

Sensitivity summation theorems for stochastic biochemical reaction systems

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ABSTRACT

We investigate how stochastic reaction processes are affected by external perturbations. We describe an extension of the deterministic metabolic control analysis (MCA) to the stochastic regime. We introduce stochastic sensitivities for mean and covariance values of reactant concentrations and reaction fluxes and show that there exist MCA-like summation theorems among these sensitivities. The summation theorems for flux variances is shown to depend on the size of the measurement time window (ϵ) within which reaction events are counted for measuring a single flux. It is found that the degree of the ϵ -dependency can become significant for processes involving multi-time-scale dynamics and is estimated by introducing a new measure of time-scale separation. This ϵ -dependency is shown to be closely related to the power-law scaling observed in flux fluctuations in various complex networks.

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1. Introduction

Metabolic control analysis (MCA) [1–3] and the closely related biochemical systems theory [4,5] have greatly enhanced our ability to understand the dynamics of cellular networks. However, these approaches are based on a deterministic picture of cellular processes and in recent years it has become clear that many networks, such as gene regulatory networks, operate with a significant degree of stochasticity [6–11]. In these situations a deterministic formalism is inadequate [12–15]. In this paper we begin the process of developing a new analysis method of control on stochastic dynamics by extending MCA to the stochastic regime. We call the extension stochastic control analysis (SCA).

MCA is an analysis that quantifies how much system variables change in response to the perturbations in system parameters. To extend MCA to the stochastic regime, we need to introduce sensitivity measures for stochastic system variables. There have been a wide variety of efforts in recent years to introduce and investigate sensitivity measures for stochastic reaction systems related to mean levels of concentrations and fluxes [16,12,17–21]. More pertinent to this paper is the work by Andrea Rocco who investigated the MCA summation and connectivity theorems related to the most-probable concentration values and their corresponding reaction rates [22]. More recently, Bruggeman et al. investigated noise propagation in reaction systems to describe the propagation in connection with network structures [23]. They expressed concen-

tration variances by using a MCA sensitivity measure, local response coefficient [24–26]. However, in both the papers, the sensitivities for noise characteristics (variance, covariance, and the higher moments) were not investigated and the summation theorems related to the noise properties were not discussed. Thus, a systematic MCA-like approach for controlling noise has not been made.

In this paper we will focus on the control coefficients [1–3], for variances, covariances, and the higher moments of concentrations and fluxes. The control coefficients quantify the global responses from one steady state to another due to (static) perturbations in the system parameters. We also introduce sensitivities for the mean levels of concentrations and fluxes, which are closely related to the MCA control coefficients. We obtain MCA-like summation theorems for the stochastic sensitivity measures. In a similar way to the deterministic MCA theorems, the SCA theorems imply that control is distributed over a reaction system satisfying special balances among the stochastic sensitivity measures.

The summation theorems for flux variances show very interesting properties: flux is measured by counting the number of reaction events within a given time window ϵ and from this we show that the sum value can be highly dependent on the measurement time window (ϵ). This in turn implies that the control of flux variances can be sensitive to the value of ϵ . The degree of such ϵ -dependency of the sum value is closely related to how wide the distribution of reaction time-scales is. We provide a simple example of a two-step cascade reaction system and generalize it to a process involving a fast reaction step where its reaction rate is under slow stochastic fluctuations.

Our study of the summation theorems for flux variances also provides a dimensionless measure for time-scale separation in

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multi-time-scale reaction systems. More precisely, the measure estimates the separability between the processes of different time-scales. Recognizing time-scale separation can be used to simplify a given model [27–33,15]. How much are the time-scales required to be separated for such simplification? The proposed time-scale separation measure provides a qualitative answer for this. The separation measure is estimated from the temporal sequences of reaction events by estimating the variances of fluxes over the different size of the time window ϵ . We show that the separability is dependent on the strength of the fluctuations that is applied to the measured flux, and the correlation time of the fluctuations. It is also shown dependent on the mean level of the reaction rate and its sensitivity (elasticity in MCA [1]).

The summation theorems for flux variances also show a close connection to the scaling relationship between flux variances and their mean values recently observed in various complex networks: the Internet, microprocessor logic networks, the World Wide Web, highway systems, river networks, and stock market [34–38]. In these systems, fluxes were defined as the number of packets processed in network routers in the Internet, activity of connections between logic gates in the microprocessors, the number of visits on sites in the World Wide Web, the number of cars in traffic at different locations in the highway systems, stream flows in the river networks, and the traded values of stocks in the stock market. Studies have been done to investigate how the standard deviation (σ) of the flux is related to the mean value of the flux ($\langle f \rangle$): $\sigma \sim \langle f \rangle^\alpha$. De Menezes and Barabási [34] claimed that the Internet and microprocessor logic networks belong to a universality class characterized by an exponent value of $\alpha = 0.5$, and the World Wide Web, highway systems, and river networks to that of $\alpha = 1$. However, stock markets such as NYSE and NASDAQ show non-universal values of α [36,37]. Meloni et al. [38] investigated a model of random diffusion to show how the value of the exponent can crossover from 0.5 to 1. They proposed a scaling crossover function describing the change in the exponent α and showed that the function depends on the number of links connected to a node, the strength of external noise and the time measurement window size ϵ . In this paper, we show a connection between the summation theorems for flux variances and the scaling crossover phenomena. We briefly discuss that the exponent crossover can take two different forms depending on the time window size ϵ relative to the correlation time of the external noise.

2. Model systems and definitions of control coefficients

The model system we will consider is a chemical reaction system described by the chemical master equation [39,40], i.e., we assume the system is spatially homogeneous (uniform concentrations throughout the time evolution of the system). We assume that it can be described by L kinds of reaction rates for M reactants. The system is composed of external and internal processes. The external process is modeled by allowing one of the species (denoted by either S_e or S_1 , see Fig. 1A) to fluctuate slowly and independently, compared to the rest. S_e is considered a source of external noise. The internal system, composed of all other species, is affected by the external noise and also by internal noise generated from the internal reactions.

We will investigate how the system responds under a parameter perturbation from one steady state to another corresponding to before and after the perturbation, respectively (i.e. steady state response, not transient). To estimate the system response we introduce sensitivity measures called control coefficients [1–3]. The system variables (y) of interest can be either mean values or coefficients of variation/covariation (CV/CCV) of concentrations and reaction fluxes at the steady state. The CV is the variance divided by the mean square and CCV is the covariance (between two vari-

ables) divided by the product of their mean values. Our system variables y are defined as the moments of stochastic state variables such as chemical concentrations and the numbers of reaction events, so the system variables are not stochastic. We define the control coefficients for the system variables y as

$$C_p^y = \frac{p}{y} \frac{dy}{dp} = \frac{d \log y}{d \log p},$$

which indicates the relative change in y due to a given relative change in a parameter p . The change in y is from one steady state to another. We note that control coefficients for different system variables – most-probable concentrations (not mean concentrations) – have been investigated in the framework of MCA, but sensitivities related to fluctuation properties have not [22].

The parameter p will be called a control parameter, which is not affected by the system's response. We restrict the set of the control parameters ($\mathbf{p} = (p_1, \dots, p_L)$) to be the proportionality constants of reaction rates [2,41]. E.g., consider a reaction rate $v = \frac{ps}{K_M + s}$ with s concentration and K_M a Michaelis constant. The parameter p , representing the total enzyme concentration or k_{cat} , can be considered a control parameter. However, K_M is not unless the concentration s is small enough that the reaction rate becomes approximately linear in s . If we represent the enzymatic reaction further in more detail by considering enzyme–substrate binding–unbinding events, all the reaction rate constants appear linearly and could in principle be considered control parameters.

Although the choice limits our application of the theorems, it can still represent various types of control. In gene regulatory or metabolic systems, controlling such a parameter can correspond to changing gene dosage, or using different alleles that have different k_{cat} . However, some potential control parameters cannot be used, e.g., for the change in promoter strength in the gene regulatory systems because the strength is related to the dissociation constant between the promoter and its specific transcription factor.

3. SCA: summation theorems for control coefficients

We have found that there exist MCA-like summation theorems among the proposed stochastic sensitivities, which are valid under any strength of noise and finite perturbations of parameters \mathbf{p} . The existence of these theorems is rooted in the fact that the stochastic measures satisfy certain scaling properties under a specific kind of scale change in time and control parameters. We emphasize that our entire analysis is confined to the study of the steady state.

