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Shared kinase fluctuations between two enzymatic reactions

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Abstract
Kinases serve crucial roles in many cellular signaling pathways that process and transfer information. When signaling kinases phosphorylate two targets, these can serve as branch points that distribute information among two pathways. Responses to stimuli transmitted by activated kinases show high levels of cell-to-cell variation that influence cellular function. We ask how fluctuations around a steady state, due to kinase fluctuations and intrinsic noise, are distributed between two reactions with substrates phosphorylated by a shared kinase. We develop the formalism to answer this question and, for a realistic set of biological constants, we illustrate various features of fluctuations and relaxation times to a steady state. We find that the steady-state response determines the size and range in enzyme concentration of phosphorylated substrate fluctuations, and that the choice of an operating point can have a large impact on how shared kinase noise is distributed among two available pathways.

1. Introduction

Signaling pathways in stimulated cells are frequently activated from a common source. Activation typically occurs through substrate phosphorylation by a kinase. When two substrates, leading to branching pathways, share an upstream kinase, they are connected, as the phosphorylation of one substrate depends on the concentration of one other substrate. Pathway branching is an important aspect of network topology, that plays a crucial role in cellular response to external stimulation. Examples are numerous: many immune cells process the information flow triggered by a receptor activation via PI3K kinase isoforms, such as $p110\delta$ and $p100\gamma$, which can simultaneously regulate two or more downstream proteins, each of which leads to different cellular outcomes [1]; in the JAK/STAT pathway stimulated by type I interferon, there is co-regulation of the phosphorylation of STAT1 and STAT2 by phosphorylated Tyk2 and phosphorylated Jak1 [2]. These are instances of known pathways originating from a common kinase. When the network topology is unknown, it is sometimes possible to infer the presence of hidden pathways. The existence of an unknown pathway was surmised in [3] from single cell ERKp responses to gonadotropin stimulated cells. The uncovering of a shared kinase of two known pathways was accomplished in [4], where the authors showed that for cells stimulated by the proinflammatory cytokine interleukin-1 (IL-1), phosphorylated TAK1 (MAP3Kp), known to be the kinase leading to activation of AP-1 through the JNK pathway, was at the same time the kinase responsible for NFκB activation through NIK and IKK.

In this work, motivated by these biological examples, we investigate the topology of two enzymatic reactions driven by the same kinase. The parallel system of the two enzymatic reactions either stands alone, or can be considered as the first stage of multistage signaling cascades, such as the Ras-Raf stage of a MAPK cascade. Cellular processes are subject to fluctuations that arise either due to intrinsic noise resulting from the stochasticity of chemical reactions or extrinsic noise due to cell-to-cell variability from other sources such as differences in the number of reactants. These fluctuations determine how faithfully an external signal is transduced, from stimulation at the cellular membrane down to transcription factor activation. Noisiness of signal transduction is thus an issue of great interest. In this context it is imperative to investigate how initial fluctuations in some kinase right after cell stimulation distribute themselves between the two

phosphorylated substrates driven by that same kinase, and what are the characteristics of the two enzymatic reactions that determine which pathway inherits what amount of input fluctuations. Previous work has dealt with noise transduction in single enzymatic reactions [5, 6] or with noise propagation through multiple stages in a signaling cascade [7, 5]. Here we give a complete treatment of small kinase fluctuations away from the steady state for a parallel system of two enzymatic reactions driven by a common kinase. Our discussion of distributed noise should be of interest to the fast evolving field of synthetic biological circuits, where recently [8] a kinase signaling pathway was modulated through engineered scaffold protein interactions. We highlight how the steady-state response determines the characteristics of fluctuations of the phosphorylated substrate due to enzyme fluctuations. We also point out that in the many cases where steady-state responses of the two substrates phosphorylated by the same kinase differ, the choice of the operating point, defined as the amount of available kinase, determines the division of kinase fluctuations between the two phosphorylated substrates. Moreover, even when the biological system is studied at one operating point only, the measurement of a gain factor across a cell population allows one to infer how noise propagates through two signaling pathways activated by the same kinase.

Our paper is organized as follows. In section 2 we present the basic equations for fluctuations around a steady state in the parallel system of figure 1 whose implications are discussed in the rest of the paper. Section 3 contains a detailed evaluation of the different sources of noise, intrinsic and extrinsic, of which a few salient features concerning relaxation times to the steady state are discussed in section 4. Section 5 gives the ingredients for numerical calculations based on numbers derived from the early stages of MAP kinase cascades. We use these parameter values to illustrate some of our results on the values of time constants describing excursions from the steady state, on kinase noise contributions to enzymatic reaction fluctuations, both for a parallel system of shared kinase and for a single reaction, and on the operating point dependence of the distribution of enzymatic noise. Section 7 contains a summary and conclusion.

2. Methods

2.1. Model and equations

In figure 1(*a*) we show the diagram of the parallel motif we study which consists of the two substrates X and Y driven by a common kinase E. The fact that activation of X and Y is due to a shared kinase, entails a form of cross-talk between the two pathways: the amount of activation in one enzymatic system depends explicitly on the amount of substrate of the second parallel reaction. We wish to determine the impact of this cross-talk on various properties of the system; in particular, on the amount of noise in the linear response of the two pathways due to noise in the input when the system is perturbed away from the steady state. Our methodology follows that used by Detwiler *et al* [5] in their study of retinal transduction as an engineering system. We represent the activation by

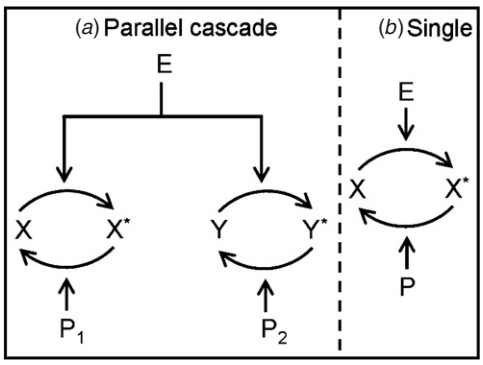

Figure 1. (*a*) Schematic of reactions for the phosphorylation of two substrates X and Y by a common kinase E and dephosphorylation by different phosphatases P_1 and P_2. (*b*) Schematic for a single substrate X of the phosphorylation by kinase E and dephosphorylation by P.

