather good agreement with the numerical calculation in the full range of λ. Instead of Eq. (1.3) we now have

$$k_0 = 4\,Da\,I_r(\lambda). \tag{2.18}$$

To this end our calculations were based on the model defined by the assumptions (i), (ii) and (iii). Let us now consider the consequences of the removal of assumption (iii). Although the general solution similar to (2.16) is difficult to get in this case, the asymptotic solutions can be considered. In the limit of low λ the analysis is not sensitive to assumption (iii), i.e. Eqs. (2.10), (2.11) and (2.14.1) hold true. In the limit of high λ the analysis is carried out in Appendix 1 and yields a result similar to (2.14.2). The only difference is that the coefficient β is lower, namely $\beta=6$. Thus, there are reasons to believe that the interpolation formula (2.17) can be still used in the general case, provided we put $\beta=6$.

3. Binding of ligands to the disc-like clusters of cell-bound receptors

In this section we apply the results obtained in Sect. 2 for the isolated cluster of receptors to describe the binding of ligands to a spherical cell that contains disc-like clusters of receptors uniformly distributed on its surface. Our consideration should be considered as a direct generalization of the theory of Berg and Purcell (1977) to the systems in which clusters of receptors can not be treated as perfect sinks, i.e. one should take into account the lateral diffusion of ligands along the surface of a cluster before it reacts with one of its receptors or goes away into the bulk of the solution.

Let us assume that the radius of clusters, a, is much less than the distance between them, l, while l is much less than the radius of the cell R. Since $a \ll l$, the flux of ligands to each of the clusters is identical to the one calculated in Sect. 2 for the isolated cluster:

$$I_f = 4\,Dan_s\,I_r(\lambda)\,N, \tag{3.1}$$

where n_s is the averaged concentration of ligands in the boundary layer adjacent to the surface of the cell. Here the boundary layer is assumed to have the thickness l_s, such that $l_s \gg l$, but $l_s \ll R$. The first inequality implies that the flux per cluster can be calculated as if the concentration of ligands at infinite separation is n_s, i.e. the results of Sect. 2 hold true with n_0 being substituted by n_s. The second inequality implies that the bulk diffusion of the ligands to the surface of the cell can be described by assuming the concentration on the surface to be identical to $n_s = n(R)$. The stationary diffusive flux of ligands to the surface of the cell is calculated in spherical coordinates on the basis of the solution of the following equation

$$\frac{1}{r}\frac{d^2}{dr^2} rn(r) = 0, \tag{3.2}$$

with boundary conditions

$$4\pi R^2 D \frac{d}{dr} n(R) = I_f = 4\,Dan(R)\,I_r(\lambda)\,N, \tag{3.3}$$

$$n(\infty) = n_0. \tag{3.4}$$

Solving (3.2) together with (3.3) and (3.4) we find I_f and calculate the forward rate constant per cluster as $k_f \equiv I_f/(N n_0)$. The resultant equation for k_f is identical to (1.4) with the forward rate constant for the second stage given by $k_1 \equiv 4\,Da\,I_r(\lambda)$. The latter constant differs from the one used by Berg and Purcell (1977) by a factor $I_r(\lambda)$.

Making use of the interpolation formula (2.17) we rewrite (1.4) in the following form

$$k_f = \frac{4\,Da\,I_r(\lambda)}{1 + \dfrac{Na}{\pi R} I_r(\lambda)} \approx \frac{4\,Da}{[1 + (\beta\lambda)^{1/2}]^2 + \dfrac{Na}{\pi R}}. \tag{3.5}$$