3.1. Summation theorems for concentrations

We note that the control parameters are chosen as the proportionality constants of reaction rates. Let us scale all control parameters by a fixed proportion α . Then the rate of each reaction changes by α : $v_i(s, \alpha \mathbf{p}) = \alpha v_i(s, \mathbf{p})$. This can be interpreted as a change in the time-scale by the amount $1/\alpha$ because the rate functions are inversely proportional to time.

Under the time-scale change, the probability distribution function of concentrations is invariant at the steady state. To understand this, consider a time evolution trajectory of a concentration at steady state. The scale change makes the trajectory compressed or stretched out in the time axis without affecting the concentration values (y -axis) [42–44]. Therefore, the concentration distribution at the steady state is independent of the time-scale change. This indicates that mean levels, CVs and CCVs of concentrations remain the same under the parameter change [43].

We can summarize these arguments with the following equation (refer to Table 1 for notation). The change in a concentration mean level from one steady state to another is expressed as

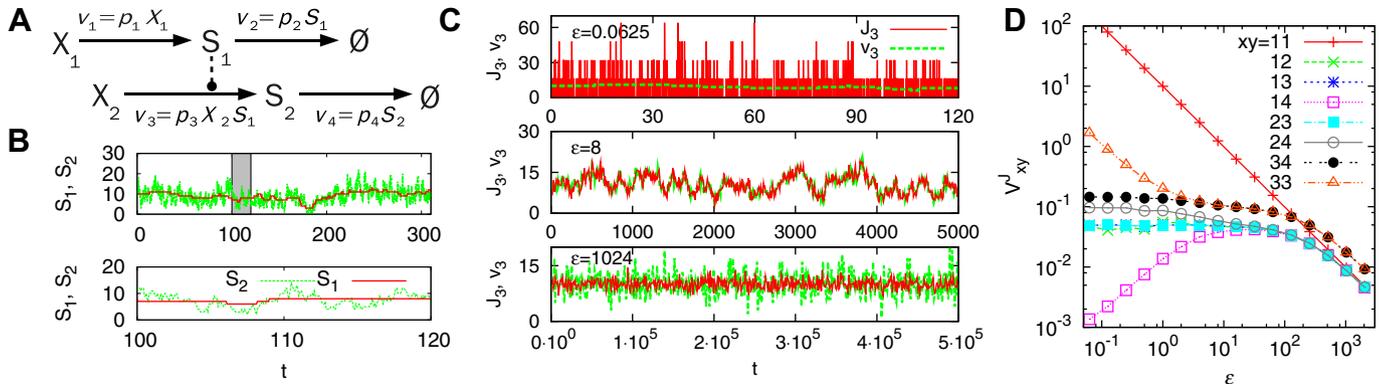


Fig. 1. Two-step cascade reaction system: S_1 up-regulates the reaction creating S_2 (A). The reaction rates involving S_1 are set 100 times slower than those involving S_2 . S_1 applies an external noise on the (internal) system of S_2 . Time evolution of S_1 and S_2 is shown (B). The region of $t = [100, 120]$ is expanded (B, bottom). The time evolution profile of S_2 follows the external noise with rapidly fluctuating internal noise (B, top). In the time-scale of the order of 1, S_2 does not fluctuate but S_1 fluctuates significantly, i.e., the internal noise becomes dominant (B, bottom). J_3 is measured with three different time window sizes, $\epsilon = 0.0625, 8, 1024$ (C). J_3 matches with v_3 for $\epsilon \approx 8$, because the internal noise is averaged out, i.e., the external noise is dominant in this time-scale (C, middle). Flux variance of J_3 decreases with the time window size ϵ (C, D). V_{33}^J shows a plateau, while V_{11}^J does not (D) (V_{22}^J overlaps with V_{11}^J , and V_{44}^J with V_{33}^J [not shown in graph]). The stochastic simulation algorithm [49] was used. Parameters: $(X_1, X_2, p_1, p_2, p_3, p_4) = (1, 1, 0.1, 0.01, 1, 1)$.

$$\frac{\delta \langle S_j \rangle}{\langle S_j \rangle} = \sum_i C_{p_i}^{(S_j)} \frac{\delta p_i}{p_i} = \sum_i C_{p_i}^{(S_j)} \frac{\alpha p_i - p_i}{p_i} = (\alpha - 1) \sum_i C_{p_i}^{(S_j)},$$

for all $j = 0, \dots, M$. Since the mean level at the steady state does not change under the scale change in the parameters: $\mathbf{p} \rightarrow \alpha \mathbf{p}$, we derive

$$\sum_{i=1}^L C_{p_i}^{(S_j)} = 0, \quad (3.1)$$

for all species j . The same argument can be applied for the concentration CVs and CCVs.

$$\sum_{i=1}^L C_{p_i}^{V_{jk}^S} = 0, \quad (3.2)$$

for all species j and k .

3.2. Summation theorems for fluxes

Before we derive the summation theorems for fluxes, it is important to clarify the difference among a propensity function, a reaction rate, and a reaction flux. All are stochastic variables. The reaction flux J is measured by counting the number of reaction events within a time window ϵ :

$$J_i = \frac{\text{Number of events of (i-th kind) reaction that occurs during } \epsilon}{\epsilon}.$$

The propensity function is a mathematical function previously denoted by v . The mean values of both v and J are equal at the steady state (refer to the Appendix). The fluctuation strengths of each can, however, be different because the variances of J are dependent on ϵ

(as will be discussed later), while those of v are not. To understand this, we consider the following process:



where α and γ are constants. X is produced at a constant rate and degrades with a lifetime of $1/\gamma$. Consider the degradation reaction at the steady state. The propensity function of the reaction is γX , where X is a stochastic variable indicating the number of molecules X at steady state. This stochastic variable X does not depend on the time window ϵ . The flux of the reaction is defined by counting the number of degradation events for a time duration ϵ (this is a counting (point) process) and dividing the number by ϵ . In the counting process, the count number is a stochastic variable dependent on the time duration ϵ , and thus the flux is also dependent on ϵ . Therefore, we express the CV/CCV of J as a function of ϵ : $V^J(\epsilon, \mathbf{p})$. We use the term, reaction rate, as either the flux or propensity function, depending on context.

To derive the summation theorems for mean fluxes, we consider that reaction systems are at steady states and undergo the same parameter scale change as before. Under this change, mean rate functions $\langle v_i \rangle$ will scale by α . Since the mean propensity function is equal to the mean fluxes $\langle J_i \rangle$ at the steady state, the mean fluxes will also scale by α . The relative change is given as

$$\frac{\delta \langle J_i \rangle}{\langle J_i \rangle} = \frac{\alpha \langle J_i \rangle - \langle J_i \rangle}{\langle J_i \rangle} = \alpha - 1.$$

Since the change in the mean flux can also be expressed as:

$$\frac{\delta \langle J_i \rangle}{\langle J_i \rangle} = \sum_{i=1}^L C_{p_i}^{(J_i)} \frac{\delta p_i}{p_i} = (\alpha - 1) \sum_i C_{p_i}^{(J_i)},$$

we obtain summation theorems for mean flux control coefficients:

$$\sum_{i=1}^L C_{p_i}^{(J_i)} = 1. \quad (3.3)$$

Now we will derive the summation theorems for flux CVs. We will use the same scaling argument as before: Scaling all parameters by α is equivalent to scaling the time by $1/\alpha$. Let us assume that the unit of V^J is $1/[\text{time}]^m$. Then under the time-scale change, V^J needs to be scaled by α^m and we obtain

$$V^J(\epsilon, \alpha \mathbf{p}) = \alpha^m V^J(\alpha \epsilon, \mathbf{p}).$$

Table 1
Notation.

