Dynamics of gradient formation by intracellular shuttling

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A number of important cellular functions rely on the formation of intracellular protein concentration gradients. Experimental studies discovered a number of mechanisms for the formation of such gradients. One of the mechanisms relies on the intracellular shuttling of a protein that interconverts between the two states with different diffusivities, under the action of two enzymes, one of which is localized to the plasma membrane, whereas the second is uniformly distributed in the cytoplasm. Recent work reported an analytical solution for the steady state gradient in this mechanism, obtained in the framework of a one-dimensional reaction-diffusion model. Here, we study the dynamics in this model and derive analytical expressions for the Laplace transforms of the time-dependent concentration profiles in terms of elementary transcendental functions. Inverting these transforms numerically, one can obtain time-dependent concentration profiles of the two forms of the protein. © 2015 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4928858>]

I. INTRODUCTION

One of the most common strategies for the regulation of protein function relies on phosphorylation, a posttranslational modification that adds bulky and negatively charged phosphate group to one or multiple aminoacids within the protein sequence.¹ Acting through changes in protein structure, phosphorylation of a protein can cause profound alterations in the spectrum of protein interactions, its stability, activity, and subcellular localization. As a rule, phosphorylation and dephosphorylation of the same residue are controlled by different enzymes, kinase and phosphatase, respectively. These enzymes may themselves be regulated at multiple levels, including tight control of their subcellular localization.

Many important cellular processes use control strategies with spatially separated kinases and phosphatases. For instance, a kinase localized to the plasma membrane may phosphorylate a protein that is later dephosphorylated by a cytoplasmic phosphatase (Fig. 1). In 1999, Brown and Kholodenko² pointed out that for a realistic set of intracellular diffusivities and reaction rates, this scenario may result in appreciable gradients in the spatial distribution of phosphorylated protein. Subsequently, such gradients, with characteristic length scales from one to multiple micrometers, have been discovered in a large number of systems and shown to play important roles in cell regulation.^{3–5}

In 2008, Lipkow and Odde⁶ considered an extended version of the model with spatially separated protein phosphorylation and dephosphorylation, in which phosphorylated and unphosphorylated forms of a protein have different diffusivities. They showed that the combination of the spatially separated kinase and phosphatase activities and strong difference in the diffusivities of the two forms of a protein substrate may establish nonuniform profiles of the total protein concentra-

tion, an effect that does not exist in the original model of Brown and Kholodenko.

Three years later, their theory was put to use in a specific experimental system, explaining the formation of a spatial gradient of the MEX-5 protein in the early *C. elegans* embryo.⁷ MEX-5 is phosphorylated by a localized kinase and dephosphorylated by a spatially uniform cytoplasmic phosphatase. Direct measurements of the diffusivities of the phosphorylated and unphosphorylated MEX-5 showed that they differ by more than an order of magnitude. This difference results in the rapid formation of pronounced gradient of total MEX-5 concentration in the single cell zygote, which is essential for guiding a number of later steps in embryogenesis.

Lipkow and Odde⁶ derived an analytical solution for the steady-state concentration profiles of the phosphorylated and unphosphorylated forms of a protein in the framework of a one-dimensional model that describes localized phosphorylation of the protein and its delocalized uniform dephosphorylation. In this paper, we study the formation dynamics of these profiles in the framework of the Lipkow-Odde model. Specifically, we derive an exact analytical solution for the Laplace transforms of the time-dependent concentration profiles of the two forms of the protein. Inverting these Laplace transforms numerically, one can find the concentration profiles as functions of time. Our results can be used as a starting point for the analysis of experimental data, as well as for the development of more complex models of intracellular gradients.

The paper is organized as follows: The formulation of the Lipkow-Odde model is presented in Section II. We derive an exact analytical solution that gives the Laplace transforms of the time-dependent concentrations of the two forms of the protein in terms of elementary transcendental functions in Section III. The solution is discussed and illustrated in Section IV.

FIG. 1. Schematic representation of spatially separated phosphorylation and dephosphorylation of a protein. A and B denote the phosphorylated and dephosphorylated forms of the protein, respectively. Phosphorylation occurs at the plasma membrane, by the localized kinases. Dephosphorylation occurs in the cytoplasm, catalyzed by uniformly distributed phosphatase. Both forms of the protein diffuse in the cytoplasm, but with different diffusivities (see text for more details).

Section V contains a summary of the obtained results and some concluding remarks.

