Propagation of Fluctuations in Biochemical Systems, I: Linear SSC Networks

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Abstract We investigate the propagation of random fluctuations through biochemical networks in which the number of molecules of each species is large enough so that the concentrations are well modeled by differential equations. We study the effect of network topology on the emergent properties of the reaction system by characterizing the behavior of variance as fluctuations propagate down chains and studying the effect of side chains and feedback loops. We also investigate the asymptotic behavior of the system as one reaction becomes fast relative to the others.

Keywords Biochemical systems · Fluctuations · Stochastic differential equations

1. Introduction

There are two different natural contexts in which stochastic dynamics arises in the study of biochemical reaction networks. In the first, the stochastic chemical dynamics arises from the randomness inherent in molecular interactions and in the formation and breaking of chemical bonds. This “intrinsic stochasticity” is particularly relevant when the numbers of molecules are small such as in gene transcription and small gene regulatory networks where the mean concentrations no longer faithfully model the chemical dynamics. There is a large literature in this field beginning with Delbruck (1940), including Gans (1960), Kurtz (1972), Gillespie (1976), and recently exemplified by Gadgil et al. (2005) and Ball et al. (2006). In this setting, the chemical species are modeled by discrete random variables and the chemical reactions by Poisson processes. This is the randomness present in the Gillespie algorithm (Gillespie, 1976). If one scales up the volume and number of molecules while keeping the initial concentrations constant, then this intrinsic stochasticity becomes negligible on the scale of concentrations and the dynamical system reduces

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to a collection of deterministic, coupled ordinary differential equations for the concentrations of the species (Kurtz, 1972). The second type of stochasticity arises naturally in this scaling limit.

In this second context, which is our focus here, one wants to investigate the response to external excitation of a large biochemical system (which is well described by differential equations for the concentrations). It is natural and theoretically useful to consider stochastic excitations and to study the emergent properties of the network as the random fluctuations propagate through the system. Here the randomness is a tool used to study the out-of-equilibrium dynamics of the biochemical system. Typically, we are interested in perturbing randomly a single (or small number of) input(s). Hence the object of study is a set of differential equations that is forced by a continuous stochastic process in one or a small number of components. We study how the stochastic fluctuations spread to the other components and, in particular, how the spreading depends on the topology of the biochemical network.

The central biological goal driving our work is to understand the behavior of biochemical systems in cells, which in vivo are exceptionally large and complicated. A metabolite can be the substrate for many different enzymes and participate in apparently unrelated reactions. Individual reactions usually have nonlinear kinetics catalyzed by enzymes that are themselves inhibited or excited by products or distant substrates in the network. Cells and tissues differ because the genes that code for certain enzymes have tissue specific expression patterns and biochemical substrates themselves also influence gene expression. Further, each cell’s environment, its inputs and outputs, and its internal state (e.g. stage of cell cycle) are not constant but vary in time. This continual variation affects both the concentrations of substrates and the expression of genes whose products catalyze particular reactions. Thus, the gene-biochemical system should not be viewed as a fixed object but as one that is continuously changing.

For each signal, either external or internal, that causes a particular cell to dramatically change its operation, there are two natural questions. First, how does the gene-biochemical system respond to accomplish the change? Second, how does the system enable the cell to maintain homeostasis in all its other operations despite the change? One would like to understand the structural and kinetic principles that allow the system to accomplish both tasks simultaneously. We take two distinct approaches to this biological goal. First, we study how fluctuations propagate through relatively simple systems. We are interested in proving theorems about how different network geometries magnify or suppress fluctuations since this may give clues as to why biochemical networks are structured as they are. Secondly, we apply fluctuations to in silico representations of specific, biological networks. By observing how fluctuations propagate we can identify reactions or subsystems that are buffered against such fluctuations, i.e., are homeostatic. Then, through in silico experimentation (e.g. removing particular reactions), we can take the system apart piece by piece to discover the regulatory mechanisms that give rise to the homeostasis. This paper takes the first approach; for an example of the second approach, see Nijhout et al. (2004, 2006a, 2006b), and Reed et al. (2004, 2006).

In this paper we study chemical reaction systems such that each chemical reaction converts one substrate into one product and we assume that the kinetics are mass action. Therefore, the corresponding differential equations are linear, so the technical difficulties involved in studying the associated stochastic processes are minimized. Thus, these systems are an excellent arena for investigating the effects of network geometry on the propagation, magnification, and suppression of fluctuations. The principles discovered then
become the natural goal for generalization to nonlinear settings (Anderson and Mattingly, 2007).

To see the kinds of questions we want to ask, consider a nonreversible chain with a side branch. The chemical species are $X_1, \ldots, X_n, X_{\text{side}}$; the corresponding concentrations are denoted by $x_1, \ldots, x_n, x_{\text{side}}$.

\[
\begin{array}{cccccccc}
I + \xi(t) & \rightarrow & X_1 & \rightarrow & X_2 & \rightarrow & X_3 & \rightarrow & \cdots & X_n & \rightarrow \\
& & k_1 & & k_2 & & k_3 & & & & \cdots & k_n \\
& & k_{i,2} & L & & & & & & & & \\
& & & & & & & x_{\text{side}} & & & & \\
\end{array}
\]

The chain has a constant input $I$, which is perturbed by some random process, $\xi(t)$. If the input is fluctuating, then each of the concentrations will fluctuate as will the fluxes, $k_j x_j$. Suppose the side chain is absent. Then, will the variations of the fluxes increase, decrease, or stay the same as we move down the chain? Does the answer depend on the rate constants $k_j$? If the side chain is present, does it affect the variances of the fluxes on the chain? If so, what is the effect of the size of $L$.

The chemical reaction diagram corresponds to a set of differential equations for the concentrations and, similarly, the diagram with stochastic forcing corresponds to a system of stochastic differential equations (SDEs):

\[
\begin{align*}
\dot{x}_1 &= I - k_1 x_1 + \xi(t), \\
\dot{x}_2 &= k_1 x_1 - L x_2 - k_2 x_2 + k_{s,2} x_{\text{side}}, \\
\dot{x}_3 &= k_2 x_2 - k_3 x_3, \\
& \vdots
\end{align*}
\]

These SDEs in turn give rise to a stochastic process on the state space $\mathbb{R}^{n+1}$. We prove that this stochastic process has a unique stationary measure. Intuitively, this means that at large times the joint distribution of values of the concentrations becomes independent of the initial condition and independent of time. That is, the statistics converge to an equilibrium distribution. The variances of the concentrations referred to above are the variances of the marginal distributions of this measure. We prove the existence of the stationary measure in Section 2.2.

The natural assumptions for the stochastic perturbation, $\xi(t)$, of an input $I$, are that they are continuous, mean zero, finite variance, stationary, and satisfy $\xi(t) \geq -I$. This last assumption guarantees that the input always remains positive and hence the concentration of each species remains nonnegative. In Section 3 we prove that the variances of the fluxes strictly decrease down a nonreversible reaction chain when the input is perturbed by such a $\xi(t)$. Since the mean of each flux is $I$, the coefficients of variation and the Fano factors of the fluxes also strictly decrease down the chain. This result is interesting from a biological point of view because it says that one way to stabilize the flux out of a chain (i.e., small variance) is to have many intervening biochemical steps between the varying input and the output. In Section 5 we show, using a comparison argument, that
side reaction systems and positive feedback loops always lower the variance of the flux out of the species to which the side reaction or feedback loop is attached. Using the results of Section 3, one can then see that the upper bound for the variances downstream from the attachment point are automatically decreased. This shows that the added complexity of a side reaction system or feedback loop buffers the output of a chain against fluctuations to the input.

The results of Sections 3 and 5 are very general in that they do not depend on detailed knowledge of the perturbations $\xi(t)$. In order to investigate how the magnitude of the decrease in variance above depends on the rate constants and the geometry of the network, we study in Sections 4 and 6 the special case where the perturbation is white noise. Of course, white noise does not satisfy the hypotheses on $\xi(t)$ above and the concentrations in systems perturbed by white noise can occasionally become negative. Nevertheless, perturbing the system by white noise makes the entire system Gaussian and enables us to make explicit calculations. In Section 4 we compute an explicit formula for the variances of each species and each flux. Using this formula we are able to show how the decrease in variance depends on the rate constants under a variety of different scenarios. In Section 6, we study a system in which one rate constant, $L$, for a reaction using a substrate $X$, is large compared to all other rate constants. We show that $\text{Var}(x) \sim O\left(\frac{1}{L}\right)$, as $L \to \infty$, so the variances of the fluxes of all other reactions utilizing $X$ go to zero at the same rate.

