Cell-to-cell communication: Time and length scales of ligand internalization in cultures of suspended cells

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A problem of cell-to-cell communication by diffusible ligands is analyzed for the case when cells are distributed in three dimensions and diffusible ligands are secreted by cells and reversibly bind to cell surface receptors. Following its binding to a receptor, the ligand can either dissociate and be released back in the medium or be absorbed by the cell in a process that is called internalization. Using an effective medium approximation, we derive analytical expressions that characterize the time and length scales associated with the ligand trajectories leading to internalization. We discuss the applicability of our approximation and illustrate the application of our results to a specific cellular system. © *2008 American Institute of Physics*. [DOI: 10.1063/1.2936845]

I. INTRODUCTION

A significant fraction of cell-cell communication in multicellular organisms relies on secreted ligands.[1,2] In a typical cell communication context, a cell secretes a protein that diffuses through the extracellular medium to the neighboring cells before binding to their cell surface receptors. Ligand-receptor binding leads to a wide range of biochemical and biophysical changes inside the cell and in this way controls cellular responses, such as differentiation and division. This type of cell-cell communication is called paracrine.[2] A ligand can also bind to receptors on the same cell that has secreted it. This mode of cell signaling is called autocrine.[2] Following its binding to a surface receptors, the ligand can either dissociate from receptor and be released back in the medium or be absorbed by the cell in a process that is called internalization.

One of the key issues in autocrine and paracrine cell communication is related to the time and length scales of secreted signals. Recently, we have derived results that characterize the time and length scales of autocrine and paracrine ligand trajectories that lead to the first binding event.[3] This problem corresponds to the case when ligand internalization is essentially instantaneous and the probability of ligand release back into the medium is zero. Here, we extend these results to the more general case, where the probability of dissociation of the ligand-receptor complex is finite.

The present note is focused on the time and length scales associated with the ligand life before internalization. Thus we have to deal with a problem of diffusion and trapping in disordered medium. This problem is too complicated to be solved exactly. We apply the effective medium approximation to overcome the difficulties. This allows us to derive an analytical solution for the Laplace transform of the ligand propagator. The propagator is then used to find the distribution of the ligand lifetime before internalization as well as the distribution of its internalization point. Note that the effective medium approximation, which we use, neglects the slowdown of trapping of the diffusing ligands due to the Poisson fluctuations in the distribution of the traps.[4]

The paper is organized as follows. We start by describing the mathematical problem that models the specific cell communication context discussed in this paper: spherical cells randomly distributed in three dimensions and communicating by diffusing ligands. Following that, we introduce the key quantities that characterize the statistical properties of ligand trajectories. Within the framework of the effective medium approximation, we derive an analytical solution for the propagator and use it to find the quantities of interest. Finally, we establish the conditions of applicability of the effective medium approximation and illustrate the application of our results for a specific biological problem.

II. STATEMENT OF THE PROBLEM

Consider a ligand released by a spherical cell of radius R located at the origin at $t=0$. This ligand diffuses in the solution containing other cells which are identical to the parent cell. Each cell contains a fixed number N of the receptors which can bind the ligand. The rate constant of ligand binding to the receptor is denoted by k_{on}. The cells are uniformly distributed in the solvent and their concentration is denoted by c. The ligand diffuses with diffusivity D until it binds to one of the receptors on the surface of either the parent cell or one of the other cells. The ligand-receptor complex may dissociate, and the ligand continues its diffusion in the solution. Alternatively, the complex may be internalized that implies

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ligand annihilation as a diffusing signal. We assume that the dissociation and internalization are monomolecular reactions and denote corresponding rate constants by k_d and k_i. Thus, the list of the problem parameters includes c, R, N, k_{on}, k_d, k_i, and D.

A ligand that survives by time t can be found either diffusing in the solution or bound to a receptor on the surface of the parent cell or one of the other cells. To describe the state of the ligand at time t we introduce a three-component propagator with the components denoted by $g_m(r,t)$, $g_{\mathrm{im}}(r,t)$, and $P_R(t)$. The first two components $g_m(r,t)$ and $g_{\mathrm{im}}(r,t)$ are the probability densities of finding the ligand at point $\mathbf{r}$ of the solution, $r>R$, in mobile (m) and immobile (im) states, respectively, at time t. The third component of the propagator $P_R(t)$ is the probability of finding the ligand bound to the parent cell at time t.

