

Cumulative Signal Transmission in Nonlinear Reaction-Diffusion Networks

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Abstract

Quantifying signal transmission in biochemical systems is key to uncover the mechanisms that cells use to control their responses to environmental stimuli. In this work we use the time-integral of chemical species as a measure of a network's ability to cumulatively transmit signals encoded in spatiotemporal concentrations. We identify a class of nonlinear reaction-diffusion networks in which the time-integrals of some species can be computed analytically. The derived time-integrals do not require knowledge of the solution of the reaction-diffusion equation, and we provide a simple graphical test to check if a given network belongs to the proposed class. The formulae for the time-integrals reveal how the kinetic parameters shape signal transmission in a network under spatiotemporal stimuli. We use these to show that a canonical complex-formation mechanism behaves as a spatial low-pass filter, the bandwidth of which is inversely proportional to the diffusion length of the ligand.

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Introduction

Cell survival hinges on the ability to respond to extracellular stimuli and self-regulate in a changing environment. Intracellular dynamics are controlled by intricate arrays of biochemical networks, and in particular, the spatiotemporal dynamics of species concentrations are key to a number of processes, including cell signalling [1], pattern formation [2] and morphogenesis [3]. Quantifying the signal transmission properties of a network is key to understand how its connectivity and parameters shape the conversion of signalling cues into cellular responses, as well as the detection of intervention points for engineering or therapeutic applications.

Our goal in this paper is to provide tools for the mathematical quantification of signal transmission in biochemical networks. We use the time-integral of species concentrations as a proxy for the ability of a network to transmit input cues. It represents the cumulative effect of external stimuli on the chemical species and has been used to discover an input amplification phenomenon in the MAPK pathway [4], and to study the activation of cell membrane receptor such as the epidermal growth factor and the erythropoietin receptors [5,6].

We focus on networks of biochemical reactions subject to molecular diffusion and spatiotemporal stimuli. We aim at obtaining *exact formulae* for the time-integrals of species concentrations. An analytic approach can reveal structural properties of the

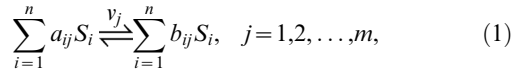
model under consideration, as opposed to simulation-based studies where it is unclear if predictions are rather a consequence of the particular parameter values examined. In the case of diffusionless systems, the work in [7] provided exact expressions for the \mathcal{L}_2 norm of a class of signalling cascades. However, similar results for reaction-diffusion systems remain elusive, owing to the fact that the vast majority of nonlinear reaction-diffusion systems are analytically intractable.

A complete solution to this problem, for any reaction-diffusion network, may require analytic solutions of the reaction-diffusion partial differential equation (PDE). We have previously identified a class of nonlinear networks in which the time-integrals of some species can be computed as a series [8]. Here we build on these results and show that in this class the time-integrals satisfy a linear inhomogeneous differential equation. Solving the derived equation leads to analytic expressions for the time-integrals without knowing the solution of the nonlinear PDE. We further provide a graphical characterization of the class of networks in terms of the Species-Reaction graph [9]. This provides a simple test to determine if a given network belongs to the derived class and to explore other network topologies that are amenable to our analysis. Applying our results to a complex-formation mechanism with sigmoidal kinetics, we show that it behaves as a spatial low-pass filter and that the temporal response can display a “waterbed effect” whereby concentrations ripple around their steady state and lead to a nil time-integral.

Results and Discussion

Exact Computation of the Time-integrals

We consider networks composed of n species interacting through m reactions:



where S_i is the i^{th} reactant or product for the j^{th} reaction. The numbers a_{ij} and b_{ij} denote the stoichiometric coefficients of the corresponding species. The reaction-diffusion model for the network is

$$\frac{\partial \mathbf{c}}{\partial t} = \mathbf{D}\nabla^2 \mathbf{c} + \mathbf{N}\mathbf{v}(\mathbf{c}) + \mathbf{B}\mathbf{u}, \quad (2)$$

where $\mathbf{c}(t, x)$ is the vector of n species concentrations, $\mathbf{u}(t, x)$ is a vector of ℓ influx/efflux rates accounting for environmental stimuli, and ∇^2 is the Laplacian operator (t and x denote time and space coordinates, respectively). The nonlinear vector function $\mathbf{v}(\mathbf{c})$ contains the m reaction rates, whereas the matrices $\mathbf{N} \in \mathbb{R}^{n \times m}$ (with $N_{ij} = b_{ij} - a_{ij}$), $\mathbf{D} = \text{diag}\{d_i\} \in \mathbb{R}^{n \times n}$ and $\mathbf{B} \in \mathbb{R}^{n \times \ell}$ describe the stoichiometry, diffusion coefficients, and which species are subject to external stimuli.

We focus on the response of the reaction-diffusion network to an initial spatial perturbation $\mathbf{c}(0, x)$ and a transient spatiotemporal stimulus $\mathbf{u}(t, x)$ such that $\lim_{t \rightarrow \infty} \mathbf{u}(t, x) = 0$ and $\int_0^\infty \mathbf{u}(t, x) dt < \infty$. For simplicity here we will focus on a 1D domain $\Omega \subseteq \mathbb{R}$ with the same boundary conditions for all species. Once the effect of the stimulus $\mathbf{u}(t, x)$ has vanished, we assume that the network reaches a unique homogeneous equilibrium $\bar{\mathbf{c}}$.