II. MODEL

The one-dimensional model proposed by Lipkow and Odde⁶ considers phosphorylation-dephosphorylation kinetics on the interval of length L , $0 < x < L$. The model assumes that the kinase is localized at the origin, whereas the phosphatase is uniformly distributed over the interval. Following Lipkow and Odde, the phosphorylated and unphosphorylated forms of the protein are referred to as A and B particles. Their concentrations at point x at time t are denoted by $c_A(x, t)$ and $c_B(x, t)$.

These concentrations satisfy reaction-diffusion equations

$$\frac{\partial c_A}{\partial t} = D_A \frac{\partial^2 c_A}{\partial x^2} - k_p c_A, \quad (2.1)$$

$$\frac{\partial c_B}{\partial t} = D_B \frac{\partial^2 c_B}{\partial x^2} + k_p c_A, \quad (2.2)$$

subject to boundary conditions

$$-D_A \frac{\partial c_A}{\partial x} \Big|_{x=0} = D_B \frac{\partial c_B}{\partial x} \Big|_{x=0} = \kappa_k c_B(0, t), \quad (2.3)$$

$$\frac{\partial c_A}{\partial x} \Big|_{x=L} = \frac{\partial c_B}{\partial x} \Big|_{x=L} = 0 \quad (2.4)$$

and initial conditions

$$c_A(x, 0) = 0, \quad c_B(x, 0) = c_0. \quad (2.5)$$

Here, D_A and D_B are the diffusivities of the A and B particles, respectively, k_p is the rate constant for the dephosphorylation reaction catalyzed by a uniformly distributed phosphatase (p), and κ_k is the rate of phosphorylation by a kinase (k) localized at the origin. It is assumed that initially ($t = 0$) all particles are in state B and that they are uniformly distributed over the interval with the concentration c_0 .

The reaction-diffusion equations in Eqs. (2.1) and (2.2) describe diffusion of A and B particles and conversion of A particles into B particles (sink and source terms in Eqs. (2.1) and (2.2), respectively). Conversion of B particles into A particles, localized at the origin, is described by the boundary conditions in Eq. (2.3), whereas Eq. (2.4) provides the reflecting boundary conditions at the end of the interval at $x = L$.

These equations model the relaxation of the uniform initial distribution of the B particles to steady-state distributions of both the A and B particles at long times ($t \rightarrow \infty$). The exact

solution for the steady-state distributions was obtained by Lipkow and Odde.⁶ In the rest of this section, we derive an exact solution for the Laplace transforms of the time-dependent concentration profiles of the A and B particles. Inverting these Laplace transforms numerically one can find the particle concentrations as functions of time, $c_A(x, t)$ and $c_B(x, t)$.

It is convenient to introduce length and time scales, $\lambda = \sqrt{D_A/k_p}$ and $1/k_p$, respectively, and to define dimensionless length and time, $x/\lambda = x$ and $k_p t = t$, denoted by the same letters as their dimensional counterparts. In addition, we use c_0 to introduce dimensionless concentrations of the A and B particles denoted by $u(x, t)$ and $v(x, t)$,

$$u(x, t) = \frac{c_A(x, t)}{c_0}, \quad v(x, t) = \frac{c_B(x, t)}{c_0}. \quad (2.6)$$

This allows us to write the initial equations, Eqs. (2.1)–(2.5), in dimensionless form,

$$\frac{\partial u}{\partial t} = \frac{\partial^2 u}{\partial x^2} - u, \quad (2.7)$$

$$\frac{\partial v}{\partial t} = \beta \frac{\partial^2 v}{\partial x^2} + u, \quad (2.8)$$

$$-\frac{\partial u}{\partial x} \Big|_{x=0} = \beta \frac{\partial v}{\partial x} \Big|_{x=0} = \alpha v(0, t), \quad (2.9)$$

$$\frac{\partial u}{\partial x} \Big|_{x=\gamma} = \frac{\partial v}{\partial x} \Big|_{x=\gamma} = 0, \quad (2.10)$$

$$u(x, 0) = 0, \quad v(x, 0) = 1. \quad (2.11)$$

Here,

$$\alpha = \kappa_k / (\lambda k_p) = \kappa_k / \sqrt{D_A k_p}, \quad (2.12)$$

$$\beta = D_B / D_A, \quad (2.13)$$

and

$$\gamma = L/\lambda = L\sqrt{k_p/D_A} \quad (2.14)$$

are dimensionless parameters of the problem, which characterize the phosphorylation rate of the B particles at the origin, the diffusivity ratio of the B and A particles, and the length of the interval, respectively.