E (see figure 1(*a*)) of $X \rightarrow X^\star$ and $Y \rightarrow Y^\star$ and the corresponding phosphatase action $X^\star \rightarrow X$ and $Y^\star \rightarrow Y$ through respectively P_1 and P_2 by the usual Michaelis–Menten equations, namely

$$\mathrm{d}X^\star/\mathrm{d}t = Q_+ X - Q_- X^\star \tag{1}$$

$$\mathrm{d}Y^\star/\mathrm{d}t = R_+ Y - R_- Y^\star, \tag{2}$$

where

$$Q_+ = \frac{k_3 {K_1}^{-1} E^t}{1 + {K_1}^{-1} X + {K_2}^{-1} Y}, \tag{3}$$

$$Q_- = \frac{k_6 \bar{K}_1^{-1} {P_1}^t}{1 + \bar{K}_1^{-1} X^\star} \tag{4}$$

$$R_+ = \frac{a_3 {K_2}^{-1} E^t}{1 + {K_1}^{-1} X + {K_2}^{-1} Y}, \tag{5}$$

$$R_- = \frac{a_6 \bar{K}_2^{-1} {P_2}^t}{1 + \bar{K}_2^{-1} Y^\star}. \tag{6}$$

Here $X + X^\star = X^t$, and $Y + Y^\star = Y^t$, X^t and Y^t represent the initial amounts of unactivated molecules of X and Y. E^t represents the available amount of enzyme E, ${P_1}^t$ and ${P_2}^t$ the initial amounts of phosphatases P_1 and P_2, respectively. The K and $\bar{K}$ are the respective Michaelis–Menten constants. Note that the two equations are coupled, since the activation of X depends on Y and that of Y on X. Thus a common source of kinase provides an explicit connection between the two systems. Its origin lies in the conservation of the original amount of kinase, which can either be free or bound in a complex with either substrate X or substrate Y.

Equations (1) and (2) can be formally derived from the set of chemical reactions

$$X + E \underset{k_1}{\overset{k_2}{\rightleftharpoons}} [XE] \overset{k_3}{\rightarrow} X^\star + E \tag{7}$$

$$Y + E \underset{a_1}{\overset{a_2}{\rightleftharpoons}} [YE] \overset{a_3}{\rightarrow} Y^\star + E \tag{8}$$

for kinase activity and

$$X^\star + P_1 \underset{k_4}{\overset{k_5}{\rightleftharpoons}} [X^\star P_1] \overset{k_6}{\rightarrow} X + P_1 \tag{9}$$

$$Y^\star + P_2 \underset{a_4}{\overset{a_5}{\rightleftharpoons}} [Y^\star P_2] \overset{a_6}{\rightarrow} Y + P_2 \tag{10}$$

for the phosphatases. In these reactions the k and a are reaction rate constants. The associated Michaelis–Menten factors are $K_1 = (k_2 + k_3)/k_1$; $\bar{K}_1 = (k_5 + k_6)/k_4$; $K_2 = (a_2 + a_3)/a_1$; $\bar{K}_2 = (a_5 + a_6)/a_4$.

Equations (1) and (2) describe these chemical reactions if it is assumed that the intermediate complexes such as $[XE]$ have reached the quasi-steady state on long time scales. Whether the assumptions are justified would have to be decided on a case-by-case basis. It is therefore preferable to look at equations (1) and (2) as effective parametrizations of the dynamics of response for the system under consideration. Mathematical biologists have studied the quasi-steady-state assumption for a long time [9], for an isolated system or a parallel system [10–12], but without the two dephosphorylation reactions. The issue of the applicability of the quasi-steady-state approximation is of much more serious concern for later stages in vertical cascades than for the parallel first stage or stand-alone system investigated in this work. The reason is that in a vertical cascade the activated substrate of one stage serves as the kinase for the subsequent stage. For the latter case steady-state approximations have been developed which go beyond the one considered here. These are reviewed and discussed in [13].

For some signaling events activation is a two-step process where a second phosphorylation follows the first one. In such a case equations (1) and (2) only apply in so far as they can be considered as providing an effective description of such a system.

2.2. *Sources of noise*

In this section we present the results of our analysis of noise around the steady state for two enzymatic reactions with shared kinase and the expression for the gain factor. Some of the details are deferred to the appendix. These expressions form the basis for the numerical results and their discussion and interpretation presented in the subsequent section. For the purpose of comparison we also summarize some results for a single reaction [5] in a subsection.

2.2.1. Shared kinase enzymatic reactions. We study the behavior of fluctuations δX^* and δY^* assuming [5] that the system has reached the steady state or the quasi-steady state, where the rate of time evolution is zero or small. In this state, the concentrations of $X^\star$ and $Y^\star$ assume values denoted respectively by $\bar{X}^\star$ and $\bar{Y}^\star$, which are solutions of equations (1) and (2) with the left-hand sides set equal to zero.

Small deviations around these steady-state values are defined by

$$X^\star(t) = \bar{X}^\star + \delta X^\star(t) \tag{11}$$

$$Y^\star(t) = \bar{Y}^\star + \delta Y^\star(t). \tag{12}$$

Fluctuations arise from two sources

(1) fluctuations in the amount of kinase E^t which activates X and Y.
(2) fluctuations due to the random nature of the two chemical reactions.

The latter can be taken into account through additive noise terms $\eta_1(t)$ and $\eta_2(t)$ in respectively equations (1) and (2) if the number of molecules is large. These uncorrelated noise terms are of 'white noise' type and characterized by the following conditions on their average and correlation, once it is assumed that the processes of equations (1) and (2) can be approximated by Poisson distributions [5]

$$\langle \eta_1(t) \rangle = \langle \eta_2(t) \rangle = 0 \tag{13}$$

$$\langle \eta_1(t)\eta_1(t') \rangle = (\bar{Q}_+ \bar{X} + \bar{Q}_- \bar{X}^\star)\delta(t - t') \tag{14}$$

$$\langle \eta_2(t)\eta_2(t') \rangle = (\bar{R}_+ \bar{Y} + \bar{R}_- \bar{Y}^\star)\delta(t - t'), \tag{15}$$

where for fluctuations around the steady state, $Q_{+,-}$ and $R_{+,-}$ (see equations (3)–(6)) depend on the steady-state values of concentrations, and are denoted by $\bar{Q}_{+,-}$ and $\bar{R}_{+,-}$. $\delta(t)$ denotes the Dirac delta function.