One way of quantifying the network response is by means of the time-integral:

$$\mathbf{I}_{\mathbf{c}}(x) = \int_0^\infty (\mathbf{c}(t, x) - \bar{\mathbf{c}}) dt, \quad (3)$$

which is finite provided that the equilibrium is exponentially stable. We relabel and partition the species and reaction rate vectors as follows:

- $\mathbf{v}^T = [\mathbf{v}_{\text{NL}}^T \quad \mathbf{v}_{\text{L}}^T]$, where \mathbf{v}_{NL} contains r nonlinear rates and \mathbf{v}_{L} contains the remaining $(m-r)$ affine rates,
- and $\mathbf{c}^T = [\mathbf{c}_{\text{NL}}^T \quad \mathbf{c}_{\text{L}}^T]$, where \mathbf{c}_{NL} contains the k species that react only in nonlinear reactions, \mathbf{c}_{L} includes the remaining $(n-k)$ species.

The affine reaction rates contain a combination of zeroth and first order terms of the form $\mathbf{v}_{\text{L}} = \mathbf{p} - \mathbf{G}\mathbf{c}_{\text{L}}$, with \mathbf{p} a vector of constant production rates and \mathbf{G} is a matrix of first order kinetic constants. The nonlinear rates typically model saturable binding kinetics such as Michaelis-Menten or Hill kinetics [10], together with linear dissociation (note that in our notation reversible reactions are taken as a single rate). If $k \geq 1$ we can find a labelling for the reaction rates so that the stoichiometric matrix has a block-triangular form

$$\mathbf{N} = \begin{bmatrix} \mathbf{N}_1 & 0 \\ \mathbf{N}_2 & \mathbf{N}_4 \end{bmatrix}, \quad (4)$$

with $\mathbf{N}_1 \in \mathbb{R}^{k \times r}$, $\mathbf{N}_2 \in \mathbb{R}^{(n-k) \times r}$ and $\mathbf{N}_4 \in \mathbb{R}^{(n-k) \times (m-r)}$.

We found that under the following conditions (see Analysis section for details):

C1 the species in \mathbf{c}_{NL} do not diffuse, and

C2 the number of species in \mathbf{c}_{NL} is equal to the number of nonlinear reactions (i.e. $r=k$), the time-integral of \mathbf{c}_{L} satisfies the differential equation

$$\mathbf{D}_4 \nabla^2 \mathbf{I}_{\mathbf{c}_{\text{L}}}(x) - \mathbf{N}_4 \mathbf{G} \mathbf{I}_{\mathbf{c}_{\text{L}}}(x) = \mathbf{F}(\mathbf{q}(x) + \mathbf{B}\mathbf{I}_{\mathbf{u}}(x)), \quad (5)$$

where $\mathbf{D}_4 \in \mathbb{R}^{(n-k) \times (n-k)}$ is the diffusion matrix of \mathbf{c}_{L} , $\mathbf{F} = [\mathbf{N}_2 \mathbf{N}_1^{-1} \quad -\mathbf{I}]$, $\mathbf{q}(x) = \mathbf{c}(0, x) - \bar{\mathbf{c}}$, and $\mathbf{I}_{\mathbf{u}}(x) = \int_0^\infty \mathbf{u}(t, x) dt$. The solution of (5) must satisfy boundary conditions consistent with those of the reaction-diffusion PDE. Equation (5) is an inhomogeneous linear differential equation with constant coefficients, and therefore depending on the spatial profile of the stimuli \mathbf{u} and initial condition $\mathbf{c}(0, x)$, it may be possible to obtain a closed-form solution for the time-integral $\mathbf{I}_{\mathbf{c}_{\text{L}}}(x)$.

In the general case when a closed-form solution is not available, equation (5) can be solved by projecting the solution on an orthonormal basis for the spatial domain Ω . To this end, we write $\mathbf{q}(x) = \sum_{i=0}^\infty \mathbf{w}_{\mathbf{q}_i} \phi_i(x)$ and $\mathbf{I}_{\mathbf{u}}(x) = \sum_{i=0}^\infty \mathbf{w}_{\mathbf{u}_i} \phi_i(x)$, where $\{\phi_i(x)\}_{i=0}^\infty$ is a complete orthonormal basis of Ω . We choose the basis as orthonormal eigenfunctions of the Laplacian, i.e. $\nabla^2 \phi_i = -\lambda_i \phi_i$ subject to the boundary conditions [11]. The time-integrals are then $\mathbf{I}_{\mathbf{c}_{\text{L}}}(x) = \sum_{i=0}^\infty \mathbf{w}_i \phi_i(x)$ with coefficients

$$\mathbf{w}_i = \mathbf{Q}(\lambda_i) (\mathbf{w}_{\mathbf{q}_i} + \mathbf{B}\mathbf{w}_{\mathbf{u}_i}), \quad (6)$$

and $\mathbf{Q}(\lambda_i) = -(\lambda_i \mathbf{D}_4 + \mathbf{N}_4 \mathbf{G})^{-1} \mathbf{F}$. The derived series is *exact* and we can use it to compute the time-integrals of \mathbf{c}_{L} without knowing the solution of the nonlinear PDE. Most importantly, the series coefficients \mathbf{w}_i are explicitly given in terms of the geometry and boundary conditions (comprised in the eigenvalues λ_i), the initial condition and the equilibrium (comprised in the function $\mathbf{q}(x)$), and the total concentration supplied to and consumed from the network (comprised in the integral of \mathbf{u}). Note that these coefficients can also be obtained by linearizing the PDE in (2), but such an approach provides no guarantee of the exactness of the solution. Linearized solutions neglect the nonlinear terms in the PDE, and therefore they are valid only for small perturbations around the equilibrium. In our case, conditions **C1** and **C2** guarantee that the derived time-integral is exact, defining a class of nonlinear networks for which the time-integral can be computed analytically for small or large perturbations.