To write the evolution equation for the propagator we use an effective medium approximation to describe diffusion and binding of the ligand in the solution and boundary homogenization to describe ligand binding to the parent cell. In these approximations the set of the evolution equations for the components of the propagator has the following form:

$$\frac{\partial g_m}{\partial t} = D\nabla^2 g_m - k_b g_m + k_d g_{\mathrm{im}} - \delta(r-R) \times \left(\kappa g_m - \frac{k_d}{4\pi R^2} P_R\right), \quad r > R, \tag{2.1}$$

$$\frac{\partial g_{\mathrm{im}}}{\partial t} = k_b g_m - (k_d + k_i) g_{\mathrm{im}}, \tag{2.2}$$

$$\frac{dP_R}{dt} = 4\pi R^2 \kappa g_m|_{r=R} - (k_d + k_i) P_R. \tag{2.3}$$

Here the effective surface trapping rate κ is[2]

$$\kappa = \frac{k_{\mathrm{on}} N}{4\pi R^2}, \tag{2.4}$$

and the binding rate k_b is given by the following Berg–Purcell–Shoup–Szabo formula.[5]

$$k_b = \frac{k_{\mathrm{Sm}} k_{\mathrm{on}} N}{k_{\mathrm{Sm}} + k_{\mathrm{on}} N} c = \frac{4\pi D R^2 \kappa}{D + R\kappa} c, \tag{2.5}$$

where $k_{\mathrm{Sm}} = 4\pi DR$ is the Smoluchowski rate constant.[6] The initial and boundary conditions which complement the set in Eqs. (2.1)–(2.3) are

$$P_R(0) = g_{\mathrm{im}}(r,0) = 0, \quad g_m(r,0) = \frac{\delta(r-R)}{4\pi R^2}, \tag{2.6}$$

and

$$\left.\frac{\partial g_m(r,t)}{\partial r}\right|_{r=R} = 0. \tag{2.7}$$

A. Quantities and functions of interest

All quantities and functions that characterize time and length scales of the ligand internalization can be expressed in terms of the two components of the propagator: $g_{\mathrm{im}}(r,t)$ and $P_R(t)$. We begin with the fractions of ligands internalized by the parent cell, autocrine fraction P_{auto}, and by other cells, paracrine fraction P_{para}. The former is given by

$$P_{\mathrm{auto}} = k_i \int_0^\infty P_R(t)\, dt, \tag{2.8}$$

while the latter is

$$P_{\mathrm{para}} = 1 - P_{\mathrm{auto}} = 4\pi k_i \int_0^\infty dt \int_R^\infty r^2 g_{\mathrm{im}}(r,t)\, dr. \tag{2.9}$$

The spatial distribution of the ligand internalization point is described by the probability density $p(r)$,

$$p(r) = \frac{\delta(r-R)}{4\pi R^2} P_{\mathrm{auto}} + k_i \int_0^\infty g_{\mathrm{im}}(r,t)\, dt H(r-R), \tag{2.10}$$

which is normalized to unity, $H(z)$ is a Heaviside step function,

$$4\pi \int_R^\infty r^2 p(r)\, dr = 1. \tag{2.11}$$

Using the probabilities P_{auto} and P_{para}, we can write $p(r)$ as a weighted sum of the probability densities that characterize distributions of the internalization points for autocrine $p_{\mathrm{auto}}(r)$ and paracrine $p_{\mathrm{para}}(r)$ ligands. The expression is

$$p(r) = p_{\mathrm{auto}}(r) P_{\mathrm{auto}} + p_{\mathrm{para}}(r) P_{\mathrm{para}}, \tag{2.12}$$

where

$$p_{\mathrm{auto}}(r) = \frac{\delta(r-R)}{4\pi R^2}, \tag{2.13}$$

and

$$p_{\mathrm{para}}(r) = \frac{\int_0^\infty g_{\mathrm{im}}(r,t)\, dt}{4\pi \int_0^\infty dt \int_R^\infty r^2 g_{\mathrm{im}}(r,t)\, dr} H(r-R). \tag{2.14}$$

One can use $p_{\mathrm{para}}(r)$ to find the mean distance from the origin where a paracrine ligand is internalized $\langle r_{\mathrm{para}} \rangle$,

$$\langle r_{para} \rangle = 4\pi \int_R^\infty r^3 p_{\mathrm{para}}(r)\, dr, \tag{2.15}$$

which is one of the important characteristics of the process.