The coupled equations for $\delta X^\star$ and $\delta Y^\star$ are derived by inserting expressions (11) and (12) into equations (1) and (2) and doing an expansion to first order in the small quantities $\delta X^\star$ and $\delta Y^\star$. One finds

$$\mathrm{d}\delta X^\star/\mathrm{d}t = -{\tau_1}^{-1}(\delta X^\star - g_1 \delta E^t - \tilde{g}_1 \delta Y^\star) + \eta_1 \tag{16}$$

$$\mathrm{d}\delta Y^\star/\mathrm{d}t = -{\tau_2}^{-1}(\delta Y^\star - g_2 \delta E^t - \tilde{g}_2 \delta X^\star) + \eta_2, \tag{17}$$

where τ_1 and τ_2 characterize the response time to fluctuations, with

$$\begin{aligned}{\tau_1}^{-1} = {} & \bar{Q}_+\big(1 + K_2^{-1}\bar{Y}\big)\big/\big(1 + K_1^{-1}\bar{X} + K_2^{-1}\bar{Y}\big) \\ & + \bar{Q}_-\big/\big(1 + \bar{K}_1^{-1}\bar{X}^\star\big)\end{aligned} \tag{18}$$

$$\begin{aligned}{\tau_2}^{-1} = {} & \bar{R}_+\big(1 + K_1^{-1}\bar{X}\big)\big/\big(1 + K_1^{-1}\bar{X} + K_2^{-1}\bar{Y}\big) \\ & + \bar{R}_-\big/\big(1 + \bar{K}_2^{-1}\bar{Y}^\star\big).\end{aligned} \tag{19}$$

The mean value of Y occurs in the first terms since the enzyme is shared and not in the second since the two phosphatases are different. The behavior of the above time constants as a function of the total enzyme concentration is important in determining different regimes of behavior and will be discussed subsequently. Moreover

$$g_1 = \tau_1 \bar{Q}_+ \bar{X}/\bar{E}^t \tag{20}$$

$$g_2 = \tau_2 \bar{R}_+ \bar{Y}/\bar{E}^t \tag{21}$$

and

$$\tilde{g_1} = \tau_1 K_2^{-1} \bar{Q}_+ \bar{X}\big/\big(1 + K_1^{-1}\bar{X} + K_2^{-1}\bar{Y}\big) \tag{22}$$

$$\tilde{g_2} = \tau_2 K_1^{-1} \bar{R}_+ \bar{Y}\big/\big(1 + K_1^{-1}\bar{X} + K_2^{-1}\bar{Y}\big). \tag{23}$$

The values of all of these quantities are determined by the steady-state concentrations of molecules, and the rate and Michaelis–Menten constants. We should note here that though equations (1)–(2) involve concentrations, equations (16)–(17) for the fluctuations should be read in terms of numbers of molecules, which are related to concentrations through cell volume.

The key expressions for the different contributions to the noise variance can be obtained from equations (16) and (17) as follows: these equations are linear in $\delta X^\star$ and $\delta Y^\star$ and can be solved by going to Fourier space. This analysis leads to an expression of the total variance $\langle \delta X^{\star 2} \rangle$, which is the sum of three terms N_1^E, N_1^1, N_1^2 , due respectively to fluctuations in δE^t, and the intrinsic noise terms η_1 and η_2. The complete expressions which form the basis for the numerical results discussed in the paper and the details of the calculation are presented in appendix A.

Gain factor $G_1(E^t)$. An important quantity characterizing the behavior of the system is the gain factor $G_1 = \mathrm{d}X^*/\mathrm{d}E^t$ which measures the response $\mathrm{d}X^*$, the change in the phosphorylated substrate, to a small change $\mathrm{d}E^t$ in the input enzyme concentration away from the steady state. For the parallel system considered, its expression can be derived from equations (16) and (17) by setting their left-hand sides equal to zero, and averaging over intrinsic noise. One thus finds for the gain factor G_1 of the enzymatic reaction $X \rightleftharpoons X^*$

$$G_1(E^t) = \frac{g_1 + \tilde{g_1} g_2}{1 - \tilde{g_1} \tilde{g_2}}. \tag{24}$$

The different g and $\tilde{g}$ on the right-hand side are given in (20)–(23). The denominator in the expression of G_1 is positive since it ensures that the system when perturbed away from the steady state decays back to it exponentially fast. We will discuss the behavior of the gain as a function of the total enzyme concentration and correlate it with that of the extrinsic noise and time scales in the results section. We note that for a parallel system, in contrast a single enzymatic reaction [5, 6] (see equations (28)–(29) below), a gain factor and extrinsic noise are not simply related except in the case where τ is much larger than τ_1 and τ_2.

The gain factor provides one important example of what can be determined by the general approach of perturbing cellular proteins away from the steady state, such as by an excursion $\mathrm{d}E^t$, and measuring the resultant change $\mathrm{d}X^*$. This approach provides a practical method for discovering network connections [14, 15]: it was used to great effect by Santos *et al* [16] for uncovering feedback in a MAPK kinase cascade in rat PC-12 cells stimulated by epidermal or neuronal growth factors.

Time constants. One way of characterizing the different regimes of behaviors of noise and gain is by the relative time scales of the relaxation to the steady state of different fluctuations. There are three time constants here, τ, the time scale of the fluctuations in E^t, and the bare, relaxation times τ_1 and τ_2. The effective relaxation times that occur in the exponential decay of X^* and Y^* can be obtained and are given in appendix A. It is more convenient to discuss the behavior in terms of τ, τ_1 and τ_2. $N_1^E/\langle \delta E^{t\,2} \rangle$ depends on all three time scales. There are two limiting cases of interest, one where $\tau \gg \tau_1$ and $\tau \gg \tau_2$, and a second one where $\tau \ll \tau_1$ and $\tau \ll \tau_2$. We discuss these in turn:

(a) $\tau \gg \tau_1$ *and* $\tau \gg \tau_2$. In this case, the cell relaxes to the steady state over much shorter time scales than that of the fluctuations of the enzyme concentration, τ. The system relaxes 'instantaneously' to the steady-state value corresponding to a change in E^t and thus $\delta X^* = G_1 \delta E^t$; thus the variance is directly connected to the gain factor of the steady-state response

$$N_1^E/\langle \delta E^{t\,2} \rangle \simeq (G_1)^2 \tag{25}$$

where the expression of G_1 is given in (24). This follows directly in this limit from the general expression (A.5). Any explicit reference to τ, τ_1, τ_2 has disappeared.

(b) $\tau \ll \tau_1$ *and* $\tau \ll \tau_2$. Here the input concentration fluctuates over time scales much shorter than those characterizing the time response of each cascade stage. In this case one finds that

$$N_1^E \simeq \frac{\tau}{\tau_1} \langle \delta E^{t\,2} \rangle \left[\frac{(g_1 + \tilde{g_1} g_2)^2}{(1 - \tilde{g_1} \tilde{g_2})} \frac{1}{1 + \tau_2/\tau_1} + \frac{g_1^2}{1 + \tau_1/\tau_2} \right]. \tag{26}$$

The contribution of input noise is now very small since it is multiplied by the small number τ/τ_1. The short time fluctuations in kinase number are averaged out by the much longer relaxation time. The system acts as a low-pass filter cutting out frequencies larger than $1/\tau_1$.