It is important to note that our goals, methods and results are different from those in classical biochemical control theory (Kacser and Burns, 1973; Crabtree and Newsholme, 1985; Heinrich and Rapoport, 1974; Westerhoff and Chen, 1984). In that theory one takes a system at a fixed steady state, makes a small perturbation in a parameter (perhaps an input), and allows the system to relax to a new steady state. By comparing the new value of a variable (a concentration or flux) to the old value, one computes the percentage change of the variable per unit percentage change in the parameter. Technically, one is computing a partial derivative. This kind of sensitivity analysis gives good information about local, linearized behavior near the initial steady state. By contrast, we are concerned with responses to continuous large scale fluctuations in inputs. Technically, this means computing properties of the distribution of each concentration or flux from properties of the stationary measure.

It is true that the classical biochemical control theory can be made “stochastic” in the following way. Suppose that the system has input $I$ and is at steady state. Consider the same system with input $I + \eta$, where $\eta$ is a random variable drawn from some density. For each $\eta$ we let the system relax to steady state and measure the value $v$, of some concentration or flux. $v$ is a random variable and comparing its variance to the variance of $\eta$ gives information about how much the steady state value of $v$ changes as $\eta$ changes. However, this modified biochemical control theory often gives completely different answers from the fluctuation theory that we are developing and the differences are biologically significant. Consider the chain (without the side chain) in the example above. If the input is $I + \eta$, then, at steady state, the flux $k_n x_n$ must equal $I + \eta$, so $\text{Var}(k_n x_n) = \text{Var}(\eta)$; thus the variance remains constant down the chain. However, as noted above, we will prove that if the input is continuously and stochastically perturbed (so the system never remains at equilibrium), then the variances of the fluxes decrease as one proceeds down the chain.
2. SSC networks and the stationary measure

In this section we introduce the class of chemical reaction systems that we will study and prove the existence of a stationary measure.

2.1. SSC systems with mass actions kinetics

In this paper we study chemical reactions networks such that each chemical reaction converts one substrate into one product. We call these SSC networks for reasons given below. Corresponding to the chemical reaction network is a directed graph where each node corresponds to a chemical substrate and a directed edge from \( X_i \) to \( X_j \) indicates that there is a chemical reaction converting \( X_i \) to \( X_j \). Let \( m \) be the number of nodes. We assume that the graph is connected and we say that the graph is weakly reversible if whenever there is a directed path from \( X_i \) to \( X_j \), then there is a directed path from \( X_j \) to \( X_i \). We assume that the rate of conversion of \( X_i \) to \( X_j \) is proportional to the concentration of \( X_i \) (mass action kinetics) with constant of proportionality \( b_{ji} \geq 0 \) where equality holds if and only if there is no directed edge from \( X_i \) to \( X_j \). Let \( B \) be the \( m \times m \) matrix \( \{b_{ij}\} \) where the diagonal coefficients are defined by \( b_{ii} = -\sum_{j \neq i} b_{ji} \). We assume each substrate \( X_i \) leaves the system at a rate proportional to its concentration with constant of proportionality \( r_{ii} \geq 0 \) and is added to the system at a constant rate of \( I_i \geq 0 \). A system with nonzero input is called weakly reversible if it is weakly reversible in the sense stated above and if there is at least one nonzero output. Let \( R \) be the diagonal matrix with diagonal entries \( r_{ii} \) and \( I \) be the vector with entries \( I_i \). Then the concentrations of the substrates are governed by the linear differential equation

\[
\dot{x}(t) = Ax(t) + I, \tag{1}
\]

where \( A = B - R \) is called the matrix of rate constants for the system.

In the notation of Horn and Jackson (1972) and Feinberg (1979) our systems are defined by the following properties: They are weakly reversible, the kinetics is mass action, there is a single linkage class, and each complex is a single substrate. It is because of the final property that we call our networks single species complex (SSC). The following Lemma follows from the deficiency zero theorem (Feinberg, 1979, 1987).

**Lemma 2.1.** If a linear SSC system with \( m \) substrates and at least one nonzero input is weakly reversible then

(a) The differential equations (1) have a unique equilibrium which is globally asymptotically stable and contained in \( \mathbb{R}^m_{\geq 0} \).

(b) The eigenvalues of the matrix of rate constants, \( A \), have strictly negative real parts.

(c) For all vectors \( v \in \mathbb{R}^m_{\geq 0} \), we have \( e^{At} v \cdot e_j \geq 0 \) for all \( j \).

**Proof:** It is simple to show that an SSC network for which there is at least one input and at least one output has the following two properties: Its deficiency is zero and the dimension of the stoichiometric compatibility class is \( m \). Part (a) then follows directly from the deficiency zero theorem. Since \( A \) is the Jacobian at the equilibrium point, (b) follows from (a) and linearity. To prove (c), consider the differential equation \( \dot{y}(t) = Ay(t) \). The only negative term in the equation governing \( \dot{y} \) contains \( y_j \). Thus, \( \mathbb{R}^m_{\geq 0} \) is invariant under the flow \( e^{At} \), which is an equivalent statement to (c). \( \square \)
2.2. The stationary measure

Consider the following weakly reversible SSC system with mass action kinetics, matrix of rate constants $A$, and nonzero input vector $I$ perturbed by a mean zero, finite variance, stationary stochastic process $\xi(t)$ such that for all $t \geq 0$ and each $i$, $\xi_i(t) \geq -I_i$:

\[
\begin{align*}
\dot{x}(t) &= Ax(t) + I + \xi(t), \\
x(0) &= x_0.
\end{align*}
\]  

(2)

**Theorem 2.2.** The process $x^*(t) = x^*(t, \xi)$ defined by

\[
x^*(t, \xi) = \int_{-\infty}^{t} e^{A(t-s)} I \, ds + \int_{-\infty}^{t} e^{A(t-s)} \xi(s) \, ds
\]

(3)

is a stationary solution to (2). Furthermore given any initial condition $x_0$, if $x(t, x_0, \xi)$ is a solution to (2) then $x(t, x_0, \xi)$ converges exponentially to $x^*(t, \xi)$ as $t \to \infty$ in that

\[
\mathbb{E}\left[\left|x(t, x_0, \xi) - x^*(t, \xi)\right|^2\right] \to 0, \quad \text{as } t \to \infty.
\]

**Proof:** Observe that for any $t, \tau \in \mathbb{R}$,

\[
x^*(t + \tau) = \int_{-\infty}^{t+\tau} e^{A(t+s)} I \, ds + \int_{-\infty}^{t+\tau} e^{A(t+s)} \xi(s) \, ds
\]

This can be written succinctly as

\[
(\theta_t x^*)(t, \xi) = x^*(t, \theta_t \xi)
\]

(4)

where the shift $\theta_t$ is defined by $(\theta_t f)(s) = f(t+s)$ for all $s, t \in \mathbb{R}$ and functions $f$ on $\mathbb{R}$. Hence for any $t_1 \leq \cdots \leq t_n$,

\[
(x^*(\tau + t_1, \xi), \ldots, x^*(\tau + t_n, \xi)) = (x^*(t_1, \theta_t \xi), \ldots, x^*(t_n, \theta_t \xi)).
\]

Since $\xi$ is a stationary process, the distribution of the right-hand side is independent of $\tau$ which proves that $x^*$ is stationary. Clearly, $x^*(t, \xi)$ is a solution in that $x(t, x^*(0, \xi), \xi) = x^*(t, \xi)$.