Probability densities for the lifetimes of autocrine $\varphi_{\mathrm{auto}}(t)$ and paracrine $\varphi_{\mathrm{para}}(t)$ ligands are given by

$$\varphi_{\mathrm{auto}}(t) = \frac{k_i P_R(t)}{P_{\mathrm{auto}}} = \frac{P_R(t)}{\int_0^\infty P_R(t)\, dt}, \tag{2.16}$$

and

$$\varphi_{\mathrm{para}}(t) = \frac{4\pi k_i \int_R^\infty r^2 g_{\mathrm{im}}(r,t)\, dr}{P_{\mathrm{para}}} = \frac{\int_R^\infty r^2 g_{\mathrm{im}}(r,t)\, dr}{\int_0^\infty dt \int_R^\infty r^2 g_{\mathrm{im}}(r,t)\, dr}. \tag{2.17}$$

The total probability density for the ligand lifetime, which does not discriminate between autocrine and paracrine ligands, $\varphi(t)$, is

$$\varphi(t) = \varphi_{\text{auto}}(t)P_{\text{auto}} + \varphi_{\text{para}}(t)P_{\text{para}}. \tag{2.18}$$

Using the probability densities $\varphi_{\text{auto}}(t)$ and $\varphi_{\text{para}}(t)$, one can find the mean lifetimes of autocrine and paracrine ligands, $\langle t_{\text{auto}}\rangle$ and $\langle t_{\text{para}}\rangle$,

$$\langle t_{\text{auto}}\rangle = \int_0^\infty t\varphi_{\text{auto}}(t)dt, \tag{2.19}$$

and

$$\langle t_{\text{para}}\rangle = \int_0^\infty t\varphi_{\text{para}}(t)dt. \tag{2.20}$$

The total mean lifetime of the ligand $\langle t\rangle$ is

$$\langle t\rangle = \int_0^\infty t\varphi(t)dt = \langle t_{\text{auto}}\rangle P_{\text{auto}} + \langle t_{\text{para}}\rangle P_{\text{para}}. \tag{2.21}$$

The mean lifetimes in Eqs. (2.19)–(2.21) are also important characteristics of the process.

III. GENERAL SOLUTION

In this section we find an exact solution for the Laplace transform of the propagator and use it in our further analysis of the quantities and functions of interest. The Laplace transform of function $f(t)$, denoted by $\hat{f}(s)$, is defined by

$$\hat{f}(s) = \int_0^\infty e^{-st}f(t)dt. \tag{3.1}$$

After the Laplace transform the set of Eqs. (2.1)–(2.3) takes the following form:

$$s\hat{g}_m - \frac{\delta(r-R)}{4\pi R^2} = D\nabla^2\hat{g}_m - k_b\hat{g}_m + k_d\hat{g}_{\text{im}} - \delta(r-R) \times\left[\kappa\hat{g}_m - \frac{k_d}{4\pi R^2}\hat{P}_R\right], \quad r > R, \tag{3.2}$$

$$s\hat{g}_{\text{im}} = k_b\hat{g}_m - (k_d + k_i)\hat{g}_{\text{im}}, \tag{3.3}$$

$$s\hat{P}_R = 4\pi R^2\kappa\hat{g}_m|_{r=R} - (k_d + k_i)\hat{P}_R. \tag{3.4}$$

Solving the last two equations we obtain

$$\hat{g}_{\text{im}} = \frac{k_b}{s + k_d + k_i}\hat{g}_m, \tag{3.5}$$

and

$$\hat{P}_R = \left.\frac{4\pi R^2\kappa}{s + k_d + k_i}\hat{g}_m\right|_{r=R}. \tag{3.6}$$

