Stochastically driven genetic circuits

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Transcriptional regulation in small genetic circuits exhibits large stochastic fluctuations. Recent experiments have shown that a significant fraction of these fluctuations is caused by extrinsic factors. In this paper we review several theoretical and computational approaches to modeling of small genetic circuits driven by extrinsic stochastic processes. We propose a simplified approach to this problem, which can be used in the case when extrinsic fluctuations dominate the stochastic dynamics of the circuit (as appears to be the case in eukaryots). This approach is applied to a model of a single nonregulated gene that is driven by a certain gating process that affects the rate of transcription, and to a simplified version of the galactose utilization circuit in yeast. © *2006 American Institute of Physics*. [DOI: 10.1063/1.2209571]

Recent experimental progress in demonstrating the importance of fluctuations in gene expression has fueled interest in the exploration of novel stochastic modeling techniques for biochemical networks. In the form of gene regulation, these networks consist of interacting transcripts, proteins, and DNA binding sites, and the governing network diagrams resemble complex electrical circuits. This complexity has led many modelers to focus on smaller modular components that perform specific cellular functions. Such a modeling emphasis dovetails with the synthetic biology thrust of engineering gene circuits, whereby small gene networks are constructed and tested in living cells using fluorescent proteins. In this work, we first review recent progress in experimental and modeling studies that parse gene expression noise into intrinsic and extrinsic components. The experiments to date have demonstrated that the major component of noise is extrinsic, yet the theoretical and modeling studies have largely focused on either the linear noise approximation, where both components are small, or situations where the intrinsic noise dominates. We present a simplified approach that can be used when extrinsic fluctuations dominate the stochastic dynamics of a genetic circuit and apply this approach to two model problems. We first investigate a single gene that is driven by a dichotomous gating process that affects the rate of transcription. We are able to make significant analytical progress, and calculate not only the variance but the full probability distribution of the reporter protein fluctuations and the cross correlation between the protein fluctuations and the extrinsic gating process. We then consider fluctuations in a simplified model of the galactose utilization pathway in yeast. Here we compare our theoretical approach with Monte Carlo simulations and observe that a driven mass-action model does an excellent job of describing the fluctuations at small inducer levels, whereas the linear noise approximation is preferred for large induction. Given the recent experiments highlighting the importance of extrinsic noise in gene expression, our novel modeling approach provides a framework for investigating the effect of extrinsic noise on large-scale gene regulatory networks.

I. INTRODUCTION

Gene networks consist of thousands of interacting regulatory components that act at both the transcriptional and translational level. While recent genomic technologies have led to significant breakthroughs in approaches aimed at deducing the connectivity of these networks, an understanding of cellular function arising from the dynamics that occurs on these networks is a central challenge in molecular and cellular biology. The mode of regulation can be at the transcriptional or post-transcriptional level, and since transcriptional regulation is relatively well characterized, modeling studies have typically focused on this form of control. Such regulation involves transcriptional activators or repressors, proteins that bind to DNA promoter sites and stimulate or repress the transcribing of genes to messenger transcripts. The mRNA is then translated to protein, and these proteins can themselves be transcription factors capable of up- or down-regulating themselves or other genes.

Since the brute-force simulation of large regulatory networks is typically impractical, a natural approach is to decouple the complexity of the genome through the utilization of synthetic biology, whereby small-scale circuits are constructed and modeled in relative isolation.[1] While this approach simplifies the problem enormously, the resulting "small scale" networks are still quite complicated. One predominant feature contributing to this complexity is the presence of noise. The biochemical reactions involved in the dy-

namics of gene regulation are inherently stochastic due to the random nature of the binding and unbinding events involved in transcription and translation, along with the regulatory processes of activation and repression. These processes often involve a small number of reactant molecules, thus leading to relatively large fluctuations.

There has been significant progress in the stochastic modeling of isolated genetic circuits.[2–5] The most accurate stochastic description is achieved using a master equation describing the state of the circuit. However, this approach quickly becomes computationally prohibitive with even a modest number of circuit connections. Luckily, in many cases the system can be simplified when some of the characteristic parameters are relatively small or large. In particular, if the number of proteins involved in a biochemical reaction is large, the master equation can be reduced to a Fokker-Planck equation, which in turn can be approximated by a corresponding Langevin equation. If the rates of some biochemical reactions are much larger than others, the fast reactions can be treated as being in local equilibrium, and thus the dimension of the system can be reduced.[6]

In reality, a synthetic gene circuit is never completely isolated from the host genome, since the native machinery required for processes such as transcription and translation is utilized by the circuit. This implies that the dynamics of a given reporter protein are driven by extrinsic variability arising from the environment. Recently, experiments have begun to address the relationship between such extrinsic variability and intrinsic noise in gene regulation. An elegant approach developed in Refs. 5 and 7 is based on detecting the expression of two different reporter genes that are controlled by identical promoters in a single cell. The variation between the levels of reporter proteins indicates the size of the intrinsic noise component, whereas the variance of the correlated component of both realizations yields the size of the extrinsic fluctuations. In Ref. 7 this method was applied in *E. coli* and comparable amounts of intrinsic and extrinsic noise were demonstrated. The same approach was recently employed in a eukaryotic setting using *S. cerevisiae*.[8] This study revealed highly correlated behavior from the reporters, which suggests that gene expression in *S. cerevisiae* is driven by a large external source of variability. Along the same lines, a second study in *S. cerevisiae* investigated the dependence of the transcriptional noise level on copy number and arrived at the same conclusion, namely that fluctuations appear to be highly correlated.[9]

In this paper we review recent progress in the theoretical analysis and computational modeling of the dynamics of gene circuits that are driven by extrinsic fluctuations. In particular, we focus on the limit when the extrinsic component is much larger than the intrinsic small-molecule source. In this limit, the problem can be reduced to solving a nonlinear stochastic differential equation with fluctuating parameters. For the case of a single intrinsic variable and dichotomous extrinsic noise (gating), this problem can be solved exactly. More generally, this approach allows for highly efficient numerical simulations, and thus provides a means for stochastic simulations describing large gene circuits. In order to ground our approach in a relatively simple context that is analytically solvable, we first consider a single gene controlled by an external gating process. We then illustrate the procedure in the context of a simplified model describing the galactose utilization circuit in *S. cerevisiae*, and compare our approximate solutions with direct Gillespie simulations.