We now turn to convergence. It follows from Lemma 2.1(b) that there are constants $\alpha, M > 0$ such that $\|e^{At}\| < Me^{-\alpha t}$ for all $t > 0$. Subtracting the solution of (2),

\[
x(t, x_0, \xi) = e^{At} x_0 + \int_0^t e^{A(t-s)} I \, ds + \int_0^t e^{A(t-s)} \xi(s) \, ds,
\]

(5)
from $x^*(t)$, squaring, and taking expected values gives,

$$
\mathbb{E} |x(t, x_0, \xi) - x^*(t, \xi)|^2 \\
\leq 3\|e^{At}\|^2|x_0|^2 + 3\mathbb{E}\left|\int_{-\infty}^{0} e^{A(t-s)} I\, ds \right|^2 + 3\mathbb{E}\left|\int_{-\infty}^{0} e^{A(t-s)} \xi(s)\, ds \right|^2 \\
\leq 3M^2|x_0|^2 e^{-2at} + \frac{3M^2|I|^2}{\alpha^2} e^{-2at} \\
+ 3\mathbb{E}\left(\int_{-\infty}^{0} \|e^{A(t-s)}\|\, ds \right)\left(\int_{-\infty}^{0} \|e^{A(t-s)}\|\, |\xi(s)|^2\, ds \right) \\
\leq 3M^2|x_0|^2 e^{-2at} + \frac{3M^2|I|^2}{\alpha^2} e^{-2at} + \frac{3M^2}{\alpha^2} e^{-2at} \text{Var}(\xi).
$$

Thus, $\mathbb{E}|x(t, x_0, \xi) - x^*(t, \xi)|^2 \to 0$ as $t \to \infty$. \hfill \Box

**Remark.** If one takes expectations on both sides of (3) and (5), one sees immediately that the model is consistent in the mean. That is, the mean of the perturbed problem is equal to the solution of the unperturbed problem.

Since $x^*(t)$ is stationary, the distribution of $x^*(t)$ is independent of $t$. More precisely, defining the measure $\mu(A) = \mathbb{P}(x^*(0) \in A)$ for all measurable $A \subset \mathbb{R}^m$, we see that $\mu$ characterizes the longtime behavior of the solution in that the distribution of $x(t, x_0, \xi)$ converges to $\mu$ as $t \to \infty$. This follows from $\mathbb{E}|x(t, x_0, \xi) - x^*(t, \xi)|^2 \to 0$ and the fact that $\mu(A) = \mathbb{P}(x^*(t) \in A)$ for all $t$.

Thus $\mu$ contains information about the average, long-term behavior of fluxes and concentrations. It will be $\mu$, therefore, which we shall probe in order to gain an understanding of how different graphical structures and asymptotic limits of biochemical reaction systems increase, decrease, and otherwise modify the exogenous fluctuations of biochemical reaction systems. Throughout the rest of this paper, it is understood that each mean or variance is computed with respect to this stationary measure.

### 3. Reaction chains

In order to study reaction chains, we begin by proving a simple general bound for the variance of the concentration of any species in an SSC system in terms of the variance of the input fluctuations. We consider the special case in which only one component of $I$ is nonzero and the fluctuations $\xi(t)$ are nonzero only in this same component. Without loss of generality, we take this component to be the first. Abusing notation slightly, we also call this nonzero scalar input $I$ and its scalar perturbation $\xi(t)$. An analogous proof works in the more general case where there are inputs to more than one species and any number of the inputs undergo independent fluctuations.

Consider the stationary solution to system (2). By taking the expected value in (3) and using that $\xi(t)$ has mean zero one sees that

$$
m_i = I \int_{-\infty}^{t} e^{A(t-s)} e_1 \cdot e_i\, ds
$$

is the mean of the $i$th species.
Theorem 3.1. Let \( x^*(t) \) be the stationary solution of an SSC system with one input, \( I \), that is perturbed by a non-constant stationary stochastic process, \( \xi(t) \), with finite variance, mean zero, and \( \xi(t) \geq -I \). Then for each \( i \),

\[
\text{Var}(x^*_i) < \left( \frac{m_i}{I} \right)^2 \text{Var}(\xi).
\]

Proof: Using Lemma 2.1(c) and the Cauchy–Schwarz inequality gives

\[
\text{Var}(x^*_i(t)) = \mathbb{E}\left( \int_{-\infty}^{t} \xi(s) e^{A(t-s)} e_1 \cdot e_i \, ds \right)^2
\]

\[
= \mathbb{E}\left( \int_{-\infty}^{t} \xi(s) \left( e^{A(t-s)} e_1 \cdot e_i \right)^{1/2} \left( e^{A(t-s)} e_1 \cdot e_i \right)^{1/2} \, ds \right)^2
\]

\[
< \mathbb{E}\left( \int_{-\infty}^{t} \xi(s)^2 e^{A(t-s)} e_1 \cdot e_i \, ds \right) \left( \int_{-\infty}^{t} e^{A(t-s)} e_1 \cdot e_i \, ds \right)
\]

\[
= \text{Var}(\xi) \left( \int_{-\infty}^{t} e^{A(t-s)} e_1 \cdot e_i \, ds \right)^2
\]

\[
= \left( \frac{m_i}{I} \right)^2 \text{Var}(\xi).
\]

The inequality is strict because \( \xi(t) \) is not a constant.

We now consider nonreversible chains with mass action kinetics:

\[
\xrightarrow{I} X_1 \xrightarrow{k_1} X_2 \xrightarrow{k_2} \cdots \xrightarrow{k_{m-1}} X_{m-1} \xrightarrow{k_m} X_m \xrightarrow{k_m}.
\]

(7)

Theorem 3.1 allows us to see that variances of the fluxes of the stationary solution decrease as one proceeds down the chain.

Theorem 3.2. Let the input, \( I \), of a nonreversible chain with mass action kinetics be perturbed by a non-constant stationary stochastic process, \( \xi(t) \), with finite variance, mean zero, and \( \xi(t) \geq -I \). Let \( x^*(t) \) denote the stationary solution for the chain. Then, for all \( i \), \( \text{Var}(k_i x^*_i) < \text{Var}(\xi) \) and

\[
\text{Var}(k_{i+1} x^*_{i+1}) < \text{Var}(k_i x^*_i).
\]

(8)

Proof: From the remark following Theorem 2.2, we know that the mean, \( m_i \), of \( x^*_i(t) \) is the equilibrium value of \( x_i \) for the unperturbed problem. This implies that \( m_i = \frac{I}{k_i} \), so the bound \( \text{Var}(k_i x^*_i) < \text{Var}(\xi) \) follows immediately from Theorem 3.1. To prove (8) note that the input to \( X_2 \) is

\[
k_1 x^*_1(t) = I + (k_1 x^*_1(t) - I)
\]

and \( k_1 x^*_1(t) - I \) is a stationary stochastic process of mean zero, finite variance, and values \( \geq -I \). Thus, by Theorem 3.1,

\[
\text{Var}(k_2 x^*_2) < \text{Var}(k_1 x^*_1 - I) = \text{Var}(k_1 x^*_1).
\]

The input to \( X_3 \) is \( k_2 x^*_2(t) \), so repeating this argument down the chain proves (8).
Remark. Notice that the variances of the fluxes and the variances of the concentrations behave differently. The variances of the fluxes strictly decrease as one moves down the chain while the mean of each flux is the constant value $I$. Thus the coefficients of variation (standard deviation divided by the mean) and the Fano factors (variance divided by the mean) of the fluxes also strictly decrease as one moves down the chain. Because $\text{Var}(k_i x_i) = k_i^2 \text{Var}(x_i)$ and $\mathbb{E} x_i = \frac{I}{k_i}$, one can easily check that: (1) the absolute variances of the concentrations do not necessarily decrease down the chain, (2) the coefficients of variation of the concentrations do necessarily decrease down the chain, and (3) the Fano factors of the concentrations do not necessarily decrease down the chain.

4. Chains with white noise

The next natural question is how much do the variances decrease down the chain? This cannot be answered without more detailed information about $\xi(t)$. To investigate it, we will perturb the input $I$ by white noise, $\sigma dB(t)$, which will allow us to use the Itô calculus and thereby make explicit calculations. We note that perturbing inputs with white noise causes the concentrations to occasionally go negative and these parts of the solutions are unphysical. However, the calculations shed light on the reasons for and magnitude of the decrease in variance down reaction chains.

By perturbing the inputs to a system with independent white noise processes, we arrive at the following system of Itô stochastic differential equations:

\[
\begin{cases}
    dx(t) = \left( Ax(t) + I \right) dt + \Sigma dB(t), \\
    x(0) = x_0,
\end{cases} \tag{9}
\]

where $\Sigma \in \mathbb{R}^{m \times p}$ and $B(t)$ is standard $p$-dimensional Brownian motion. The following theorem is proved in the same manner as Theorem 2.2.