Using Eqs. (3.5) and (3.6) we can write Eq. (3.2) as

$$\frac{D}{r^2}\frac{d}{dr}\left(r^2\frac{d\hat{g}_m}{dr}\right) - K(s)\hat{g}_m = -\frac{\delta(r-R)}{4\pi R^2}\left[1 - \frac{4\pi R^2\kappa(s+k_i)}{s + k_d + k_i}\hat{g}_m\right], \quad r > R, \tag{3.7}$$

where function $K(s)$ is defined by

$$K(s) = \frac{s^2 + s(k_b + k_d + k_i) + k_bk_i}{s + k_d + k_i}. \tag{3.8}$$

Equation (3.7) should be solved with the following boundary condition:

$$\left.\frac{d\hat{g}_m}{dr}\right|_{r=R} = 0, \tag{3.9}$$

which follows from Eq. (2.7). The solution is

$$\hat{g}_m(r,s) = \frac{\exp[-\sqrt{K(s)/D}(r-R)]H(r-R)}{4\pi Dr\left[1 + R\sqrt{K(s)/D} + \frac{R\kappa}{D}\frac{s+k_i}{s+k_d+k_i}\right]}. \tag{3.10}$$

Substituting this into Eqs. (3.5) and (3.6) we obtain

$$\hat{g}_{\text{im}}(r,s) = \frac{k_b\exp[-\sqrt{K(s)/D}(r-R)]H(r-R)}{4\pi Dr(s+k_d+k_i)\left[1 + R\sqrt{K(s)/D} + \frac{R\kappa}{D}\frac{s+k_i}{s+k_d+k_i}\right]}, \tag{3.11}$$

and

$$\hat{P}_R(s) = \frac{R\kappa/D}{(s+k_d+k_i)\left[1 + R\sqrt{K(s)/D} + \frac{R\kappa}{D}\frac{s+k_i}{s+k_d+k_i}\right]}. \tag{3.12}$$

Equations (3.10)–(3.12) provide an exact solution for the Laplace transform of the propagator, which we use in our further analysis.

A. Quantities and functions of interest

Using the definitions in Eqs. (2.8) and (2.9) we can find the autocrine and paracrine fractions which are given by

$$P_{\text{auto}} = k_i\hat{P}_R(0) = \frac{P_iR/\ell_\kappa}{1 + R/\ell_{\text{para}} + P_iR/\ell_\kappa}, \tag{3.13}$$

$$P_{\text{para}} = 4\pi k_i\int_R^\infty r^2\hat{g}_{\text{im}}(r,0)dr = \frac{1 + R/\ell_{\text{para}}}{1 + R/\ell_{\text{para}} + P_iR/\ell_\kappa}. \tag{3.14}$$

Here we have introduced the internalization probability P_i for a ligand bound to a receptor,

$$P_i = \frac{k_i}{k_i + k_d}, \tag{3.15}$$

and the two lengths, ℓ_{para} and ℓ_κ, defined as

$$\ell_{\text{para}} = \sqrt{D/(P_i k_b)}, \quad \ell_\kappa = D/\kappa. \tag{3.16}$$

The former provides a length scale for the distance traveled by a paracrine ligand before internalization [see Eq. (3.19)].

Based on the definitions of $p_{\text{para}}(r)$ and $\langle r_{\text{para}}\rangle$ in Eqs. (2.14) and (2.15) we obtain

$$p_{\text{para}}(r) = \frac{\hat{g}_{\text{im}}(r,0)}{4\pi\int_R^\infty r^2 \hat{g}_{\text{im}}(r,0)dr} = \frac{\exp[(r-R)/\ell_{\text{para}}]H(r-R)}{4\pi r \ell_{\text{para}}^2(1+R/\ell_{\text{para}})}, \tag{3.17}$$

and

$$\langle r_{\text{para}}\rangle = R + \ell_{\text{para}}\left(1 + \frac{1}{1+R/\ell_{\text{para}}}\right). \tag{3.18}$$

This can be used to estimate the mean distance traveled by a paracrine ligand before internalization,

$$\langle r_{\text{para}}\rangle - R = \ell_{\text{para}}\left(1 + \frac{1}{1+R/\ell_{\text{para}}}\right). \tag{3.19}$$