It is quite obvious that Eq. (3.5) can be immediately obtained from Eq. (1.4) by substituting Eq. (2.18). One can see that Eq. (3.5) is identical to the original equation of Berg and Purcell (1977) if $\lambda \ll 1$ or $\beta\pi\lambda R/(Na) \ll 1$. In the opposite limiting case, Eq. (3.5) reads:

$$k_f \approx \frac{4}{\beta} d\,a^3\,t_c^{-1} \tag{3.6}$$

where $d \equiv \tilde{D}/D$. In some experimental applications it is more convenient to make use of the rate constant per receptor, $k_{f\mathrm{rec}} \equiv k_f/N_{\mathrm{rec}}$, where $N_{\mathrm{rec}} \equiv N_s/N = \phi(a/c_r)^2$ is the total number of receptor sites in the cluster, ϕ is the packing factor, i.e. the fraction of the surface occupied by the projections of receptors on the plane of the disc-like cluster. Making use of Eq. (3.5) we write:

$$k^* = k_{f\mathrm{rec}}/D\,c_r = \frac{4X/\phi}{(X + (\beta d\lambda_r)^{1/2})^2 + X\dfrac{\zeta}{\pi\phi}}, \tag{3.7}$$

where $X \equiv a/c_r$, $\lambda_r D\,t_c/c_r^2$, $\zeta \equiv N_s c_r/R$. In Figs. 5 and 6 we have plotted the relative forward rate constant per receptor, k^*, vs. relative cluster radius, x, assuming $\beta=6$. The following values of parameters were chosen:

$$R = 4\ \mu\mathrm{m},\ D = 1.0 \cdot 10^{-5}\ \mathrm{cm^2/s},\ N_s \sim 5.4 \cdot 10^5,\ c_r = 5\ \mathrm{nm}, \tag{3.8}$$

as found experimentally for the system of RBL cells (Erickson et al. 1987; Goldstein 1989; Posner et al. 1992). For the same system on the basis of some experimental data (see below) other parameters can be estimated as follows:

$$k_{f\mathrm{rec}} = 4 \cdot 10^{-14}\ \mathrm{cm^3/s},\ t_c = 3 \cdot 10^{-6}\ \mathrm{s},\ c = 0.2\ \mathrm{nm}, \tag{3.9}$$

where c is the radius of ligands. There are two parameters of our model, (relative diffusion coefficient, d, and packing factor, ϕ) which were not determined experimentally. Since the viscosity of the liquid in the vicinity of the membrane can be higher than the bulk viscosity, we assume d to be in the range 0.1 to 1. For dense compact packing of receptors one has $\phi \approx 0.91$, but in reality the packing factor can be lower owing to loose packing or higher owing to the roughness of the cell surface (Ryan et al. 1988) which makes the actual area of the disc higher than its projection on the plane tangent to the sphere at a give point. Anyway one expects ϕ to be close to unity, so that we assume ϕ to be in the range 0.5 to 1.5.

In Figs. 5 and 6 we have plotted the results of calculations according to the original theory of Berg and Purcell

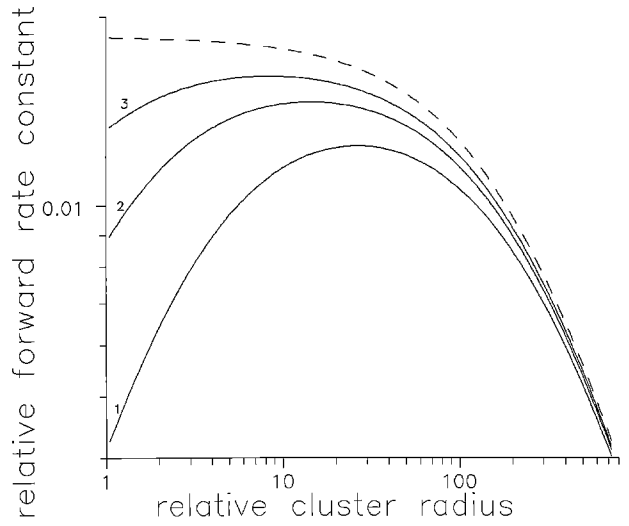

Fig. 5. Relative forward rate constant, k^*, is plotted vs relative radius of the cluster, x, at $\phi=1.0$. *Solid curves* 1–3 are calculated according to Eq. (3.7) with $\beta=6$, $d=1.0$ (1), 0.3 (2), 0.1 (3); *dashed curve* corresponds to the original theory of Berg and Purcell (1977), i.e. $d=0$