$\langle f \rangle_{(x)}$	Ensemble average of f (over x) at a steady state
p	Control parameter
s	Concentration of a species S
S	Molecule number of a species S
v	Reaction propensity function
J	Reaction flux
V_{ij}	Coefficient of co-variation (CCV) between i and j
V_{jj}^J	Coefficient of variation (CV) of J
$\text{Var}(x)$	Variance of x

However, the flux CVs that are flux variances are unitless in time since $J/\langle J \rangle$ is unitless in time and its second moment is the flux CV. Therefore, m is equal to 0 and we obtain

$$V^j(\epsilon, \alpha \mathbf{p}) = V^j(\alpha \epsilon, \mathbf{p}). \quad (3.4)$$

By using the left hand side of Eq. (3.4), the relative change in V^j can be expressed as

$$\frac{\delta V^j}{V^j} = \frac{V^j(\epsilon, \alpha \mathbf{p}) - V^j(\epsilon, \mathbf{p})}{V^j(\epsilon, \mathbf{p})} = \sum_{i=1}^L C_{p_i}^{V^j} \frac{\delta p_i}{p_i} = (\alpha - 1) \sum_i C_{p_i}^{V^j}.$$

By using the right hand side of Eq. (3.4), the relative change can also be expressed as

$$\begin{aligned} \frac{\delta V^j}{V^j} &= \frac{V^j(\alpha \epsilon, \mathbf{p}) - V^j(\epsilon, \mathbf{p})}{V^j(\epsilon, \mathbf{p})} = \left[\frac{V^j(\alpha \epsilon, \mathbf{p}) - V^j(\epsilon, \mathbf{p})}{V^j(\epsilon, \mathbf{p})} \frac{\epsilon}{\alpha \epsilon - \epsilon} \right] \frac{\alpha \epsilon - \epsilon}{\epsilon} \\ &= \frac{\epsilon}{V^j} \frac{\partial V^j}{\partial \epsilon} (\alpha - 1) = (\alpha - 1) \frac{\partial \log V^j}{\partial \log \epsilon}. \end{aligned}$$

Therefore, we obtain the summation theorem for V^j :

$$\sum_{i=1}^L C_{p_i}^{V^j} = \frac{\partial \log V^j}{\partial \log \epsilon}, \quad (3.5)$$

for all reactions j, k . This equation means that the sum value is equal to the slope of a log–log plot of flux CV and CCV vs. ϵ . Since the flux CV and CCV depend on ϵ , the sum value can also depend on ϵ .

The summation theorems derived above can be generalized to all the higher moments. At the steady state the probability distribution functions for the concentrations are invariant under the time-scale change that has been used for obtaining Eqs. (3.1) and (3.2). This means that all the higher moments of the concentrations are also invariant. Therefore, the sum values for the summation theorems for any concentration moments are equal to zero. For the flux summation theorems, the same argument that has been used for deriving Eq. (3.4) can be applied: $J/\langle J \rangle$ is unitless in time and any moments of this is also unitless. The same scale relationship as Eq. (3.4) holds for any moments. Therefore, the same summation theorem as Eq. (3.5) can be derived for the moments.

3.3. Summation theorems for flux CVs show ϵ -dependency

In this section, we will investigate in more detail the summation theorem for flux CVs, Eq. (3.5). We have found an interesting fact that the sum value in the theorem can vary significantly with the change in ϵ when the system shows wide distributions of reaction time-scales.

We consider a simple reaction system: a two-step reaction cascade as shown in Fig. 1A. S_1 is created with a rate v_1 and degrades with a rate v_2 . S_1 enhances the conversion of X_2 to S_2 (X_2 is fixed). We assume that the creation and degradation processes of S_1 are much slower than those of S_2 . S_1 is the source of external noise. The reaction process involving S_2 is considered an internal system. The time evolution trajectory of S_2 shows a mixture of two different kinds of noise (slow and fast) as shown in Fig. 1B.

We investigate how flux CVs depend on the time window size ϵ . We have plotted all internal and external flux CVs and CCVs vs. ϵ (Fig. 1D). The slope of the plotted graph can change significantly over different values of ϵ . Since the right hand side of Eq. (3.5) corresponds to the slope, the sum of the control coefficients for flux CVs and CCVs can change significantly. We have graphed the slope of the log–log plot of the CV of J_3 vs. ϵ (see Fig. 2). This slope is -1 for the small values of ϵ and rises near to 0 for the intermediate values of ϵ and comes down to -1 again for the large values of ϵ . The region for the sum value close to 0 becomes wider and the value becomes closer to 0, as p_2 decreases (Fig. 2). This means that

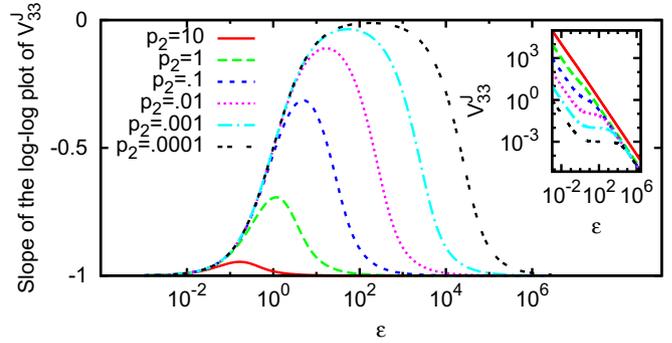


Fig. 2. Two-step cascade reaction system (Fig. 1A): the sum value of the control coefficients for the coefficient of variation of J_3 (V_{33}^j) is plotted for different values of ϵ and p_2 . The sum value corresponds to the slope of a log–log plot for V_{33}^j vs. ϵ (the inset graph). The exact analytic function for V_{33}^j , Eq. (3.6), is used.

the plateau region in the log–log plot becomes flatter and wider (the inset graph of Fig. 2). We have shown that the sum value can change significantly depending on the value of p_2 .

3.4. Mechanism for the ϵ -dependency

We will discuss the mechanism of the change in the sum value of Eq. (3.5). First, we discuss the flux CVs: V_{ii}^j . For the fluxes corresponding to the fast reactions ($i = 3, 4$), a plateau region appears, and for the fluxes corresponding to the slow reactions ($i = 1, 2$) they don't. The plateau region appears due to the fact that the internal fast noise sufficiently averages out, as the value of ϵ increases to reach the plateau region, while the external slow noise does not. This tendency is clearly shown in Fig. 1C and Fig. 3B.

The reason that the internal and external noise average out at different values of ϵ is as follows. First, the internal noise, caused by the stochastic events of the production of S_2 , averages out when ϵ is larger than the time duration taken for one molecule of S_1 to produce one molecule of S_2 : $\epsilon \gtrsim 1/p_3 X_2$. Second, the external noise averages out when ϵ is larger than the correlation time of S_1 ($1/p_2$), which corresponds to the lifetime of S_2 : $\epsilon \gtrsim 1/p_2$. Since the external noise fluctuates much more slowly than the internal noise, $1/p_2$ is much larger than $1/p_3 X_2$. Within the region of the value of ϵ : $1/X_2 p_3 \lesssim \epsilon \lesssim 1/p_2$, the internal noise becomes averaged out while the external noise does not, and the flux J_3 fluctuates only due to the external noise originated from S_1 . Therefore, the approximate equality $J_3 \simeq v_3$ holds for the region of the value of ϵ . In Fig. 3B, we have used $1/X_2 p_3 = 1$ and $1/p_2 = 100$, and these values roughly correspond to the beginning and end of the plateau region. By using $J_3 \simeq v_3 = p_3 X_2 S_1$, we can estimate the value of the flux CV at the plateau region: $V_{33}^j \simeq V_{33}^v = V_{11}^S = 1/\langle S_1 \rangle = 0.1$. If the plateau region is wide enough, V_{33}^j can be equal to $1/\langle S_1 \rangle$ for most of the region, and the slope of V^j becomes close to zero, which means that the sum value of the flux CV is also close to zero.