II. INTRINSIC AND EXTRINSIC NOISE IN GENE REGULATION

There are many potential mechanisms for variations in gene expression that can lead to correlations within the same cell: variations in cell size, cell cycle stage, or common signaling, chromatin remodeling, etc. All of these factors may contribute to the observed genetic variability, however, they may have different "signatures" in the output noise characteristics. Swain *et al.*[5] recently introduced a general framework for theoretically parsing the intrinsic and extrinsic noise components. If $P(\mathbf{I},\mathbf{E})$ is the protein concentration that depends on both intrinsic $\mathbf{I}$ and extrinsic $\mathbf{E}$ variables, then the overall noise variance is obtained by a double averaging:

$$V = \overline{\langle P(\mathbf{I},\mathbf{E})^2\rangle} - (\overline{\langle P(\mathbf{I},\mathbf{E})\rangle})^2, \tag{1}$$

where the angular brackets stand for the averaging over intrinsic noise sources and the overbar denotes averaging over extrinsic fluctuations. This expression can be rewritten as the sum of two components:

$$\begin{aligned} V &= \overline{\langle P(\mathbf{I},\mathbf{E})^2\rangle - \langle P(\mathbf{I},\mathbf{E})\rangle^2} + \overline{\langle P(\mathbf{I},\mathbf{E})\rangle^2} - (\overline{\langle P(\mathbf{I},\mathbf{E})\rangle})^2 \\ &= V_{\text{int}} + V_{\text{ext}}. \end{aligned} \tag{2}$$

The first component denotes the variance of the protein concentration at a particular set of values of extrinsic variables and then averaged over all possible values of these variables,

$$V_{\text{int}} = \int p(\mathbf{E})d\mathbf{E}\left[\int P(\mathbf{I},\mathbf{E})^2 p(\mathbf{I}|\mathbf{E})d\mathbf{I} - \left(\int P(\mathbf{I},\mathbf{E})p(\mathbf{I}|\mathbf{E})d\mathbf{I}\right)^2\right]. \tag{3}$$

The second component denotes the variance of the protein concentration when the internal fluctuations are averaged out,

$$V_{\text{ext}} = \int p(\mathbf{E})d\mathbf{E}\left(\int P(\mathbf{I},\mathbf{E})p(\mathbf{I}|\mathbf{E})dI\right)^2 - \left[\int p(\mathbf{E})d\mathbf{E}\int P(\mathbf{I},\mathbf{E})p(\mathbf{I}|\mathbf{E})d\mathbf{I}\right]^2, \tag{4}$$

where $p(\mathbf{E})$ and $p(\mathbf{I}|\mathbf{E})$ are the probability distributions for the extrinsic and intrinsic variables conditioned by the extrinsic variables. Thus defined, the intrinsic and extrinsic contributions to the overall noise strength are always additive.

Note that the definitions (3) and (4) implicitly assume that the processes involved in gene regulation are Markovian, i.e., the protein concentration at time t, $P(t)$, is determined by the values of the intrinsic and extrinsic variables at the same time t. This is a strong assumption that may be violated in many experimentally relevant situations (see Ref. 10 for modeling approaches for systems that are non-

Markovian). Furthermore, it is difficult to envision a situation when an experimenter is able to artificially keep the extrinsic variables fixed in order to measure the intrinsic noise. Swain *et al.*[5] proposed an elegant approach that overcomes this difficulty. Suppose that there are two identical copies of the intrinsic circuits driven by the same extrinsic variables **E**, and that these two circuits produce two independently measurable protein concentrations $P_1(t)$ and $P_2(t)$. Measuring the statistics of the difference $P_1(t)-P_2(t)$, one can find the magnitude of the intrinsic noise, and calculate the extrinsic noise variance using the total noise strength and Eq. (2). This approach has been implemented experimentally in *E. coli* by Elowitz *et al.*[7] and in *S. cerevisiae* by Raser and O'Shea.[8]

Elowitz *et al.*[7] were the first to experimentally address the issue of intrinsic versus extrinsic noise in gene expression. They developed a dual-reporter technique that employs two different fluorescent proteins for simultaneous measurements of the activity of two identical promoters. They inserted plasmids carrying two promoters followed by cyan and yellow fluorescent proteins (CFP and YFP) and measured fluctuations in the CFP and YFP expression using flow cytometry and single-cell techniques. These measurements allowed for the quantification of the noise that is common (correlated) to both promoters, along with the component that is uncorrelated. Figure 1(a) shows the scatter plot of CFP and YFP values for each cell in one of the experiments.

Along the same lines, Raser and O'Shea[8] studied the stochasticity in the gene expression in yeast using the dual-reporter technique. They constructed yeast strains that express cyan and yellow fluorescent proteins (CFP and YFP) from identical promoters integrated at the same locus. Using two-color flow cytometry, they found that the expression of the genes is highly correlated [Fig. 1(b)]. For two PHO5 promoters, the intrinsic noise was between 2% and 20% of the total noise, and for the two GAL1 promoters, the intrinsic component of noise did not exceed 3% of the total fluctuations observed in the system.