The above limits are applicable even when the difference between τ and τ_1 is only a factor of two or three. Thus we have checked that the noise $N_1^E/\langle \delta E^{t\,2} \rangle$ decreases by a factor of 2.5 when τ goes from 100 s to 20 s for the reaction rate values of section 5.1, which corresponds to case 1,1 in figure 3. For these values τ_1 varies over a narrow range in E^t with a maximum of 45 s (see figure 3). Thus $\tau = 100$ s is larger than τ_1 by at least a factor of 2 over the whole range of kinase values, whereas $\tau = 20$ s is below τ_1 over the region in E^t where the latter varies significantly. As expected from the consideration of the two limits above, the noise is smaller when $\tau = 20$ s ($\tau < \tau_1$), whereas—as we have checked as well—G_1^2 provides a good description of noise when $\tau = 100$ s ($\tau > \tau_1$).

2.2.2. Single enzymatic reaction. For comparison with the parallel system, we now give for a single enzymatic reaction (see figure 1(*b*)), studied by Detwiler *et al* [5] the expressions of relaxation time τ_{1s}, of the fluctuations N_{1s}^E of X^* due to kinase fluctuations and of the gain factor G_{1s} (for a single enzymatic reaction $X \rightleftharpoons X^*$, the relevant equation is equation (1) with the Y term in the denominator of Q_+ deleted).

$$\begin{aligned} {\tau_{1s}}^{-1} &= k_3 E^t \big/ \left[K_1 \left(1 + K_1^{-1} \bar{X} \right)^2 \right] \\ &\quad + k_6 {P_1}^t \big/ \left[\bar{K_1} \left(1 + \bar{K_1}^{-1} \bar{X^\star} \right)^2 \right] \end{aligned} \tag{27}$$

$$N_{1s}^E \big/ \langle \delta E^{t\,2} \rangle = g_1^2/(1 + \tau_1/\tau). \tag{28}$$

In the case of the single enzymatic reaction the steady-state gain G_{1s} is identical to g_1 (in contrast to the parallel cascade case) and is given by

$$G_{1s}^{-1} = E^t \left[\frac{1}{\bar{X} \left(1 + K_1^{-1} \bar{X} \right)} + \frac{1}{\bar{X^\star} \left(1 + \bar{K}_1^{-1} \bar{X^\star} \right)} \right]. \tag{29}$$

The method for deriving the single reaction expressions (34)–(36) is identical to the method used above for the parallel system. If the above expression of τ_{1s} is used, G_{1s} assumes the form $G_{1s} = \tau_{1s} k_3 \bar{X}/(K_1 + \bar{X})$.

3. Results and discussion

3.1. Reaction rate values

In this section we illustrate the general results presented in the previous section and the appendix with numerical calculations in some realistic cases. We solved the nonlinear equations that determine the steady-state concentrations and evaluated the analytic expressions using Mathematica [17]. In some cases, we simulated the effective Michaelis–Menten equations using the Gillespie algorithm in order to check the validity of the linear noise approximation. We draw inferences of a general nature about the distribution of input noise between two parallel systems.

In order to have a consistent set of parameter values for our parallel system, we use the work of Fujioka *et al* [18], where the rate constants for a Ras/ERK MAPK cascade were determined with fluorescent probes, and compared with a number of results from other groups. For system X (see figure 1) we take from [18] as kinase associated parameters those of the early stage, the phosphorylation of MEK by the complex Ras.Raf, and for system Y those of the phosphorylation of ERK by phosphorylated MEK. The phosphorylation values are for system X (see reactions (7))

$$\begin{aligned} k_1 &= 0.65\ \mathrm{s}^{-1}\mu\ \mathrm{M}^{-1}; \\ k_2 &= 0.065\ \mathrm{s}^{-1}; \\ k_3 &= 0.18\ \mathrm{s}^{-1} \end{aligned} \tag{30}$$

and for system Y (see reactions (8))

$$\begin{aligned} a_1 &= 0.88\ \mathrm{s}^{-1}\ \mu\mathrm{M}^{-1}; \\ a_2 &= 0.088\ \mathrm{s}^{-1}; \\ a_3 &= 0.22\ \mathrm{s}^{-1}. \end{aligned} \tag{31}$$

The corresponding Michaelis–Menten constants are

$$K_1 = (k_2 + k_3)/k_1 = 0.38\ \mu\mathrm{M} \tag{32}$$

$$K_2 = (a_2 + a_3)/a_1 = 0.35\ \mu\mathrm{M}. \tag{33}$$

For the phosphatase reactions (see (9)–(10)), there is more of a disagreement about parameter values (see the tables in [18], supplementary material): the values of k_6 (or a_6) can vary by a factor of 100 depending on the phosphatase and the cell line. For simplicity we take the same values for the two phosphatases, namely [18]

$$k_6 = a_6 = 0.3\ \mathrm{s}^{-1} \tag{34}$$

and

$$\bar{K}_1 = \bar{K}_2 = 0.07\ \mu\mathrm{M}. \tag{35}$$

We now take the above eight parameter values, $k_3, k_6, K_1, \bar{K}_1$ for reaction X, and $a_3, a_6, K_2, \bar{K}_2$ for reaction Y as effective parameters for substrate phosphorylation and dephosphorylation according to equations (1)–(6).

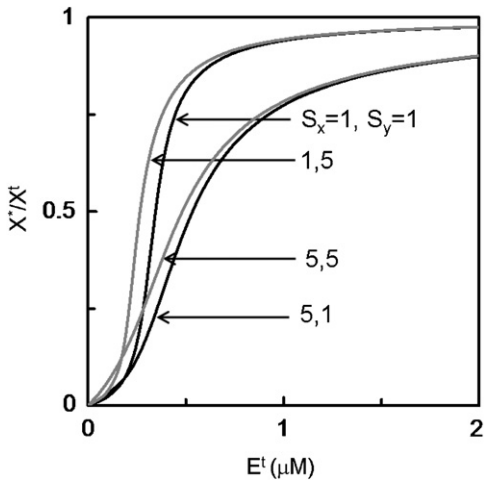

Figure 2. Dependence of the steady-state concentrations of X^*/X^t on the total enzyme concentration E^t for various combinations of the values of S_X and S_Y. S_X denotes the scaling factor for the Michaelis–Menten K_1, $\bar{K}_1$ constants of the phosphorylation of X and dephosphorylation of X^*. Thus $S_X = 1$ refers to the values of the Michaelis–Menten K_1 and $\bar{K}_1$ given in section 5.1. S_Y is a similar scaling factor for $Y \rightleftharpoons Y^*$.