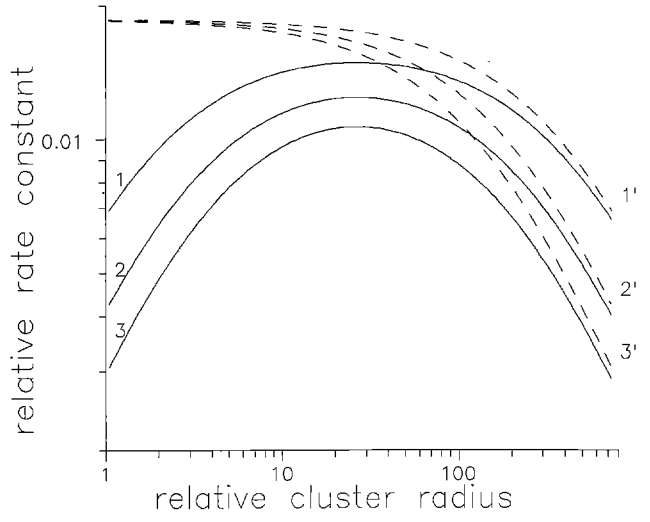

Fig. 6. Relative forward rate constant, k^*, is plotted vs relative radius of the cluster, x. *Solid curves* 1–3 are calculated according to Eq. (3.7) with $\beta=6$, $d=1.0$; *dashed curves* 1′–3′ correspond to the original theory of Berg and Purcell (1977), i.e. $d=0$, with $\phi=0.5$ (1,1′), 1.0 (2,2′), 1.5 (3,3′)

(1977) (see also Shoup and Szabo 1982), which correspond to the limit $d=0$. One can see that over almost the entire range of x in which the theory is valid, the approximation of Berg and Purcell (1977) does not work. There is an important quantitative difference between our calculations and their theory. Indeed, Berg and Purcel (1977) predict k^* to be a monotonously decreasing function of x, while our theory predicts k^* to be non-monotonous function of x with its maximum value corresponding to the transition from a predominantly diffusion-limited to a predominantly reaction-limited regime of ligand binding at $\beta\lambda<1$ (or $a>[\beta\tilde{D}t_c]^{1/2}$) and $\beta\lambda>1$ (or $a<[\beta\tilde{D}t_0]^{1/2}$), respectively.

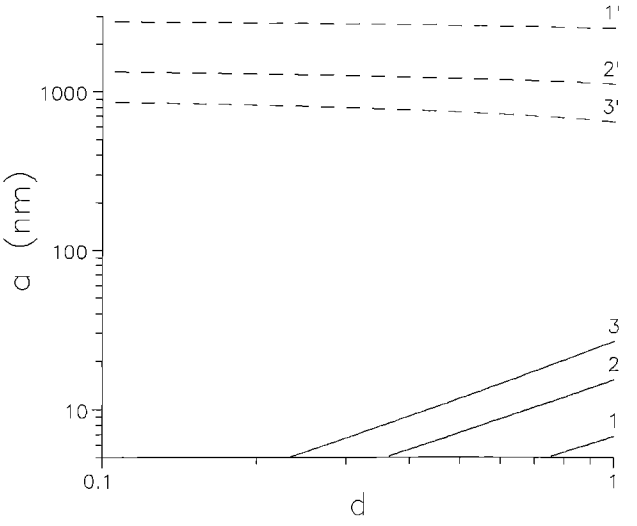

Fig. 7. Calculated values of cluster radius, a, is plotted vs ratio of 2D and 3D diffusion coefficients, d, at $\beta=6$, $k_{free}=4\cdot10^{-14}$ cm^3/s. *Solid curves* 1–3 are calculated according to Eq. (3.7) *dashed curves* 1′–3′ correspond to the original theory of Berg and Purcell (1977), i.e., $d=0$, with $\phi=0.5$ (1,1′), 1.0 (2,2′), 1.5 (3,3′)