**Theorem 4.1.** The process $x^*(t) = x^*(t, B)$ defined by

\[
x^*(t, B) = \int_{-\infty}^{t} e^{A(t-s)} I \, ds + \int_{-\infty}^{t} e^{A(t-s)} \Sigma dB(s) \tag{10}
\]

is a stationary solution to (9). Furthermore given any $x_0$, if $x(t, x_0, B)$ is a solution to (9) then $x(t, x_0, B)$ converges exponentially to $x^*(t, B)$ as $t \to \infty$ in that

$$\mathbb{E} \left| x(t, x_0, B) - x^*(t, B) \right|^2 \to 0, \quad \text{as } t \to \infty.$$  

**Proof:** The proof is identical to that of Theorem 2.2, except that the Itô isometry (Oksendal, 2003) is used to control the expected value of the square of the Itô integral term. □

**Theorem 4.2.** Let $x^*(t)$ be the stationary solution of the linear chain (7) where the input is perturbed by white noise. Then for each $i$,

\[
\text{Var}(k_{i+1} x^*_{i+1}) < \text{Var}(k_i x^*_i). \tag{11}
\]
Further, if we assume that the rate constants, \( k_i \), are distinct. Then

\[
\text{Var}(x^*_i) = \sigma^2 \sum_{j=1}^{i} \sum_{r=1}^{i} p_{ij} p_{ir} \frac{1}{k_j + k_r},
\]

where

\[
p_{ij} = \begin{cases} 
\frac{(i-1)!}{\prod_{n=1}^{i} k_n} \prod_{n=1, n \neq j}^{i} (k_n - k_j), & i \geq j, \\
0, & i < j.
\end{cases}
\]

**Proof:** The proof of inequality (11) is identical to that in Theorem 3.2. To prove (12), we note that the matrix of rate constants, \( A \), give

\[
A = \begin{bmatrix}
-k_1 & 0 & \ldots & 0 \\
k_1 & -k_2 & \ldots & 0 \\
\vdots & \vdots & \ddots & \vdots \\
0 & \ldots & k_{m-1} & -k_m
\end{bmatrix}.
\]

Let \( P = \{ p_{ij} \} \). A straightforward calculation shows that the \( j \)th column of \( P \) is the eigenvector of \( A \) corresponding to eigenvalue \(-k_j\). Thus, \( D = P^{-1} A P \) is diagonal. In addition, \( P \) takes the vector \((1, 1, \ldots, 1)^T\) to the vector \((1, 0, \ldots, 0)^T\). Using these facts, the formula (10) for \( x^*(t) \), and the Itô isometry,

\[
\text{Var}(x^*_i) = \sigma^2 \left[ \int_{-\infty}^{t} (P e^{D(t-s)} e_1 \cdot e_i) dB_s \right]^2 = \sigma^2 \int_{-\infty}^{t} (P e^{D(t-s)} e_1 \cdot e_i)^2 ds
\]

\[
= \sigma^2 \int_{-\infty}^{t} \left( P e^{D(t-s)} \begin{bmatrix} 1 \\ \vdots \\ 1 \end{bmatrix} \cdot e_i \right)^2 ds = \sigma^2 \int_{-\infty}^{t} \left( P \begin{bmatrix} e^{-k_1(t-s)} \\ \vdots \\ e^{-k_m(t-s)} \end{bmatrix} \cdot e_i \right)^2 ds
\]

\[
= \sigma^2 \int_{-\infty}^{t} \left( \sum_{j=1}^{i} p_{ij} e^{-k_j(t-s)} \right)^2 ds = \sigma^2 \sum_{j=1}^{i} \sum_{r=1}^{i} p_{ij} p_{ir} \frac{1}{k_j + k_r}. \quad \square
\]

We assumed that the \( k_i \)'s were distinct so that the explicit formulas above make sense. It can be shown that the variances of the concentrations are continuous functions of the rate constants (Anderson, 2005). This fact, together with the bound given by (8) allows us to conclude that formula (12) has finite limits as various subsets of the \( k_i \)'s become identical.

We can use the explicit formula (12) to answer several natural questions.

**Example 4.3 (Magnitude of decrease).** Theorem 3.2 shows that variances of fluxes are strictly decreasing as one moves down a chain. To investigate how much they decrease,
consider the chain (7) where \( m = 2 \) and the input is perturbed by white noise. Using (12) we see that \( \text{Var}(k_1 x_1^*) = \frac{\sigma^2 k_1}{2} \) and \( \text{Var}(k_2 x_2^*) = \frac{\sigma^2 k_1 k_2}{2(k_1 + k_2)} \). Thus,

\[
\frac{\text{Var}(k_2 x_2^*)}{\text{Var}(k_1 x_1^*)} = \frac{k_2}{k_1 + k_2}.
\]

This simple example shows that the ratio of successive variances can be any number between zero and one.

Example 4.4 (Long chains). Assume that \( k_i = k \) for some fixed \( k > 0 \) and all \( i \). Taking the limit of (12) is difficult. Instead, since all the \( k_i \)'s are equal, an induction proof shows that

\[
x_i^*(t) = \frac{I}{k} + \sigma \frac{k^{i-1}}{(i-1)!} \int_{-\infty}^{t} (t-s)^{i-1} e^{-k(t-s)} dB(s).
\]

Using the Itô isometry, it follows that

\[
\text{Var}(x_i^*) = \sigma^2 \frac{2(2i - 2)!}{4^{i-1} (i-1)!^2}.
\]

Using Stirling’s formula, we see that

\[
\text{Var}(k x_i^*) \sim \sigma^2 \frac{k}{2 \sqrt{\pi} \sqrt{i}} + O(i^{-3/2}), \quad \text{as} \quad i \to \infty.
\]

Thus the variances decrease to zero in a regular fashion if all of the rate constants are the same.

Example 4.5 (A small rate constant). Suppose that one rate constant, \( k_i \), in a chain is very small. Using the explicit formula (12), one can easily compute that

\[
\text{Var}(k_i x_i^*) \sim \sigma^2 \frac{1}{2} k_i + O(k_i^2), \quad \text{as} \quad k_i \to 0,
\]

\[
\text{Var}(k_j x_j^*) \sim \sigma^2 \frac{1}{2} k_i + O(k_j^2), \quad \text{as} \quad k_i \to 0, \quad \text{for} \quad j > i.
\]

Notice that the small rate constant has the effect of significantly decreasing the variances of the \( i \)th and all subsequent fluxes while the means of the fluxes remain unchanged. Therefore a small rate constant is not “rate limiting” but instead is “variance limiting.”

Example 4.6 (A large rate constant). Suppose that one rate constant, \( k_i \), in a chain is very large. Again, using (12), one can compute that

\[
\text{Var}(k_i x_i^*) \to \text{Var}(k_{i-1} x_{i-1}^*), \quad \text{as} \quad k_i \to \infty.
\]

Furthermore, for all \( j > i \),

\[
\text{Var}(k_j x_j^*) \to \text{Var}(k_j x_j^*), \quad \text{as} \quad k_i \to \infty,
\]
where $\tilde{x}_j$ is from the process arising from the following system:

$$I + \sigma dB(t) \rightarrow \tilde{X}_1 \xrightarrow{k_1} \tilde{X}_2 \xrightarrow{k_2} \cdots \xrightarrow{k_{i-1}} \tilde{X}_{i-1} \xrightarrow{k_i} \tilde{X}_{i+1} \xrightarrow{k_{i+1}} \cdots.$$  

This shows that in the asymptotic limit where $k_i \to \infty$ one can replace the original chain by the chain with the substrate $X_i$ removed. Here we implicitly use the fact that since the kinetics are linear, and hence the concentrations are Gaussian, the statistics are determined by the means and variances.