For initial concentrations we use

$$\begin{aligned} X^t &= 1.4\ \mu\mathrm{M}; \\ Y^t &= 0.8\ \mu\mathrm{M}; \\ P_1^t &= P_2^t = 0.1\ \mu\mathrm{M}. \end{aligned} \tag{36}$$

3.2. Numerical calculations

We continue to focus our attention on system X of the shared kinase parallel system and investigate the behavior, the shape and magnitude, of the fluctuations of the phosphorylated substrate. We claim that the behavior can be understood from the shape of the steady-state response. Therefore, we first look at steady-state behavior as a function of E^t, and illustrate our claim with a discussion of figures 2 and 3. Thereafter we study the distribution of enzyme noise among the two phosphorylated substrates. We make the point that the choice of the operating point E^t can have a significant impact on the level of fluctuations in either activated substrate. These results should be relevant to the construction of synthetic biological systems.

3.2.1. Steady state and noise. In figure 2 we show the ratio of steady state phosphorylated X, namely $\bar{X}^*$, to the total substrate concentration X^t as E^t varies. We use the rate and Michaelis–Menten constants of section 5.1. Since response sensitivity depends greatly on the values of Michaelis–Menten constants [19], we also vary the latter. We call the corresponding scaling factor S_X. For example in figure 2 we consider the case ($S_X = 5$) where the two Michaelis–Menten constants K_1 and $\bar{K}_1$ of system X, given in section 5.1., are multiplied by 5. Similarly for reaction Y we have scaling factor S_Y. We note that the influence of system Y on system X is proportional to K_1/K_2 since this quantity in equation (3) measures the contribution of Y to the phosphorylation of X.

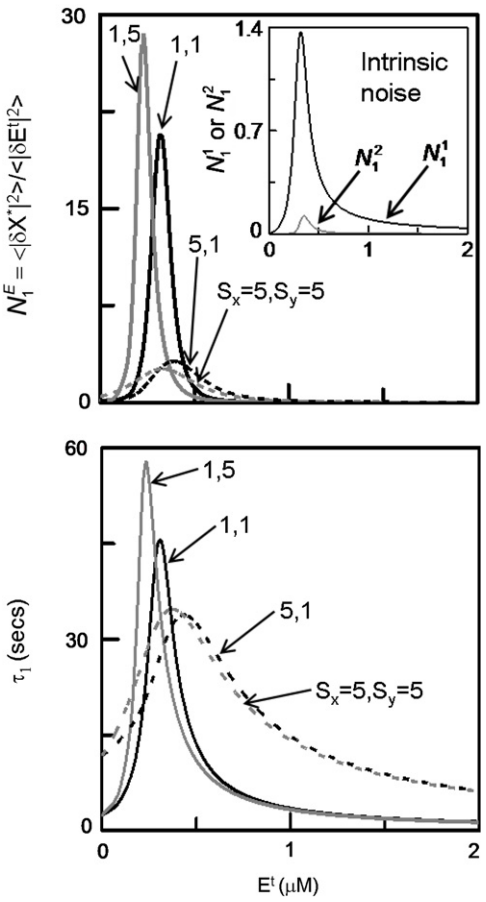

Figure 3. Dependence of (*a*) $N_1^E/\langle\delta E^{t2}\rangle$ (equation (A.5)) and (*b*) relaxation time τ_1 (equation (18)) on the total enzyme concentration E^t for various scaling factor (defined in figure 1 caption) S_X and S_Y combinations. Inset: dependence of intrinsic noises N_1^1, N_1^2 on the total enzyme concentration E^t for $S_X = S_Y = 1$. The results are shown for $\tau = 100$ s.

From figure 2 we learn that for the parameters above (relations (A.3)–(A.9)), where $S_X = S_Y = 1$, the response is abrupt, the fraction of $\bar{X}^\star$ going from a few percent to 80% over a very narrow range of E^t. The response becomes even more abrupt if the impact of the presence of system Y diminishes, as the comparison of $S_X = S_Y = 1$ with $S_X = 1$, $S_Y = 5$ shows. Conversely, the response of system X typically becomes more gradual when its Michaelis–Menten constants K_1 and $\bar{K}_1$ are larger as the comparison of cases $S_X = 5, S_Y = 1$ and $S_X = S_Y = 5$ with $S_X = S_Y = 1$ and $S_X = 1, S_Y = 5$ in figure 2 shows.

These features of the steady-state response determine the amplitude and shape of fluctuations and relaxation time for X^*. For the same range of scaling factors S_X and S_Y, consider now in figure 3 fluctuations $N_1^E/\langle\delta E^{t2}\rangle$ of X^* relative to those of E^t, and the corresponding relaxation time τ_1. For both the maximum occurs close to where the steady-state response, shown in figure 2, is steepest ($\bar{X}^*/X^t \approx 0.55$). Moreover the narrower fluctuation and relaxation time responses are in E^t and larger their amplitudes at maximum, the steeper the steady-state response. The comparison in figure 3 of, for example, the cases $S_X = S_Y = 1$ and $S_X = 5, S_Y = 1$ highlights these interesting facets of the connection between fluctuations

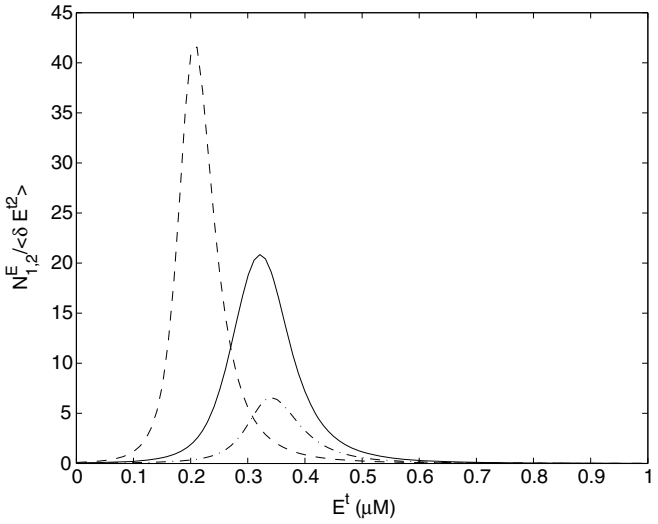

Figure 4. Comparison of noise, namely $N_1^E/\langle\delta E^{t2}\rangle$ (solid line) and $N_2^E/\langle\delta E^{t2}\rangle$ (dash-dotted line), as a function of the kinase E^t level, for the parallel system of two enzymatic reactions and the single reaction (dashed line)(equation (28)), as represented in figure 1. The rate and Michaelis–Menten constant values are those of section 5.1 ($S_X = S_Y = 1$) for both the parallel system and the single enzymatic reaction. The value of $\tau = 100$ s.

and the steady-state response [20, 21]. These [20, 21] point out that zero-order ultra-sensitivity is accompanied by long relaxation times and large random fluctuations; our choice of parameters (based on one set of experimental values) leads to sensitivity but not ultra-sensitivity. In particular, when the Michaelis–Menten constants K_1, $\bar{K}_1$ are multiplied by 5, noise is considerably reduced. Typical relaxation times (see figure 3) are of the order of a minute and less, which are comparable to dynamical time scales in MAPK cascades which are measured to be of the order of a few minutes [16, 22].