Graph Interpretation of the Network Conditions

Conditions **C1** and **C2** are structural (hence independent of the functional form of the nonlinearities) and can be interpreted in terms of a graph. We use the Species-Reaction graph (Fig. 1 A), composed of two sets of nodes [9]: species nodes, denoted as S-nodes, and reaction nodes, denoted as R-nodes. The graph is bipartite—so that reaction nodes only link to species nodes, and *vice versa*—and is defined as follows: an S-node c_i is connected to an R-node v_j if v_j depends on c_i (i.e. $\partial v_j / \partial c_i \neq 0$). As shown in Fig. 1 A, we color the S-nodes black (red) if they are diffusive (nondiffusive) species, and the R-nodes black (red) if they correspond to linear (nonlinear) reaction rates. With these definitions, conditions **C1** and **C2** amount to:

C* all S-nodes that are not connected to black R-nodes are red, and their number equals the number of red R-nodes.

As illustrated by the examples in Fig. 1 A, under condition **C*** the network graph contains a red subgraph that corresponds to the

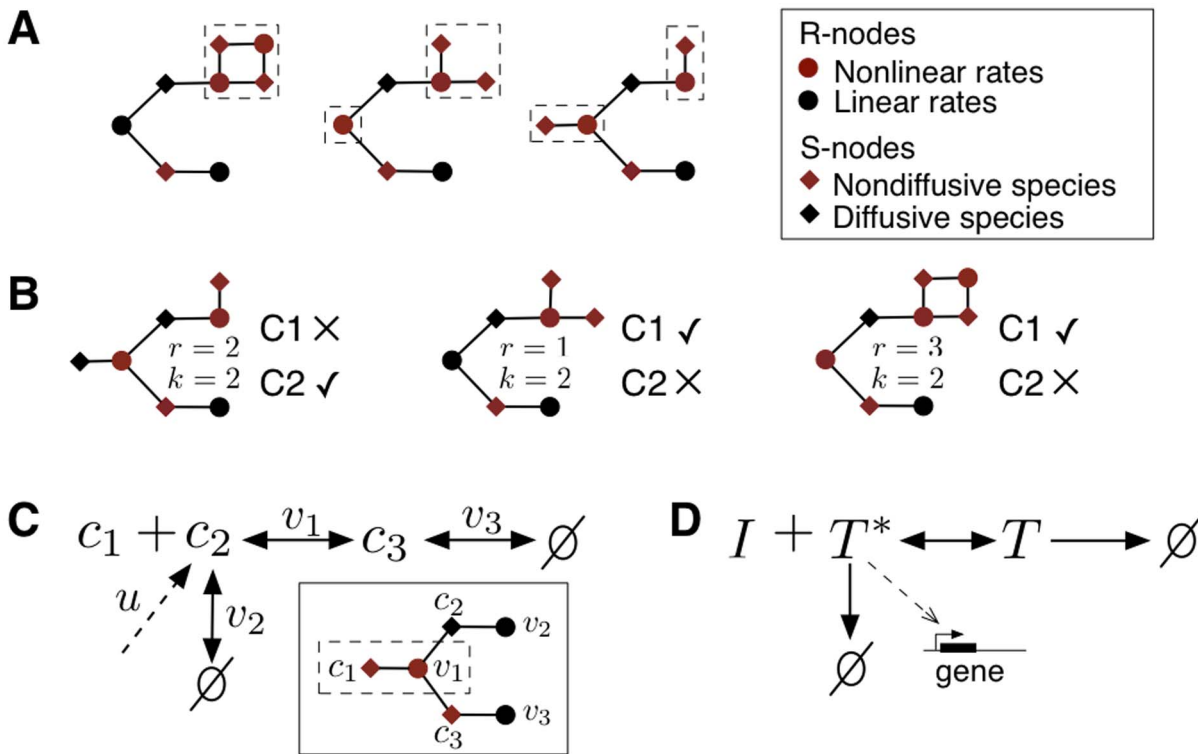


Figure 1. Conditions for the computation of the species' time-integrals in terms of the Species-Reaction graph. The conditions amount to the graph having a (possibly disjoint) subgraph containing every red R-node with all their adjacent red S-nodes not linked with any black R-nodes; in this subgraph, the number of red R-nodes and red S-nodes must be the same. **(A)** Networks with two nonlinear reactions ($r=k=2$) satisfying the conditions. The red subgraphs are marked with dashed boxes. **(B)** Networks that do not satisfy the conditions. **(C)** A generic complex-formation mechanism satisfying the conditions. A spatially-fixed molecule c_1 binds a diffusible ligand c_2 to form a complex c_3 . Species c_2 and c_3 are synthesized at a constant rate and linearly degraded. External stimuli of ligand can be modeled via a spatiotemporal influx $u(t,x)$. The Species-Reaction graph is shown in the inset. **(D)** Genetic regulation via protein sequestration [12] is an instance of the mechanism in **C**. A nondiffusive inhibitor I sequesters a transcriptional activator T^* to form an inactive complex T , causing the downregulation of gene expression. doi:10.1371/journal.pone.0062834.g001

nonlinear and nondiffusive portion of the network that is not directly connected to any linear reactions (see Fig. 1 B for examples where the conditions are not met). Under these conditions we can use our formula to compute the time-integrals of all the species outside the subgraph.

