Early signatures of regime shifts in gene expression dynamics

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Recently, a large number of studies have been carried out on the early signatures of sudden regime shifts in systems as diverse as ecosystems, financial markets, population biology and complex diseases. Signatures of regime shifts in gene expression dynamics are less systematically investigated. In this paper, we consider sudden regime shifts in the gene expression dynamics described by a fold-bifurcation model involving bistability and hysteresis. We consider two alternative models, Models 1 and 2, of competence development in the bacterial population *B. subtilis* and determine some early signatures of the regime shifts between competence and vegetative state. We use both deterministic and stochastic formalisms for the purpose of our study. The early signatures studied include the critical slowing down as a transition point is approached, rising variance and the lag-1 autocorrelation function, skewness and a ratio of two mean first passage times. Some of the signatures could provide the experimental basis for distinguishing between bistability and excitability as the correct mechanism for the development of competence.

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I. INTRODUCTION

Complex dynamical systems, ranging from ecosystems and the climate to gene regulatory networks and financial markets, are known to exhibit abrupt shifts from one dynamical regime to a contrasting one at critical parameter values [1–4]. Examples of sudden regime shifts include the collapse of vegetation when semi-arid conditions prevail, the transition from a clear lake to a turbid one, sudden changes in fish/wildlife populations [4–6], distinct changes in the climate and patterns of oceanic circulation [4–7], financial markets undergoing global crashes [4], spontaneous systemic failures such as asthma attacks [4], or epileptic seizures [6] etc. In a gene regulatory network, a sudden transition may occur from one stable gene expression state to another at a critical parameter value [6–12]. The induction of the *lac* operon in *E. coli* results in the synthesis of the protein β-galactosidase required for breaking up sugar molecules and releasing energy to the cell. There is now experimental evidence [7] that an abrupt transition from the uninduced (β-galactosidase level low) state to the induced (β-galactosidase level high) state of the *lac* operon occurs at a critical value of an inducer concentration.

The regime/state shifts in the examples mentioned above are mostly a consequence of the fold-bifurcation (or the fold-catastrophe), well-characterized in dynamical systems theory [1, 2, 6, 13]. Figure 1 illustrates a specific type of the fold-bifurcation based on bistability and hysteresis, which provides a physical understanding of the features associated with sudden regime shifts. The plot represents the steady states of a dynamical system versus a specific parameter. The state of the dynamical system at time *t* is defined by the magnitudes of the relevant variables at *t*. In the steady state, the net rate of change in the magnitude of a variable is zero so that there is no further time evolution. The solid lines in Figure 1 denote stable steady states separated by a branch (dotted line) of unstable steady states. The steady state curve is folded backwards giving rise to bistability, i.e., the existence of two stable steady states in the shaded region. The parameter values *u*₁ and *u*₂ represent the bifurcation points at which the abrupt regime shifts from one stable steady state to another occur. On crossing the bifurcation point, the system loses bistability and becomes monostable. The transition from one branch of stable steady states to the other is not reversible but exhibits hysteresis. This implies that if a transition takes place at a bifurcation point, say the upper one, the reverse transition from the upper to the lower branch occurs at the lower bifurcation point and not at the upper bifurcation point itself. Bistability owes its origin to the presence of one or more positive feedback loops governing the system dynamics with the added condition that the dynamics be sufficiently nonlinear [11, 12]. Each stable steady state has its own basin of attraction in the state space, i.e., the space of all states. In the case of more than one stable steady state, the location of the initial state of the system in a basin of attraction determines the steady state attained by the system in the course of time.

The stability of a steady state indicates that the system returns to the state on being weakly perturbed from it. This is shown by arrow directions in Figure 1. If the perturbation is sufficiently strong so that a transition takes place from one basin of attraction to the other, a switch occurs between the stable steady states even before the bifurcation point is reached. Closer the system is to the bifurcation point, the lesser is the magnitude of the perturbation needed to bring about the switch. In the example of Figure 1, the branch of unstable steady states constitutes the border between the two basins of attraction. The distance of a stable steady state from the corresponding unstable steady state is a measure of the resilience (robustness against perturbation) of the system. The resilience is gradually weakened as the system approaches a bifurcation point. The dynamics of natural systems, in general, have a stochastic component due to the presence of random external influences and the inherently probabilistic nature of the processes involved in the dynamics. Consider the dynamics of gene expression in a gene regulatory network. Gene expression consists of two major steps: transcription and translation during

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which messenger RNA (mRNA) and protein molecules respectively are synthesized. The biochemical events (e.g., the binding of a RNA polymerase at the promoter region of the DNA to initiate transcription) constituting gene expression are inherently stochastic in character resulting in fluctuations (noise) around mean mRNA and protein levels \[14, 15\]. Extrinsic influences like variability in the number of regulatory molecules also contribute to the noise. Instead of a single steady state protein level, as in the case of deterministic time evolution, one now has a steady state probability distribution in the protein levels reflecting the stochastic nature of the time evolution. In the presence of low/moderate amounts of noise, the physical picture underlying sudden regime shifts still remains valid. As in the case of applied perturbations, fluctuations of sufficiently strong magnitude can bring about regime shifts before the bifurcation point is crossed. A number of early signatures of regime shifts have been proposed so far \[1, 6, 16, 17\] in the scenario of the fold-bifurcation. These are the critical slowing down (CSD) and its associated effects, namely, rising variances and the lag-1 autocorrelation function as the critical transition point is approached, increased skewness in the steady state probability distribution and the presence of flickering transitions between the stable steady states. The utility of such signals in the cases of ecological and financial systems \[1\] and complex deteriorating diseases \[18\] is significant, specially, in developing appropriate risk-aversion/management strategies. Also, in the absence of physical models capturing the essential dynamics, one can obtain quantitative measures of the early signatures by analyzing time-series data.

In this paper, we compute quantities providing early signatures of abrupt regime shifts in gene expression dynamics. We specifically focus on the phenomenon of competence development in the soil bacteria \textit{B. subtilis} involving binary gene expression, i.e., the existence of two expression states. We consider two alternative models of competence development based on bistable and excitable dynamics. In Section 2, the two models are described and their different dynamics highlighted. In Section 3, we define the quantitative measures of the early signatures of sudden regime shifts. Section 4 contains the results of our computations on these early signatures. Section 5 contains concluding remarks.