One can infer from the inset of figure 3 that for the values of rate and Michaelis–Menten constants and concentrations considered, for which gain factors are sizable, the contributions from noise intrinsic to the enzymatic reactions are generally small [6]. We will therefore concentrate on extrinsic, i.e. kinase-induced fluctuations in the following.

The choice of the operating point, i.e. the amount of E^t, of the parallel system of two coupled enzymatic reactions is thus crucial for regulating the amount of enzymatic noise. It determines both the size of fluctuations and the quickness of response to excursions from the steady state. For example, if one is in the saturating part of the steady-state response for the case $S_X = S_Y = 1$ for which $E^t > 0.5$ (see figure 2), fluctuations are practically non-existent, and relaxation takes only a few seconds (see figure 3).

The main point is that the steady-state response illuminates the salient aspects of fluctuations in X^*. We will focus next on the distribution of noise between the two enzymatic reactions activated by the same kinase.

3.2.2. Noise distribution. We start by comparing the level of noise for the two reactions of the parallel system with that of a single reaction for the parameters of section 5.1 for which $S_X = S_Y = 1$. The comparison in figure 4 is instructive.

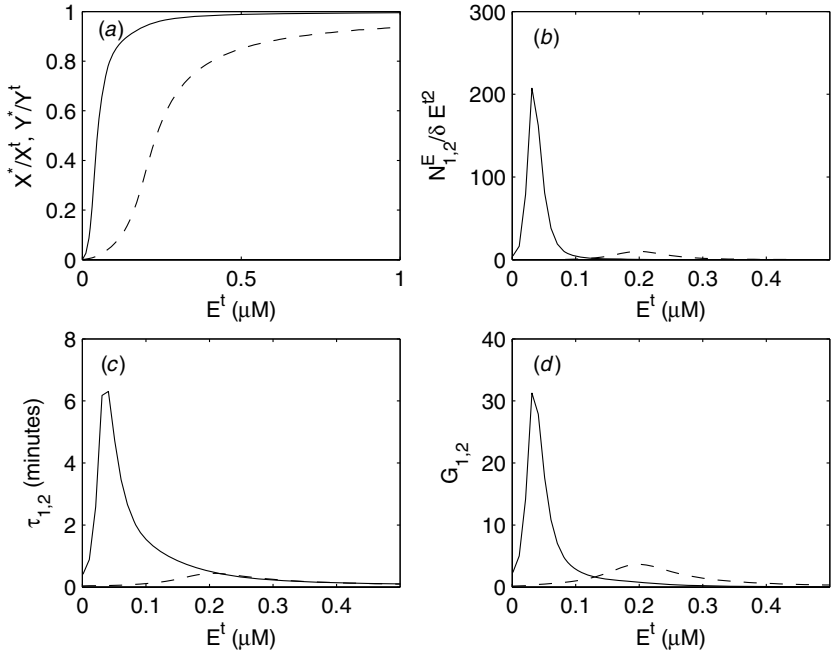

Figure 5. Comparison of reactions $X \rightleftharpoons X^*$ and $Y \rightleftharpoons Y^*$ of figure 1 for parameter values of section 5.1 ($S_X = S_Y = 1$) but with the dephosphorylation rate k_6 of X^* (see equation (4)) divided by ten. The solid line and subscript 1 refer to reaction X, the dashed line and subscript 2 to reaction Y. In figures 5(*a*)–(*d*) the x-axis represents the total amount of enzyme concentration. (*a*) Steady-state values X^* and Y^* normalized by their respective total amounts, (*b*) $N^E_{1,2}/\delta {E^t}^2$, fluctuations of phosphorylated substrates normalized by enzyme fluctuations, (*c*) relaxation times τ_1 and τ_2, and (*d*) gain factors G_1 and G_2. The value of $\tau = 100$ s.

Enzymatic reaction fluctuations for reaction X are, as expected, considerably higher when this reaction is single (dashed curve) than when it shares a kinase with a second one (solid curve). When a kinase is shared, its fluctuations are distributed among the two reactions, such that the fraction of kinase noise in any shared kinase reaction decreases relative to the single reaction case. The other point worth mentioning is that fluctuations in X^* can be (for steep steady-state responses) many times larger than kinase fluctuations. This amplification is the result of sizable gain factors [6]. Gain factors play as well a role in the propagation of noise down a signaling cascade [7, 5]. But the situation here is more interesting than in the single reaction case, since the original amount of kinase fluctuations now breaks up into two components of unequal weight (see figure 4), which entails very different fluctuations downstream from each reaction. In figure 4 the fluctuations in Y^* are much smaller than those in X^*, despite the fact that the reaction rate and Michaelis–Menten constants for both reactions are close (see section 5.1). The reason is that the total substrate concentrations differ, namely $X^t = 1.4\mu$ M and $Y^t = 0.8\ \mu$M. Thus for two enzymatic reactions with dynamics determined by similar Michaelis–Menten constants, it is the relative size of total substrate concentrations which determines how kinase fluctuations are distributed between the two. Fluctuations are several times larger for the reaction with higher substrate values. As in figure 3 fluctuations in figure 4 are maximum at a value of E^t which corresponds to the middle of the fast rising part of the steady-state response in figure 2. The maximum for the parallel system occurs at a larger value of the enzyme concentration than for the single enzyme system since the enzymes are shared between the two systems and this makes the steady-state response of X somewhat less steep.

Let us now ask what would happen if the relaxation time τ_1 of reaction X were slower by a factor of say ten. This situation is realized when dephosphorylation slows down, i.e. rate k_6 in equation (4) decreases. As the discussion in section 5.1. of the value of k_6 makes clear, this is a possible occurrence. The same result could be achieved through a smaller total phosphatase ${P_1}^t$ concentration (see equation (4)), since ${\tau_1}^{-1} \sim k_6 {\bar{K}_1}^{-1} P_1^t$ (see equation (18)). When k_6 is reduced, $X^\star$ reaches its steady state much faster, and saturation as a function of E^t obtained for much smaller values of the kinase. This is clear from comparing $\bar{X}^*(E^t)$ of figure 1 for $S_X = S_Y = 1$ with that in figure 5(*a*), where the values of rate and Michaelis–Menten constants are the same, except for the reduction by a factor of 10 of k_6. Figure 5(*a*) shows as well the steady-state response of $\bar{Y}^*(E^t)$ (dashed line), which is less steep than that of $\bar{X}^*$ (solid line). We have already learned that from the steady-state behavior one can guess how fluctuations and relaxation times vary as a function of E^t. This is confirmed from the shapes and magnitudes of the fluctuations of X^* and Y^* relative to kinase noise in figure 5(*b*) and from the corresponding relaxation times in figure 5(*c*). Fluctuations in X^* (solid line) are much larger, over a narrower range of E^t, centered at smaller E^t, than those of Y^* (dashed line), reflecting the difference in steepness of the respective steady-state responses of figure 5(*a*). As expected, for smaller k_6, relaxation times $\tau_1(E^t)$ for reaction X can now reach values of the order of many minutes. Large relaxation times entail large gain factors, as is clear from expression (24) and the fact that $g_1, \tilde{g_1}$ are

proportional to τ_1 (equations (20), (22)), and illustrated in figure 5(*d*) (solid curve).