Equation (3.7) can be easily solved with respect to X to provide the dependence of the cluster radius upon other parameters. We find:

$$X=\frac{1}{2}\Bigg[-2(\beta\lambda_r d)^{1/2}-\frac{\zeta}{\pi\phi}+\frac{4}{\phi k^*} \pm\left(\left(\frac{\zeta}{\pi\phi}\right)^2+\frac{16}{(\phi k^*)^2}+4(\beta\lambda_r d)^{1/2}\frac{\zeta}{\pi\phi} -\frac{16}{(\phi k^*)}(\beta\lambda_r d)^{1/2}-8\frac{\zeta}{\pi\phi^2 k^*}\right)^{1/2}\Bigg]. \tag{3.10}$$

According to Eqs. (3.7) and (3.10), in the general case there are two different values of the cluster radius which correspond to the same k^* (two solutions given by (3.10)). However, not every solution has physical meaning, since there are conditions which should be satisfied for the theory to be valid, i.e.: $R\gg a\gg c_r$, $N\gg1$. For parameters given by (3.8), (3.9) and $\beta=6$ both solutions are plotted in Fig. 7 as functions of d with different ϕ. The upper solution corresponds to the diffusion-limited process ($\beta\lambda\ll1$), while the lower solution corresponds to the reaction-limited process ($\beta\lambda\gg1$).

Now let us compare the results of calculations according to Eq. (3.5) with the experimental data of Posner et al. (1992) for binding (dissociation) of a monovalent ligand DCT (2,4-dinitrophenyl (DNP)-aminocaproyl-L-tyrosine) to (from) aggregated IgE-Fc$_\varepsilon$ receptor complexes associated with the surface of RBL cells. Here IgE-Fc$_\varepsilon$ are clustered by monoclonal anti-IgE antibody, B1E3, and polyclonal anti-IgE as a secondary antibody. This system is characterized by the parameters (3.8) (see Erickson et al. 1987; Goldstein 1989; Posner et al. 1992). Radius of ligands, c, was estimated as 0.21 nm by making use of the Einstein formula for the bulk diffusion coefficient, $D=kT/(6\pi c\eta)$, where η is the viscosity of the media.

Special comments are necessary concerning the choice of $k_{f\mathrm{rec}}$ and t_c. In most experiments reverse rate constants were determined. Three types of these constants were measured: for free receptors in solution, k_{rs}, for unclustered cell-bound receptors, k_{rcu}, and for clustered cell-bound receptors, k_{rcc}. As found by Goldstein (1989) and Erickson et al. (1987) $k_{\mathrm{rs}}/k_{\mathrm{rcu}} \approx 2$, while Posner et al. (1992) determined $k_{\mathrm{rcu}}/k_{\mathrm{rcc}} \approx 2-3$. Since the equilibrium rate constants in both cases were equal, we assume that the same ratios hold true for the forward rate constants.

Since for free receptors in solution the forward rate constant was determined as $k_{\mathrm{fs}} = 2 \cdot 10^{-13}$ cm^3/s (Goldstein 1989), we find an estimate for the forward rate constant for cell-bound clustered receptors: $k_{f\mathrm{rec}} \approx 4 \cdot 10^{-14}$ cm^3/s, as used in (3.9). The information about t_c can be obtained from the forward rate constant k_{fs} determined for binding of the same ligand by the same receptor in solution. Since in this case the receptors are not clustered and not cell-bound, they can be approximately described as spheres of radius c_r with the diffusion coefficient D_r, interacting with ligands of radius c according to the process (1.1). In the steady state approximation for the encounter complex the following equations can be used to relate k_{fs} to other rate constants (Eigen 1974):

$$k_{fs} = \frac{k_+ k_-}{k_1 + k_-}. \tag{3.11}$$

The diffusive rate constants can be estimated as $k_+ = 4\pi(D+D_r)(c+c_r)$, $k_- = 3(D+D_r)/(c+c_r)^2$ (see, e.g., Shoup and Szabo 1982), while k_1 can be identified with t_c^{-1}. Then we find

$$\begin{aligned} k_1 = t_c^{-1} &= k_f k_- /(k_+ - k_f) \\ &= \frac{3k_{fs}}{4\pi(c+c_r)^3}\left(1 - \frac{k_{fs}}{4\pi(D+D_r)(c+c_r)}\right)^{-1}. \end{aligned} \tag{3.12}$$

Substituting the experimental value of k_{fs} and other parameters into (3.8) we find $t_c = 3 \cdot 10^{-6}$ s as used in (3.9). It is worth noting that this value of t_c is close to the upper limit of the range of estimates of t_c for typical antibody-hapten interactions, $10^{-9}-10^{-5}$ s, given by Pecht and Lancet (1977) and Bell (1978).