II. MODELS OF COMPETENCE DEVELOPMENT

Microorganisms like bacteria have to cope with a number of stresses during their lifetime. The bacteria adopt a number of strategies to enhance their chances of survival under changed circumstances \[12, 19\]. One of these is the generation of phenotypic heterogeneity (no genetic change) in the bacterial population so that a fraction of the population, even if small, may survive under stressful conditions. In the \textit{B. subtilis} population, only a part of the population, in which the level of a key transcription factor ComK is high, develops competence \[12, 20\]. The rest of the population is in the so-called vegetative state. The ComK protein acts as a master regulator activating the transcription of several genes including those essential for the uptake of foreign DNA from the environment. The incorporation of the new DNA into the bacterial genome confers favorable traits on the bacterial subpopulation with high ComK level, enabling the subpopulation to adapt to stress. The ComK activity resulting in the development of competence is in turn controlled
by a host of other proteins. The core module of the complex regulatory network consists of an autoregulatory positive feedback loop in which the ComK proteins promote their own production. The positive feedback gives rise to binary gene expression in the cell population, i.e., two distinct subpopulations with low and high ComK levels respectively. Two independent experiments [21, 22] provide confirmation that an autoregulatory positive feedback loop of ComK production is by itself sufficient to establish binary gene expression in a bacterial population. Considering deterministic time evolution, the steady state ComK level versus an appropriate gene expression parameter exhibits a hysteresis curve, resulting from the fold-bifurcation, similar to the one shown in Figure 1 [24]. In this scenario, if the cells in a population are prepared to be in the same initial state, all the cells evolve to the same final state giving rise to a homogeneous cell population. The generation of heterogeneity in the form of two distinct subpopulations is brought about by fluctuation-driven transitions between the low and high ComK expression states, the fluctuations being associated with the ComK levels. This gives rise to the experimentally observed [12, 23] bimodal distribution in the ComK levels, i.e., a distribution with two prominent peaks. There is now experimental evidence [24] that a lower fraction of the B. subtilis population develops competence with reduced noise in the low ComK level. An alternative physical mechanism underlying competence development has been proposed by Suel et al. [25] in terms of excitability in the dynamics of the genetic circuit. The excitable core module consists of both positive and negative feedback loops which bring about a transient activation to the high ComK state. In this case, there is only one stable steady state (low ComK level) and two unstable steady states the lower of which, in terms of the ComK level, sets a threshold for switching [25]. Fluctuations in the low ComK level activate the switch to expression states in the neighborhood of the state with high ComK level which constitutes an unstable steady state. The transient activation is followed by an ultimate return to the stable low ComK state. At any instant of time, the population divides into two subpopulations with low and high ComK levels respectively, signifying a different origin for the bimodal distribution. Quantitative time lapse fluorescence microscopy provides experimental evidence [25] of the probabilistic and transient differentiation into competence.

We first consider Model 1 in which the protein product of a single gene autoactivates its own production via a positive feedback loop. The genetic circuit is shown in Figure 2(a) and, as mentioned earlier, constitutes a core module of the complex network resulting in competence development. The proteins synthesized by the comK gene form dimers. The dimer molecules bind at the promoter region of the DNA and activate gene expression. The gene also synthesizes proteins at a basal level. A kinetic scheme of the model is as follows [23]:

\[
P_2 + G \xrightarrow{k_s} GP_2 \xrightarrow{k_d} G^* \\
G \xrightarrow{J_0} P \\
G^* \xrightarrow{J_1} P \\
P + P \xrightarrow{K_a} P_2 \\
P \xrightarrow{k_p} \phi
\]

(1)

The gene can be in two possible states: inactive (G) and active (G*). Proteins are synthesized with rate constant \(J_1(J_0)\) in the state \(G^*(G)\) with \(J_0 < J_1\). The synthesized proteins dimerize with \(K_a\) as the equilibrium dissociation constant. The protein dimer \(P_2\) binds to the gene in state \(G\) and forms the complex \(GP_2\) with \(k_1\) and \(k_2\) being the rate constants for binding and unbinding. The complex \(GP_2\) in turn is activated to the state \(G^*\), the rate constants \(k_a\) and \(k_d\) being the activation and deactivation rate constants. The synthesized proteins are degraded with a rate constant, \(k_p\), \(\phi\) denoting the degradation product. As shown by Karmakar and Bose [23], the kinetic scheme displayed in Equation (1) can be mapped onto a simpler scheme

\[
G \xrightarrow{k'_a} G^* \\
G \xrightarrow{J_1} P \\
G^* \xrightarrow{J_1} P \\
P + P \xrightarrow{K_a} P_2 \\
P \xrightarrow{k_p} \phi
\]

(2)

The effective activation and deactivation rate constants \(k'_a(x)\) and \(k'_d\) are:

\[
k'_a(x) = k_a \frac{(x/k_s)^2}{1 + (x/k_s)^2}, \quad k'_d = k_d
\]

(3)

In Equation (3), \(x\) denotes the protein concentration and \(k_s = \sqrt{k_1 K_a}\). In the simplified scheme, the rate of change of the protein concentrations:

\[
\frac{dx}{dt} = \frac{J_1 k'_a}{k'_a + k_d} + \frac{J_0 k_d}{k'_a + k_d} - k_p x
\]

(4)

The steady state condition \(\frac{dx}{dt} = 0\) yields three solutions in a specific parameter regime corresponding to two stable steady states separated by an unstable steady state. The model dynamics exhibit the fold-bifurcation of the type shown in Figure 1. The rate constants \(J_0, J_1,\) and \(k_a\) serve as the bifurcation parameters. Figure 2(b) shows the genetic circuit (Model 2), proposed in Ref. [25], as governing the dynamics of competence development via excitability. The circuit contains the autoregulatory positive feedback loop of Figure 2(a). In addition there is a negative feedback loop in which the ComK protein represses the expression of the comS gene whereas the ComS protein inhibits the degradation of ComK by the MecA-ClpP-ClpC complex. The repression of comS by ComK is however,
The stochastic dynamics of the model are investigated using the formulations based on the Langevin and Fokker-Planck (FP) equations. The steady state analysis of a bistable system in these formalisms is described in Refs. 26, 28, 31. The one-variable Langevin equation (LE) including both multiplicative and additive noise terms is given by

$$\frac{dx}{dt} = f(x) + g_1(x)\varepsilon_1(t) + \Gamma(t)$$

(7)

where \(\varepsilon(t)\) (multiplicative noise) and \(\Gamma(t)\) (additive noise) are Gaussian white noises with mean zero and correlations:

$$\langle \varepsilon(t)\varepsilon(t') \rangle = 2d_1\delta(t-t')$$

$$\langle \Gamma(t)\Gamma(t') \rangle = 2d_2\delta(t-t')$$

$$\langle \varepsilon(t)\Gamma(t') \rangle = \langle \Gamma(t)\varepsilon(t') \rangle = 2\lambda_d\sqrt{d_1d_2}\delta(t-t')$$