Condition **C1** is generally valid for species with large molecular weight or that are spatially fixed, such as membrane-bound receptors or molecules anchored to the cytoskeleton. Condition **C2** is more restrictive because it requires the nonlinear and nondiffusive part of the network to have as many reactions as species. A particularly relevant system that meets the conditions is the generic complex-formation network in Fig. 1 C, where a diffusible ligand forms a complex with an immobile molecule. This mechanism can be found, for example, in ligand-receptor interactions [5] and protein sequestration [12]. In the latter case, if the sequestered protein is a transcription factor for a specific gene (Fig. 1 D), cells can use sequestration to downregulate gene expression in response to intracellular signals.

If we write the perturbation (both the initial spatial perturbation $\mathbf{e}(0,x)$ and the spatiotemporal stimulus \mathbf{u}) in the basis of Ω , the coefficients \mathbf{w}_i in (6) can be seen as the product of those of the perturbation and the coefficients comprised in the matrix $\mathbf{Q}(\lambda_i)$. For a number of spatial geometries, the eigenfunctions of the Laplacian are typically sine and/or cosine functions [13], and thus the resulting time-integral can be understood as a filtered version of the perturbations. The form of the coefficients in (6) also indicates that if a column of B is orthogonal to a row of $\mathbf{Q}(\lambda_i)$ for

all i , then the corresponding input will generate a nil time-integral. We can use this property to detect input channels that generate a “waterbed effect”, where concentrations ripple around the equilibrium and lead to a nil time-integral.

Complex-formation Network under Spatiotemporal Stimuli

To illustrate the utility of our approach we use it in the complex-formation network in Fig. 1 C. The reaction-diffusion PDE for this network in the domain $\Omega=[0,L]$ is

$$\frac{\partial c_1}{\partial t} = -\sigma(c_2)c_1 + k_{1b}c_3, \tag{7}$$

$$\frac{\partial c_2}{\partial t} = d_2 \nabla^2 c_2 - \sigma(c_2)c_1 + k_{1b}c_3 - \gamma_2 c_2 + k_2 + u, \tag{8}$$

$$\frac{\partial c_3}{\partial t} = \sigma(c_2)c_1 - k_{1b}c_3 - \gamma_3 c_3 + k_3, \tag{9}$$

where $\sigma(c_2) = k_{1f}c_2^h / (\theta^h + c_2^h)$, $\theta > 0, h > 0$ is a sigmoidal nonlinearity describing the binding between c_1 and c_2 , k_{1b} is their dissociation rate constant, k_i are the synthesis rates, and γ_i are the degradation rate constants.

We partition the rate vector into its nonlinear and linear components, i.e. $\mathbf{v}_{\text{NL}} = \sigma(c_2)c_1 - k_{1b}c_3$ and $\mathbf{v}_{\text{L}} = [k_2 - \gamma_2c_2 \quad k_3 - \gamma_3c_3]^T$, and the species as $\mathbf{c}_{\text{NL}} = c_1$ and $\mathbf{c}_{\text{L}} = [c_2 \quad c_3]^T$. For homogeneous Neumann boundary conditions (i.e. $\partial c_i / \partial x|_{x=0,L} = 0$ for $i=1,2,3$), it can be shown that the network has a unique homogeneous equilibrium point at $\bar{c}_1 = k_{1b}k_3 / (\gamma_3\sigma(k_2/\gamma_2))$, $\bar{c}_2 = k_2/\gamma_2$ and $\bar{c}_3 = k_3/\gamma_3$. The blocks of the stoichiometric matrix are $\mathbf{N}_1 = -1$, $\mathbf{N}_2 = [-1 \quad 1]^T$ and $\mathbf{N}_3 = \mathbf{I}$, whereas the matrix of first order kinetic constants is $\Gamma = \text{diag}\{\gamma_2, \gamma_3\}$ and $\mathbf{B} = [0 \quad 1 \quad 0]^T$. The eigenfunctions of the Laplacian in $[0, L]$ are $\phi = \{\sqrt{1/L}, q \cos(\pi x/L), q \cos(2\pi x/L), \dots\}$, with $\lambda_i = (i\pi/L)^2$ and $q = \sqrt{2/L}$. From (5) we can obtain an ordinary differential equation for the integral of c_2 and an explicit expression for the one of c_3 (note that c_3 does not diffuse):

$$d_2 \frac{\partial^2 \mathbf{I}_{c_2}(x)}{\partial x^2} - \gamma_2 \mathbf{I}_{c_2}(x) = c_1(0, x) - c_2(0, x) - (\bar{c}_1 - \bar{c}_2) - \mathbf{I}_{\mathbf{u}}(x), \quad (10)$$

$$\mathbf{I}_{c_3} = \frac{1}{\gamma_3} (c_1(0, x) + c_3(0, x) - \bar{c}_1 - \bar{c}_3), \quad (11)$$

with $\mathbf{I}_{\mathbf{u}}(x) = \int_0^\infty u(t, x) dt$ and subject to boundary conditions $\partial \mathbf{I}_{c_2} / \partial x|_{x=0,L} = 0$. We next consider the network response to two types of perturbations: a purely spatial perturbation and a spatiotemporal influx of ligand.