Now if we put $d=1$ and $\phi=1$ in Eq. (3.7) and (3.10) we find that the value of $k_{f\mathrm{rec}} = 4 \cdot 10^{-14}$ cm^3/s which we determined on the basis of experimental data corresponds to two possible values of the cluster radius: $a_1 \simeq 15.4$ nm ($X=3.4$, $\beta\lambda \approx 74$) and $a_2 \simeq 1$ μm ($X=228.9$, $\beta\lambda \approx 0.013$). The upper value ($a_2$) corresponds to the diffusion-limited process and is close to the one which is given by the original theory of Berg and Purcell (1977), i.e. at $\lambda=0$. However, this value turns out to be too high. The lower value (a_1) is, by an order of magnitude, closer to the values $a \approx 40$ nm, which were determined experimentally (Ryan et al. 1986, 1988). One can see that the lower value of the cluster radius, a_1, corresponds to $N_{\mathrm{rec}} \sim 10$. Since in this case λ is high, we can conclude that the binding of the ligands in this system is reaction-limited.

It seems interesting to determine the range of the values of the ratio of the forward rate constants for free and clustered receptors, $\gamma \equiv k_{fs}/k_{f\mathrm{rec}}$. This is important for optimizing of experimental parameters to check the predictions of our model and for the discrimination of the cell systems with respect to the possible influence of receptor clustering on the regulation of their functional response. Making use of Eqs. (3.7) and (3.11) and assuming $c_r \gg c$, we estimate γ as:

$$\gamma = \frac{\pi\phi}{X} \frac{[X + (\beta d \lambda_r)^{1/2}]^2 + \dfrac{N_s c_r}{\pi\phi R}}{1 + 3\lambda_r}. \tag{3.13}$$

In the general case γ is a complicated function of c_r, a etc. If we consider the system with parameters defined by (3.8), (3.9) and put $d=1$ and $\phi=1$, we find that γ is a non-monotonous function of X with its minimum value, $\gamma=2.8$, at $X=27$.

4. Conclusion and further perspectives

We have presented a theoretical model to describe the binding of ligands to cell-bound clustered receptors. As distinct from the models proposed previously by other authors (Berg and Purcell 1979; Shoup and Szabo 1982) we have taken into account the lateral diffusion of ligands over the surface of the cluster of receptors by assuming the reaction between the ligand and the receptor to be characterized by some finite time, t_c. We have calculated the number of ligands bound by the receptor in the cluster per unit time (which is proportional to the forward rate constants k_f or $k_{f\mathrm{rec}}$) as a function of the dimensionless lateral diffusion coefficient, $\lambda \equiv D t_c/a$. In the limit $\lambda \to 0$ our theory describes the diffusion-limited process (i.e. the ligand is bound by the receptor immediately after it hits the cluster), while for $\lambda \geq 1$ the process is not purely reaction-limited (i.e. after the ligand hits the cluster, it is either bound by the receptor of the cluster or leaves the cluster after travelling over its surface for some time). We have demonstrated that the agreement with the experimental data is achieved at $\lambda \gg 1$ so that the dependence of the rate constants upon t_c or λ can not be neglected and the interaction of the ligand with the clustered receptors is far from being pureley diffusion-limited.

Despite the relative simplicity of our model it turns out to be in rather good agreement with the experimental data. However, it seems worthwhile to make some comments concerning further improvements and modifications which can be introduced into our model in the future.

Our present model is based on the assumption that the ligands can be captured by the cell only if they hit the internal part of the clusters and leave the surface of the cell when they reach the edge of the cluster. More general models should take into account the adsorption of the ligands by the whole surface of the cell and their desorption from any of its parts (although the probabilities of the adsorption and desorption are different for different parts of the surface). However, such generalization would require more detailed information about the adhesion of ligands to different parts of the cell surface and more complicated mathematical treatment.