(8)

In Equation (8), \(d_1\) and \(d_2\) denote the strengths of the noises \(\varepsilon(t)\) and \(\Gamma(t)\) respectively and \(\lambda_d\) is the degree of correlation between them. The first term in Equation (7) describes the deterministic dynamics. In the case of Model 1, \(f(x)\) is given by the right hand side expression in Equation (4). The FP equation is a rate equation for the probability distribution \(P(x,t)\), obtained from the LE as

\[
\frac{\partial P(x,t)}{\partial t} = -\frac{\partial}{\partial x}[A(x)P(x,t)] + \frac{\partial^2}{\partial x^2}[B(x)P(x,t)]
\]

(9)

where

\[
A(x) = f(x) + d_1g_1(x)g_1'(x) + \lambda_d\sqrt{d_1d_2}g_1(x)
\]

(10)

and

\[
B(x) = d_1[g_1(x)]^2 + 2\lambda_d\sqrt{d_1d_2}g_1(x) + d_2
\]

(11)

III. EARLY SIGNATURES OF REGIME SHIFTS

The deterministic dynamics of Model 1 are governed by the rate equation shown in Equation (4). A simple stochastic version of the model has been studied in Ref. 23. In this model, the only stochasticity considered is associated with the random transitions of the gene between the inactive (G) and active (G*) states. The rest of the processes undergo deterministic time evolution. The simple model yields bimodal protein distributions in the steady state in certain parameter regions. In this paper, the stochastic dynamics of the model are investigated using the formulations based on the Langevin and Fokker-Planck (FP) equations. The steady state analysis of a bistable system in these formalisms is described in Refs. 26, 28, 31. The one-variable Langevin equation (LE) including both multiplicative and additive noise terms is given by

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\]

(10)

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\[
B(x) = d_1[g_1(x)]^2 + 2\lambda_d\sqrt{d_1d_2}g_1(x) + d_2
\]

(11)
The steady state probability distribution (SSPD) is given by

$$P_s(t) = \frac{N}{B(x)} \exp \left[ \int^t A(x) \, dx \right]$$

$$= \frac{N}{\left( d_1 |g_1(x)|^2 + 2 \lambda d \sqrt{d_1 d_2 g_1(x)} + d_2 \right)}$$

$$\times \exp \left[ \int^{x_f} \frac{f(x') \, dx'}{d_1 |g_1(x')|^2 + 2 \lambda d \sqrt{d_1 d_2 g_1(x')} + d_2} \right]$$

where $N$ is the normalization constant. Equation (12) can be rewritten in the form

$$P_{st}(x) = Ne^{-\phi_F(x)}$$

(13)

with

$$\phi_F(x) = \frac{1}{2} \ln \left( d_1 |g_1(x)|^2 + 2 \lambda d \sqrt{d_1 d_2 g_1(x)} + d_2 \right)$$

$$- \int^x \frac{f(y) \, dy}{d_1 |g_1(y)|^2 + 2 \lambda d \sqrt{d_1 d_2 g_1(y)} + d_2}$$

(14)

$\phi_F(x)$ defines the “stochastic potential” of the dynamics. Once the SSPD is determined, quantities like the variance $\phi_M$ can be rewritten in the form

$$\phi_M = \int P_s(x) \, dx$$

The solution of the Equation (15) is

$$\eta(t) = \eta(0) e^{\lambda t}$$

(16)

where $\eta(0)$ is the initial value of $\eta(t)$ at time $t = 0$. The sign of the parameter $\lambda$ indicates the stability of the steady state. If $\lambda < 0$, the system is stable and if $\lambda > 0$, the system is unstable. We designate $\lambda$ as the parameter $\lambda$ associated with the steady state losing stability, becomes zero.

In the case of Model 1, one can check that $\lambda = 0$ at the two bifurcation points. The return time $T_R = 1/|\lambda|$ thus diverges at a bifurcation point. If the dynamical system is described by more than one variable, the stability of a steady state is determined by the eigenvalues of the Jacobian matrix governing the dynamics of the perturbed system.

Let $\lambda_{\text{max}}$ be the real part of the dominant eigenvalue of the Jacobian matrix (for stability, the real parts of all the $\lambda$’s should be negative). The dominant eigenvalue is the one with the largest real part. The return time $T_R$ is given by $T_R = 1/|\lambda_{\text{max}}|$. Examples of the experimental observations of the CSD include the transition from the G2 growth phase to the mitotic phase.
of the eukaryotic cell division cycle [31], a direct observation of the CSD in a laboratory population of budding yeast before population collapse occurs at a critical experimental condition [32] and the demonstration of the CSD in a population of cyanobacteria [33].

In the presence of noise, the variance $\sigma^2$ is determined from the fluctuation-dissipation (FD) relation (Equation (A.3)) in Appendix A. In the case of a one-variable model (Model 1), the matrix $A$ consists of a single element $\lambda$, the stability parameter defined in Equation (15). The steady state covariance matrix $C$ reduces to a single element, the variance $\sigma^2$, which is determined as

$$\sigma^2 = \frac{D}{2 | \lambda |}$$

with $\lambda$ negative. Also, the lag-1 autocorrelation in the stationary state (Equations (A.8) and (A.9)) is given by

$$\rho(1) = e^\lambda$$

The variance $\sigma^2$ diverges and the lag-1 autocorrelation $\rho(1)$ reaches its maximum value at the bifurcation point since the stability parameter $\lambda$ is zero at this point. Rising variance and autocorrelation thus provide early signatures of impending regime shifts. The CSD close to the bifurcation point implies that the system’s intrinsic rates of change are decreased so that the state of the system at time $t$ resembles closely the state at time $t-1$. This increased memory is measured by the lag-1 autocorrelation function. Also, because of a flatter basin of attraction close the transition point, a given perturbation brings about a greater shift in the system’s state, i.e., an increasing variance.