What is clear from figure 5 is how the choice of an operating point impacts on the amount of fluctuation in the two phosphorylated substrates. By increasing E^t from zero, figures 5(*b*) shows that one encounters first fluctuations of that phosphorylated substrate which has the steepest steady-state response, which is X^* in figure 5(*a*) (solid curve). The range in kinase concentration of these fluctuations is typically narrow. Thus one goes rapidly from a region of large fluctuations to an operating point where fluctuations in both reactions are small, to a region of E^t where fluctuations in the steepest system are very small and those of the less steep system are important. We know however from our previous considerations that the latter fluctuations are smaller and sometimes much smaller (see figure 5(*b*)) than the former. Finally at large E^t where the steady-state responses saturate, fluctuations in either phosphorylated substrate are gone. Enzymatic fluctuations are not transmitted by either reaction.

From the discussion of figure 5 it is clear that the choice of operating point determines to what extent enzymatic noise propagates through what can be the first stage of signaling cascades activated by a common enzyme. The operating point is set by the cellular machinery in response to a stimulus, and depending on its value there exist several options for enhancing or mitigating enzymatic noise in one or the other or both enzymatic reactions.

If cellular behavior is studied at a single operating point rather than over a range of kinase values, how can one judge what situation avails for the parallel system of figure 1? As can be seen from figure 5, the behavior of gain factors mimics that of fluctuations. However, while fluctuations entail averages over single cells, gain factors can be measured for a population of cells. In [16] siRNA were used selectively to move certain kinases away from steady state and measure the resulting changes in others through western blot assays. Imagine then that for a given operating point, both gain factors are small, small meaning closer to 1 (locally linear steady-state response) than to 10. This entails in all cases that fluctuations in X^* and Y^* are small and relaxation times fast. It also means that in terms of the steady-state response the operating point corresponds to saturation, or else that responses to changes in enzyme concentration are very gradual for both enzymatic reactions. With similar reasoning in other situations, much can be inferred, simply measuring the gain at some operating point, about how common enzymatic fluctuations impact on the two phosphorylation reactions that can initiate two separate signaling pathways.

4. Conclusion and outlook

Signaling pathways sharing a common kinase frequently occur in the topology of cellular networks. We have developed here the formalism for the shared kinase fluctuations around the steady state among two enzymatic reactions. We imagine the kinase in question to be at the first level of activation of cellular components once a cell is stimulated. Since for each cell early kinase fluctuations can propagate through signaling pathways down to the activation of transcription factors, our formalism for the early stage of shared kinase-induced fluctuations should be helpful in designing synthetic biology modules for cell-to-cell variability. The general formalism applies to single cell noise. As techniques for single cell measurements improve, one can envisage experiments, such as those on relaxation toward the steady state, through which one could measure time constants and also disentangle intrinsic noise and the extrinsic noise due to kinase fluctuations. The prospect of actually measuring a quantity such as the relaxation time τ_1 appears nevertheless remote, although recently single enzyme fluctuations have been measured [23, 24] that enable one to determine kinase rate and Michaelis–Menten constants [23], or the statistics of bursts [24].

Our work illustrates the wide range of possible behaviors available to a cell as its steady-state operating point shifts according to, for example, the type and the amount of a stimulus. We have highlighted the fact that a knowledge of steady-state responses gives insight into many aspects of phosphorylated substrate fluctuations, such as their magnitude and the range of kinase concentrations over which they are important. But even when the biological system is studied at one operating point only, we have shown that the measurement of gain factors across the cell population at this single point gives useful indications of how much of the shared kinase noise gets transmitted through the two phosphorylation reactions, and down which one of the two pathways. What about the role of feedback? In the work of Santos *et al* [16] the existence of a feedback loop, negative or positive according to the type of stimulus, in a MAPK kinase cascade was established. Imagine then that for some stimulus the situation is that described in figure 5, but for another stimulus a feedback loop gets activated that at steady state changes the amount of phosphatase in reaction X, all this without any change in the operating point. The change in phosphatase will entail a change in the steady-state response which will be steeper if phosphatase concentration decreases. Compared to the case of the first stimulus the result will be to displace for system X the curves for fluctuations, relaxation time and gain factor as a function of E^t (figure 5), and alter their relative position to the corresponding curves for reaction Y. The feedback loop can thus alter, if the operating point is the same, the balance of enzymatic fluctuations between the two reactions, a feature with implications for the functioning of real and synthetic systems.

Our numerical studies are based on a range of kinetic and Michaelis–Menten constants derived from MAPK kinase studies. For different situations or different ranges the systems would need to be re-examined with the help of the formalism provided.

Acknowledgments

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Appendix A. Calculation of the different noise contributions to the variance of X^*

Equations (16) and (17) which are linear in $\delta X^\star$ and $\delta Y^\star$ are solved by going to Fourier space. They lead to an expression of the total variance $\langle \delta X^{\star 2}\rangle$, which is the sum of three terms, originating from fluctuations δE^t, and η_1 and η_2, namely

$$\langle \delta X^{\star 2}\rangle = \int \frac{\mathrm{d}\omega}{2\pi}|g_1(\omega)|^2\langle|\delta E^t(\omega)|^2\rangle + (\bar{Q}_+\bar{X} + \bar{Q}_-\bar{X}^\star)\int \frac{\mathrm{d}\omega}{2\pi}|a_1(\omega)|^2 + (\bar{R}_+\bar{Y} + \bar{R}_-\bar{Y}^\star)\int \frac{\mathrm{d}\omega}{2\pi}|b_1(\omega)|^2. \quad (A.1)$$

The second term with its coefficient coming from (14) and the third one with its coefficient coming from (15) represent the contributions of intrinsic noise (due to randomness of the reactions) η_1 and η_2, respectively. The expressions in (24) of $g_1(\omega)$, $a_1(\omega)$ and $b_1(\omega)$ follow from the equations in Fourier space. They are

$$g_1(\omega) = (g_1 + \tilde{g}_1 g_2 + \mathrm{i}\omega\tau_2 g_1)/[1 - \tilde{g}_1\tilde{g}_2 + \mathrm{i}\omega(\tau_1+\tau_2) - \omega^2\tau_1\tau_2] \quad (A.2)$$

$$a_1(\omega) = (\mathrm{i}\omega\tau_1\tau_2 + \tau_1)/[1 - \tilde{g}_1\tilde{g}_2 + \mathrm{i}\omega(\tau_1+\tau_2) - \omega^2\tau_1\tau_2] \quad (A.3)$$

$$b_1(\omega) = \tau_2\tilde{g}_1/[1 - \tilde{g}_1\tilde{g}_2 + \mathrm{i}\omega(\tau_1+\tau_2) - \omega^2\tau_1\tau_2]. \quad (A.4)$$

These expressions show that the enzymatic reactions act as low-pass filters ($|g_1(\omega)|^2 \sim \omega^{-2}$ for large ω) on the input fluctuations in δE^t.