III. SOLUTION

It is convenient to write the solution for the concentrations $u(x, t)$ and $v(x, t)$ in terms of the A and B particle propagators (Green's functions, transition probabilities), denoted by $G_A(x, t)$ and $G_B(x, t|x')$. $G_A(x, t)$ is the probability density of finding an A particle at point x at time t , on condition that it was at the origin ($x = 0$) at $t = 0$ and has not been dephosphorylated during time t . $G_B(x, t|x')$ is the probability density of finding a B particle at point x at time t , on condition that it was at point x' at $t = 0$ and has not been phosphorylated during time t . These propagators satisfy

$$\frac{\partial G_A}{\partial t} = \frac{\partial^2 G_A}{\partial x^2} - G_A, \quad (3.1)$$

$$\frac{\partial G_A}{\partial x} \Big|_{x=0} = \frac{\partial G_A}{\partial x} \Big|_{x=\gamma} = 0, \quad G_A(x, 0) = \delta(x), \quad (3.2)$$

and

$$\frac{\partial G_B}{\partial t} = \beta \frac{\partial^2 G_B}{\partial x^2}, \quad (3.3)$$

$$\beta \frac{\partial G_B}{\partial x} \Big|_{x=0} = \alpha G_B(0, t|x'), \quad \frac{\partial G_B}{\partial x} \Big|_{x=\gamma} = 0, \quad (3.4)$$

$$G_B(x, 0|x') = \delta(x - x').$$

Let $j(t) = \alpha v(0, t)$ be the flux of A particles injected at the origin due to phosphorylation of the B particles at time t . Using this flux and the propagator $G_A(x, t)$, concentration $u(x, t)$ can be written as

$$\begin{aligned} u(x, t) &= \int_0^t G_A(x, t - t') j(t') dt' \\ &= \alpha \int_0^t G_A(x, t - t') v(0, t') dt'. \end{aligned} \quad (3.5)$$

Concentration $v(x, t)$ can be written in terms of the concentration $u(x, t)$ and the propagator $G_B(x, t|x')$,

$$\begin{aligned} v(x, t) &= \int_0^\gamma \left[G_B(x, t|x') \right. \\ &\quad \left. + \int_0^t G_B(x, t - t'|x') u(x', t') dt' \right] dx'. \end{aligned} \quad (3.6)$$

This equation gives $v(x, t)$ as the sum of two terms. The first term is the contribution due to the initial uniform distribution of the B particles, whereas the second term is due to new B particles injected at points x' at time t' .

After the Laplace transformation, defined in the conventional way (the Laplace transform of function $f(t)$, denoted

by $\hat{f}(s)$, is defined as $\hat{f}(s) = \int_0^\infty e^{-st} f(t) dt$, where s is the Laplace parameter), Eqs. (3.5) and (3.6) take the form

$$\hat{u}(x, s) = \alpha \hat{G}_A(x, s) \hat{v}(0, s) \quad (3.7)$$

and

$$\hat{v}(x, s) = \int_0^\gamma \hat{G}_B(x, s|x') (1 + \hat{u}(x', s)) dx', \quad (3.8)$$

where $\hat{u}(x, s)$, $\hat{v}(x, s)$, $\hat{G}_A(x, s)$, and $\hat{G}_B(x, s|x')$ are the Laplace transforms of the concentrations, $u(x, t)$ and $v(x, t)$, and the propagators, $G_A(x, t)$ and $G_B(x, t|x')$, respectively.

Using Eq. (3.7), we can write Eq. (3.8) as

$$\begin{aligned} \hat{v}(x, s) &= \alpha \left(\int_0^\gamma \hat{G}_B(x, s|x') \hat{G}_A(x', s) dx' \right) \hat{v}(0, s) \\ &= \int_0^\gamma \hat{G}_B(x, s|x') dx'. \end{aligned} \quad (3.9)$$

This equation can be easily solved at $x = 0$. The solution for $\hat{v}(0, s)$ is given by

$$\hat{v}(0, s) = \frac{\int_0^\gamma \hat{G}_B(0, s|x') dx'}{1 - \alpha \int_0^\gamma \hat{G}_B(0, s|x') \hat{G}_A(x', s) dx'}. \quad (3.10)$$

Having in hand $\hat{v}(0, s)$, we can find $\hat{u}(x, s)$ by Eq. (3.7),

$$\begin{aligned} \hat{u}(x, s) &= \alpha \hat{G}_A(x, s) \hat{v}(0, s) \\ &= \frac{\alpha \hat{G}_A(x, s) \int_0^\gamma \hat{G}_B(0, s|x') dx'}{1 - \alpha \int_0^\gamma \hat{G}_B(0, s|x') \hat{G}_A(x', s) dx'}. \end{aligned} \quad (3.11)$$