Two other early signatures of a regime shift which are not related to the CSD are skewness and flickering [13]. The skewness $\gamma$ of a probability distribution $P(x)$ is a dimensionless measure of its degree of asymmetry and is defined as

$$\gamma = \frac{\int (x - \mu)^3 P(x)dx}{\sigma^3}$$

Figure 6. For Model 1, the plot of $|\gamma|$, where $\gamma$ is the skewness of the normalized steady SSPD, versus the strength of additive noise $d_2$ for the parameter values $k_a = 1.5$, $k_d = 1.5$, $k_p = 0.03$, $J_0 = 0.01$, $J_1 = 1$, and $d_1 = 0$ (no multiplicative noise). The value $k_a = 1.5$ falls in the region of bistability.

Figure 7. For Model 1, the variation of the stability parameter $\lambda$ (Equation (15)), the return time $T_R$, the lag-1 autocorrelation $\rho_1(1)$ and the variance $\sigma^2$ (Equations (17) and (18)) as a function of the bifurcation parameter. The plots exhibit characteristic features at the bifurcation points $k_{a,1} = 1.344$ and $k_{a,2} = 5.176$. The parameter values are $k_d = 1$, $k_a = 15$, $k_p = 0.03$, $J_0 = 0.01$, $J_1 = 1$ and $\sigma_d = 0.25$.

Figure 8. The computed data points in the $\ln T_R$ versus (a) $\ln |k_a - k_{a,1}|$ and (b) $\ln |k_a - k_{a,2}|$ plots are fitted by straight lines (a) $y = A + Bx$ and (b) $y = C + Dx$ respectively. The parameter values are the same as in the case of Figure 7. The values of $A$ and $C$ are $A = 3.53401 \pm 0.00008$ and $C = 4.35482 \pm 0.00049$. The exponents have values very close to $-\frac{1}{2}$ ($B = -0.49752 \pm 0.00013$ and $D = -0.49901 \pm 0.00099$).

where $\mu$ and $\sigma$ are respectively the mean and standard deviation of $P(x)$. The variance $\sigma^2$, the coefficient of variation $C_v$ and the third moment $M_3$ of $P(x)$ are expressed as

$$\sigma^2 = \int (x - \mu)^2 P(x)dx$$

$$M_3 = \int (x - \mu)^3 P(x)dx$$

$$C_v = \frac{\sigma}{\mu}$$

The skewness of a probability distribution increases as the bifurcation point is approached. This is because of the asymmetry in fluctuations with a system exhibiting greater amplitude deviations in the direction of the state it is fated to switch to than in the other direction. The
phenomenon of flickering is observed in the region of bistability with the system switching back and forth between the two attractor states before the bifurcation point is reached. We propose a quantitative measure of flickering which serves as an early signature of regime shift. In the region of bistability, the stability landscape has two minima corresponding to the two stable steady states. Noise-induced transitions take the system from one valley to the other. The mean first passage time (MFPT) refers to the induced transitions take the system from one valley to the other. The mean first passage time (MFPT) refers to the time-series data of \(x(t)\) versus \(t\) obtained by solving the deterministic rate equation, Equation (4). The parameter values are \(k_d = 1, k_s = 15, k_p = 0.03, J_0 = 0.01, J_1 = 1\) and \(d_2 = 0.25\).

IV. RESULTS ON EARLY SIGNATURES

A. Model 1 (one variable)

We first present the results on early signatures in the case of Model 1. In the region of bistability, the SSPD \(P_{st}(x)\) is bimodal with two distinct peaks. \(P_{st}(x)\) may be obtained via a solution of the FP equation (Equation 12) or obtained by a numerical solution of the LE (Equation 7). Figure 3 shows an example of a bimodal distribution with two distinct peaks at \(x = x_1\) and \(x_3\). The inset of the figure shows the corresponding stochastic potential profile (Equation 13) with \(x_2\) denoting the location of the hill and \(x_1, x_3\) denoting the minima of the left and right valley respectively. We find the normalized probability distributions, \(P^l_{st}(x)\) and \(P^u_{st}(x)\), corresponding to the low and high expression states respectively, using the following procedure:

1. From the stochastic potential profile \(\phi_F(x)\), determine the position of the hill at \(x_2\) and compute \(P_{st}(x_2)\). The point \(x_2\) corresponds to the minimum of the probability distribution between its two peaks at \(x_1\) and \(x_3\). The point \(x_2\) is chosen to be the left cut-off point, \(x^l_{st}\), for \(P^u_{st}(x)\). The right cut-off point, \(x^u_{st}\), of \(P^u_{st}(x)\) is obtained as a solution of the equation

\[
P_{st}(x) = P_{st}(x^u_{st}), \quad x > x_3
\]

2. If \(P_{st}(0) < P_{st}(x_2)\), then the lower cut-off point, \(x^l_{st}\), of \(P^l_{st}(x)\) is determined from the solution of the equation

\[
P_{st}(x) = P_{st}(x^l_{st}), \quad x < x_1
\]

where \(x^l_{st}\), the right cut-off point of \(P^l_{st}(x)\), is given by \(x^l_{st} = x^l_{st}\). If \(P_{st}(0) > P_{st}(x_2)\), \(x^l_{st} = 0\).

Other cut-off procedures, e.g., that in Ref. 5, have been proposed to isolate the low and high expression probability distributions but the general results on the early signatures are qualitatively similar.

In the case of Model 1, we first consider only an additive noise in the LE (Equation 7, \(g_1(x) = 0\)). The additive noise \(\Gamma(t)\) represents noise arising from an external perturbative influence or originating from some missing information because of rate equation approximations [31]. The SSPD, \(P_{st}(x)\), in the region of bistability is obtained from Equation 12 putting \(g_1(x), g_1(x') = 0\). Following the prescription already given, one determines the cut-off points of the distribution \(P^l_{st}(x)\). The normalized distributions are obtained from

\[
P^l_{st}(x) = \frac{P^l_{st}(x)}{\int_{x^l_{st}}^{x^l_{st}} P_{st}(x)dx} \quad \text{for} \quad x^l_{st} < x < x^l_{st}
\]

\[
P^u_{st}(x) = \frac{P^u_{st}(x)}{\int_{x^u_{st}}^{x^u_{st}} P_{st}(x)dx} \quad \text{for} \quad x^u_{st} < x < x^u_{st}
\]