Further generalization would also require taking into account the lateral mobility of cell receptors. This in-

cludes several different phenomena: (1) lateral diffusion of clusters (Torney and Bell 1986); (2) regular movement of clusters due to endocytosis and shedding (Timoshenko and Cherenkevich 1990); (3) change of cluster size, since receptors in some cases can join the cluster or leave it (Kaprelyants 1988).

More accurate description of ligand interaction with clustered receptors is also necessary. The heterogeneous structure of the clusters should be taken into account (see e.g. the data on fractal properties of clusters given by Dewey and Datta (1989)). Strengthening of RL-complexes with time due to rebuilding of the intracellular cytoskeletal structures (Tozeren 1990) may be taken into account in terms of our model by introducing time dependence of t_c.

Thus, the theory developed in this paper can be used to describe those processes of ligand interaction with disc-like clustered receptors which can not be described as purely diffusion-limited.

Appendix 1

In this Appendix we consider the adsorption of ligands by a disk-like cluster of receptors by taking into account the possible return of ligands to this cluster (recapturing) after they leave from its edge. Thus, we in some sense generalize the solution given in Sect. 2 by removing assumption (iii). However, as we will see below, such a generalization is possible at the expense of confining the analysis to the limit of high λ.

To start with we need to modify Eqs. (2.1) and (2.3) so that they will take into account the fact that every capture ligand becomes free as soon as it reaches the edge of the disc, thus being able to rejoin the disk again.
Instead of Eq. (2.1) we consider the following equation:

$$D\nabla^2 n(r,z) \equiv D\left[\frac{1}{r}\frac{\partial}{\partial r} r \frac{\partial}{\partial r} n(r,z) + \frac{\partial^2}{\partial z^2} n(r,z)\right] = D\left.\frac{\partial \tilde{n}(r)}{\partial r}\right|_{r=a} \delta(z)\,\delta(r-a), \tag{A.1}$$

where in the right-hand side we introduce a flux of free ligands emerging on the edge of the disc.
Instead of Eq. (2.3) we have:

$$\tilde{D}\frac{1}{r}\frac{d}{dr} r \frac{d}{dr}\tilde{n}(r) - \frac{1}{t_c}\tilde{n}(r) = -j(r) - j_r(r), \tag{A.2}$$

where we introduced into the right-hand side an additional contribution into the flux, $j_r(r)$, which is responsible for recapturing of ligands. Our next goal is to determine $j_r(r)$. Let us write $n(r,z)$ in the following form:

$$n(r,z) = n_p(r,z) + n_r(r,z), \tag{A.3}$$

where $n_p(r,z)$ is the solution of (2.1) with boundary conditions (2.2) which we have already found in Sect. 2, $n_r(r,z)$ is the solution of (A.1) with the following boundary conditions

$$n_r(r<a,0) = 0,\ \nabla n_r(r>a,0),\ n_r(\infty,\infty) = 0. \tag{A.4}$$

To find n_r we employ the electrostatic analogy already mentioned in Sect. 2. In the framework of this analogy n_r is found as the potential of the charged circular ring at the edge of a conducting disk of radius a, provided the charge of the ring, Q, is formally indentified with $-(ad/2)[\partial\tilde{n}(r)/\partial r]|_{r=a}$. Making use of the solution for a point charge outside the conducting oblate ellipsoid (see Morse and Feschbach 1953), we find the general form of $n_p(r,z)$. Below we will be interested only to know $n_r(r,z)$ in the vicinity of the disk surface, i.e. in the limit $\xi\to 0$ or $z\to 0$. Keeping in mind (2.8), we find:

$$n_r(r,z) = -d\left.\frac{\partial\tilde{n}(r)}{\partial r}\right|_{r=a} \sum_{l=0}^{\infty}(4l+1)P_{2l}(v)\,\varepsilon(2l) \cdot \{P_{2l}(i\xi)Q_{2l}(0) - P_{2l}(0)Q_{2l}(i\xi)\},\ \text{at } z\to 0 \text{ or } \xi\to 0, \tag{A.5}$$