Another experimentally feasible method for parsing intrinsic and extrinsic noise has been proposed in Ref. 9. When multiple copies of the same circuit contribute to the production of the same protein, the statistics of the protein fluctuations depend on the correlations among the circuits. If the circuits are fully correlated (as it would be if there were no intrinsic noise, and all fluctuations were extrinsic), the variance of the observed protein fluctuations would scale as M^2, where M is the copy number of the circuit. In the opposite limit, when the circuits are completely decorrelated (no extrinsic noise), the variance scales as M. In the intermediate regime, the variance scales as $V=Mv+M(M-1)c$, where $v=\langle P_i^2\rangle-\langle P_i\rangle^2$ is the variance of a single copy, and $c=\langle P_iP_j\rangle-\langle P_i\rangle^2$ is the cross correlation between two copies. By measuring this scaling, one can estimate the amount of extrinsic versus intrinsic noise in the system. This algorithm has been applied for the study of genetic variability in yeast.[9] Specifically, the stochastic variability of GAL1 production in yeast was explored using multiple promoter-gene inserts in one or several different loci in the *S. cerevisiae* genome. Up to five pairs of the native GAL1 promoters driving yEGFP (yeast-enhanced green fluorescent protein) were integrated into the GAL1-10 locus on chromosome II, and the fluorescence fluctuations were quantified using flow cytometry data. Figure 2 illustrates the results of these measurements. Figures 2(c)–2(f) demonstrate that both the mean and standard deviation scale linearly with the copy number, so the coefficient of variation $V^{1/2}/\langle P\rangle$ is independent of the copy number M. Furthermore, the fluorescence probability distributions $P_M(f)$ showed a striking collapse after rescaling to $MP_M(f/M)$ [Figs. 2(a) and 2(b)]. This collapse suggests that the fluctuations in the yGFP expression are strongly dominated by a common extrinsic factors rather than independent stochastic events during GFP transcription and translation. This scaling also implies that not only the mean and the standard deviation are proportional to the copy number M, but generally the nth moment of the fluorescence distribution is proportional to M^n.

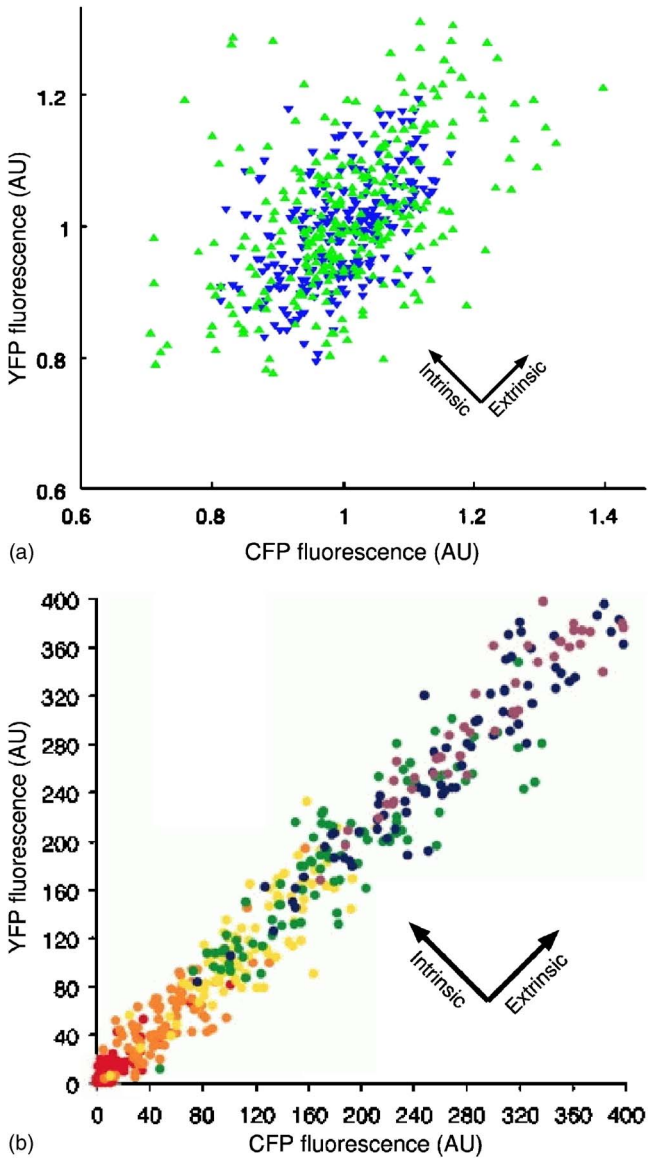

FIG. 1. Separation of intrinsic and extrinsic noise in bacteria (Ref. 7) (a) and yeast (Ref. 8) (b) using a scatter plot of CFP and YFP values for each cell. Intrinsic noise is manifested as scatter perpendicular to the diagonal, and the scatter along the diagonal is generated by both intrinsic and extrinsic noise.