In Equations 23 and 24, \(P'_{st}(x)\) is not normalized. With a knowledge of the normalized probability distributions \(P^l_{st}(x)\) and \(P^u_{st}(x)\), one can compute the skewness \(\gamma\), variance \(\sigma^2\), third moment \(M_3\) and the coefficient of variation \(C_v\) using Equations 19 and 20. Figure 5 shows plots of these quantities (solid lines) versus the bifurcation parameter \(k_a\) for the probability distribution \(P^u_{st}(x)\), i.e., considering the system to be in the high expression state. The sudden regime shift in the deterministic case occurs at the lower bifurcation point \(k_{a,1} = 1.334\). The parameter values used for the computation are: \(k_d = 1, k_s = 15, k_p = 0.03, J_0 = 0.01\) and \(J_1 = 1\). The strength \(d_2\) of the additive noise is \(d_2 = 0.25\). One finds that all the four quantities \(\gamma, \sigma^2, M_3\) and \(C_v\) increase as the lower bifurcation point is approached thus providing early signatures of a regime shift. The quantities, however, reach their maxima before the deterministic bifurcation point is reached and then start decreasing. The quantities, though providing early signatures, cannot provide knowledge of the bifurcation point. We next include an additional mul-
The multiplicative noise is associated with the protein synthesis rate constant \( J_1 \) in the active state, i.e., \( J_1 \rightarrow J_1 + \varepsilon(t) \). The origin of multiplicative noise lies in the fact that the rate constants are expected to fluctuate in time due to the inherently stochastic nature of gene expression as well as due to stochastic influences like fluctuations in the number of regulatory molecules and RNA polymerases. With both the additive and multiplicative noise terms present, the SSPDs \( P_n^a(x) \) and \( P_n^u(x) \) are computed following the procedure already described. The dotted curves in Figure 5 show the variations of \( \gamma \), \( \sigma^2 \), \( M_3 \) and \( C_v \), associated with the probability distribution \( P_n^u(x) \), as a function of the bifurcation parameter \( k_a \). The parameter values are the same as before with \( d_1 \), the strength of the multiplicative noise term, having the value \( d_1 = 0.25 \). The cross-correlation coefficient \( \lambda_d \) in Equation (5) is taken to be zero. Figure 6 shows how the skewness of \( P_n^u(x) \) changes as a function of the additive noise strength \( d_2 \) for the parameter values \( k_a = 1.5, k_b = 1, k_s = 15, k_p = 0.03, J_0 = 0.01, J_1 = 1 \) and \( d_1 = 0 \) (no multiplicative noise). The value \( k_a = 1.5 \) is greater than the value of \( k_a,1 = 1.344 \). The rising skewness is thus a signature of noise-induced regime shift. In the case of the SSPD \( P_n^a(x) \), one obtains early signatures of the upper bifurcation point \( k_{a,2} = 5.176 \) similar to the ones shown in Figures 4 and 5 in the case of \( P_n^u(x) \), though the quantitative measures exhibit less prominent variation. We find that the early signatures of regime shifts are obtained in both the cases, (i) only additive noise is present and (ii) additive as well as multiplicative types of noise are present.

Considering Model 1, we next calculate the stability parameter \( \lambda \) (Equation 13), with \( f(x) \) given by the expression on the r.h.s. of Equation (4), in the region of bistability. Knowing \( \lambda \), the return time \( T_R(= \frac{1}{\lambda}) \) as well as the lag-1 autocorrelation \( \rho(1) \) and the variance \( \sigma^2 \) (Equations 17 and 18) are also determined. Figure 7 shows the variation of \( \lambda \), \( T_R \), \( \rho(1) \) and \( \sigma^2 \) as a function of the bifurcation parameter \( k_a \) and for the parameter values \( k_a = 1, k_s = 15, k_p = 0.03, J_0 = 0.01, J_1 = 1 \) and \( \sigma_d = 0.25 \). The stability parameter \( \lambda \) becomes zero at the lower \( (k_{a,1} = 1.344) \) and the upper \( (k_{a,2} = 5.176) \) bifurcation points as expected. The associated return time \( T_R \) diverges at the bifurcation points, a characteristic feature of the CSD. The lag-1 autocorrelation \( \rho(1) \) reaches the maximum value and the variance \( \sigma^2 \) diverges at the bifurcation points. These quantities are good indicators of regime shifts and carry distinct signatures of the bifurcation points. The return time \( T_R \) is known to satisfy a general scaling law \( T_R \sim |B - B_1|^{-\frac{3}{2}} \) where \( B \) and \( B_1 \) stand for the bifurcation parameter and point respectively. In Figure 8, the scaling is demonstrated for the bifurcation parameter \( k_a \) and the bifurcation points \( k_{a,1} = 1.344 \) and \( k_{a,2} = 5.176 \). The exponent is very close to \(-\frac{3}{2}\) in each case.

Figure 9 shows the variation of \( r = T_1/T_2 \) versus the bifurcation parameter \( k_a \). The ratio is seen to diverge at the lower bifurcation point \( k_{a,1} = 1.344 \). The parameter values are \( k_d = 1, k_s = 15, k_p = 0.03, J_0 = 0.01, J_1 = 1 \) and \( d_2 = 0.25 \) (only additive noise is considered). The time-series data shown in the inset of Figure 9 is obtained via numerical solution of the LE (Equation 7) using the algorithm described in Ref. 39. In the limit of large times, the steady state is assumed to be reached. The solid lines in the inset mark the stable expression states \( x^\alpha \) and \( x^\beta \) and the dotted line corresponds to the unstable steady state \( x^\gamma \). The values are obtained from a solution of the deterministic rate equation. The MFPTs \( T_1 \) and \( T_2 \) are computed using the method outlined in Ref. 57. Let us consider a bistable potential with the stable steady states at \( x_1 \) and \( x_3 \) \( (x_1 < x_3) \) which are separated by an unstable steady state at \( x_2 \), termed the barrier state (Figure 9). The MFPT \( T(x; a, b) \) is the average time of the first exit from the interval \((a, b)\) and satisfies the equation (25)

\[
-1 = A(x) \frac{dT(x)}{dx} + \frac{1}{2} B(x) \frac{d^2T(x)}{dx^2}
\]

(26)

where \( A(x) \) and \( B(x) \) appear in the associated FP equation (Equation 9). The MFPT \( T(x_1) (= T_1) \) for exit from the basin of attraction of the stable steady state at \( x_1 \) is obtained as a solution of Equation (25) with the interval \((a, b) = (0, x_2)\) and boundary conditions given by

\[
T'(a; a, b) = 0 \quad \text{and} \quad T'(b; a, b) = 0
\]

(27)