where $P_{2l}(..)$ and $Q_{2l}(..)$ are Legendre functions, $i\equiv(-1)^{1/2}$, $\varepsilon(0)=1$, $\varepsilon(2l>0)=2$. We calculate j_r as:

$$j_r(r) = -D(\nabla n_r(r,z))_\xi\Big|_{\xi=0} = -D\frac{1}{a\tilde{\nu}}\left.\frac{\partial n_r(r,z)}{\partial\xi}\right|_{\xi=0} = -\tilde{D}\frac{1}{a\tilde{\nu}^2}[1-\tilde{\nu}^2]^{1/2}\left.\frac{\partial\tilde{n}(r)}{\partial\tilde{\nu}}\right|_{r=a} F(\tilde{\nu}). \tag{A.6}$$

where $F(\tilde{\nu}) \equiv \sum_{l=0}^{\infty}(4l+1)\varepsilon(2l)P_{2l}(0)P_{2l}(\tilde{\nu})$. Now to calculate $\tilde{n}(\tilde{\nu})$ we substitute (A.6) into (A.2). We make further simplification by assuming that the second term in the left-hand side of Eq. (A.2) can be neglected, which is true in the limit of high λ. Then Eq. (A.2) is reduced to:

$$\frac{d}{d\nu}\frac{1-\tilde{\nu}^2}{\tilde{\nu}}\frac{d\tilde{n}(\tilde{\nu})}{d\tilde{\nu}} = -\alpha x_1^2 + CF(\tilde{\nu}), \tag{A.7}$$

where $C \equiv \left.\frac{1}{\tilde{\nu}}\frac{d\tilde{n}(\tilde{\nu})}{d\tilde{\nu}}\right|_{\tilde{\nu}=0}$. Equation (A.7) must be integrated by taking into account boundary conditions (2.4) and considering C as some undefined parameter. First integration yields:

$$\frac{1-\tilde{\nu}^2}{\tilde{\nu}}\frac{d\tilde{n}(\tilde{\nu})}{d\tilde{\nu}} = -\alpha x_1^2(\tilde{\nu}-1) + C\int_1^{\tilde{\nu}} F(\tilde{\nu})\,d\tilde{\nu}. \tag{A.8}$$

To find C we put in (A.8) $\tilde{\nu}=0$. Taking into account that $\int_0^1 F(\tilde{\nu})\,d\tilde{\nu}=0$, we find:

$$C = \alpha x_1^2/2. \tag{A.9}$$

Taking into account (A.9) we now solve (A.7) and find:

$$\tilde{n}(\tilde{\nu}) = \alpha x_1^2[\tilde{\nu} - \ln(1+\tilde{\nu})] + \alpha x_1^2\frac{1}{2}\int_0^{\tilde{\nu}} d\tilde{\nu}'\frac{\tilde{\nu}'}{1-\tilde{\nu}'^2}\int_1^{\tilde{\nu}} d\tilde{\nu}''\,F(\tilde{\nu}''). \tag{A.10}$$

The first term on the right-hand side of (1.10) is identical to (2.12), while the second term represents a contribution due to recapturing. Integration (similar to (2.13)) no yields:

$$I = \pi a^2\int_0^1 t_c^{-1}\tilde{n}(\tilde{\nu})\,d\tilde{\nu},\ I_r = \frac{I}{4Dan_0} = \frac{1}{\beta\lambda},$$

$$\beta^{-1} = \frac{1}{12} + \int_0^1 d\tilde{\nu}\,\tilde{\nu}\int_0^{\tilde{\nu}} d\tilde{\nu}'\frac{\tilde{\nu}'}{1-\tilde{\nu}'^2}\int_1^{\tilde{\nu}} d\tilde{\nu}''\,F(\tilde{\nu}'') = \frac{1}{6}. \tag{A.11}$$

Thus, we come to the result discussed in the end of Sect. 2. The physical meaning of this result is as follows. The possibility for ligands to be receptured by the cluster of receptors increases the total number of captured ligands by a factor of two, thus doubling the number of ligands which are bound per unit time.

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