One can see that this distance is between ℓ_{para} and $2\ell_{\text{para}}$. Thus, ℓ_{para} provides a scale for the distance traveled by a paracrine ligand. Finally, the probability density of the internalization points in Eq. (2.12) is given by

$$p(r) = \frac{\frac{P_i\delta(r-R)}{4\pi R\ell_\kappa} + \left(1+\frac{R}{\ell_{\text{para}}}\right)\exp\left(-\frac{r-R}{\ell_{\text{para}}}\right)H(r-R)}{1+\frac{R}{\ell_{\text{para}}}+\frac{P_iR}{\ell_\kappa}}. \tag{3.20}$$

The Laplace transform of the probability density for the lifetime of an autocrine ligand, according to the definition in Eq. (2.16), is given by

$$\varphi_{\text{auto}}(s) = \frac{\hat{P}_R(s)}{\hat{P}_R(0)} = \frac{(k_d+k_i)\left(1+\frac{R}{\ell_{\text{para}}}+\frac{P_iR}{\ell_\kappa}\right)}{(s+k_d+k_i)\left[1+R\sqrt{\frac{K(s)}{D}}+\frac{R(s+k_i)}{\ell_\kappa(s+k_d+k_i)}\right]}. \tag{3.21}$$

Here we have used $\hat{P}_R(s)$ in Eq. (3.12). We use this Laplace transform of the probability density to find the mean lifetime of an autocrine ligand defined in Eq. (2.19),

$$\langle t_{\text{auto}}\rangle = -\left.\frac{d\hat{\varphi}_{\text{auto}}(s)}{ds}\right|_{s=0} = \frac{1+\frac{R[k_b(k_d+2k_i)+(k_d+k_i)^2]}{2\ell_{\text{para}}k_bk_i}+\frac{R}{\ell_\kappa}}{(k_d+k_i)\left(1+\frac{R}{\ell_{\text{para}}}+\frac{P_iR}{\ell_\kappa}\right)}. \tag{3.22}$$

The Laplace transform of the probability density for the lifetime of a paracrine ligand can be found from the definition in Eq. (2.17),

$$\varphi_{\text{para}}(s) = \frac{\int_R^\infty r^2\hat{g}_{\text{im}}(r,s)dr}{\int_R^\infty r^2\hat{g}_{\text{im}}(r,0)dr}. \tag{3.23}$$

Substituting $\hat{g}_{\text{im}}(r,s)$ given in Eq. (3.11) and carrying out the integrations we obtain

$$\varphi_{\text{para}}(s) = \frac{k_bk_i\left(1+\frac{R}{\ell_{\text{para}}}+\frac{P_iR}{\ell_\kappa}\right)\left(1+R\sqrt{\frac{K(s)}{D}}\right)}{\left(1+\frac{R}{\ell_{\text{para}}}\right)[s^2+s(k_b+k_d+k_i)+k_bk_i]\left[1+R\sqrt{\frac{K(s)}{D}}+\frac{R(s+k_i)}{\ell_\kappa(s+k_d+k_i)}\right]}. \tag{3.24}$$

The mean lifetime of a paracrine ligand, defined in Eq. (2.20), can be found using the Laplace transform in Eq. (3.24). The result is

$$\langle t_{\text{para}}\rangle = -\left.\frac{d\hat{\varphi}_{\text{para}}(s)}{ds}\right|_{s=0} = \frac{(k_b+k_d+k_i)\left[\left(1+\frac{R}{\ell_{\text{para}}}\right)^2+\frac{P_iR}{\ell_\kappa}F\right]}{k_bk_i\left(1+\frac{R}{\ell_{\text{para}}}\right)\left(1+\frac{R}{\ell_{\text{para}}}+\frac{P_iR}{\ell_\kappa}\right)}, \tag{3.25}$$

where factor F is given by

$$F = \frac{(k_d + k_i)^2\left(1 + \frac{R}{2\ell_{\text{para}}}\right) + k_b k_d\left(2 + \frac{3R}{2\ell_{\text{para}}}\right) + k_b k_i\left(1 + \frac{R}{\ell_{\text{para}}}\right)}{(k_d + k_i)(k_b + k_d + k_i)}. \tag{3.26}$$

Finally, one can use the results in Eqs. (3.13), (3.14), (3.21), (3.22), (3.24), and (3.25) to find the Laplace transform of the total probability density of the ligand lifetime [Eq. (2.18)] as well as the mean ligand lifetime [Eq. (2.21)].