Finally, we find $\hat{v}(x, s)$ by substituting $\hat{u}(x, s)$ above into Eq. (3.8). The result is

$$\hat{v}(x, s) = \frac{\int_0^\gamma \hat{G}_B(x, s|x') \{1 + \alpha [\hat{G}_A(x', s) \int_0^\gamma \hat{G}_B(0, s|x'') dx'' - \int_0^\gamma \hat{G}_B(0, s|x'') \hat{G}_A(x'', s) dx'']\} dx'}{1 - \alpha \int_0^\gamma \hat{G}_B(0, s|x') \hat{G}_A(x', s) dx'}. \quad (3.12)$$

The last two equations provide the solutions for the Laplace transforms of the concentrations $u(x, t)$ and $v(x, t)$ in terms of the Laplace transforms of the propagators $G_A(x, t)$ and $G_B(x, t|x')$.

As shown in Appendices A and B, the Laplace transforms of the propagators are given by

$$\hat{G}_A(x, s) = \frac{1}{M_A(s)} \cosh((\gamma - x) \sqrt{s + 1}) \quad (3.13)$$

and

$$\hat{G}_B(x, s|x') = \frac{1}{M_B(s)} \begin{cases} a(x', s) b(x, s), & 0 < x < x' \\ a(x, s) b(x', s), & x' < x < \gamma \end{cases}, \quad (3.14)$$

where functions $a(x, s)$, $b(x, s)$, $M_A(s)$, and $M_B(s)$ are

$$a(x, s) = \cosh((\gamma - x) \sqrt{s/\beta}), \quad (3.15)$$

$$b(x, s) = \sqrt{s\beta} \cosh(x \sqrt{s/\beta}) + \alpha \sinh(x \sqrt{s/\beta}), \quad (3.16)$$

$$M_A(s) = \sqrt{s + 1} \sinh(\gamma \sqrt{s + 1}), \quad (3.17)$$

$$M_B(s) = \sqrt{s\beta} [\alpha \cosh(\gamma \sqrt{s/\beta}) + \sqrt{s\beta} \sinh(\gamma \sqrt{s/\beta})]. \quad (3.18)$$

The expressions for the Laplace transforms of the concentrations $u(x, t)$ and $v(x, t)$, Eqs. (3.11) and (3.12), contain the integrals of the Laplace transforms of the propagators. Introducing for these integrals, the notations

$$I_1(x, s) = \int_0^\gamma \hat{G}_B(x, s|x') dx' \quad (3.19)$$

and

$$I_2(x, s) = \int_0^\gamma \hat{G}_B(x, s|x') \hat{G}_A(x', s) dx', \quad (3.20)$$

we can write $\hat{u}(x, s)$ and $\hat{v}(x, s)$ as

$$\hat{u}(x, s) = \frac{\alpha \hat{G}_A(x, s) I_1(0, s)}{1 - \alpha I_2(0, s)} \quad (3.21)$$

and

$$\hat{v}(x, s) = \frac{I_1(x, s) + \alpha [I_2(x, s) I_1(0, s) - I_1(x, s) I_2(0, s)]}{1 - \alpha I_2(0, s)}. \quad (3.22)$$

Performing the integrations in Eqs. (3.19) and (3.20), we obtain

$$I_1(x, s) = \frac{K_1(x, s)}{\sqrt{s/\beta} M_B(s)} \quad (3.23)$$

and

$$I_2(x, s) = \frac{\sqrt{s/\beta} K_2(x, s)}{(s + 1 - s/\beta) M_A(s) M_B(s)}, \quad (3.24)$$

where

$$K_1(x, s) = \sqrt{s\beta} \sinh(\gamma\sqrt{s/\beta}) + \alpha [\cosh(\gamma\sqrt{s/\beta}) - \cosh((\gamma - x)\sqrt{s/\beta})], \quad (3.25)$$

$$K_2(x, s) = \beta\sqrt{s+1} N_1(x, s) - \sqrt{s\beta} N_2(x, s) - \alpha N_3(x, s), \quad (3.26)$$

$$N_1(x, s) = \sinh(\gamma\sqrt{s+1}) \cosh((\gamma - x)\sqrt{s/\beta}), \quad (3.27)$$

$$N_2(x, s) = \sinh(\gamma\sqrt{s/\beta}) \cosh((\gamma - x)\sqrt{s+1}), \quad (3.28)$$

$$N_3(x, s) = \cosh(\gamma\sqrt{s/\beta}) \cosh((\gamma - x)\sqrt{s+1}) - \cosh(\gamma\sqrt{s+1}) \cosh((\gamma - x)\sqrt{s/\beta}). \quad (3.29)$$