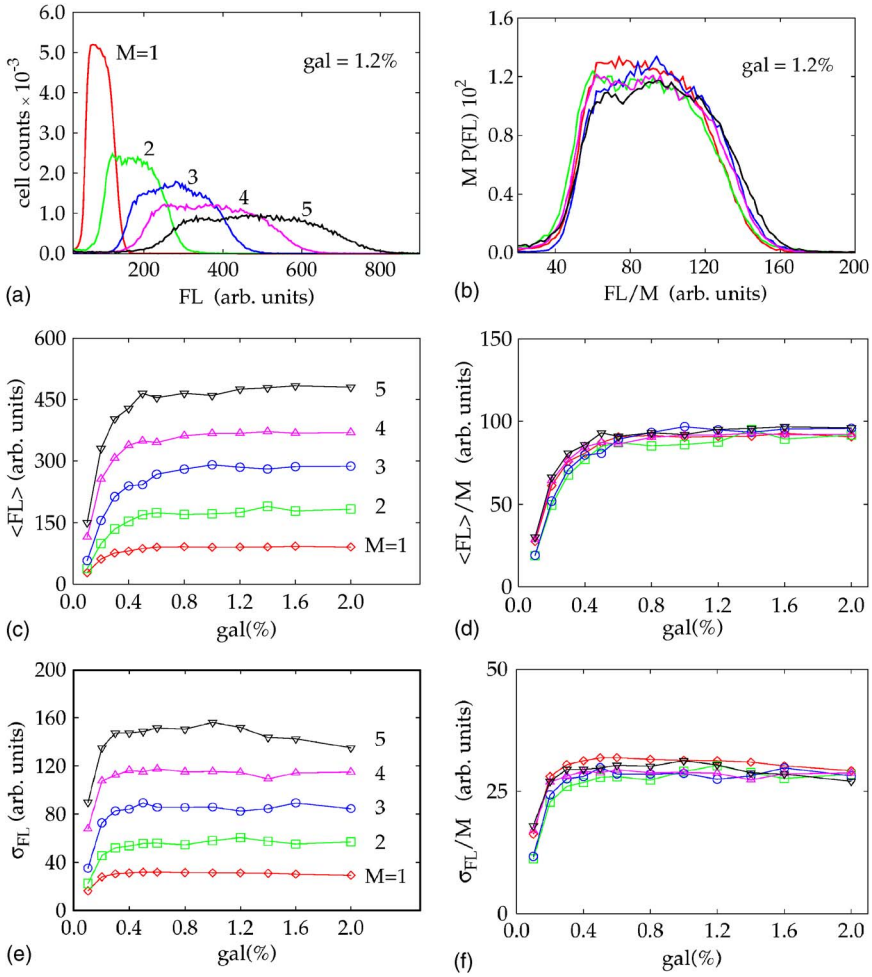

FIG. 2. Experimental results for GFP expression in yeast (Ref. 9) for different copy numbers and galactose concentrations. (a) Histograms of GFP measurements for copy numbers from $M=1$ to $M=5$ above the saturation (gal=1.2%). (b) Collapse of GFP distributions under the transformation $F \to F/M$, $P(F) \to MP(F/M)$. (c) Induction curves for copy numbers from $M=1$ to $M=5$. (d) Collapse of the induction curves. (e) Standard deviations of GFP corresponding to induction curves. (f) Collapse of the dependencies shown in (e).

In the following sections we develop theoretical methods of modeling stochastic dynamics of gene regulatory systems dominated by extrinsic noise fluctuations and apply these methods to two simple genetic circuits.

III. GENERAL FORMULATION

A. Multivariate stochastic dynamics of gene networks

Even the simplest genetic circuits usually involve multiple reacting species (DNA, mRNA, proteins, chemical inducers) and corresponding reaction channels. In the following,[11] we introduce a set of N molecular species $\{S_1, \cdots, S_N\}$ and M reaction channels $\{R_1, \cdots R_M\}$ in a cell of fixed volume Ω. The instantaneous state of the system is specified by the vector of copy numbers of all species, $\mathbf{X}=(X_1, \cdots, X_N)^T \in \mathbb{Z}^N$. The reactions

$$\mathbf{R}: \mathbf{X} \xrightarrow{\mathbf{W}(\mathbf{X})} \mathbf{X} + \mathbf{A}, \tag{5}$$

are characterized by the stoichiometric matrix $\mathbf{A}=\{a_{ij}\} \in \mathbb{Z}^{N\times M}$, where a_{ij} is the change of the copy number of S_i after a single firing of the reaction R_j. The frequency of occurrence of individual reactions is given by the propensity function $\mathbf{W}(\mathbf{X})=\{W_1(\mathbf{X}), \cdots, W_M(\mathbf{X})\}^T \in \mathbb{R}^M$, where $W_j(\mathbf{X})dt$ is defined as the probability that the R_j reaction occurs within an infinitesimal time interval dt when the system is in the state $\mathbf{X}$.

Since in a finite system the copy numbers change stochastically, their evolution can be described by the chemical master equation (CME),[12]

$$\frac{dP(\mathbf{X},t)}{dt} = \sum_{j=1}^{M} [W_j(\mathbf{X}-\mathbf{a}_j)P(\mathbf{X}-\mathbf{a}_j,t) - W_j(\mathbf{X})P(\mathbf{X},t)], \tag{6}$$

where $\mathbf{a}_j=(a_{1,j}, \cdots, a_{N,j})^T$ is the vector of stoichiometric coefficients corresponding to the reaction channel R_j.