Thus, we have derived general solutions for the functions and quantities that characterize spatial and temporal ranges of ligand internalization in cultures of suspended cells. Our derivation is based on the exact solution for the propagator given in Eqs. (3.10)–(3.12) obtained in the framework of the effective medium approximation. We discuss the conditions of applicability of this approximation in the next section. Finally, we indicate that in the limiting case of instantaneous internalization, $k_i \to \infty$, $P_i = 1$, and the results derived above reduce to the corresponding results derived in our recent work.[3]

IV. DISCUSSION

The theory developed in the last two sections is based on the effective medium approximation, which replaces the solution with randomly located cells by an effective, uniformly absorbing homogeneous medium. This approximation is applicable when the characteristic length ℓ_{para} [Eq. (3.16)] is much greater than the cell radius and the intercell distance, $\ell_{\text{para}} \gg R$, $c^{-1/3}$. Below we show that the condition of applicability of this approximation is given by

$$P_i \frac{R}{\ell_\kappa} \ll 1. \tag{4.1}$$

Being written in terms of the initial parameters of the system, this inequality takes the following form:

$$\frac{k_{\text{on}} N}{4\pi D R} \ll 1 + \frac{k_d}{k_i}. \tag{4.2}$$

This reduces to the requirement of the weak ligand capture by an individual cell,

$$\frac{k_{\text{on}} N}{4\pi D R} \ll 1, \tag{4.3}$$

when $k_i \gg k_d$. However, if $k_i \ll k_d$, the inequality in Eq. (4.2) can be fulfilled even when the individual cells operate in the strong capture regime so that

$$\frac{k_{\text{on}} N}{4\pi D R} \gg 1. \tag{4.4}$$

To prove the inequality in Eq. (4.1) consider the radio $(R/\ell_{\text{para}})^2$, in which we use the definition of ℓ_{para} in Eq. (3.16) to obtain

$$\left(\frac{R}{\ell_{\text{para}}}\right)^2 = P_i \frac{R^2 k_b}{D}. \tag{4.5}$$

Substituting here the definition of k_b in Eq. (2.5) we arrive at

$$\left(\frac{R}{\ell_{\text{para}}}\right)^2 = P_i \frac{3\omega R/\ell_\kappa}{1 + R/\ell_\kappa} \ll 1, \tag{4.6}$$

where we have introduced the cell volume fraction ω,

$$\omega = \tfrac{4}{3}\pi R^3 c. \tag{4.7}$$

The inequality in Eq. (4.6) is a consequence of the inequality in Eq. (4.1) and the fact that the cell volume fraction is smaller than unity, $\omega < 1$, since the cells do not overlap.

Next, to prove the inequality

$$\ell_{\text{para}} \gg c^{-1/3}, \tag{4.8}$$

we consider the quantity $(c^{1/3}\ell_{\text{para}})^{-2}$. Using the definition of ℓ_{para} in Eq. (3.16) we can write

$$\frac{1}{c^{2/3}\ell_{\text{para}}^2} = \frac{P_i k_b}{c^{2/3} D} = \left(\frac{R}{\ell_{\text{para}}}\right)^2 \left(\frac{4\pi}{3\omega}\right)^{2/3}, \tag{4.9}$$

where we have used the relation in Eq. (4.5). Substituting here the expression for $(R/\ell_{\text{para}})^2$ in Eq. (4.6) we obtain

$$\frac{1}{c^{2/3}\ell_{\text{para}}^2} = P_i \frac{(4\pi\sqrt{3})^{2/3}\omega^{1/3} R/\ell_\kappa}{1 + R/\ell_\kappa}, \tag{4.10}$$

where the factor $(4\pi\sqrt{3})^{2/3}$ is approximately equal to 7.7. Thus, one can see that the inequality in Eq. (4.8) is fulfilled when the quantity $P_i R/\ell_\kappa$ satisfies the inequality in Eq. (4.1).