Expressions in Eqs. (3.21) and (3.22) together with the expressions for the integrals in Eqs. (3.23) and (3.24) provide solutions for the Laplace transforms of the concentrations $u(x, t)$ and $v(x, t)$ in terms of elementary transcendental functions. These expressions are the main results of this paper.

IV. DISCUSSION

The derived expressions can be used to investigate the solutions as functions of the model parameters. First, one can use the small- s asymptotic behavior of $\hat{u}(x, s)$ and $\hat{v}(x, s)$ to find the steady-state concentration profiles $u_{ss}(x)$ and $v_{ss}(x)$. The results are

$$u_{ss}(x) = \lim_{s \rightarrow 0} s \hat{u}(x, s) = \frac{\alpha\beta\gamma \cosh(\gamma - x)}{(\alpha\beta + \beta\gamma - \alpha) \sinh \gamma + \alpha\gamma \cosh \gamma} \quad (4.1)$$

and

$$v_{ss}(x) = \lim_{s \rightarrow 0} s \hat{v}(x, s) = \frac{\gamma \{ \beta \sinh \gamma + \alpha [\cosh \gamma - \cosh(\gamma - x)] \}}{(\alpha\beta + \beta\gamma - \alpha) \sinh \gamma + \alpha\gamma \cosh \gamma}. \quad (4.2)$$

This recovers the steady-state profiles $u_{ss}(x)$ and $v_{ss}(x)$, obtained by Lipkow and Odde.

The total dimensionless concentration, denoted by $w(x, t)$ is

$$w(x, t) = \frac{1}{c_0} (c_A(x, t) + c_B(x, t)) = u(x, t) + v(x, t). \quad (4.3)$$

As follows from the initial conditions in Eq. (2.11), initially, at $t = 0$, this concentration is equal to unity, $w(x, 0) = 1$. As $t \rightarrow \infty$, this concentration approaches a steady state given by

$$w_{ss}(x) = u_{ss}(x) + v_{ss}(x). \quad (4.4)$$

Substituting here $u_{ss}(x)$ and $v_{ss}(x)$ given in Eqs. (4.1) and (4.2), we can write $w_{ss}(x)$ as

$$w_{ss}(x) = \frac{\gamma(\beta \sinh \gamma + \alpha \cosh \gamma) + \alpha\gamma(\beta - 1) \cosh(\gamma - x)}{\gamma(\beta \sinh \gamma + \alpha \cosh \gamma) + \alpha(\beta - 1) \sinh \gamma}. \quad (4.5)$$

FIG. 2. Steady states profiles for the unphosphorylated (red), phosphorylated (blue), and total (black) concentrations, for three representative sets of model parameters. For profiles in (a): $\alpha = 1$, $\beta = 1$, $\gamma = 3$. For profiles in (b): $\alpha = 1$, $\beta = 30$, $\gamma = 3$. For profiles in (c): $\alpha = 1$, $\beta = 1/30$, $\gamma = 3$.

This shows that there is a gradient of the total concentration only when the diffusivities of the A and B particles are different and $\beta \neq 1$.

The steady-state concentration profiles for three representative sets of parameters are shown in Fig. 2. When the diffusivities of the phosphorylated and unphosphorylated forms of the protein are equal ($\beta = 1$), the total concentration is uniform (Fig. 2(a)), which can be readily seen from Eq. (4.5). When the phosphorylated form diffuses slower than the unphosphorylated form ($\beta > 1$), the profile of the total concentration has a maximum at the membrane where the kinase is localized (Fig. 2(b)). The opposite is true, when the phosphorylated form diffuses more rapidly than the unphosphorylated form ($\beta < 1$). In this regime, which corresponds to the mechanism established for the MEX-5 protein in the early *C. elegans* embryo, a newly phosphorylated particle may establish a uniform distribution over the interval before it is dephosphorylated. As a consequence, it returns to the unphosphorylated state with low diffusivity at any point of the interval with equal probability. Note that in this case the steady profile of

FIG. 3. Dynamics of concentration profiles. Panels (a) and (b) show the spatial distributions of the unphosphorylated (red) and phosphorylated (blue) species, respectively. Dynamics of the spatial profiles for the total concentration (black) is shown in panel (c). These results correspond to the following set of parameters: $\alpha = 1$, $\beta = 0.1$, $\gamma = 5$. In all panels, the spatial profiles are plotted for $t = 0.001, 0.01, 0.1, 1$, and 100 .

the total concentration is graded throughout the domain; it is lower than one at the boundary where the kinase is localized and is clearly greater than one at the opposite boundary (Fig. 2(c)).