We now prove a comparison result that allows us to generalize the asymptotic behavior seen in Example 4.4. Combining Theorem 4.7 with Example 4.4 shows that if the rate constants of a chain are bounded away from infinity and bounded away from zero, then there exist $m$ and $M$ so that, for all $i$,

$$\frac{m}{\sqrt{i}} \leq \text{Var}(k_i x_i^*) \leq \frac{M}{\sqrt{i}}. \quad (14)$$

**Theorem 4.7** (Bounding variances). Let $k_i$ be a sequence of rate constants of a reaction chain that satisfy

$$0 < m \leq k_i \leq M < \infty.$$  

Let $x^*$ be the stationary solution to the chain with rate constants $k_i$, and let $x^{*,m}$, $x^{*,M}$ be the stationary solutions to the chains with all rate constants equal to $m$, $M$, respectively. Then for all $i$,

$$\text{Var}(mx_i^{*,m}) \leq \text{Var}(k_i x_i^*) \leq \text{Var}(M x_i^{*,M}).$$

**Proof:** The crux of the proof is to prove that the variance of each flux is a strictly monotone increasing function of the rate constants earlier in the chain. The theorem then follows directly from this fact. The ODE governing the concentration of $X_i$ is:

$$\dot{x}_i(t) = I - k_i x_i(t) + \xi(t), \quad (15)$$

where $\xi(t) = k_{i-1} x_{i-1} - I$. Through a change of variables it can be shown that

$$\text{Var}(k_i x_i) = \mathbb{E}(k_i x_i(t) - I)^2 = \int_0^\infty \int_0^\infty e^{-u-v} \mathbb{E} \left[ \xi(t) - \frac{1}{k_i} u \right] \mathbb{E} \left[ \xi(t) - \frac{1}{k_i} v \right] du dv.$$

By Proposition 3.6.4 and the proof of Proposition 3.1.1 in (Anderson, 2005), there is a strictly positive, strictly monotone decreasing function $f : \mathbb{R}_{\geq 0} \to \mathbb{R}_{>0}$ such that for all $t, s \in \mathbb{R}$, $\mathbb{E}[\xi(t)\xi(s)] = f(|t-s|)$. Therefore,  

$$\text{Var}(k_i x_i) = \mathbb{E}(k_i x_i(t) - I)^2 = \int_0^\infty \int_0^\infty e^{-u-v} f \left( \frac{|u-v|}{k_i} \right) du dv. \quad (16)$$

Thus,

$$\frac{\partial}{\partial k_i} \text{Var}(k_i x_i) > 0.$$
The formula for $\text{Var}(k_i x_i)$ obtained by multiplying (12) by $k_j^2$ is symmetric in the $k_j$‘s for $j \in [1, \ldots, i]$. Therefore, $\frac{\partial}{\partial k_j} \text{Var}(k_i x_i) = \frac{\partial}{\partial k_j} \text{Var}(k_i x_i)$ for each $j < i$ and so

$$\frac{\partial}{\partial k_j} \text{Var}(k_i x_i) > 0,$$

which was the desired result. \hfill \Box

5. Side reaction systems and feedback loops

A side reaction system on a chain is any SSC system that gets its input from a species on the chain and has output that flows back into the same species; see Fig. 1 below.

Note that there must be a species within the side reaction system whose output flows to $X_1$ with some rate constant, $k_3$. Define $Y$ to be that species and let $y(t)$ denote its concentration. The differential equation governing the behavior of $x_1(t)$ is then

$$\frac{d}{dt} x_1(t) = I - k_1 x_1(t) - k_2 x_1(t) + k_3 y(t) + \xi(t). \quad (17)$$

If $\tilde{x}_1$ is the solution to the above system when there is no side reaction system (i.e. $k_2 = k_3 = 0$), then

$$\frac{d}{dt} \tilde{x}_1(t) = I - k_1 \tilde{x}_1(t) + \xi(t). \quad (18)$$

For the theorems in this section we assume that the covariance of the perturbation is strictly positive and decays as the difference in time increases.

**Assumption 5.1.** For $s < t$, $\mathbb{E} \xi(t) \xi(s) > 0$ and $\mathbb{E} \xi(t) \xi(s)$ is an increasing function in $s$.

**Theorem 5.2** (Side reactions lower variance). Let $x_1^*$ and $\tilde{x}_1^*$ be the stationary solutions to (17) and (18), respectively, where $\xi(t)$ is a finite variance, mean zero, non-constant stationary stochastic process that satisfies Assumption 5.1 and such that $\xi(t) \geq -I$ for all $t \geq 0$. Then,

$$\text{Var}(k_1 x_1^*) < \text{Var}(k_1 \tilde{x}_1^*).$$

![Fig. 1 A side reaction on a linear chain.](image-url)
\textbf{Proof:} By (17), \((k_{1} x_{1}(t) - I)^{2}\) satisfies the differential equation
\[
\frac{d}{dt}(k_{1} x_{1}(t) - I)^{2} = -2k_{1}(k_{1} x_{1}(t) - I)^{2} + 2k_{1}(k_{1} x_{1}(t) - I)(k_{3} y(t) - k_{2} x_{1}(t)) \\
+ 2k_{1}(k_{1} x_{1}(t) - I)\xi(t).
\]
Integrating, taking expected values and differentiating with respect to \(t\) gives:
\[
\frac{d}{dt}\mathbb{E}(k_{1} x_{1}(t) - I)^{2} = -2k_{1}\mathbb{E}(k_{1} x_{1}(t) - I)^{2} + 2k_{1}\mathbb{E}[(k_{1} x_{1}(t) - I)(k_{3} y(t) - k_{2} x_{1}(t))] \\
+ 2k_{1}\mathbb{E}[(k_{1} x_{1}(t) - I)\xi(t)].
\]
Thus, by the stationarity of \(x_{1}^{*}(t)\) and \(\xi(t)\),
\[
\mathbb{E}(k_{1} x_{1}^{*}(t) - I)^{2} = \mathbb{E}[(k_{1} x_{1}^{*}(t) - I)(k_{3} y^{*}(t) - k_{2} x_{1}^{*}(t))] + k_{1}\mathbb{E}x_{1}^{*}(t)\xi(t). \tag{19}
\]
By a similar argument, \(\text{Var}(k_{1} x_{1}^{*}(t) - I)^{2}\) satisfies
\[
\mathbb{E}(k_{1} \tilde{x}_{1}^{*}(t) - I)^{2} = k_{1}\mathbb{E}\tilde{x}_{1}^{*}(t)\xi(t). \tag{20}
\]
The remainder of the proof consists of showing \(\mathbb{E}[(k_{1} x_{1}^{*}(t) - I)(k_{3} y^{*}(t) - k_{2} x_{1}^{*}(t))] < 0\) and \(\mathbb{E}x_{1}^{*}(t)\xi(t) \leq \mathbb{E}\tilde{x}_{1}^{*}(t)\xi(t)\), which, combined with (19) and (20), proves the result.

Because SSC systems are consistent in the mean, \(\mathbb{E}x_{1}^{*}(t) = I/k_{1}\) and
\[
\mathbb{E}k_{3} y^{*}(t) = \mathbb{E}k_{2} x_{1}^{*}(t). \tag{21}
\]
Equation (21) together with Theorem 3.1 yields \(\mathbb{E}(k_{3} y^{*}(t))^{2} < \mathbb{E}(k_{2} x_{1}^{*}(t))^{2}\) and so by the Cauchy–Schwarz inequality
\[
|\mathbb{E}(k_{2} x_{1}^{*}(t)k_{3} y^{*}(t))| \leq \left(\mathbb{E}k_{2}^{2} x_{1}^{*}(t)^{2}\right)^{1/2}\left(\mathbb{E}k_{3}^{2} y^{*2}(t)\right)^{1/2} < \mathbb{E}k_{2}^{2} x_{1}^{*}(t)^{2}. \tag{22}
\]
Combining (21) and (22) gives the desired result:
\[
\mathbb{E}[(k_{1} x_{1}^{*}(t) - I)(k_{3} y^{*}(t) - k_{2} x_{1}^{*}(t))] = \mathbb{E}[k_{1} k_{3} x_{1}^{*}(t)y^{*}(t) - k_{1} k_{2} x_{1}^{*}(t)] \\
= \frac{k_{1}}{k_{2}}\mathbb{E}[k_{2} x_{1}^{*}(t)k_{3} y^{*}(t) - k_{2}^{2} x_{1}^{*}(t)^{2}] < 0,
\]
In order to show that \(\mathbb{E}x_{1}^{*}(t)\xi(t) \leq \mathbb{E}\tilde{x}_{1}^{*}(t)\xi(t)\) we will first show that for all \(s < t\), \(\mathbb{E}\xi(t) x_{1}^{*}(s)\) is an increasing function in \(s\). Let \(A\) be the matrix of rate constants for the system consisting of \(X_{1}\) and the side reaction where the first row of \(A\) represents the rate constants defining the rate of change for the concentration of species \(X_{1}\). Then for any \(s\)
\[
x_{1}^{*}(s) = I \int_{-\infty}^{s} e^{A(s-r)} e_{1} \cdot e_{1} dr + \int_{-\infty}^{s} \xi(r)e^{A(s-r)} e_{1} \cdot e_{1} dr. \tag{23}
\]
Let \(t > s\) and \(0 < \Delta < t - s\). Multiplying (23) by \(\xi(t)\), taking expected values, and applying Assumption 5.1 and Lemma 2.1(c) gives
\[
\mathbb{E} \xi(t) x_1^*(s) = \int_{-\infty}^{s} \mathbb{E}[\xi(t) \xi(r)] e^{A(s-r)} e_1 \cdot e_1 \, dr
\]
\[
= \int_{0}^{\infty} \mathbb{E} \left[ \xi(t) \xi(s - u) \right] e^{A u} e_1 \cdot e_1 \, du
\]
\[
\leq \int_{0}^{\infty} \mathbb{E} \left[ \xi(t) \xi(s + \Delta - u) \right] e^{A u} e_1 \cdot e_1 \, du
\]
\[
= \int_{-\infty}^{s+\Delta} \mathbb{E} \left[ \xi(t) \xi(r) \right] e^{A(s+\Delta-r)} e_1 \cdot e_1 \, dr = \mathbb{E} \xi(t) x_1^*(s + \Delta).
\]