The CME is difficult to solve unless the system is relatively simple.[3] However, it can be used to calculate the dynamics of the moments of the probability distribution $P(\mathbf{X},t)$.[12] In particular, multiplying (6) by $\mathbf{X}$ and summing over $\mathbf{X}$ yields an equation for the mean $\langle \mathbf{X} \rangle$,

$$\langle \dot{\mathbf{X}} \rangle = \langle \mathbf{A}\mathbf{W}(\mathbf{X}) \rangle \equiv \langle \mathbf{F}(\mathbf{X}) \rangle. \tag{7}$$

Similarly, multiplying by $\mathbf{X}\mathbf{X}^T$ and summing leads to an equation for the second moment:

$$\frac{d}{dt}\langle \mathbf{X}\mathbf{X}^T \rangle = \langle \mathbf{F}(\mathbf{X})\mathbf{X}^T \rangle + \langle \mathbf{X}\mathbf{F}^T(\mathbf{X}) \rangle + \langle D(\mathbf{X}) \rangle \tag{8}$$

where the diffusion matrix $\mathbf{D}(\mathbf{X}) \in \mathbb{R}^{N\times N}$ is given by

$$\mathbf{D}(\mathbf{X}) = \sum_{j=1}^{M} \mathbf{a}_j \mathbf{a}_j^T W_j(\mathbf{X}). \tag{9}$$

For reactions of zeroth and first order, the moment equations decouple and can be solved directly. However, in general, the moment equations, albeit linear, form an infinite set where the lower moments depend on the higher-order moments. Further progress in solving Eqs. (7) and (8) can be made when the noise is small, i.e., the probability distribution $P(\mathbf{X},t)$ is highly peaked near its mean value $\mathbf{x} \equiv \langle \mathbf{X} \rangle$. Then the propensity function W can be linearized near $\mathbf{X}=\mathbf{x}$ (the so-called linear noise approximation, which can be rigorously derived from a general "system-size expansion"[13])

$$\frac{d\boldsymbol{\mu}}{dt} = \mathbf{K}(\mathbf{x})\boldsymbol{\mu}, \tag{10}$$

$$\frac{d\boldsymbol{\Sigma}}{dt} = \mathbf{K}(\mathbf{x})\boldsymbol{\Sigma} + \boldsymbol{\Sigma}\mathbf{K}^T(\mathbf{x}) + \mathbf{D}(\mathbf{x}) + \left.\frac{\partial \mathbf{D}(\mathbf{y})}{\partial \mathbf{y}}\right|_{\mathbf{y}=\mathbf{x}} \boldsymbol{\mu}, \tag{11}$$

where $\boldsymbol{\mu}=\langle \mathbf{X}-\mathbf{x} \rangle$ and $\boldsymbol{\Sigma}=\langle (\mathbf{X}-\mathbf{x})(X-x)\rangle^T - \langle \mathbf{X}-\mathbf{x}\rangle\langle \mathbf{X}-\mathbf{x}\rangle^T$. Equation (10) coincides with a linearized deterministic equation for the mean concentration of molecular species that can be obtained in the thermodynamic limit $\Omega \to \infty$. If this equation has a stable fixed point $\mathbf{x}_0$, then the asymptotic solution of Eq. (10) is $\boldsymbol{\mu}=0$, and the corresponding variance of the stochastic fluctuations is found from the following Lyapunov equation:

$$\mathbf{K}(\mathbf{x}_0)\boldsymbol{\Sigma} + \boldsymbol{\Sigma}\mathbf{K}^T(\mathbf{x}_0) + \mathbf{D}(\mathbf{x}_0) = 0. \tag{12}$$

This set of N^2 linear equations can always be solved numerically. However, in certain cases this solution can be substantially simplified using methods developed in control theory,[14] (see also Refs. 11 and 15). In particular, great simplification can be achieved if either the Jacobian matrix $\mathbf{K}$ or the diffusion matrix $\mathbf{D}$ is diagonalizable. For example, if the Jacobian matrix can be written as $\mathbf{K}=\mathbf{Q}\boldsymbol{\Lambda}\mathbf{Q}^{-1}$, where $\boldsymbol{\Lambda}$ is a diagonal matrix with eigenvalues $(\lambda_1, \cdots, \lambda_N)$ and $\mathbf{Q}$ is a $N \times N$ matrix with eigenvectors of $\mathbf{K}$ as columns, the solution of (12) is of the form

$$\tilde{\Sigma}_{ij} = -\frac{\tilde{D}_{ij}}{\lambda_i + \lambda_j}, \tag{13}$$

where the $\tilde{\Sigma}_{ij}$ are components of the transformed covariance matrix $\mathbf{Q}^{-1}\boldsymbol{\Sigma}\mathbf{Q}^{-1T}$ and $\tilde{\mathbf{D}}=\mathbf{Q}\mathbf{D}\mathbf{Q}^{-1T}$.

More detailed information about the dynamics of the joint probability distributions of different molecular species in the limit of small noise (or large system size) can be generated by reducing the chemical master equation (6) to a corresponding multidimensional Fokker-Planck equation via the Kramers-Moyal expansion[13]

$$\partial_t p(\mathbf{X},t) = -\sum_{i=1}^{N} \partial_i [V_i(\mathbf{X})p(\mathbf{X})] + \frac{1}{2}\sum_{i,j=1}^{N} \partial_i \partial_j [D_{ij}(\mathbf{X})p(\mathbf{X},t)], \tag{14}$$

where the operator ∂_i denotes partial differentiation with respect to a particular variable $X_i \in \mathbf{X}$, $V_i(\mathbf{X})$ is an ith component of the vector $\mathbf{V}(\mathbf{X})=\Sigma_{j=1}^{M}\mathbf{a}_j W_j \cdots(\mathbf{X})$ and $D_{ij}(\mathbf{X})$ are components of the diffusion matrix (9).

We do not discuss here the conditions of applicability and limitations of the described methods of reduction of the basic CME for multivariate stochastic processes; for a detailed exposition of these issues, see, for example, Refs. 12 and 13.