The prime denotes differentiation with respect to \( x \), with reflecting and absorbing boundary conditions prevailing at \( a \) and \( b \) respectively (28, 53). Following the same procedure, the MFPT \( T(x_3)(= T_2) \) for exit from the basin with the stable steady state at \( x_3 \) can be calculated from Equation (25). The interval now is \((a, b) = (x_2, \infty)\) with \( x_2 \) and \( \infty \) serving as absorbing and reflecting boundary points respectively, i.e.,

\[
T(a; a, b) = 0 \quad \text{and} \quad T'(b; a, b) = 0
\]

(28)

Figure 10. The phase diagram of Model 2 in the \( b_n - b_s \) plane. It shows two regions of monostability, one region of bistability and one region of excitation. The boundaries shown as solid lines correspond to saddle-node (SN) bifurcations. Lines 1 and 2 mark the values of the parameter \( k_a \) for which computations are carried out. The other parameter values are the same as in Ref. 22, namely, \( a_k = 0.004, k_0 = 0.2, k_1 = 0.222, n = 2 \) and \( p = 5 \).
drawn in the phase diagram at the points SN bifurcation \[13\]. Two vertical lines 1 and 2 are types: monostable low (low ComK level) and monostable gram shows three different regions: monostable, bistable between the mid-ComK and high-ComK levels. For the is a saddle point, (iv) only one fixed point exists which characteris- tic of an unstable spiral and the mid-ComK fixed point is a saddle point, (iv) only one fixed point exists which is unstable. The system exhibits limit cycle oscillations between the mid-ComK and high-ComK levels. For the purpose of our study, a part of the \(b_k - b_s\) phase diagram has been recomputed and shown in Figure 11. The diagram shows three different regions: monostable, bistable and excitable. The monostable region is again of two types: monostable low (low ComK level) and monostable high (high ComK level). The boundaries between the regions depicted by solid lines correspond to the saddle-node (SN) bifurcation \[13\]. Two vertical lines 1 and 2 are drawn in the phase diagram at the points \(b_{s,1} = 0.0875\) and \(b_{s,2} = 0.15\) respectively. Line 1 intersects the phase boundaries at the three points \(b_{s,1} = 0.7799, b_{s,2} = 0.7868\) and \(b_{s,3} = 0.8094\). Line 2 has two points of intersection: \(b_{s,1} = 0.6175\) and \(b_{s,2} = 0.7504\). Figure 11 shows the plots of \(\lambda_{\text{max}}\), the real part of the dominant eigenvalue of the Jacobian matrix computed from Equations \[3\] and \[6\], and the return time \(T_R = \frac{1}{\lambda_{\text{max}}}\) versus \(b_s\) along line 1 ((a) and (b)) and along line 2 ((c) and (d)). For a specific steady state, the Jacobian matrix \(J\) has the following structure \[13\]:

\[
J = \begin{pmatrix} f_{11} & f_{12} \\ f_{21} & f_{22} \end{pmatrix}_{SS}
\]  

(29)

The suffix \(SS\) stands for steady state, i.e., the matrix elements are to be computed at the fixed point \((x^*, y^*)\). A matrix element \(f_{ij}\) is given by

\[
f_{ij}(x_1, x_2) = \frac{\partial f_i}{\partial x_j} (i = 1, 2, j = 1, 2)
\]  

with \(x_1 = K, x_2 = S, n = 2\) and \(p = 5\). The parameter values are the same as in Ref. \[25\]: \(a_k = 0.005, b_0 = 0.2, k_1 = 0.222\). The eigenvalue of \(J\) are \(\lambda_1\) and \(\lambda_2\) and \(\lambda_{\text{max}}\) is the real part of the dominant eigenvalue. In Figure 11 one notes that \(\lambda_{\text{max}}\) becomes zero at the SN bifurcation points as expected and the corresponding return time \(T_R\) diverges at a bifurcation point. The shaded regions in the Figure denote the regions of bistability in which the two stable steady states correspond to low and high ComK levels respectively. The level of ComS is anticorrelated with that of ComK. The solid (dotted) lines in Figures 11 are associated with the stable steady states representing low (high) ComK levels. Along line 2 and at the bifurcation point \(b_{s,1} = 0.6176\) (Figures 11(c) and (d)) the stable steady state corresponding to the high ComK level loses stability \((\lambda_{\text{max}} = 0, T_R\) diverges). At the boundary point \(b_{s,2} = 0.7504\), the state representing the low ComK level loses stability. A steady state is stable if the real parts of both \(\lambda_1\) and \(\lambda_2\) are negative. At the point \(b_{s,1} = 0.7799\) along line 1 (Figures 11(a) and (b)), there is no loss of stability of a steady state and hence no CSD with diverging \(T_R\) is observed at this point. The low ComK level of the monostable region continues to remain stable as one enters the region of excitability at the point \(b_{s,1}\) along line 1. At the point \(b_{s,2} = 0.7868\), the system enters the region of bistability from a region of excitability. The high ComK stable steady state loses its stability at this point with \(\lambda_{\text{max}} = 0\) and a divergent \(T_R\) (dotted branch in Figures 11(a) and (b)). The low ComK state loses stability at the point \(b_{s,3} = 0.8094\) when the system passes from a region of bistability to a region of monostable high ComK level. The eigenvalue \(\lambda_{\text{max}} = 0\) and \(T_R\) diverges at this point. The main point to note from the results is that there is no CSD and diverging return time \(T_R\) at the transition point \(b_{s,1}\) along line 1 between the regions of monostability and excitability. On the other hand, at the point \(b_{s,1}\) along line 2, separating the regions of monostability and bistability, one of the expression states, namely, the high ComK state, loses stability with a divergent \(T_R\).