For $\epsilon \ll \tau(=1/p_2)$, S_1 does not fluctuate compared to S_2 in this time-scale (Fig. 1B). S_2 can be considered to be synthesized with a constant rate, v_3 . The probability $P(n; \epsilon)$ of having the number n of events of reaction v_3 during time ϵ satisfies a Poisson distribution:

$$P(n; \epsilon) = e^{-v_3 \epsilon} \frac{(v_3 \epsilon)^n}{n!}.$$

One of the properties of the Poisson distribution is that the variance is equal to the mean. By using this property, the flux CV can be shown to become inversely proportional to ϵ (Fig. 3A):

$$V_{33}^j = \frac{\langle J_3^2 \rangle - \langle J_3 \rangle^2}{\langle J_3 \rangle^2} = \frac{\langle n^2 \rangle - \langle n \rangle^2}{\langle n \rangle^2} = \frac{1}{\langle n \rangle} = \frac{1}{\epsilon \langle J_3 \rangle}.$$

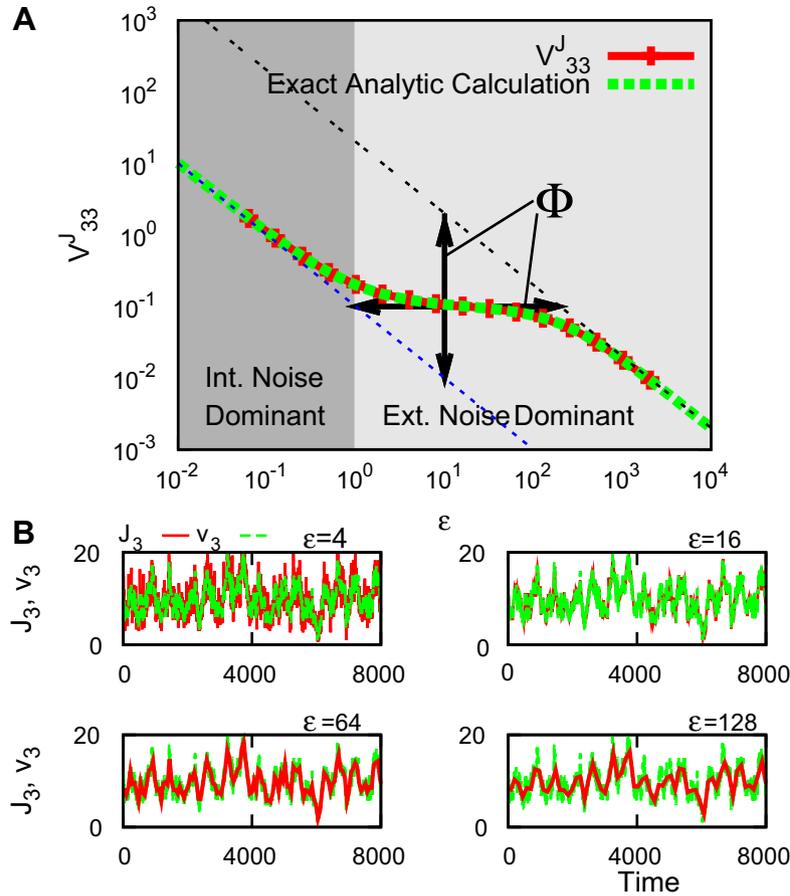


Fig. 3. Two-step cascade reaction system (Fig. 1A): the estimate of V_{33}^J from the simulations is compared with the exact analytic result (Eq. (3.6)) and its asymptotic forms corresponding to $\epsilon \ll \tau(=1/p_2)$ and $\epsilon \gg \tau$ (A). The two asymptotic lines have slope -1 and their vertical and horizontal separations (at the log–log scale) are same. The separation is denoted by Φ , the time-scale separation measure (Eq. (4.2)) (A). J_3 matches well with v_3 for the plateau region corresponding to the intermediate value of ϵ (B).

Thus, the sum value of the flux CV control coefficients is -1 for $\epsilon \ll \tau$.

Consider that ϵ is a couple of times the correlation time τ of the external noise S_1 . Let us assume that we estimate the mean values of $s_1(t)$ over the following time intervals: $[0, \epsilon]$, $[\epsilon, 2\epsilon]$, $[2\epsilon, 3\epsilon]$, \dots . The mean values become sufficiently independent in time because ϵ is larger than τ . This indicates that J_3 , estimated by using this ϵ value, also becomes independent in time. We denote the minimum of ϵ satisfying the independence by ϵ_{ind} . For the value of $\epsilon \gg \epsilon_{ind}$, the flux estimate J^ϵ can be considered an average of independent samples of $J^{\epsilon_{ind}}$ with a sample size ϵ/ϵ_{ind} . Therefore, $V^{J^\epsilon} = V^{J^{\epsilon_{ind}}} \frac{1}{\epsilon/\epsilon_{ind}} \propto \frac{1}{\epsilon}$. (From Fig. 3A, ϵ_{ind} is roughly 200.) This explains intuitively why the flux CV scales as $1/\epsilon$ for large ϵ values (Fig. 3A). Therefore, the sum value of the flux CV control coefficients is -1 for $\epsilon \gg \tau$.

For each different pair of fluxes, the asymptotic form of its coefficient of covariation for $\epsilon \ll \tau$, is different: either a plateau or a straight line proportional to ϵ (Fig. 1D). A detailed discussion on this is provided in the Appendix.

We can obtain the exact functional form of V_{33}^J (we refer to the Appendix for the derivation):

$$V_{33}^J = \frac{1}{\epsilon \langle J_3 \rangle} + \frac{2}{\langle S_1 \rangle} \frac{p_2 \epsilon - 1 + e^{-p_2 \epsilon}}{(p_2 \epsilon)^2}. \quad (3.6)$$

For $p_2 \epsilon \ll 1$, V_{33}^J can be approximated as

$$V_{33}^J \simeq \frac{1}{\epsilon \langle J_3 \rangle} + \frac{1}{\langle S_1 \rangle},$$

where we have used the following Taylor series expansion: $x - 1 + e^{-x} \simeq x - 1 + 1 - x + \frac{1}{2}x^2 = \frac{1}{2}x^2$. This equation explains both

-1 slope for the small value of ϵ and the crossover to a plateau region for the intermediate value of ϵ . For $p_2 \epsilon \gg 1$, the flux variance V_{33}^J becomes asymptotically

$$V_{33}^J \simeq \left(\frac{1}{\langle J_3 \rangle} + \frac{2}{\langle S_1 \rangle p_2} \right) \frac{1}{\epsilon},$$

showing that the slope for the large value of ϵ becomes again -1 . In this section, we have shown that when a flux shows a mixture of slow and fast fluctuations, the ϵ -dependency becomes significant due to the fact that the fast fluctuations average out first and the slow ones later as the value of ϵ increases.

3.5. ϵ -dependency in more general systems

The previous results on the ϵ -dependency can be generalized for more general reaction systems showing flux fluctuations with two different time-scale dynamics. A plateau region (for intermediate ϵ) and two regions of -1 slope (for small ϵ and large ϵ) can appear for CVs of such fluctuations.

We consider a reaction system with its rate function given by $\nu(s_e, s)$ (or, simply $\nu(s_e)$), showing a dependence on an external noise s_e and a substrate concentration s (see Fig. 4). The fluctuations in s are considered negligible. We assume that the fluctuations in s_e are confined to the linear region of $\nu(s_e)$. Such an assumption is widely used to estimate the approximate noise level of stochastic reaction processes, and is called the linear noise approximation [45].

Under these assumptions, the counting process of the reaction events can be approximately described by a doubly-stochastic

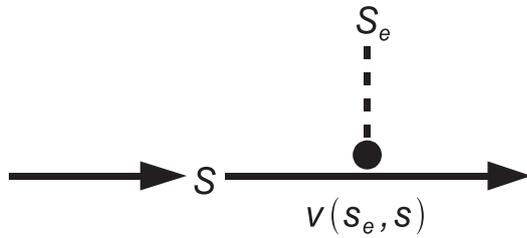


Fig. 4. A reaction step influenced by a slowly-fluctuating noise s_e . The propensity v is a function of an intermediate species s and an external regulator s_e .

Poisson process [46], and the following asymptotic functions for the flux CV of the reaction v can be obtained (refer to the Appendix for the derivation):

$$V^J \simeq \frac{1}{\epsilon \langle J \rangle} + \left(\frac{v'_{s_e}}{\langle J \rangle} \right)^2 \text{Var}(s_e), \quad (3.7)$$

for $\epsilon \ll \tau$, and

$$V^J \simeq \frac{1}{\epsilon \langle J \rangle} + \frac{2\tau}{\epsilon} \left(\frac{v'_{s_e}}{\langle J \rangle} \right)^2 \text{Var}(s_e), \quad (3.8)$$

for $\epsilon \gg \tau$, where

$$v'_{s_e} = \left. \frac{\partial v(s_e)}{\partial s_e} \right|_{s=(s), s_e=(s_e)}$$

Eqs. (3.7) and (3.8) show that V^J is inversely proportional to ϵ for $\epsilon \ll \tau$ and reaches a plateau region as ϵ increases. V^J becomes again inversely proportional to ϵ for $\epsilon \gg \tau$.