B. Extrinsic and intrinsic fluctuations in the linear noise approximation

Let us now turn to a case when the system can be decomposed into the extrinsic and intrinsic components that are both small. Suppose that the genetic circuit can be described by the N_I-dimensional vector of (discrete) intrinsic variables $\mathbf{I}$ and the N_E-dimensional vector of extrinsic variables $\mathbf{E}$. The set of intrinsic variables includes the numbers of protein and mRNA molecules corresponding to genes constituting the circuit, as well as the states of the operators controlling the transcription. Extrinsic variables may include the numbers of other transcription factors, the RNA polymerase concentration, cell volume, etc. We divide the set of M biochemical reactions into two categories: M_I intrinsic reactions that only affect the values of the intrinsic variables,

$$\mathbf{R}^I : \mathbf{I} \xrightarrow{\mathbf{W}^I(\mathbf{I},\mathbf{E})} \mathbf{I} + \mathbf{A}^I, \tag{15}$$

and M_E extrinsic reactions that only change the values of the extrinsic variables,

$$\mathbf{R}^E : \mathbf{E} \xrightarrow{\mathbf{W}^E(\mathbf{E})} \mathbf{E} + \mathbf{A}^E. \tag{16}$$

By assumption, there are no reactions that affect both intrinsic and extrinsic variables simultaneously. Also by definition, the propensities of the intrinsic reactions $\mathbf{W}^I(\mathbf{I},\mathbf{E})$ may in general depend both on $\mathbf{I}$ and $\mathbf{E}$, whereas the transition rates of the individual extrinsic reactions $\mathbf{W}^E(\mathbf{E})$ are independent of $\mathbf{I}$. Thus the overall stoichiometric matrix $\mathbf{A}$ and the Jacobian matrix $\mathbf{K}$ from the previous section have the following block form:

$$\mathbf{A} = \begin{pmatrix} \mathbf{A}^E & 0 \\ 0 & \mathbf{A}^I \end{pmatrix}, \quad \mathbf{K} = \begin{pmatrix} \mathbf{K}^{EE} & 0 \\ \mathbf{K}^{IE} & \mathbf{K}^{II} \end{pmatrix} \tag{17}$$

where $\mathbf{K}^{II}=\partial \mathbf{A}^I \mathbf{W}^I / \partial \mathbf{I}, \mathbf{K}^{EE}=\partial \mathbf{A}^E \mathbf{W}^E / \partial \mathbf{E}, \mathbf{K}^{IE}=\partial \mathbf{A}^I \mathbf{W}^I / \partial \mathbf{E}$.

Similarly, we can introduce the block form of the symmetric covariance matrix $\boldsymbol{\Sigma}$,

$$\boldsymbol{\Sigma} = \begin{pmatrix} \boldsymbol{\Sigma}^{EE} & \boldsymbol{\Sigma}^{EI} \\ (\boldsymbol{\Sigma}^{EI})^T & \boldsymbol{\Sigma}^{II} \end{pmatrix}, \tag{18}$$

where $\boldsymbol{\Sigma}^{EE} \equiv \langle (\mathbf{E}-\mathbf{e})(\mathbf{E}-\mathbf{e})^T \rangle - \langle \mathbf{E}-\mathbf{e}\rangle\langle \mathbf{E}-\mathbf{e}\rangle^T$ is $N_E \times N_E$ covariance matrix of extrinsic fluctuations, $\boldsymbol{\Sigma}^{II} \equiv \langle (\mathbf{I}-\mathbf{i}) \times (I-i)^T \rangle - \langle \mathbf{I}-\mathbf{i}\rangle\langle \mathbf{I}-\mathbf{i}\rangle^T$ is $N_I \times N_I$ covariance matrix of extrinsic fluctuations, and $\boldsymbol{\Sigma}^{EI} \equiv \langle (\mathbf{E}-\mathbf{e})(I-i)^T \rangle - \langle \mathbf{E}-\mathbf{e}\rangle\langle I-i\rangle^T$ is the $N_E \times N_I$ covariance matrix of extrinsic-intrinsic fluctuations.

The diffusion matrix $\mathbf{D}$ introduced above has a block-diagonal form

$$\mathbf{D} = \begin{pmatrix} \mathbf{D}^E & 0 \\ 0 & \mathbf{D}^I \end{pmatrix}, \tag{19}$$

where $\mathbf{D}^E$ is an $N_E \times N_E$ matrix with coefficients

$$\mathbf{D}^E = \sum_{j=1}^{M_E} \mathbf{a}_E \mathbf{a}_E^T W_j^E(\mathbf{E}) \tag{20}$$

and similarly for $\mathbf{D}^I \in \mathbb{R}^{N_I \times N_I}$,

$$\mathbf{D}^I = \sum_{j=1}^{M_I} \mathbf{a}_I \mathbf{a}_I^T W_j^I(\mathbf{I},\mathbf{E}). \tag{21}$$

The multidimensional probability distribution $p(\mathbf{I},\mathbf{E},t)$ obeys the following chemical master equation:

$$\begin{aligned} \partial_t p(\mathbf{I},\mathbf{E},t) &= \sum_{j=1}^{M_I} [W_j^I(\mathbf{I}-\mathbf{a}_{Ij},\mathbf{E}) p(\mathbf{I}-\mathbf{a}_{Ij},\mathbf{E},t) - W_j^I(\mathbf{I},\mathbf{E}) p(\mathbf{I},\mathbf{E},t)] \\ &\quad + \sum_{j=1}^{M_E} [W_j^E(\mathbf{E}-\mathbf{a}_{Ej}) p(\mathbf{I},\mathbf{E}-\mathbf{a}_{Ej},t) - W_j^E(\mathbf{E}) p(\mathbf{I},\mathbf{E},t)]. \end{aligned} \tag{22}$$