Spatial Perturbation

We first consider the case of an initial spatial perturbation in c_1 of the form $c_1(0, x) = \bar{c}_1 + f(x)$, where $f(x)$ is the spatial profile of the perturbation and there is no stimulus ($u=0$). The other species are initially at equilibrium, i.e. $c_i(0, x) = \bar{c}_i$ for $i=2,3$, so that $\mathbf{q}(x) = [f(x) \quad 0 \quad 0]^T$ in (5). We can write the perturbation in the basis as

$$f(x) = \frac{\tilde{f}_0 \sqrt{L}}{2} \phi_0 + \sqrt{\frac{L}{2}} \sum_{i=1}^{\infty} \tilde{f}_i \phi_i(x), \quad x \in [0, L], \quad (12)$$

with $\tilde{f}_i = (1/L) \int_{-L}^L \tilde{f}(x) \cos(i\pi x/L) dx$ and $\tilde{f}(x)$ is the even $2L$ -periodic extension of $f(x)$. Note that the expansion in (12) converges to $f(x)$ only in the domain $[0, L]$. Using (6) we get the time-integrals

$$\mathbf{I}_{c_2}(x) = -\frac{\tilde{f}_0}{2\gamma_2} - \sum_{i=1}^{\infty} \frac{\tilde{f}_i}{(i\pi/L)^2 d_2 + \gamma_2} \cos(i\pi x/L), \quad (13)$$

and $\mathbf{I}_{c_3}(x) = f(x)/\gamma_3$. In Fig. 2 we show the response of the network to a Gaussian spatial perturbation under zero flux boundary conditions, together with the time-integrals \mathbf{I}_{c_2} and \mathbf{I}_{c_3} .

The coefficients \tilde{f}_i describe the frequency content of the perturbation (plus spurious harmonics arising from the periodic extension). The expressions for \mathbf{I}_{c_2} and \mathbf{I}_{c_3} therefore indicate that the time-integral of the diffusible species is a filtered version of the spatial perturbation, and this filtering effect disappears in the case of the immobile species. Diffusion of c_2 acts as a spatial filter with a low-pass characteristic [14], and the magnitude of its frequency response is $\mathcal{K}_2(\omega) = 1/(\omega^2 d_2 + \gamma_2)$, representing the attenuation factor for a spatial harmonic of frequency $\omega = i\pi/L$.

An important parameter of reaction-diffusion systems is the *diffusion length* $\kappa_2 = \sqrt{d_2 \tau}$, where τ is the species half-life. It represents the distance a molecule typically diffuses over its lifetime and determines the length scale of the diffusion process [15]. The cutoff frequency of the spatial filter (the frequency above which harmonics are attenuated by at least 50%) is $\omega_c = \sqrt{\gamma_2/d_2}$, thus inversely proportional to the diffusion length. This indicates that in the mechanism of Fig. 1 C, ligands with a short diffusion length behave as high-bandwidth filters that encode a rich harmonic content in their time-integral.

Conversely, molecules with long diffusion lengths may suppress all harmonics and lead to a spatially homogeneous time-integral. This attenuation can be drastic, for example, in cytosolic proteins of *S. cerevisiae*, whose mean diffusion coefficient and diffusion length have been recently estimated at $d \sim 4 \mu\text{m}^2/\text{s}$ and $\kappa \sim 160 \mu\text{m}$ (based on 1400 proteins [16]). Considering the typical diameter of *S. cerevisiae* $L = 4 \mu\text{m}$, we conclude from $\mathcal{K}_2(\omega)$ that the first harmonic of the perturbation is subject to $\sim 90\%$ attenuation, and any higher harmonic will be attenuated by a larger factor. In these cases, we suggest that information encoded in the perturbation may be more faithfully transmitted through transient-dependent features, such as the peak value or the response time of the species concentrations. These quantities have been extensively used in diffusionless models for biochemical networks [4,6], but remain largely unexplored when molecular diffusion is not negligible.

Spatiotemporal Influx

We now consider that all species are initially in equilibrium, i.e. $c_i(0, x) = \bar{c}_i$ for $i=1,2,3$, and a spatiotemporal influx of ligand $u(t, x) = g(t)f(x)$. In this case we have $\mathbf{q}(x) = 0$ and from (6) we get

$$\mathbf{I}_{c_2}(x) = \frac{G \tilde{f}_0}{2\gamma_2} + G \sum_{i=1}^{\infty} \frac{\tilde{f}_i}{(i\pi/L)^2 d_2 + \gamma_2} \cos(i\pi x/L), \quad (14)$$

and $\mathbf{I}_{c_3} = 0$ with $G = \int_0^\infty g(t) dt$. In Fig. 3 we show the network response to a spatiotemporal Gaussian pulse of ligand influx under zero flux boundary conditions, together with the time-integral \mathbf{I}_{c_2} .

The nil time-integral of c_3 indicates that the areas above and below the equilibrium cancel out, leading to a waterbed effect. In Fig. 3 A we effectively observe that c_3 peaks and then undershoots below its equilibrium, subsequently recovering back to its pre-stimulus level. The time-integral of c_3 is zero for all points in space and reveals a fundamental tradeoff in the response: its peak can be amplified only at the expense of a deeper valley under the equilibrium. This type of tradeoff arises from the network structure and is independent of the parameter values, emphasizing the role of model analysis in applications that require a precise control of biological responses, such as the delivery of growth factors in tissue engineering [17] or the control of pattern formation [18].

Concluding Remarks

In this work we discussed the analytic computation of time-integrals in nonlinear reaction-diffusion systems. We found conditions under which the time-integrals of some species satisfy a linear differential equation, the solution of which can be written as a function of the kinetic parameters, the geometry and the spatiotemporal stimuli. The derived conditions represent constraints on the interaction topology between the nonlinear rates and nondiffusive species. They depend only on the network topology and are independent of the functional form of the kinetic nonlinearities.