B. Model 2 (two variables)

We next consider Model 2 the dynamics of which are governed by the set of two equations (5) and (6). In Ref. \[25\], the phase diagram of the model in the \(b_k - b_s\) plane has been determined which has four different regions: (i) monostability with only one fixed point, (ii) bistability with three fixed points, two stable and one unstable, (iii) excitation involving three fixed points only one of which, corresponding to low ComK value, is stable. The competent fixed point (high ComK level) has the characteristic of an unstable spiral and the mid-ComK fixed point is a saddle point, (iv) only one fixed point exists which is unstable. The system exhibits limit cycle oscillations between the mid-ComK and high-ComK levels.
the elements calculated from Equations (30) and (31) using the relationship \( f_{ij} = \frac{\partial f_i}{\partial x_j} \) \((i = 1, 2, j = 1, 2)\). The stoichiometric matrix is given by

\[
S = \begin{pmatrix} 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 \end{pmatrix}
\] (32)

The first and second rows of \( S \) correspond to \( \text{ComK} \) and \( \text{ComS} \) molecular numbers respectively. The “elementary complex reactions” [26, 27] considered for the computations are the composite reactions (Equations (6) and (8) with \( x_1 = K \) and \( x_2 = S \)),

\[
\begin{align*}
X_1 \xrightarrow{a_k + \frac{b_k x_k^p}{x_1 + x_1}} & X_1 + 1 \\
X_1 \xrightarrow{1 + \tau_1 / x_2} & X_1 - 1 \\
X_2 \xrightarrow{1 + \tau_2 / x_2} & X_2 + 1 \\
X_2 \xrightarrow{1 + \tau_3 / x_2} & X_2 - 1
\end{align*}
\] (33)

The reaction propensity vector (Equation (A.2)) is given by

\[
f(x) = \begin{pmatrix} a_k + \frac{b_k x_k^p}{x_1 + x_1} & 0 & 0 & 0 \\ 0 & \frac{x_1}{1 + x_1 + x_2} & 0 & 0 \\ 0 & 0 & \frac{b_k}{1 + \frac{x_k^p}{x_2}} & 0 \\ 0 & 0 & 0 & \frac{1 + \tau_3 / x_2}{1 + \tau_3 / x_2 + x_2} \end{pmatrix}
\] (34)

With knowledge of the stoichiometric matrix \( S \) and the reaction propensity vector \( f(x) \), the diffusion matrix \( D \) in the FD relation can be calculated using Equation (A.7). Substituting the computed \( A \) and \( D \) matrices in the FD relation, the variances and the covariances are determined. Similarly, with the help of Equations (A.8) and (A.9), the lag-1 autocorrelation function \( \rho_{11}(\tau) = \langle \delta x_1(t + \tau) \delta x_1(t) \rangle \) with \( \tau = 1 \) is calculated. Figures 12 (a) and (b) exhibit the plots of the variance \( \sigma^2 = \langle \delta x_1^2 \rangle \), i.e., the variance of the \( \text{ComK} \) fluctuations, as a function of the bifurcation parameters \( b_s \). Figures 12 (c) and (d) exhibit the lag-1 autocorrelation function \( \rho_{11}(1) \), associated with the \( \text{ComK} \) fluctuations, as a function of the bifurcation parameter \( b_s \). The parameter values used in the computations are the same as in the case of Figure 11. In the cases of Figures 12(a) and (b), the computations are carried out along the line \( b_{s,1} = 0.0875 \) which traverses successively through the regimes of monostability, excitability, bistability and monostability. In the cases of Figures 12(c) and (d), the calculations are carried out along the line \( b_{s,2} = 0.15 \). The shaded regions in Figure 12 represent the regions of bistability with the solid (dotted) lines associated with low (high) \( \text{ComK} \) levels. From the plots one finds that along the line \( b_{s,2} = 0.15 \), the high \( \text{ComK} \) level loses stability at the bifurcation point \( b_{s,1} = 0.6176 \) (Figures 12(c) and (d)). The sudden regime shift from the high to the low \( \text{ComK} \) state is signaled by a diverging variance as the bifurcation point is approached and the lag-1 autocorrelation function attaining its maximum value at the point. Similar signatures are obtained at the other bifurcation point \( b_{s,2} = 0.7504 \) where the low \( \text{ComK} \) state loses stability. In Figures 12(a) and (b), at the point \( b_{s,1} = 0.7799 \) along line \( 1 \) (\( b_{k,1} = 0.0875 \)), as one enters a region of excitability from a region of monostability, the low \( \text{ComK} \) level of the monostable region continues to be stable. Since no state loses stability at the point, the variance \( \sigma^2 \) and the lag-1 autocorrelation function \( \rho_{11}(1) \) do not provide any signatures of regime change. At the point \( b_{s,2} = 0.7868 \), the system enters the region of bistability from a region of excitability. The high \( \text{ComK} \) state loses its stability at this point signaled by a diverging variance and with \( \rho_{11}(1) \) attaining a maximum at this point. The low \( \text{ComK} \) level loses stability at the point \( b_{s,3} = 0.8094 \) when the system transitions from a region of bistability to a region of monostability with high \( \text{ComK} \) level. Experimentally, the entry into a region of excitability/bistability from a region of monostability (low \( \text{ComK} \) level) is identified by the appearance of a bimodal distribution in the \( \text{ComK} \) levels as observed in single cell flow-cytometry measurements. In the case of excitability, however, there are no accompanying signatures in the measurable quantities like variance and lag-1 autocorrelation function. In the case of bistability, sharp rises in \( \sigma^2 \) and \( \rho_{11}(1) \) indicate an impending regime shift.

V. CONCLUDING REMARKS

In this paper, we investigate the early signatures of sudden regime shifts occurring at the bifurcation points associated with a fold-bifurcation model. The basic concepts and methodology are applicable to a general class of bifurcation phenomena occurring in diverse systems. Our focus, however, is on obtaining quantitative estimates of the early signatures of regime shifts in the gene expression dynamics of competence development in \( B. subtilis \). While quantities like \( \lambda_{\text{max}} \) and \( T_R \) are computed with a knowl-
edge of the deterministic rate equations, stochastic formalisms based on the LE, the FP equation and the LNA have been employed to calculate the other quantities. The early indicators of regime shifts, which include the CSD, variance, lag-1 autocorrelation, skewness and the ratio $r$ of MFPTs, are shown to exhibit distinctive variations as a bifurcation point is approached. Though the literature on the early signatures of regime shifts in ecosystems, financial markets and complex diseases is extensive [41,42], the issue has not been systematically addressed in the case of gene expression dynamics, a fundamental activity in the living cell. Our study is the first in this direction and illustrates how the early signatures provide knowledge in advance of an impending regime shift. Some of these signatures may also provide clues on the physical principles underlying gene expression dynamics. As already pointed out, $\lambda_{\text{max}}$ and $T_R$ do not exhibit any distinctive features(Figures 11(a) and (b)) when the system passes from a region of excitability (bimodal distributions in protein levels) to a region of monostability (unimodal distribution of low ComK levels). This is in contrast to the case when the system passes from a region of bistability to one of monostability. The quantity $\lambda_{\text{max}}$, associated with the steady state which loses stability, becomes zero at the bifurcation point and the return time $T_R$ diverges (Figures 11(c) and (d)). Experiments detecting the CSD, as the bifurcation point is approached, are difficult to carry out. Observation of the CSD in recent experiments [25,26] provides pointers for carrying out further such experiments. The CSD experiment, if designed in the appropriate manner, would be able to distinguish between the bistability versus excitability paradigm in the case of competence development in B.subtilis. Experiments based on flow-cytometry and time-lapse fluorescence microscopy are easier to carry out and provide estimates of the variance $\sigma^2$ and the lag-1 autocorrelation function $\rho_1(1)$ [25,26]. Similar to Figure 11, no distinctive signatures are obtained in terms of $\sigma^2$ and $\rho_1(1)$ as one crosses from a region of excitability to that of monostability (Figures 12(a) and (b)) whereas prominent signatures indicate the passage from bistability to monostability (Figures 12(c) and (d)). Thus flow-cytometry and time-lapse fluorescence microscopy measurements for a range of values of the bifurcation parameter would be able to distinguish between bistability and excitability as the physical mechanism underlying competence development.