Substituting the matrices (17) and (18) into the Lyapunov equation (12), we obtain the following set of equations for the covariance matrices in the linear noise approximation:

$$\mathbf{K}^{EE}\mathbf{\Sigma}^{EE} + \mathbf{\Sigma}^{EE}(\mathbf{K}^{EE})^T + \mathbf{D}^E = 0, \tag{23}$$

$$\mathbf{K}^{EE}\mathbf{\Sigma}^{EI} + \mathbf{\Sigma}^{EI}(\mathbf{K}^{II})^T + \mathbf{\Sigma}^{EE}(\mathbf{K}^{IE})^T = 0, \tag{24}$$

$$\mathbf{K}^{II}\mathbf{\Sigma}^{II} + \mathbf{\Sigma}^{II}(\mathbf{K}^{II})^T + \mathbf{K}^{IE}\mathbf{\Sigma}^{EI} + (\mathbf{\Sigma}^{EI})^T(\mathbf{K}^{IE})^T + \mathbf{D}^I = 0. \tag{25}$$

The first equation describes the isolated dynamics of the extrinsic reactions, and the other two equations can be used to find the intrinsic covariance matrix $\mathbf{\Sigma}^{II}$ and cross-covariance matrix $\mathbf{\Sigma}^{EI}$. Notice that the calculation of the cross-covariance matrix $\mathbf{\Sigma}^{EI}$ involves knowledge of the Jacobian of the extrinsic system. This somewhat counterintuitive result can be explained by the fact that the amount of extrinsic noise "transferred" into the intrinsic subsystem depends on the spectral properties of the extrinsic noise which, in turn, are determined by the Jacobian $\mathbf{K}^{EE}$.

Equations (24) and (25) can be solved sequentially: first the cross-covariance matrix $\mathbf{\Sigma}^{EI}$ can be found from Eq. (24), and then it can be substituted in Eq. (25). Note that Eq. (25) has the same structure as Eq. (12), where the cross-covariance term $(\mathbf{\Sigma}^{EI})^T(\mathbf{K}^{IE})^T$ is added to the intrinsic diffusion term $\mathbf{D}^I$. It can be solved using the orthogonalization procedure in the same way as the general Lyapunov equation (see the previous section).

A significant simplification can be achieved if there is only one component in the extrinsic subsystem, $N_E = 1$. Then $\mathbf{K}_{EE}$ is a scalar K^{EE} describing the relaxation rate of the extrinsic fluctuations, and the solution of Eq. (24) can be written explicitly,

$$\mathbf{\Sigma}^{EI} = -\mathbf{\Sigma}^{EE}(\mathbf{K}^{IE})^T[(\mathbf{K}^{II})^T + K^{EE}\mathbf{I}]^{-1}, \tag{26}$$

where I is the identity matrix. If additionally, there is only one intrinsic variable, we obtain

$$\begin{aligned} &\Sigma^{EE} = -\frac{D_E}{2k_{EE}}, \quad \Sigma^{EI} = \Sigma_{EE}\frac{k_{IE}}{k_{II}+k_{EE}}, \\ &\Sigma^{II} = -\frac{D_I}{2k_{II}} - \Sigma^{EI}\frac{k_{IE}}{k_{II}} = -\frac{D^I}{2k_{II}} - \frac{D^E}{2k_{EE}}\frac{k_{IE}^2}{k_{II}^2}\frac{k_{II}}{k_{II}+k_{EE}}. \end{aligned} \tag{27}$$

The last expression coincides with the formula obtained by Paulsson.[16] The first term in (27) represents the intrinsic noise contribution, and the last term is due to the extrinsic noise filtered by the intrinsic subsystem.

C. Small intrinsic noise, arbitrary extrinsic noise

To date, experiments aimed at parsing the noise into intrinsic and extrinsic components have demonstrated that the extrinsic component is large and often dominates gene expression variability. In such cases, the linear noise approximation of the preceding section is not applicable. However, a significant simplification of the general stochastic system can nevertheless be obtained. The intrinsic noise is small when abundances of corresponding reactants are large. Then the transition rates $W^I(\mathbf{I},\mathbf{E})$ and probability distribution $p(\mathbf{I},\mathbf{E},t)$ change weakly on the scale of individual intrinsic reactions $\mathbf{r}_i$. Then we can perform the Kramers-Moyal expansion[13] along the intrinsic variables only, obtaining

$$\begin{aligned} \partial_t p(\mathbf{I},\mathbf{E},t) &= -\sum_{i=1}^{N_I} \partial_i [F_i^I(\mathbf{I},\mathbf{E}) p(\mathbf{I},\mathbf{E})] + \tfrac{1}{2}\sum_{i,j} \partial_i\partial_j [D_{ij}(\mathbf{I},\mathbf{E}) p(\mathbf{I},\mathbf{E},t)] \\ &\quad + \sum_{j=1}^{M_E} [W_j^E(\mathbf{E}-\mathbf{a}_{Ej}) p(\mathbf{I},\mathbf{E}-\mathbf{a}_{Ej},t) - W_j^E(\mathbf{E}) p(\mathbf{I},\mathbf{E},t)], \end{aligned} \tag{28}$$

where the operator ∂_i denotes partial differentiation with respect to a particular intrinsic variable $I_i \in \mathbf{I}$, $F_i^I(\mathbf{I},\mathbf{E})$ is the ith component of the vector

$$\mathbf{F}^I(\mathbf{I},\mathbf{E}) = \sum_{j=1}^{M_I} \mathbf{a}_j^I W_j^I(\mathbf{I},\mathbf{E})$$

and $D_{ij}(\mathbf{I},\mathbf{E})$ is a component of the intrinsic diffusion tensor (21).