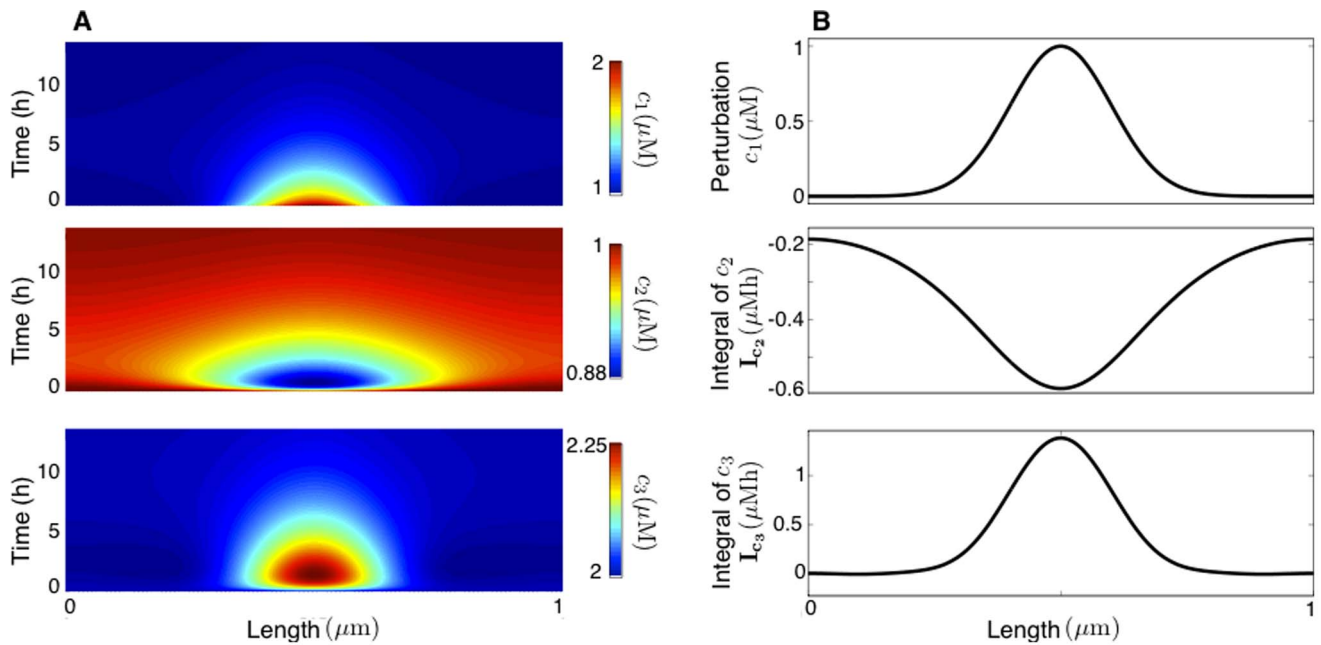


Figure 2. Response of the network in Fig. 1 C to a spatial perturbation in c_1 . (A) Species concentrations. (B) Gaussian perturbation $f(x) = e^{-(x-L/2)^2/0.02}$ and the time-integrals of species c_2 and c_3 . The parameters are $L = 1\mu\text{m}$, $k_{1f} = 4 \cdot 10^{-4}\text{s}^{-1}$, $k_{1b} = 1 \cdot 10^{-4}\text{s}^{-1}$, $k_i = \{2,4\} \cdot 10^{-4}\mu\text{M}/\text{s}$, $d_2 = 10^{-5}\mu\text{m}^2/\text{s}$, $\gamma_{2,3} = 2 \cdot 10^{-4}\text{s}^{-1}$, $\theta = 1\mu\text{M}$, and $h = 2$. doi:10.1371/journal.pone.0062834.g002

We recast the conditions in terms of a graph that provides a simple test to check their validity in any given network and a means to find other topologies where our analysis can be applied. The graph interpretation suggests the conditions are well suited for systems with a small number of nonlinear reactions and whose diffusive reactants appear also in first order reactions. This

narrows down the class of networks amenable to our result, albeit this is not surprising since analytic solutions for nonlinear PDEs are rarely available. Moreover, typical reaction-diffusion models have a small number of species and reactions, as their analysis can become increasingly complex in high dimensions (even in low dimensional cases they can display a wide range of complex