Dynamics of the concentration profiles in the model can be readily obtained by numerical inversion of the Laplace transforms given in Eqs. (3.21) and (3.22). Figures 3 and 4 show the results for several sets of model parameters, in the regime when the phosphorylated form has higher diffusivity ($\beta < 1$). In all cases, the system starts from the initial condition when all of the protein is unphosphorylated and evolves to the steady state in which the spatial profile of the total concentration has a minimum at the boundary where the kinase is localized. Importantly, depending on the value of model parameters and position within the interval, approach to the steady state value can proceed with an overshoot (Fig. 3). In this case, the phosphorylated form of the protein first accumulates near the membrane ($x = 0$), where it is produced and then later

FIG. 4. Time courses of concentrations at selected locations. (a), (b), and (c) show the time courses for the unphosphorylated (red), phosphorylated (blue), and total (black) concentrations, respectively. These results correspond to the following set of parameters: $\alpha = 1$, $\beta = 0.1$, $\gamma = 5$. Concentrations are plotted for $x = 0, 1$, and 2 (locations noted near the curves). Note that dynamics can be nonmonotonic.

it spreads over the interval. Whether or not such overshoots occur for the MEX-5 gradient is still unclear.

V. CONCLUDING REMARKS

This paper is devoted to the dynamics of the concentration profiles of phosphorylated and unphosphorylated forms of a protein. It is assumed that the protein is phosphorylated by kinases localized to the plasma membrane and dephosphorylated by phosphatases uniformly distributed in the cytoplasm, as schematically shown in Fig. 1. In addition, it is assumed that the two forms of the protein diffuse in the cytoplasm with different diffusivities. We have derived analytical solutions for

the Laplace transforms of the time-dependent concentrations of the two forms of the protein. Inverting these transforms numerically, one can find concentration profiles of the two forms of the protein as functions of time. Our results extend the work of Lipkow and Odde,⁶ who derived exact solutions for steady-state concentrations in this model. It is worth noting that sharp localization of the kinase plays a crucial role in finding our analytical solutions. If the kinase is not sharply localized, the solution can only be obtained numerically. In the future, it will be interesting to compare the predictions of the one-dimensional model with numerical solutions for the time-dependent concentration profiles obtained for a more realistic model of a three-dimensional cell with protein phosphorylation not sharply localized to the plasma membrane, but distributed in the cytoplasm.

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APPENDIX A: DERIVATION OF EQ. (3.12)

The propagator $G_A(x, t)$ satisfies Eq. (3.1), subject to the boundary and initial conditions in Eq. (3.2). After the Laplace transformation, we find that the Laplace transform of the propagator, $\hat{G}_A(x, s)$, satisfies

$$s\hat{G}_A - \delta(x) = \frac{d^2\hat{G}_A}{dx^2} - \hat{G}_A, \quad (\text{A1})$$

subject to the boundary conditions

$$\left. \frac{d\hat{G}_A}{dx} \right|_{x=0} = \left. \frac{d\hat{G}_A}{dx} \right|_{x=\gamma} = 0. \quad (\text{A2})$$

Solving the equation, we obtain the expression for the Laplace transform of the propagator in Eq. (3.12).

APPENDIX B: DERIVATION OF EQ. (3.14)

The propagator $G_B(x, t|x')$ satisfies Eq. (3.3) subject to the boundary and initial conditions in Eq. (3.4). After the Laplace transformation, we find that the Laplace transform of the propagator, $\hat{G}_B(x, s|x')$, satisfies

$$s\hat{G}_B - \delta(x - x') = \frac{d^2\hat{G}_B}{dx^2}, \quad (\text{B1})$$

subject to the boundary conditions

$$\beta \left. \frac{d\hat{G}_B}{dx} \right|_{x=0} = \alpha \hat{G}_B(0, t|x'), \quad \left. \frac{d\hat{G}_B}{dx} \right|_{x=\gamma} = 0. \quad (\text{B2})$$

Solving the equation, we obtain the expression for the Laplace transform of the propagator in Eq. (3.12).

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