One utility of obtaining knowledge of the early signatures is that specific risk aversion strategies may be developed if the sudden transition is to a regime which is undesirable due to various considerations. Examples of such regime shifts include asthma attacks [3], epileptic seizures [3] and the sudden deterioration of complex diseases [12]. One may add the development of persistence of pathogens like M. tuberculosis in the human lung granulomas to the list. Recent experiments [40,41] on a sister species M. smegmatis provide evidence that a fraction of the mycobacterial population (the population of persisters) is able to survive under nutrient depletion. To achieve this, the mycobacteria adopt the strategy of generating phenotypic heterogeneity in the form of two distinct subpopulations. The concentration of a key regulatory protein Rel is high in one subpopulation (which develops into the persister subpopulation) and low in the other. In the subpopulation with high Rel level, the stringent response pathway is initiated which help the mycobacteria to avoid death and adapt to nutrient depletion. The experiments [40,41] show that the principle underlying the development of phenotypic heterogeneity is based on bistability and noise-induced transitions between the expression states corresponding to low and high Rel levels. The problem of persisters is that they are not killed by antibiotic drugs and wait for the opportune moment to restart an infection [12]. Early signatures of a regime shift from the normal to the persistent state would help in developing measures preventing the switch to persistence. Similar studies could be carried out on other systems in which bifurcation phenomena are responsible for the development of heterogeneity, choice of cell fate [10] or regime shifts leading to new types of dynamical behaviour.

Appendix: A. Linear Noise Approximation

In the Appendix A, we describe briefly the linear noise approximation (LNA) to the Chemical Master Equation (CME) [26,27] and introduce the notations for the relevant quantities. We consider an intracellular biochemical system with volume $\Omega$ and $N$ different chemical components. The concentrations of the components are represented in the form of the vector $x = (x_1, x_2, \ldots, x_N)^T$ where $T$ denotes the transpose. The chemical constitutents take part in $R$ elementary reactions. The state of the system is given by $x$ which changes due to the occurrence of any one of the $R$ reactions. We define the integers $S_{ij}, i = 1, 2, \ldots, N, j = 1, 2, \ldots, R$ to be the elements of a stoichiometric matrix $S$. The number of molecules of the chemical components $i$ changes from $X_i$ to $X_i + S_{ij}$ when the $j$th reaction takes place.

The deterministic dynamics of the system are described by the rate equations

\[
\frac{dx_i}{dt} = \sum_{j=1}^{R} S_{ij} f_j(x) \quad (i = 1, 2, \ldots, N) \quad (A.1)
\]

In a compact notation

\[
x = Sf(x), \quad f(x) = (f_1(x), \ldots, f_R(x))^T \quad (A.2)
\]

where $f(x)$ defines the reaction propensity vector. In the steady state, $\dot{x} = 0$ with the state vector $x_0$ determined from the condition $f(x_0) = 0$. Let $\delta x$ denote a weak perturbation applied to the steady state, i.e., the new state vector $x = x_0 + \delta x$. On Taylor expansion of the rate vector $\frac{dx}{dt}$ about the steady state and retaining only terms linear in $\delta x$, one gets

\[
\frac{d}{dt}\delta x = A\delta x \quad (A.3)
\]

where $A$ is the Jacobian matrix the elements of which are given by
\[ A_{ij} = \sum_{k=1}^{R} S_{ik} \frac{\partial f_k}{\partial x_j} \]  

(A.4)

The state \( x_s \) is stable if all the eigenvalues of \( A \) have negative real parts. A deterministic dynamical model, as described above, is appropriate for describing the dynamics of a system when the number of molecules, \( X_i \), \( i = 1, 2, ..., N \) is large. In reality, the biomolecules participating in cellular reactions are mostly small in number so that a stochastic description of the dynamics is more valid.

The CME describes the rate of change of the probability distribution \( P(X_1, X_2, ..., X_N, t) \) of the numbers of the different chemical components. The CME is not exactly solvable in most cases and one has to take recourse to various approximate methods in order to solve the equation. The LNA provides an approximation to the CME via a large volume (\( \Omega \)) expansion around the macroscopic steady state. Noise in the form of fluctuations around the steady state is expected to be small in the large \( \Omega \) limit. Noise in the form of fluctuations around the steady state is given by a multivariate Gaussian distribution. One can compute at the stationary state \( \langle \delta x_i \rangle = 0 \), \( i = 1, 2, ..., N \). Once \( A \) and \( D \) are determined, one can determine the elements of the covariance matrix (specially, the variances) from the FD relation (A.5). The time correlation matrix for \( \delta x \) is given by

\[ \langle \delta x(t + \tau)(\delta x(t))^T \rangle = \exp(\mathbf{A} \tau) \mathbf{C} \]

with

\[ \langle \delta x_i(t + \tau)(\delta x_j(t))^T \rangle = \sum_{m} \sum_{n} e^{\lambda_m \tau} (T^{-1})_{mn} T_{nm} C_{nj} \]  

(A.8)

In Equation (A.8), \( \lambda_m \)'s are the eigenvalues of the Jacobian matrix \( A \) and \( T \) is a matrix the columns of which represent the right eigenvectors of \( A \). The diagonal elements of the matrix are the autocovariances and \( \tau \) defines the lag time. The lag-\( \tau \) autocorrelation for the \( i \) th chemical component is

\[ \rho(\tau) = \frac{\langle \delta x_i(t + \tau)(\delta x_j(t)) \rangle}{\sqrt{\text{var}(x_i(t + \tau)) \text{var}(x_i(t))}} \]  

(A.9)

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