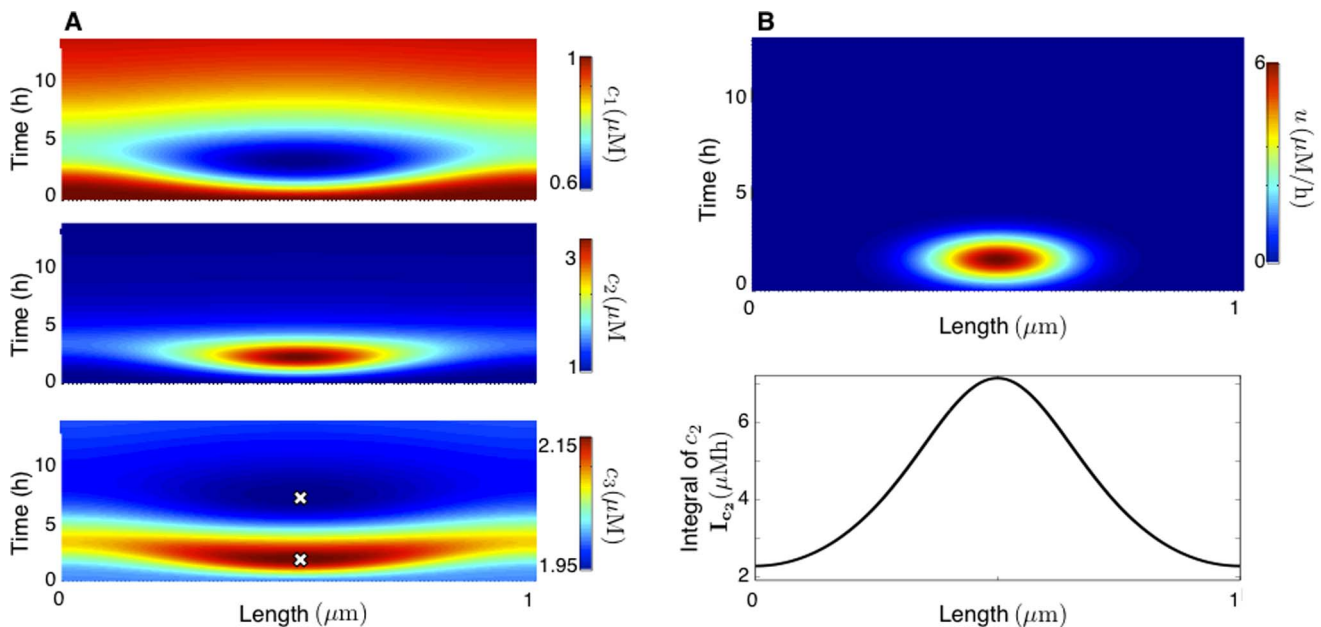


Figure 3. Response of the network in Fig. 1 C to a spatiotemporal influx of ligand c_2 . (A) Species concentrations; the white crosses mark the peak and valley of c_3 . (B) Gaussian influx $u = 0.1e^{-(t-t_{on})^2/5 \cdot 10^3} e^{-(x-L/2)^2/0.02}$ and time-integral of the ligand. Parameter values are the same as in Fig. 2. doi:10.1371/journal.pone.0062834.g003

dynamics [19]). In those models that do satisfy the required conditions, the analytic relationship between the time-integrals and the model parameters can reveal substantial insights into the network dynamics.

We showed that a model for protein sequestration—a ubiquitous mechanism in cell regulation—can be readily analyzed with our theory. Other relevant mechanisms amenable to our approach include membrane receptor systems [20] and calcium sequestration by immobile buffers [21]. We illustrated our results in a canonical complex-formation mechanism with sigmoidal binding kinetics. This is a non-trivial and biologically relevant system where the reaction-diffusion PDE has no known analytic solution. We showed that this mechanism behaves as a low-pass filter and displays a waterbed effect [22] in the dynamic response of the complex for all parameter values and a wide range of spatiotemporal stimuli. Analytic approaches such as the one presented here can shed light on the mechanisms by which living cells modulate their responses to environmental cues. This can ultimately lead to the identification of key control parameters that can be targeted to modify cellular responses, for example, with the use of therapeutic drugs.

Analysis

Here we show how to obtain the differential equation for the time-integrals in (5), and the series coefficients in (6). With the chosen partitions for the reaction rates and species concentrations, we can write

$$\mathbf{N} = \begin{bmatrix} \mathbf{N}_1 & 0 \\ \mathbf{N}_2 & \mathbf{N}_4 \end{bmatrix}, \quad \frac{\partial \mathbf{v}}{\partial \mathbf{c}} \Big|_{\bar{\mathbf{c}}} = \begin{bmatrix} \mathbf{J}_1 & \mathbf{J}_2 \\ 0 & \mathbf{J}_4 \end{bmatrix}, \quad (15)$$

with $\mathbf{N}_1 \in \mathbb{R}^{k \times r}$, $\mathbf{N}_2 \in \mathbb{R}^{(n-k) \times r}$, $\mathbf{N}_4 \in \mathbb{R}^{(n-k) \times (m-r)}$, $\mathbf{J}_1 \in \mathbb{R}^{r \times k}$, $\mathbf{J}_2 \in \mathbb{R}^{r \times (n-k)}$, and $\mathbf{J}_4 \in \mathbb{R}^{(m-r) \times (n-k)}$. Exponential stability of the equilibrium $\bar{\mathbf{c}}$ implies that the matrix

$$\mathbf{A} = \frac{\partial \mathbf{Nv}}{\partial \mathbf{c}} \Big|_{\bar{\mathbf{c}}} = \begin{bmatrix} \mathbf{N}_1 \mathbf{J}_1 & \mathbf{N}_1 \mathbf{J}_2 \\ \mathbf{N}_2 \mathbf{J}_1 & \mathbf{N}_2 \mathbf{J}_2 + \mathbf{N}_4 \mathbf{J}_4 \end{bmatrix} \quad (16)$$

is invertible [23], which means that \mathbf{N}_1 is full column rank (note that otherwise, if there exists a vector $\mathbf{w} \neq 0$ such that $\mathbf{w}^T \mathbf{N}_1 = 0$, then $[\mathbf{w}^T \ 0] \mathbf{A} = 0$, which implies that $\det \mathbf{A} = 0$ and contradicts the invertibility of \mathbf{A}). In addition, by Condition C2 the matrix \mathbf{N}_1 is square and therefore \mathbf{N}_1^{-1} is well defined. By Condition C1 the reaction-diffusion PDE in (2) can be written as

$$\frac{\partial \mathbf{c}_{\text{NL}}}{\partial t} = \mathbf{N}_1 \mathbf{v}_{\text{NL}} + \mathbf{B}_1 \mathbf{u}, \quad (17)$$

$$\frac{\partial \mathbf{c}_{\text{L}}}{\partial t} = \mathbf{D}_4 \nabla^2 \mathbf{c}_{\text{L}} + \mathbf{N}_2 \mathbf{v}_{\text{NL}} + \mathbf{N}_4 \mathbf{v}_{\text{L}} + \mathbf{B}_2 \mathbf{u}, \quad (18)$$

where \mathbf{D}_4 is the diffusion matrix of \mathbf{c}_{L} and $\mathbf{B}^T = [\mathbf{B}_1^T \ \mathbf{B}_2^T]$.