3.6. Mechanism for the ϵ -dependency revisited

We will provide more detailed explanations on the ϵ -dependency based on Eqs. (3.7) and (3.8). In Eq. (3.7), the first term corresponds to the contribution of the internal noise, and the second term to that of the external noise. The first term is due to a Poisson distribution in the flux that would be obtained without any external noise. The second term indicates the fluctuation strength in the rate function relative to the mean due to the external noise [23]. When the contribution of the external noise is larger than that of the internal one, the flux J becomes dominantly affected by the external noise and can be approximated to the propensity function v .

In Eq. (3.8), the first term is again the effect of the internal noise and the second that of the external. The only difference from Eq. (3.7) is the extra factor: $2\tau/\epsilon$. Why does this factor appear? As we have explained in the previous subsection, the flux J becomes independent in time when ϵ is roughly larger than the external noise correlation time τ . The minimum value of ϵ satisfying the independence was denoted by ϵ_{ind} . When ϵ is much larger than ϵ_{ind} , the external noise contribution to V^J is reduced by the number of independent samples averaged over ϵ/ϵ_{ind} . This indicates that ϵ_{ind} is 2τ from Eq. (3.8).

4. Estimation of time-scale separation

As presented previously, the plateau region in Fig. 3A appears due to the time-scale separation between fast and slow system dynamics. If the separation is not wide enough, the plateau region can be tilted (Fig. 2). In this case, the sum value of the flux CV control coefficients will deviate from zero in the region of the plateau (Fig. 2). To identify such deviations, we propose a measure for time-scale separation, or more precisely noise separability.

The separation measure (Φ) quantifies the horizontal (or vertical because the slope is -1) distance between the two asymptotic

linear lines for the log-log plot of flux CV vs. ϵ corresponding to $\epsilon \rightarrow 0$ and ∞ as shown in Fig. 3A. The larger the measure Φ , the wider the plateau region and the smaller its slope, i.e., the sum value of flux CV control coefficients becomes closer to zero.

Consider again the reaction system shown in Fig. 4. The asymptotic form of the flux CV for $\epsilon \ll \tau$ is

$$V^J = 1/\epsilon \langle J \rangle, \quad (4.1)$$

by keeping the dominant term only in Eq. (3.7). We obtain the vertical distance between the two asymptotic lines by taking the logarithms on Eqs. (3.8) and (4.1) and taking their difference, and propose the difference as the separation measure:

$$\Phi = \log \left[1 + 2\tau \frac{(v'_{s_e})^2 \text{Var}(s_e)}{\langle v \rangle} \right], \quad (4.2)$$

where we used $\langle J \rangle = \langle v \rangle$. The functional expression of Φ contains the variance and correlation time of s_e , and the local sensitivity of the rate v on s_e . The estimates of these quantities will roughly quantify the noise separability.

We have tested the dependency of Φ on the factors (τ , v'_{s_e} , $\text{Var}(s_e)$, and $\langle v \rangle$ in Eq. (4.2)) by considering the case of $v(s_e) = p_3 + \frac{p_4 s_e^n}{K_m + s_e^n}$. We have reduced Φ by perturbing one or more of the factors (dotted lines in Fig. 5). Among the performed perturbations, we note the perturbations in $\langle v \rangle$ (“ p_3 ” and “ p_4 ” in Fig. 5) affect Φ in a different way depending on whether v'_{s_e} changes or not. If p_3 is increased with the other parameters fixed, the mean flux $\langle v \rangle$ will increase without changing the sensitivity v'_{s_e} , and Φ is shown to decrease for this choice of control (Fig. 5 “ p_3 ”). However, if p_4 is increased while $p_3 = 0$, not only the mean flux but also its sensitivity increases and this leads to an increase in Φ (Fig. 5 “ p_4 ”) via the change in the sensitivity; when p_4 is increased by $x\%$, both the sensitivity and mean flux increase by $x\%$, and as a net effect Φ can increase for this choice of control.

For the two-step cascade reaction system as shown in Fig. 1A, the time-scale separation measure for this system can be exactly estimated, by using Eq. (3.6), as

$$\Phi = \log \left[1 + \frac{2 \langle J_3 \rangle}{\langle J_1 \rangle} \right].$$

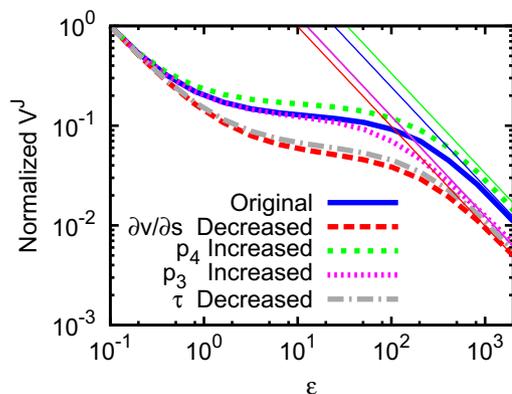


Fig. 5. Time-scale separation measure Φ (Eq. (4.2)) is verified with numerical simulations. External noise s_e , generated by $X_0 \xrightarrow{p_1 X_0} S_e \xrightarrow{p_2 S_e} \emptyset$, is applied onto a reaction: $v(s_e) = p_3 + \frac{p_4 s_e^n}{K_m + s_e^n}$. To estimate the CV of the reaction flux of $u(s_e)$, we obtained the trajectories of s_e by using the stochastic simulation algorithm [49], and then applied them to estimate $\int_0^\epsilon dt v(s_e(t))$ appearing in Eq. (B.2). We normalized V^J such that its normalized value for $\epsilon = 0.1$ equals 1 for ease of comparison. Φ given by Eq. (4.2) is shown to predict the separation well (thin solid lines: $\log_{10}(\text{Normalized})V^J = \Phi - 1 - \log_{10}(\epsilon)$). Parameters used: $X_0 = 1$ for all the cases, $(p_1, p_2, p_3, p_4, K_m, n) = (0.2, 0.01, 0, 100, 400, 2)$ for “Original”, $(0.2, 0.01, 0, 100, 20, 1)$ for “ $\partial v/\partial s$ ”, $(0.2, 0.01, 0, 200, 400, 2)$ for “ p_4 ”, $(0.2, 0.01, 100, 100, 400, 2)$ for “ p_3 ”, and $(0.4, 0.02, 0, 100, 400, 2)$ for “ τ ”.

Φ increases with the internal reaction flux (J_3): The time-scale separation gets larger as the internal dynamics becomes faster.

5. Power-law scaling in flux fluctuations

We will now briefly show how changes in the slope of the log-log plot of flux CV is related to power-law scaling that is observed in flow fluctuations in other complex networks [34–38]. In the scaling studies, it was investigated how the flux CV is related to mean flux (actually, rather than flux (J), the number of events (n) that occurs within ϵ was investigated). As shown previously, depending on how far the value of ϵ is away from the correlation time τ of the external noise, the scaling crossover takes the two different forms (Eq. (3.7) for $\epsilon \ll \tau$ and Eq. (3.8) for $\epsilon \gg \tau$). We propose here that the scaling crossover that appears in other complex networks can also depend on the interplay between the external noise correlation time (τ) and the flux measurement time window size (ϵ).

We consider the case that the reaction rate function $v(s_e)$ is linear with s_e :

$$v(s_e) = \alpha s_e.$$

From Eq. (3.7), we obtain the variance of the number of events that occurs within ϵ :

$$\begin{aligned} \text{Var}(n) &= V^J \langle n \rangle^2 = \langle n \rangle \left(1 + \epsilon \alpha^2 \frac{\text{Var}(s_e)}{\alpha \langle s_e \rangle} \right) \\ &= \langle n \rangle \left(1 + \langle n \rangle \frac{\text{Var}(s_e)}{\langle s_e \rangle^2} \right) \end{aligned} \quad (5.1)$$

for $\epsilon \ll \tau$, where we used $\langle n \rangle = \epsilon \langle J \rangle = \epsilon \alpha \langle s_e \rangle$. From Eq. (3.8), we obtain:

$$\text{Var}(n) = \langle n \rangle \left(1 + \frac{2\tau}{\epsilon} \langle n \rangle \frac{\text{Var}(s_e)}{\langle s_e \rangle^2} \right) \quad (5.2)$$

for $\epsilon \gg \tau$.