Using the latter inequality [Eq. (4.1)], we can simplify the expressions for the quantities and functions of interest derived in the last section. From this inequality together with Eqs. (3.13) and (3.14) it follows that $P_{\text{auto}} \simeq 0$ while $P_{\text{para}} \simeq 1$. According to Eq. (3.18) we have $\langle r_{\text{para}} \rangle = \ell_{\text{para}}$ and according to Eqs. (2.12) and (3.17) we can write

$$p(r) \simeq p_{\text{para}}(r) \simeq \frac{\exp(-r/\ell_{\text{para}})}{4\pi\ell_{\text{para}}^2 r}. \tag{4.11}$$

The mean lifetime $\langle t_{\text{para}} \rangle$ [Eq. (2.23)] also simplifies and takes the following form:

$$\langle t_{\text{para}} \rangle = \frac{k_b + k_d + k_i}{k_b k_i}. \tag{4.12}$$

The simplified versions of the Laplace transforms $\hat{\varphi}(s)$ and $\hat{\varphi}_{\text{para}}(s)$ are given by

$$\hat{\varphi}(s) \simeq \hat{\varphi}_{\text{para}}(s) \simeq \frac{k_b k_i}{s^2 + s(k_b + k_d + k_i) + k_b k_i}, \tag{4.13}$$

where we have used the relations in Eqs. (2.18) and (3.24). Inverting this transform we obtain

$$\varphi(t) \simeq \varphi_{\text{para}}(t) \simeq \frac{k_b k_i}{s_2 - s_1}(e^{s_1 t} - e^{-s_2 t}), \tag{4.14}$$

where s_1 and s_2 are negative roots of the denominator in Eq. (4.13),

$$s_{1,2} = \tfrac{1}{2}[k_b + k_d + k_i \mp \sqrt{(k_b + k_d + k_i)^2 - 4k_b k_i}]. \tag{4.15}$$

Note that the results in Eqs. (4.12)–(4.15) can be obtained from consideration of the two-state model,

$$m \underset{k_d}{\overset{k_b}{\rightleftarrows}} \text{im} \overset{k_i}{\rightarrow}, \tag{4.16}$$

which describes transitions of the ligand between the mobile (m) and immobile (im) states and its annihilation in the immobile state.

To illustrate the results derived above we discuss the spatial and temporal ranges of Interferon (IFN) β signaling in experiments on early response of cultured human dendritic cells to viral infection.[3,7] In response to viral infection dendritic cells secrete IFN β molecules. When bound to cell surface receptor, IFN β induces a variety of biochemical and transcriptional effects. To determine the special and temporal ranges of IFN β signaling we use the following values of the problem parameters: $c=10^6$ cm^{-3}, $R=2.5\times10^{-3}$ cm, $N=5\times10^4$, $k_{\text{on}}=10^7$ mol^{-1} s^{-1}, $k_d=10^{-3}$ s^{-1}, $k_i=5\times10^{-5}$ s^{-1}, and $D=10^{-6}$ cm^2 s^{-1}.[3] One can check that for this set of the parameter values the inequality in Eq. (4.2) is fulfilled and, hence, the applicability of the effective medium approximation is justified. As a consequence, we have $P_{\text{auto}}=0$ and $P_{\text{para}}=1$. Using Eq. (3.16) we find that $\langle l_{\text{para}}\rangle \approx 0.16$ cm and, hence, $\langle l_{\text{para}}\rangle/R$ and $\langle l_{\text{para}}\rangle c^{1/3} \gg 1$. Using the definition in Eq. (2.5) we obtain $k_b=0.8\times10^{-3}$ s^{-1}. Then the mean ligand lifetime [Eq. (4.2)] is $\langle t_{\text{para}}\rangle \approx 12.5$ h. Because the values of k_b and k_d are close, the ligand spends in the mobile state about half of its lifetime.

Finally, we note that here we have considered the case when cells are distributed in three-dimensions. The problem can also be posed for the case when the cells are distributed in two-dimensions (a tissue-culture dish covered by adherent cells under a layer of a liquid medium).[8] This case can be analyzed using a combination of Brownian dynamics simulations and a homogenization approach for problems with patchy boundary conditions.[9]

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