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Note that setting (17) and (18) to zero for $\mathbf{u} = 0$, we get that any homogeneous equilibrium $\bar{\mathbf{c}}$ satisfies $\mathbf{v}_{\text{NL}}(\bar{\mathbf{c}}) = 0$ (because \mathbf{N}_1 is a nonsingular matrix) and $\mathbf{N}_4 \mathbf{v}_{\text{L}}(\bar{\mathbf{c}}_{\text{L}}) = 0$. Using the form of the affine rates $\mathbf{v}_{\text{L}} = \mathbf{p} - \Gamma \mathbf{c}_{\text{L}}$, we conclude that the homogeneous equilibrium for \mathbf{c}_{L} satisfies $\mathbf{N}_4 \Gamma \bar{\mathbf{c}}_{\text{L}} = \mathbf{N}_4 \mathbf{p}$. From (17) we can solve for \mathbf{v}_{NL} to get $\mathbf{v}_{\text{NL}} = \mathbf{N}_1^{-1} (\partial \mathbf{c}_{\text{NL}} / \partial t - \mathbf{B}_1 \mathbf{u})$, which after substituting in (18) and rearranging terms yields

$$\mathbf{D}_4 \nabla^2 \mathbf{c}_{\text{L}} - \mathbf{N}_4 \Gamma \mathbf{c}_{\text{L}} + \mathbf{N}_4 \mathbf{p} = -\mathbf{F} \left(\frac{\partial \mathbf{c}}{\partial t} - \mathbf{B} \mathbf{u} \right), \quad (19)$$

with $\mathbf{F} = [\mathbf{N}_2 \mathbf{N}_1^{-1} \quad -\mathbf{I}]$. Using the relation $\mathbf{N}_4 \Gamma \bar{\mathbf{c}}_{\text{L}} = \mathbf{N}_4 \mathbf{p}$, the equation in (19) can be rewritten as

$$\mathbf{D}_4 \nabla^2 (\mathbf{c}_{\text{L}} - \bar{\mathbf{c}}_{\text{L}}) - \mathbf{N}_4 \Gamma (\mathbf{c}_{\text{L}} - \bar{\mathbf{c}}_{\text{L}}) = -\mathbf{F} \left(\frac{\partial \mathbf{c}}{\partial t} - \mathbf{B} \mathbf{u} \right). \quad (20)$$

The differential equation for $\mathbf{I}_{\mathbf{c}_{\text{L}}}(x)$ in (5) can be obtained by integrating (20) from $t = 0$ to $t = \infty$. To get the coefficients for the series in (6), we substitute the series for $\mathbf{I}_{\mathbf{c}_{\text{L}}}(x)$, $\mathbf{q}(x)$ and $\mathbf{I}_{\mathbf{u}}(x)$ in (5):

$$(\mathbf{D}_4 \nabla^2 - \mathbf{N}_4 \Gamma) \sum_{j=0}^{\infty} \mathbf{w}_j \phi_j(x) = \mathbf{F} \sum_{j=0}^{\infty} (\mathbf{w}_{\mathbf{q}_j} + \mathbf{B} \mathbf{w}_{\mathbf{u}_j}) \phi_j(x). \quad (21)$$

Since the basis satisfies the eigenvalue problem $\nabla^2 \phi_i = -\lambda_i \phi_i$, from (21) we get

$$\sum_{j=0}^{\infty} (\lambda_j \mathbf{D}_4 + \mathbf{N}_4 \Gamma) \mathbf{w}_j \phi_j(x) = -\mathbf{F} \sum_{j=0}^{\infty} (\mathbf{w}_{\mathbf{q}_j} + \mathbf{B} \mathbf{w}_{\mathbf{u}_j}) \phi_j(x), \quad (22)$$

Postmultiplying (22) by $\phi_i(x)$ and integrating over the spatial domain leads to one equation for each coefficient \mathbf{w}_i

$$(\lambda_i \mathbf{D}_4 + \mathbf{N}_4 \Gamma) \mathbf{w}_i = -\mathbf{F} (\mathbf{w}_{\mathbf{q}_i} + \mathbf{B} \mathbf{w}_{\mathbf{u}_i}), \quad (23)$$

for $i = 0, 1, \dots$. To obtain (23) we used the orthonormality of the basis (i.e. $\int_{\Omega} \phi_i \phi_j dx = 1$ for $i = j$ and zero otherwise). The final expression for the coefficients in (6) can be obtained directly from (23).

Author Contributions

Conceived and designed the experiments: DAO FLC MRG RHM AYW. Performed the experiments: DAO FLC MRG RHM AYW. Analyzed the data: DAO FLC MRG RHM AYW. Wrote the paper: DAO FLC MRG RHM AYW.

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