We note that only in the case for $\epsilon \ll \tau$, the variance of n , given as Eq. (5.1), shows the same crossover function as shown in Eq. (7) in [38]; the relative noise strength, δ^2/W^2 , appearing in Eq.(7) in [38], is expressed by $\text{Var}(s_e)/\langle s_e \rangle^2$ in Eq. (5.1). However, in the case for $\epsilon \gg \tau$, the variance of n takes a different functional form: Eq. (5.2) has an extra factor $2\tau/\epsilon$ in the crossover term. In this section, we have shown a connection between power-law scaling and flux fluctuations in reaction networks, and proposed the two different scaling crossover functions for the different values of the time window size ϵ relative to the external noise correlation time τ .

6. Conclusion

In this paper we describe extensions of metabolic control analysis into the stochastic regime for general biochemical reaction networks. We have shown that there exist MCA-like summation theorems for stochastic sensitivity measures for mean values and coefficients of variation/covariation (CV/CCV) for concentrations and reaction fluxes. The summation theorems for the reaction fluxes have shown that the sum values of control coefficients for flux CVs/CCVs depend on the size of the measurement time window (ϵ). Such dependency becomes stronger as the reaction systems show multi-time-scale dynamics, i.e. the time-scale separation between slow and fast modes becomes larger. We have provided a measure to quantify such separation. We have shown a connection between the summation theorems and the power-law scaling studies on various complex networks, and proposed two different power-law scaling functions depending on the value of ϵ relative to the time-scale of dynamics.

Acknowledgments

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Appendix A. Proof of $\langle v \rangle = \langle J \rangle$ at the steady state

We prove that

- (A) $\langle J \rangle$ is independent of the time interval size ϵ at the steady state.
- (B) $\lim_{\epsilon \rightarrow 0} \langle J \rangle = \langle v \rangle$.

We prove A. first. At the steady state, the ensemble average of the number of events that occurs during the interval $t_0 < t \leq t_0 + \epsilon$ must be independent of the time t_0 at which the ensemble starts to be taken. Thus, the average number of events occurring during ϵ can be denoted by $\langle N(\epsilon) \rangle$. Consider the number of events occurring during the twice longer time interval, 2ϵ . The average number of events occurring during 2ϵ , $\langle N(2\epsilon) \rangle$, will be twice the average number of events occurring during ϵ : $\langle N(2\epsilon) \rangle = 2\langle N(\epsilon) \rangle$. The mean flux $\langle J \rangle$ for the interval ϵ is given by $\langle N(\epsilon) \rangle / \epsilon$ and so is that for the interval 2ϵ . This proof can be generalized for the arbitrary interval: $3\epsilon, 4\epsilon, \dots, n\epsilon$. Therefore, the mean flux is independent of ϵ .

Now we prove B. The number of events occurred during the infinitesimal time interval ϵ can be zero or one, since any higher numbers of events are less likely to occur. Thus, the mean flux for this time interval can be expressed as

$$\lim_{\epsilon \rightarrow 0} \langle J \rangle = \frac{1 \times P(n=1; \epsilon)}{\epsilon},$$

where $P(n=1; \epsilon)$ denotes the probability that one event has occurred during ϵ . To express $P(n=1; \epsilon)$ in terms of reaction rates, we introduce the probability that one event occurs at the interval $[t, t+dt]$ and no event occurs for the rest of the duration $[0, t]$ and $[t+dt, \epsilon]$:

$$P_{[t, t+dt]} = e^{-v_{\text{tot}}^b t} (v^b dt) e^{-v_{\text{tot}}^a (\epsilon-t)},$$

where the first factor in the integrand is the probability that no reaction occurs until time t , the second factor is the probability that a reaction occurs between t and $t+dt$, and the last factor is the probability that no reaction occurs for the rest of the duration. $v^{b(a)}$ denotes the reaction rate before (after) the reaction occurs. v_{tot} is the sum of all the reaction rates. $P(n=1; \epsilon)$ can be obtained by taking the sum of $P_{[t, t+dt]}$ for all possible value of t and applying an ensemble average:

$$P(n=1; \epsilon) = \left\langle \int_0^\epsilon e^{-v_{\text{tot}}^b t} (v^b dt) e^{-v_{\text{tot}}^a (\epsilon-t)} \right\rangle \simeq \langle v^b \rangle \epsilon.$$

Therefore, the flux becomes

$$\lim_{\epsilon \rightarrow 0} \langle J \rangle = \langle v_b \rangle = \langle v \rangle,$$

where in the last step we have used the fact that the mean reaction rates before and after a reaction occurs are equal because we are at the steady state.

Appendix B. Derivation of CV of J

In this section we derive the CV of the flux of the reaction v in Fig. 4. We will start from a simple case and generalize it.

Consider the case that S and S_e are fixed: the source of the reaction is constant. The number n of events of the reaction v occurred

during a time interval ϵ is therefore a Poisson process, satisfying the following distribution:

$$P(n; \epsilon) = \frac{1}{n!} (\nu \epsilon)^n e^{-\nu \epsilon}.$$

One of the properties of the Poisson distribution is that the variance of the distribution is equal to its mean. By using this property, one can obtain the flux CV:

$$V^J = \frac{\text{Var}(n)}{\langle n \rangle^2} = \frac{1}{\langle n \rangle} = \frac{1}{\epsilon \langle J \rangle},$$

where at the last step we have used the definition of J .

Consider the case that S is fixed while S_e fluctuates *non-stochastically*. The reaction rate $\nu(s_e(t))$ varies in time and the number of its reaction events is described by a non-homogeneous Poisson process [45], satisfying the following distribution:

$$P(n; \epsilon) = \frac{1}{n!} \left(\int_0^\epsilon dt \nu(s_e(t)) \right)^n e^{-\int_0^\epsilon dt \nu(s_e(t))}. \quad (\text{B.1})$$

Since this is also a Poisson distribution, it satisfies that the variance is equal to the mean, and the flux CV can be obtained as:

$$V^J = \frac{\text{Var}(n)}{\langle n \rangle^2} = \frac{1}{\langle n \rangle} = \frac{1}{\epsilon \langle J \rangle}.$$

Consider the case that S is fixed and S_e fluctuates *stochastically*. The reaction rate $\nu(s_e(t))$ depends on a stochastic variable $s_e(t)$ and the number of the reaction events is described by a doubly-stochastic Poisson process [46]. For a given time trajectory of $s_e(t)$ for $0 \leq t \leq \epsilon$, the probability of having n reactions is also given as a Poisson distribution by Eq. (B.1) [47]. However, to estimate the probability of having n reactions for all possible trajectories, we apply an ensemble average over all the trajectories:

$$P(n; \epsilon) = \frac{1}{n!} \left\langle \left(\int_0^\epsilon \nu_t dt \right)^n e^{-\int_0^\epsilon \nu_t dt} \right\rangle_{s_e},$$

where we denote the rate by ν_t for simplicity. Since the inside of the ensemble average is a Poisson distribution, the mean value of n is obtained as

$$\langle n \rangle = \sum_n n P(n; \epsilon) = \left\langle \int_0^\epsilon \nu_t dt \right\rangle_{s_e} = \int_0^\epsilon \langle \nu_t \rangle_{s_e} dt = \epsilon \langle \nu_t \rangle_{s_e} = \epsilon \langle J \rangle,$$

where in the last step we used $\langle \nu_t \rangle = \langle J \rangle$ at the steady state, and the second moment of n is obtained as

$$\langle n^2 \rangle = \left\langle \left(\int_0^\epsilon \nu_t dt \right)^2 + \left(\int_0^\epsilon \nu_t dt \right) \right\rangle_{s_e}.$$

Thus, the flux CV is given as

$$V^J = \frac{\langle n \rangle^2 - \langle n \rangle^2}{\langle n \rangle^2} = \frac{1}{\epsilon \langle J \rangle} \left(1 + \frac{\text{Var}(\int_0^\epsilon \nu_t dt)_{s_e}}{\epsilon \langle J \rangle} \right), \quad (\text{B.2})$$

where $\text{Var}(x)_{s_e}$ means the variance over all possible trajectories of $s_e(t)$ for $0 \leq t \leq \epsilon$: $\text{Var}(x)_{s_e} \equiv \langle x^2 \rangle_{s_e} - \langle x \rangle_{s_e}^2$. The first term on the right hand side of Eq. (B.2) appears due to the intrinsic noise generated by reaction event firings and the second term due to the stochastic noise in $s_e(t)$.