Therefore, for all \( s < t \), \( \mathbb{E} \xi(t) x_1^*(s) \) is an increasing function in \( s \).

Solving (17) and (18) with integrating factors gives:
\[
x_1^*(t) = \frac{I}{k_1} + \int_{-\infty}^{t} \left( k_3 y^*(s) - k_2 x_1^*(s) \right) e^{-k_1(t-s)} \, ds + \int_{-\infty}^{t} \xi(s) e^{-k_1(t-s)} \, ds
\]
\[
\bar{x}_1^*(t) = \frac{I}{k_1} + \int_{-\infty}^{t} \xi(s) e^{-k_1(t-s)} \, ds.
\]

Therefore,
\[
\mathbb{E} x_1^*(t) \xi(t) = \int_{-\infty}^{t} \mathbb{E} \left[ \xi(t) \left( k_3 y^*(s) - k_2 x_1^*(s) \right) \right] e^{-k_1(t-s)} \, ds + \int_{-\infty}^{t} \mathbb{E} \left( \xi(t) \xi(s) \right) e^{-k_1(t-s)} \, ds
\]
\[
= \int_{-\infty}^{t} \mathbb{E} \left[ \xi(t) \left( k_3 y^*(s) - k_2 x_1^*(s) \right) \right] e^{-k_1(t-s)} \, ds + \mathbb{E} \bar{x}_1^*(t) \xi(t).
\]

Thus, to complete the proof it is sufficient to show that for all \( s < t \), the following inequality holds:
\[
\mathbb{E} \left[ \xi(t) \left( k_3 y^*(s) - k_2 x_1^*(s) \right) \right] \leq 0. \tag{24}
\]

Consider the side reaction system as its own reaction system with input \( k_2 x_1^*(t) = \psi(t) + k_2 I / k_1 \) and output \( k_3 y^*(t) \), where \( \psi(t) \) is a stationary, mean zero, finite variance, stochastic process such that \( \psi(t) \geq -k_2 I / k_1 \). Let \( B \) be the matrix of rate constants that governs just the side reaction. We may now solve for \( y^*(s) \) by looking at this subsystem and conclude that for some integer \( j \),
\[
y^*(s) = \frac{k_2 I}{k_1} \int_{-\infty}^{s} e^{B(s-r)} e_1 \cdot e_j \, ds + \int_{-\infty}^{s} \psi(r) e^{B(s-r)} e_1 \cdot e_j \, ds.
\]

Because the solutions are consistent in the mean, we must have \( \mathbb{E} k_3 y^*(s) = k_2 I / k_1 \). Thus,
\[
\int_{-\infty}^{s} e^{B(s-r)} e_1 \cdot e_j \, ds = 1 / k_3.
\]

Combining this with Proposition 2.1(c) and the fact that for \( r < t \), \( \mathbb{E} \xi(t) x_1^*(r) \) is an increasing function of \( r \) gives
\[
k_3 \mathbb{E} \xi(t) y^*(s) = k_3 \int_{-\infty}^{s} \mathbb{E} \left[ \xi(t) \psi(r) \right] e^{B(s-r)} e_1 \cdot e_j \, dr
\]
\[
= k_3 \int_{-\infty}^{s} \mathbb{E} \left[ \xi(t) \left( k_2 x_1^*(r) - \frac{k_2 I}{k_1} \right) \right] e^{B(s-r)} e_1 \cdot e_j \, dr
\]
\[ k_2 k_3 \int_{-\infty}^{s} \mathbb{E}[\xi(t)x_1^*(r)]e^{B(s-r)}e_1 \cdot e_j \, dr \leq k_2 k_3 \mathbb{E}[\xi(t)x_1^*(s)] \int_{-\infty}^{s} e^{B(s-r)}e_1 \cdot e_j \, dr \]
\[ = k_2 \mathbb{E}[\xi(t)x_1^*(s)]. \]

This is equivalent to (24) and so the proof is complete. \( \square \)

**Remark.** Theorem 5.2 still holds if instead of \( \xi(t) \) the perturbation is assumed to be white noise. The proof in the white noise case is simpler than that given above because one gets martingales (which become zero when expected values are taken) instead of the \( x_1(t)\xi(t) \) terms in the above proof (Anderson, 2005).

We now turn our attention to feedback loops on chains. A feedback loop on a chain is an SSC system together with an input from one species on the chain, \( X_n \), and an output to an earlier species, \( X_1 \); see Fig. 2. As in the case of side reactions, the following theorem also holds if the perturbations are white noise.

**Theorem 5.3 (Feedback loops lower variance).** Let \( \tilde{x}(t) \) be the vector of species concentrations for the chain (7) and let \( x(t) \) be the vector of species concentrations for the chain with feedback loop (Fig. 2), where \( \xi(t) \) is a finite variance, mean zero, non-constant stationary stochastic process that satisfies Assumption 5.1 and such that \( \xi(t) \geq -I \) for all \( t \geq 0 \). Then,
\[ \text{Var}(k_nx_n^*) < \text{Var}(k_n\tilde{x}_n^*). \]

**Proof:** Let \( \{V_i\} \) be the substrates and \( B \) be the matrix of rate constants of the SSC subsystem in Fig. 2. We suppose that \( V_j \) is the species which gives input to \( X_1 \) with rate constant \( \alpha \). Then the input to \( X_1 \) from the feedback loop is
\[ f_1(t) = \alpha e^{Bt}v(0) \cdot e_j + \alpha c \int_{0}^{t} x_n(s)e^{B(t-s)}e_1 \cdot e_j \, ds, \]
which depends explicitly only on \( x_n \). If we let \( R(t) = k_{n-1}x_{n-1}(t) \) then the differential equation for \( x_n(t) \) is \( \dot{x}_n(t) = R(t) - cx_n(t) - k_nx_n(t) \).

\[ I + \xi(t) \quad \xrightarrow{k_1} \quad X_1 \quad \xrightarrow{k_{n-1}} \quad \ldots \quad \xrightarrow{k_n} \quad X_n \quad \xrightarrow{c} \quad \text{Subsystem} \]

**Fig. 2** A chain with a feedback loop.
Consider the chain with side reaction system given in Fig. 3 where the subsystem is the same as in Fig. 2 and the flux to $Y_1$ comes from $V_j$ with rate constant $\alpha$. Let $Q(t) = k_{n-1}x_{n-1}(t)$ and $P(t) = k_{n-1}y_{n-1}(t)$ be the inputs to $X_n$ in Fig. 3. Since the input to the $Y$-chain is $f_1(t)$ and the rate constants for the two chains are the same, $R(t) = Q(t) + P(t)$ because the differential equations are linear. Thus, the differential equation governing $x_n(t)$ in Fig. 2 is the same as the differential equation governing $x_n(t)$ in Fig. 3. Since the system in Fig. 3 is a chain with a side reaction system, the result follows from Theorem 5.2.