In order to compute $\langle \delta X^{\star 2}\rangle$ we need an expression for $\delta E^t(\omega)$ in the first term of (24). If kinase E itself is activated through a phosphorylation–dephosphorylation process with extrinsic noise, and relaxes back to the steady state with a time τ, one has $\langle|\delta E^t(\omega)|^2\rangle = 2\tau\langle\delta E^{t2}\rangle/(1+\omega^2\tau^2)$ [5]. One finds after performing the first integral on the right-hand side of (24) using expression (25) for $g_1(\omega)$

$$\frac{N_1^E}{\langle\delta E^{t2}\rangle} = \frac{1}{\left[1 + \frac{\tau^2}{\tau_1\tau_2}(1-\tilde{g}_1\tilde{g}_2)\right] + (\tau/\tau_1 + \tau/\tau_2)}(A_1 + B_1) \quad (A.5)$$

where

$$A_1 = \frac{(g_1+\tilde{g}_1 g_2)^2}{1-\tilde{g}_1\tilde{g}_2}\frac{\tau}{\tau_1+\tau_2}\left(1 + \frac{\tau}{\tau_1} + \frac{\tau}{\tau_2}\right) \quad (A.6)$$

$$B_1 = \frac{\tau\tau_2}{\tau_1(\tau_1+\tau_2)}g_1^2. \quad (A.7)$$

The subscript refers to system 1 of X and $X^\star$. N_1^E (equation (A.5)) represents the contribution to the fluctuations of $X^\star$ around its steady-state value due to fluctuations in the input kinase. The second and third term in (24), which we shall denote by N_1^1 and N_1^2, represent the contribution to $\langle\delta X^{*2}\rangle$ from fluctuations of respectively η_1 and η_2. These terms can be written in the following form:

$$N_1^1 = \frac{(\bar{Q}_+\bar{X} + \bar{Q}_-\bar{X}^\star)\tau_1}{2} + \frac{(\bar{Q}_+\bar{X} + \bar{Q}_-\bar{X}^\star)\tau_1}{2}\left(\frac{\frac{1}{\tau_2}}{\frac{1}{\tau_1}+\frac{1}{\tau_2}}\right)\left(\frac{\tilde{g}_1\tilde{g}_2}{1-\tilde{g}_1\tilde{g}_2}\right) \quad (A.8)$$

$$N_1^2 = \frac{(\bar{R}_+\bar{Y} + \bar{R}_-\bar{Y}^\star)\tau_2}{2}\left(\frac{\frac{1}{\tau_1}}{\frac{1}{\tau_1}+\frac{1}{\tau_2}}\right)\left(\frac{\tilde{g}_1{}^2}{1-\tilde{g}_1\tilde{g}_2}\right). \quad (A.9)$$

The expressions can be interpreted following Paulsson [25]. The first term in N_1^1 is the variance $\langle(\delta X^*)^2\rangle$ in the non-interacting case and is just the Einstein fluctuation dissipation result. The second term arises due to the coupling resulting from the sharing of the enzyme by the two reactions. In this term the free variance is multiplied by the second factor which arises from time averaging and a third factor that corresponds to an interaction coefficient. Analogous interpretations apply to the result for N_1^2. Fluctuations $\langle\delta Y^{\star 2}\rangle$ are similar to those of $\langle\delta X^{\star 2}\rangle$, and are given by similar expressions with subscripts 1 and 2 interchanged. For completeness we presently have the result for the covariance $\langle\delta X^*\delta Y^*\rangle$

$$\langle\delta X^*\delta Y^*\rangle = \frac{(\bar{Q}_+\bar{X} + \bar{Q}_-\bar{X}^\star)\tau_1}{2}\frac{\frac{1}{\tau_2}}{\frac{1}{\tau_1}+\frac{1}{\tau_2}}\frac{\tilde{g}_2}{1-\tilde{g}_1\tilde{g}_2} + \frac{(\bar{R}_+\bar{Y} + \bar{R}_-\bar{Y}^\star)\tau_2}{2}\frac{\frac{1}{\tau_1}}{\frac{1}{\tau_1}+\frac{1}{\tau_2}}\frac{\tilde{g}_1}{1-\tilde{g}_1\tilde{g}_2}. \quad (A.10)$$

The results for the intrinsic noise with delta-function correlations can be also easily obtained by employing a linear algebra formalism [26].

In the subsection on time constants we discussed the relative values of τ_1, τ_2 and τ. The time scales τ_1 and τ_2 appear explicitly in the rate equations and determine the relaxation rates of the uncoupled systems. The autocorrelation functions $\langle\delta X^*(t)\delta X^*(0)\rangle$, etc, of the coupled reactions can be computed explicitly and lead to a sum of exponential terms with effective relaxation (autocorrelation) times $\tau_\pm$. These can be obtained directly from the linearized equations or from the poles of $|a_1(\omega)|^2$. We give the expressions below

$$\tau_\pm^{-1} = \frac{1}{2}\left(\frac{1}{\tau_1}+\frac{1}{\tau_2} \pm \sqrt{\left(\frac{1}{\tau_1}-\frac{1}{\tau_2}\right)^2 + 4\frac{\tilde{g}_1\tilde{g}_2}{\tau_1\tau_2}}\right). \quad (A.11)$$

The values of $\tau_\pm$ are of the order of τ_1 and τ_2; τ_- is larger than τ_1 and $\tau_+ < \tau_2$ for $\tau_1 > \tau_2$.

Glossary

Kinase. Enzyme that catalyses phosphorylation of a substrate protein.
Phosphatase. Enzyme that catalyses dephosphorylation of a substrate protein.

Gain factor. Measures the change in reponse of a system's output to a (small) change in its input.

Operating point. The amount of available kinase for substrate phosphorylation.

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