In the rest of this section, we will investigate the second term in the asymptotic limits of ϵ : (1) ϵ is much smaller than the correlation time τ of $s_e(t)$ and (2) ϵ is much larger than τ . We assume that the noise strength of s_e is small enough that the linear noise approximation can be applied. Then, the fluctuations in the reaction rate with respect to the mean can be expressed as

$$\delta \nu_t \simeq \left. \frac{\partial \nu_t}{\partial s_e} \right|_{s_e = \langle s_e \rangle} \delta s_e,$$

with $\delta x \equiv x - \langle x \rangle_{s_e}$. For simplicity, we denote $\left. \frac{\partial \nu_t}{\partial s_e} \right|_{s_e = \langle s_e \rangle}$ by ν'_{s_e} . Then, we derive

$$\begin{aligned} \text{Var} \left(\int_0^\epsilon \nu_t dt \right)_{s_e} &= \int_0^\epsilon dt_1 \int_0^\epsilon dt_2 \langle \delta \nu_{t_1} \delta \nu_{t_2} \rangle_{s_e} \\ &\simeq (\nu'_{s_e})^2 \int_0^\epsilon dt_1 \int_0^\epsilon dt_2 \langle \delta s_e(t_1) \delta s_e(t_2) \rangle_{s_e} \end{aligned} \quad (\text{B.3})$$

In the asymptotic limit of $\epsilon \ll \tau$, Eq. (B.3) can be simplified to

$$\text{Var} \left(\int_0^\epsilon \nu_t dt \right)_{s_e} \simeq \epsilon^2 (\nu'_{s_e})^2 \text{Var}(s_e),$$

and, in the opposite limit of $\epsilon \gg \tau$, simplified to

$$\begin{aligned} \text{Var} \left(\int_0^\epsilon \nu_t dt \right)_{s_e} &\simeq 2 (\nu'_{s_e})^2 \int_0^\epsilon dt_1 \int_0^{t_1} dt_2 \langle \delta s_e(t_1) \delta s_e(t_2) \rangle_{s_e} \\ &= 2 (\nu'_{s_e})^2 \int_0^\epsilon dt_1 \int_0^{t_1} dt' \langle \delta s_e(t') \delta s_e(0) \rangle_{s_e} \\ &\simeq 2 (\nu'_{s_e})^2 \int_0^\epsilon dt_1 \int_0^\infty dt' \langle \delta s_e(t') \delta s_e(0) \rangle_{s_e} \\ &\simeq 2 \epsilon \tau (\nu'_{s_e})^2 \text{Var}(s_e). \end{aligned} \quad (\text{B.4})$$

Thus, we obtain two asymptotic forms of the flux CV:

$$V^J \simeq \frac{1}{\epsilon \langle J \rangle} \left(1 + \epsilon \frac{(\nu'_{s_e})^2 \text{Var}(s_e)}{\langle J \rangle} \right),$$

for $\epsilon \ll \tau$, and

$$V^J \simeq \frac{1}{\epsilon \langle J \rangle} \left(1 + 2 \tau \frac{(\nu'_{s_e})^2 \text{Var}(s_e)}{\langle J \rangle} \right),$$

for $\epsilon \gg \tau$. Depending on the value of ϵ relative to the noise correlation time τ , either of the asymptotic forms is taken by exchanging ϵ to 2τ inside the parenthesis and vice versa.

Finally, consider the case that there are multiple extrinsic noise sources $S_{e1}, S_{e2}, \dots, S_{eN}$. Eq. (B.2) is derived in the same way as before except the ensemble average is applied over all the sources of extrinsic noise. We assume that the noise strength is small enough that the linear noise approximation can be applied. Then, the reaction rate can be expressed as

$$\delta \nu(s, S_e) \simeq \sum_{i=1}^N \nu'_{s_{ei}} \delta s_{ei}.$$

In the linear noise approximation, the contribution from each noise source is expressed *additively*. If we further assume that each s_{ei} represents an independent source of noise, the contribution to the flux CV will also appear *additively* (because the covariance between any two different noise sources vanishes). Depending on the value of ϵ relative to the correlation time of each noise, a different asymptotic form needs to be taken and summed up for all noise sources. For example, consider three independent sources of noise: s_{e1} , s_{e2} , and s_{e3} , which have correlation times τ_1 , τ_2 , and τ_3 , respectively. For the case of $\epsilon \ll \tau_1$, $\epsilon \ll \tau_2$ and $\epsilon \gg \tau_3$, the flux CV becomes

$$\begin{aligned} V^J \simeq \frac{1}{\epsilon \langle J \rangle} \left(1 + \frac{1}{\langle J \rangle} \left[\epsilon (\nu'_{s_{e1}})^2 \text{Var}(s_{e1}) + \epsilon (\nu'_{s_{e2}})^2 \text{Var}(s_{e2}) \right. \right. \\ \left. \left. + 2 \tau (\nu'_{s_{e3}})^2 \text{Var}(s_{e3}) \right] \right). \end{aligned}$$

In summary, we have derived the CV of a flux that is under external noise. The CV takes two different asymptotic forms depending on the value of ϵ with respect to the correlation time of an external noise.

B.1. Derivation of Eq. (3.6)

The flux CV of v_3 in Fig. 1A can be exactly derived by using Eqs. (B.2) and (B.4). Since the reaction rate v_3 is linear in s_1 , Eq. (B.4) becomes exact:

$$\begin{aligned} \text{Var}\left(\int_0^\epsilon v_t dt\right)_{s_e} &= 2\left(\frac{\partial v_3}{\partial s_1}\right)^2 \int_0^\epsilon dt_1 \int_0^{t_1} dt' \langle \delta s_e(t') \delta s_e(0) \rangle_{s_e} \\ &= 2(p_3 X_2)^2 \int_0^\epsilon dt_1 \int_0^{t_1} dt' \left(\frac{p_1 X_1}{p_2}\right) e^{-p_2 t'} \\ &= 2(p_3 X_2)^2 \frac{p_1 X_1}{p_2^3} (p_2 \epsilon - 1 + e^{-p_2 \epsilon}). \end{aligned}$$

By substituting this into Eq. (B.2), we obtain Eq. (3.6).

Appendix C. Coefficients of covariance of fluxes vs. ϵ

In this section, we will investigate how the sum value of Eq. (3.5) changes with ϵ for the coefficients of covariation (CCV) between two different fluxes by investigating the slope of the log-log plot of flux CCV vs. ϵ . For ease of presentation, we will consider covariances of fluxes rather than the coefficients of covariation. A covariance between two different fluxes is defined as

$$\sigma_{ij}^J = \langle (J_i - \langle J_i \rangle)(J_j - \langle J_j \rangle) \rangle = \langle J_i J_j \rangle - \langle J_i \rangle \langle J_j \rangle,$$

where the ensemble average $\langle \cdot \rangle$ is performed over the steady states obtained by independent runs of stochastic simulations.

Consider a two-step cascade reaction system as shown in Fig. 1A. First, we will investigate how the flux covariance behaves in the limit of $\epsilon \rightarrow 0$. Flux covariances show different asymptotic behaviors in the limit of $\epsilon \rightarrow 0$ depending on the different pairs of fluxes (see Fig. 6). We will explain the mechanisms that generate the different behaviors.

First, we investigate the flux covariance between J_1 and J_2 . If we assume that J_1 and J_2 become independent in the limit of $\epsilon \rightarrow 0$, the covariance σ_{12}^J vanishes. This, however, is not what we observed by simulation. This indicates that there is a correlation between them. The correlation is due to the fact that one reaction of v_1 will increase S_1 by one, resulting in the increase of v_2 and affecting the probability that the reaction v_2 will occur. We take into account this causal correlation to estimate the flux covariance. For a sufficiently small value of ϵ , the dominant contributions to the flux covariance come from two cases: first, reactions of v_1 and v_2 occur once for each within the time interval ϵ , with the reaction v_1 first and then the reaction v_2 , and second, each reaction occurs in the opposite order. The contribution of the first case to the estimation of $\langle J_1 J_2 \rangle$ is, for sufficiently small ϵ ,

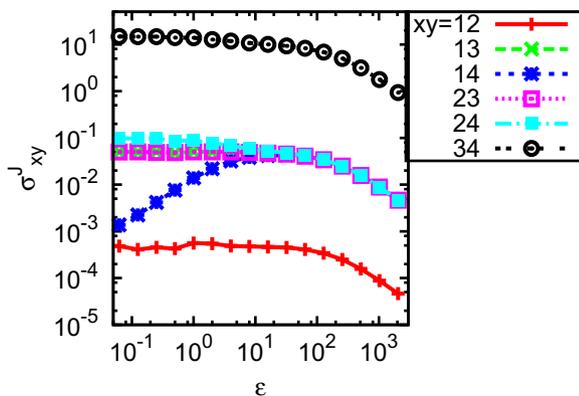


Fig. 6. Flux covariances of different pairs of reactions in the two step cascade reaction system Fig. 1A. Parameters: $(X_1, X_2, p_1, p_2, p_3, p_4) = (1, 1, 0.1, 0.01, 1, 1)$.