Remark. Similarly to Theorem 3.2, Theorems 5.2 and 5.3 can be reformulated to state the same results in terms of Fano factors and coefficients of variations.

6. The effect of one large rate constant

We now consider a general weakly reversible SSC system with input perturbed by white noise. Our goal is to characterize the effect of one large rate constant.

**Theorem 6.1.** Suppose that independent white noise processes perturb the inputs to a weakly reversible SSC system with $m$ substrates. Let $X_a$ be a particular substrate and suppose that the rate constant $L$ for one flux out of $X_a$ is large. Then,

$$\text{Var}(x_a^*) \sim O\left(\frac{1}{L}\right), \text{ as } L \to \infty.$$  \hspace{1cm} (25)

**Proof:** We will assume that one of the perturbed inputs goes directly to $X_a$. The proof of the general case is similar. The stochastic differential equation governing $x_a(t)$ is given by

$$dx_a(t) = \left(C + \sum_{i=1}^{m} c_i x_i(t) - (L + K)x_a(t)\right) dt + \sigma dB(t),$$  \hspace{1cm} (26)
where \( L + K > 0 \) is equal to the sum of all the rate constants for reactions leaving \( X_a \), \( C > 0 \) is the input flux to \( X_a \), \( \sigma > 0 \), and \( c_i \geq 0 \) is the rate constant associated with the reaction \( X_i \to X_a \). Solving (26) for \( x_i^* \) in terms of the \( x_i^* \) and using the Itô isometry, one can easily bound \( \operatorname{Var}(x_a^*) \),

\[
\operatorname{Var}(x_a^*) \leq \frac{\beta}{2(L + K)} + \beta \sum_{i=1}^{m} c_i^2 \frac{\operatorname{Var}(x_i^*)}{(L + K)^2},
\]

for some constant \( \beta \). To complete the proof we will show that \( \operatorname{Var}(x_i^*) \leq O(L) \).

Let \( A \) be the matrix of rate constants for the SSC system. Using the formula (10) for the stationary solution and the Itô isometry, one easily calculates:

\[
\operatorname{Var}(x_i^*) = \sigma^2 \int_{-\infty}^{t} (e^{A(t-s)}e \cdot e_i)^2 ds,
\]

for some vector \( e \). By Lemma 2.1(b) we know that the real parts of the eigenvalues of \( A \), \( \{\lambda_i\} \), are strictly negative; let \( \lambda = \inf \{|\lambda_i|\} \). There exist positive constants \( c \) and \( M \) so that for all \( t - s > 0 \), we have \( \|e^{A(t-s)}\| \leq ce^{-M\lambda(t-s)} \). Using this inequality in (27), we have

\[
\operatorname{Var}(x_i^*) \leq \frac{\sigma^2 c^2 |e|}{2M} \frac{1}{\lambda}.
\]

In Appendix we prove that \( \lambda \geq O(1/L) \), so \( \operatorname{Var}(x_i^*) \leq O(L) \), which concludes the proof. \( \Box \)

**Example 6.2** (A side chain with a large rate constant). To illustrate the theorem, we consider the linear chain with a side reaction given in the diagram in the Introduction. If the input is perturbed by white noise, then, as the rate constant \( L \) becomes large, Theorem 6.1 tells us that \( \operatorname{Var}(x_2^*) \leq O(1/L) \). Therefore the flux out of \( X_2 \) down the chain has variance \( \operatorname{Var}(k_2x_2^*) \leq O(1/L) \). By Theorem 3.1,

\[
\operatorname{Var}(k_i x_i^*) \leq \operatorname{Var}(k_2 x_2^*) \leq O(1/L) \quad \text{for all } i \geq 2.
\]

Thus, for all \( i \geq 2 \), the means of the fluxes remain equal to \( I \), while the variances of the fluxes go to zero as \( L \to \infty \).

**7. Discussion**

We have developed a theory of propagation of fluctuations in biochemical systems for the special case of linear SSC networks and proved theorems relating variances to network structure. Variances (Fano factors, coefficients of variation) of fluxes decrease down a chain and the presence of side reactions and feedback loops always lowers the upper bound of the variances further down the chain. These results are very general. As shown in Sections 2, 3, and 5, they hold, independent of the choice of rate constants, for linear SSC networks whose inputs are perturbed by quite arbitrary continuous stochastic processes, \( \xi(t) \), that satisfy mild assumptions (such as \( \xi(t) \geq -I \), so concentrations remain nonnegative). These results also hold if the inputs are perturbed by white noise, which enables us
to use the Itô calculus to make explicit calculations and comparison estimates (Sections 4 and 6). Some of these results generalize to cases in which the kinetics are nonlinear and reactions can have more than one substrate (Anderson and Mattingly, 2007). It is tempting to speculate that biochemical systems evolved to be as complicated as they are partly because of the homeostasis of exit fluxes achieved by having many intermediate steps, side reactions, or feedback loops.

In cell metabolism, networks are exceptionally complicated and the kinetics are highly nonlinear. In addition, substrates often inhibit or activate the enzymes that catalyze distant reactions in the network (see, e.g., Nijhout et al. 2006a, 2006b; Reed et al. 2004, 2006). It is unlikely that one can prove general theorems that apply to such a wide range of dynamical systems, although theorems about special subclasses may be possible. However, these systems can be investigated using the ideas of fluctuation theory by numerical computation. By running Monte Carlo simulations, one can compute numerically the stationary measure (if it exists) and thus compute the variances of the substrate concentrations and fluxes that are of interest, for example, finding fluxes that are exceptionally stable in the face of (possibly large) input fluctuations. By removing individual reactions or altering other biochemical influences, one can investigate the structural and kinetic reasons that give rise to the observed emergent behavior. This is the program carried out in Nijhout et al. (2006a) where we investigate the long range interactions in the methionine and folate cycles that stabilize the flux of the reaction by which methyl groups from S-adenosylmethionine (SAM) are attached to DNA. Large swings in methionine input cause even larger swings in SAM concentration, but the DNA methylation rate has exceptionally small variance.

For simplicity of exposition, we have discussed the special case where a single input to a biochemical system is varied. The same ideas can be used to introduce fluctuations in a concentration, a flux, or in several places, and then study how the fluctuations propagate throughout the system. Understanding the consequences of fluctuations in kinetic parameters is also important because kinetic parameters depend on enzyme concentrations and other properties that are variable and themselves dependent on time-varying genetic regulation. Analyzing this case requires some technical extensions of this work.

In the Introduction we contrasted two contexts in which stochastic dynamics occurs naturally in the study of biochemical systems. In the first, the stochastic behavior arises from the making and breaking of chemical bonds and plays a fundamental role for biochemical systems with relatively small numbers of molecules. In the second, considered in this paper, the stochastic behavior comes from random external forcing at the scale of concentrations. There are, however, intermediate models in which both kinds of stochastic behavior play a role. Consider a linear chain with pseudospecies input:

\[ X_0 \xrightarrow{k_0} X_1 \xrightarrow{k_1} X_2 \xrightarrow{k_2} \cdots \xrightarrow{k_n} X_n. \]

We denote by \( N_i \) the number of molecules of species \( X_i \); \( N_0 \) is held fixed. The rate constant \( k_i \) is the probability per unit time that a given \( X_i \) molecule becomes an \( X_{i+1} \) molecule. Gadgil et al. (2005) show that the steady state distribution of each \( N_i \) is Poisson with mean \( k_0 N_0 / k_i \). Thus the variance of the \( i \)th flux is \( \text{Var}(k_i N_i) = k_i^2 \mathbb{E}[N_i] = k_i k_0 N_0 \). Therefore, in this (context 1) model, the variances of the fluxes can increase or decrease down a chain depending on the rate constants.
Now suppose that the number of molecules of the pseudospecies is fluctuating in time at the scale of its concentration. That is, we replace \( N_0 \) by \( N_0 + V\xi(t) \), where \( V \) is the volume. We now scale the system as in Kurtz (1972) letting both \( N_0 \) and \( V \) get large with the ratio fixed. In this limit the system is well described by differential equations for the concentrations \( N_i/V \) and the part of the variance due to the making and breaking of chemical bonds becomes negligible. The stochastic external forcing \( \xi(t) \) remains, and so we are left with the type of (context 2) dynamical system considered in this paper, for which we have proven that the variances of the fluxes decrease down the chain.