$$\frac{1}{\epsilon^2} \int_0^\epsilon dt \int_t^\epsilon dt' v_1 v_2 (S_1 + 1) P_s(S_1, S_2) \simeq \frac{\langle v_1 v_2 (S_1 + 1) \rangle}{2},$$

where P_s is the probability distribution function of S_1 and S_2 at the steady state. The contribution of the second case is

$$\frac{\langle v_1 v_2 (S_1) \rangle}{2}.$$

Thus, we obtain the covariance:

$$\sigma_{12}^J \simeq \frac{\langle v_1 v_2 (S_1 + 1) + v_1 v_2 (S_1) \rangle}{2} - \langle v_1 \rangle \langle v_2 \rangle.$$

Since v_1 is constant ($v_1 = p_1$) and $v_2 = p_2 S_1$, we obtain

$$\sigma_{12}^J \simeq \frac{1}{2} v_1 p_2 = \frac{1}{2} p_1 X_1 p_2.$$

We have verified this result with the simulation data as shown in Fig. 7A.

The flux covariance between J_1 and J_3 in the limit of $\epsilon \rightarrow 0$ can be also estimated in the same way as above:

$$\sigma_{13}^J \simeq \frac{1}{2} v_1 p_3 = \frac{1}{2} p_1 X_1 p_3.$$

The covariance is estimated at 0.05 (see Fig. 7B).

σ_{14}^J converges to 0 linearly with ϵ as $\epsilon \rightarrow 0$. This is because an event of reaction v_1 does not make any change in the number of S_2 . The only way to make a correlation between J_1 and J_4 is through an event of reaction v_3 . By taking into account such indirect effects, the contribution to $\langle J_1 J_4 \rangle$ becomes

$$\begin{aligned} \frac{1}{\epsilon^2} \int_0^\epsilon dt \int_t^\epsilon dt' \int_{t'}^\epsilon dt'' v_1 v_3 (S_1 + 1) v_4 (S_2 + 1) P_s(S_1, S_2) \\ = \frac{1}{6} p_1 p_3 p_4 \langle (S_1 + 1)(S_2 + 1) \rangle \epsilon. \end{aligned}$$

Since the non-zero effect on σ_{14}^J comes from the three-event correlation, we obtain

$$\sigma_{14}^J = \frac{1}{6} p_1 p_3 p_4 \epsilon,$$

and this result is verified with the simulation data as shown in Fig. 7C.

The covariance between J_2 and J_3 shows a plateau region for the small value of $\epsilon \lesssim 1$ and this occurrence is due to the fact that J_2 and J_3 are causally correlated and also that they share a common source of noise. $\langle J_2 J_3 \rangle$ are estimated by considering two cases of event sequences: one event of v_1 comes first and then v_2 later, and these events occur in the opposite order. By taking into account both the cases, we can estimate $\langle J_2 J_3 \rangle$ as

$$\langle J_2 J_3 \rangle = \frac{1}{2} \langle v_2 (s_1) v_3 (s_1 - 1) \rangle + \frac{1}{2} \langle v_3 (s_1) v_2 (s_1) \rangle,$$

where the first term represents the case that an event of reaction v_2 occurs first, resulting in the decrease in S_1 by one, and then an event of reaction v_3 occurs. The second term is for the other case that the reactions occur in the opposite order. Therefore, we obtain the flux covariance:

$$\sigma_{23}^J \simeq p_2 p_3 \left[\sigma_{11}^s - \frac{1}{2} \langle S_1 \rangle \right].$$

The first term on the right hand side is due to the common source of noise, in this case S_1 , and the second due to the causal correlation. The above expression can be further simplified to $\sigma_{23}^J \simeq \frac{1}{2} p_2 p_3 \langle S_1 \rangle$. The height of the plateau is well estimated at 0.05 (graph is not shown).

The covariance between J_2 and J_4 also shows a plateau region for the small value of $\epsilon \lesssim 1$, and the height of the plateau can be estimated by

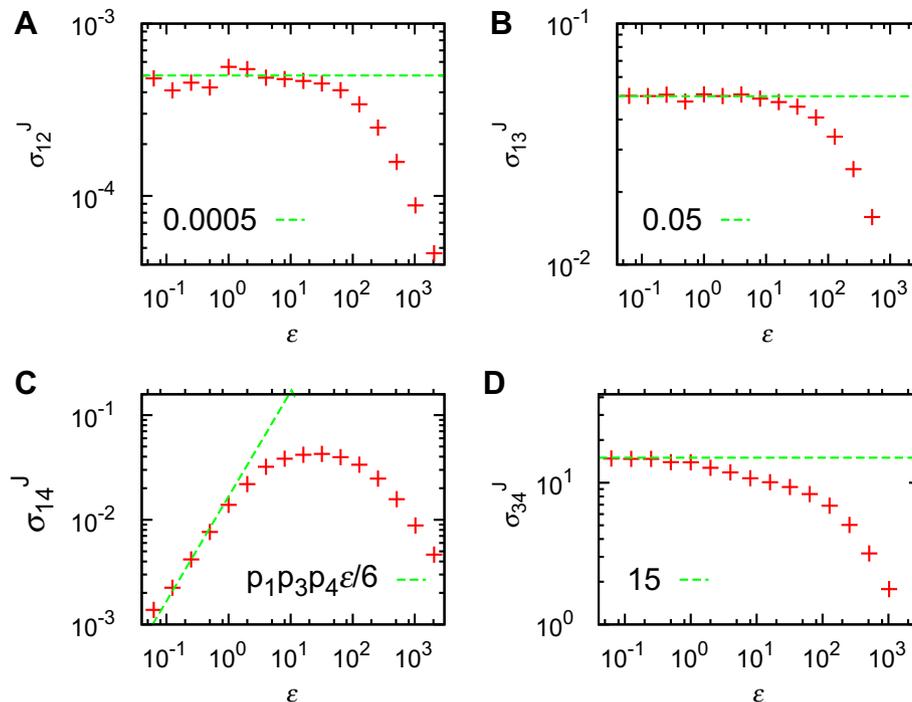


Fig. 7. Flux covariance of different pairs of reactions in the two-step cascade reaction system Fig. 1A are compared with theoretical estimates. Parameters: $(X_1, X_2, p_1, p_2, p_3, p_4) = (1, 1, 0.1, 0.01, 1, 1)$.

$$\sigma_{24}^J = \langle v_2(S_1)v_4(S_2) \rangle - \langle v_2 \rangle \langle v_4 \rangle = p_2 p_4 \sigma_{12}^s.$$

This estimates the plateau height well (graph is not shown). The reason for the occurrence of the plateau region is that J_2 and J_4 have a common source of noise, resulting in the flux covariance: e.g., an event of reaction v_2 can be correlated with that of reaction v_4 by events of reaction v_1 that has occurred previously.

σ_{34}^J can be estimated by following the similar estimation procedure to the one for σ_{23}^J :

$$\sigma_{34}^J = p_3 p_4 \left[\sigma_{12}^s + \frac{1}{2} \langle S_1 \rangle \right]$$

The first term is due to the noise propagation [48] from common sources of noise and the second due to the causal correlation. The flux covariance is estimated at 15 (see Fig. 7D).

Finally, for the intermediate and large value of ϵ , i.e., $\epsilon \gtrsim 50$, four different covariance quantities match with one another: σ_{13}^J , σ_{23}^J , σ_{24}^J , σ_{14}^J , because $J_1 \simeq J_2$ and $J_3 \simeq J_4$.

In summary, the sum value of the flux CV summation theorem depends on which reaction pairs to choose as well as the value of ϵ . The asymptotic forms of flux CCVs in the limit of $\epsilon \rightarrow 0$ are independent of ϵ , i.e., plateau regions appear, if (1) the two reaction steps are affected by the noise propagated from common sources or (2) they are directly connected such that one reaction event leads to the direct change in the probability that the other reaction occurs.

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