Clearly, there exists an intermediate regime where both kinds of stochasticity play an important role. In this intermediate regime, whether the variances of the fluxes decrease down the chain will depend on the rate constants and on the balance between the two types of stochasticity. Understanding and characterizing this phenomenon is an interesting and important mathematical and biological goal that would build on the foundations in Gans (1960), Gadgil et al. (2005), Ball et al. (2006), and the present paper.

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Appendix

We derive the bound used in Theorem 6.1. There are two cases which need consideration:

1. The flux out of \( X_a \) with rate constant \( L \) goes to another species. This case is handled in Theorem A.1 below.
2. The flux out of \( X_a \) with rate constant \( L \) leaves the system. The proof of the result in this case is similar to the proof of the theorem below and so the details are omitted.

**Theorem A.1.** Let \( A = \{a_{ij}\} \) be an \( n \times n \) matrix with the following properties:

1. For each \( i \), \( a_{ii} < 0 \) and \( |a_{ii}| \geq \sum_{j \neq i} |a_{ji}| \).
2. \( a_{11} = -L + \alpha_{11} \) and \( a_{21} = L + \alpha_{21} \) for some \( \alpha_{11} < 0 \) and \( \alpha_{21} \in \mathbb{R} \).
3. For every \( L > 0 \), the real parts of the eigenvalues of \( A \) are all strictly negative.

Denote the eigenvalues of \( A \) by \( \{\lambda_i\} \) and let \( \lambda = \inf \{|\Re(\lambda_i)|\} \). Then

\[
\lambda \geq O(1/L), \quad \text{as } L \to \infty.
\]

**Proof:** Let \( B = \frac{1}{L} A \). The eigenvalues of \( B \) are \( \{\frac{1}{L} e_i : e_i \text{ is an eigenvalue of } A\} \). We will use the characteristic polynomials of \( A \) and \( B \) to show that the magnitude of the real parts of the eigenvalues of \( B \) are no smaller than \( O(1/L^2) \), which implies our result.

Because \( L \) only appears in the first column of \( A \), all \( O(1) \) terms of \( B \) occur in the first column. Expanding the determinant of \( B \) by cofactor expansion along the first column then shows that \( \det(B) \) must be of order \( O(1/L^n) \) or \( O(1/L^{n-1}) \). Similarly, the cofactors of \( B \) must be of order \( O(1/L^{n-1}) \) or \( O(1/L^{n-2}) \). Therefore, computing the inverse of \( B \)
(which exists by assumption (3) above) by cofactors, we see that the possible order of the entries of $B^{-1}$ are 1, $L$, and $L^2$. Therefore, $\|B^{-1}\| \leq O(L^2)$.

One may view $B$ as a $1/L$ matrix perturbation of the matrix $C = \{c_{ij}\}$, where $c_{11} = -1$, $c_{21} = 1$, and $c_{ij} = 0$ for all other entries. Therefore, each eigenvalue, $\rho$, of $B$ is an analytic functions of $1/L$:

$$\rho = \rho_0 + \frac{1}{L} \rho_1 + \frac{1}{L^2} \rho_2 + O\left(\frac{1}{L^3}\right), \quad \text{(A.1)}$$

where $\rho_0$ is $-1$ or 0. If $\rho_0 = -1$ there is nothing to prove; so we assume $\rho_0 = 0$. If $\rho_1 = \rho_2 = 0$ then $\rho = O(1/L^3)$. However, this would imply that $O(1/\rho) = O(L^3)$. Since $1/\rho$ is an eigenvalue of $B^{-1}$, this would contradict the norm bound for $B^{-1}$, above. Thus $\rho_1$ and $\rho_2$ can not both be zero. It remains to be shown that the leading order term in (A.1) can not be purely imaginary. We will do this through asymptotic matching.

Consider two different formulations for the characteristic polynomial of $A$, $p_A(x)$:

$$p_A(x) = \det(xI_n - A) \quad \text{(A.2)}$$

$$= x^n + Lu(x) + v(x) \quad \text{(A.3)}$$

$$= x^n + c_{1,n-1}Lx^{n-1} + c_{0,n-1}x^{n-1} + \cdots + c_{1,2}Lx^2 + c_{0,2}x^2$$

$$+ c_{1,1}Lx + c_{0,1}x + c_{1,0}L + c_{0,0}, \quad \text{(A.4)}$$

where $u(x)$ and $v(x)$ are polynomials of degree $n - 1$ that are independent of $L$, and $c_{i,j} \in \mathbb{R}$ for $i = 1, 2$ and $j = 1, \ldots, n - 1$ ($i$ gives the power of $L$ and $j$ gives the power of $x$ for the term $c_{ij}L^ix^j$). We note that we can not have $c_{1,0} = c_{0,0} = 0$, for then there would be a zero eigenvalue, which would contradict assumption (3).

To show that the leading order term in (A.1) is not purely imaginary we will consider two cases: $\rho_1 = 0$ and $\rho_1 \neq 0$. We begin by supposing $\rho_1 = 0$ and $\rho_2 \neq 0$. Then $\rho = O(1/L^2)$ and there is a solution to (A.4) which is $O(1/L)$. Putting $x = \rho_2/L$ into (A.4) and setting the equation equal to zero gives us:

$$O\left(\frac{1}{L^3}\right) + \frac{c_{1,2}\rho_2^2}{L} + \frac{c_{0,2}\rho_2^2}{L^2} + c_{1,1}\rho_2 + \frac{c_{0,1}\rho_2}{L} + c_{1,0}L + c_{0,0} = 0.$$

Matching like terms in $L$ tells us that $c_{1,0} = 0$, $c_{0,0} \neq 0$, and $c_{1,1} \neq 0$. Solving for $\rho_2$ gives us $\rho_2 = -c_{0,0}/c_{1,1} \in \mathbb{R}$. Therefore, $\rho_2$ has a nonzero real part.

We now suppose that $\rho_1 \neq 0$. Because finding an $O(1/L)$ solution to (A.1) is equivalent to finding an $O(1)$ solution to (A.3), $\rho_1$ must satisfy $u(\rho_1) = 0$. Let $D(x) = xI_n - A$. Then $u(x) = D(x)_{11} + D(x)_{21}$, where $D(x)_{ij}$ is the $i$, $j$th cofactor of $D(x)$. $D(x)_{11}$ and $D(x)_{21}$ differ only in the first row, so we may combine the determinants by adding the first two rows. We conclude that

$$u(x) = \begin{vmatrix}
-a_{22} - a_{12} + x & -a_{13} - a_{23} & -a_{14} - a_{24} & \cdots & -a_{1n} - a_{2n} \\
-a_{32} & -a_{33} + x & -a_{34} & \cdots & -a_{3n} \\
-a_{42} & -a_{43} & -a_{44} + x & \cdots & -a_{4n} \\
\vdots & \vdots & \ddots & \ddots & \vdots \\
-a_{n2} & -a_{n3} & -a_{n4} & \cdots & -a_{nn} + x
\end{vmatrix}.$$
Solving \( u(x) = 0 \) for nonzero solutions is therefore equivalent to finding the nonzero eigenvalues of the matrix

\[
\tilde{A} = \begin{bmatrix}
    a_{22} + a_{12} & a_{13} + a_{23} & a_{14} + a_{24} & \ldots & a_{1n} + a_{2n} \\
    a_{32} & a_{33} & a_{34} & \ldots & a_{3n} \\
    a_{42} & a_{43} & a_{44} & \ldots & a_{4n} \\
    \vdots & \vdots & \vdots & \ddots & \vdots \\
    a_{n2} & a_{n3} & a_{n4} & \ldots & a_{nn}
\end{bmatrix}.
\]

By assumption (1), the diagonal entries of \( \tilde{A} \) are nonpositive and have magnitudes that are greater than or equal to the sums of the magnitudes of all the other terms in that column. Therefore, Gershgorin’s theorem says that the nonzero eigenvalues of \( \tilde{A} \), and hence the nonzero solutions of \( u(x) = 0 \), have strictly negative real part. Thus, \( \text{Re}(\rho_1) \neq 0 \). This completes the proof. \( \square \)

If the flux out of \( X_a \) with rate constant \( L \) leaves the system, the only change in the statement of the above theorem is that \( a_{21} \) is independent of \( L \). The proof is identical except that \( u(x) = D(x)_{11} \) and so we no longer have to add two determinants together to simplify \( u(x) \).

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