Equation (28) has a mixed form; it is a Fokker-Planck equation for the intrinsic reactions and a master equation for extrinsic reactions. Unlike Eq. (22), here the intrinsic variables are continuous, $\mathbf{I} \in \mathbb{R}^{N_I}$, while $\mathbf{E}$ are still discrete, $\mathbf{I} \in \mathbb{Z}^{N_E}$. This equation corresponds to a coupled system of Langevin equations for the intrinsic reactions

$$\dot{I}_i = F_i^I(\mathbf{I},\mathbf{E}) + \sum_j g_{ij}(\mathbf{I},\mathbf{E})\xi_j(t), \tag{29}$$

along with the independent extrinsic reactions (16). In the Langevin equation (29), $\xi_i(t)$ is white Gaussian noise with unit variance: $\langle \xi_i(t) = 0 \rangle$, $\langle \xi_i(t)\xi_{i'}(t') \rangle = \delta_{ii'}\delta(t-t')$, and the

noise magnitude $g_{ij}(\mathbf{I},\mathbf{E})$ is related to $D_{ij}(\mathbf{I},\mathbf{E})$ by

$$D_{ij}(\mathbf{I},\mathbf{E}) = g_{ik}(\mathbf{I},\mathbf{E})g_{jk}(\mathbf{I},\mathbf{E}).$$

The intrinsic dynamics are driven by both white Gaussian intrinsic noise and extrinsic noise, which is colored since it is "filtered" by the extrinsic dynamics. As we have seen in Sec. II, the intrinsic noise is often small as compared to the extrinsic one. Thus, the simplest approximation to be made is to neglect the small intrinsic noise altogether and arrive at the stochastic equation for the intrinsic variables $\mathbf{I}$:

$$\dot{\mathbf{I}} = \mathbf{F}^I(\mathbf{I},\mathbf{E}), \tag{30}$$

which is driven by the colored noise that is specified by the extrinsic reactions (16).

This problem is much simpler than the original problem, which included both intrinsic and extrinsic stochastic components. It can be simulated relatively easily, however, it is amenable to analytical treatment only in certain special cases. First, even when $\mathbf{E}$ is large but enters $\mathbf{F}^I(\mathbf{I},\mathbf{E})$ linearly, $\mathbf{V}(\mathbf{I},\mathbf{E})=\mathbf{F}_0^I(\mathbf{I})+\mathbf{F}_1^I(\mathbf{I})\mathbf{E}$, and the correlation time of the extrinsic noise τ_e is much smaller than the smallest characteristic time of the intrinsic reactions, the extrinsic processes can be approximated by white Gaussian noise. In this limit, Eq. (30) can be converted to a multidimensional Fokker-Planck equation for $p(\mathbf{I})$,

$$\partial_t p(\mathbf{I},t) = -\sum_{i\in\mathcal{I}} \partial_i[F_{1i}^I(\mathbf{I})p(\mathbf{I})] + \tfrac{1}{2}\sum_{i,i'} \partial_i\partial_{i'}[B_{ii'}(\mathbf{I})p(\mathbf{I},t)], \tag{31}$$

where $B_{ii'}=F_{1ik}^I(\mathbf{I})F_{1jk}^I(\mathbf{I})$. Various approximate methods for solving Eq. (31) are given in Ref. 13.

If, on the other hand, the extrinsic noise is slow compared with the slowest time scale of the intrinsic processes, the *switching-curve approximation*[18] can be employed. In this approximation, the stationary probability distribution $p(\mathbf{I},\mathbf{E})=p_e(\mathbf{E})\delta(\mathbf{I}-\mathbf{f}(\mathbf{E}))$, where $p_e(\mathbf{E})$ is the stationary probability density function (pdf) of the extrinsic processes, and $\mathbf{f}(\mathbf{E})$ is defined by $V(\mathbf{f},\mathbf{E})=0$. However, one has to be careful when using this approximation in situations where the deterministic dynamics exhibit multistability.[4]

In many cases relevant to gene regulation, the intrinsic dynamics of even the simplest genetic circuits involve vastly different time scales. Therefore, one may often encounter situations when the extrinsic noise is slow compared with the "fast" intrinsic reactions, but also fast or comparable with the "slow" reactions. In these situations, one can simplify the problem by assuming that fast reactions are in quasiequilibrium, perform adiabatic elimination of fast variables, and recast the problem in terms of slow variables only. This approach has been discussed in several recent publications both for deterministic[3] and stochastic systems.[6,17] Here we discuss adiabatic elimination in general terms for deterministic mass-action equations. Let us assume that the model can be written in the form

$$\dot{\mathbf{I}} = \mathbf{F}_f(\mathbf{I},\mathbf{E}) + \epsilon\mathbf{F}_s(\mathbf{I},\mathbf{E}), \tag{32}$$

where $\mathbf{F}_f$ represents the fast reactions, $\mathbf{F}_s$ the slow reactions, and $\epsilon \ll 1$. If the functions $\mathbf{F}_f(\mathbf{I},\mathbf{E})$ are linearly independent [the Wronskian W of the set of functions $F_f(\mathbf{I},\mathbf{E})$ with respect to any of the intrinsic variables is nonzero], then there are no slow variables in the system, and hence the slow reactions can be ignored. However, if the Wronskian is zero, then the null space of the corresponding Wronskian matrix $\{\mathbf{h}^i(\mathbf{E}), i=1,\ldots,n_s\}$ gives the slow variables of the full system $I_s^i=\mathbf{h}_s^i(\mathbf{E})^T\cdot\mathbf{I}$ (the number of slow variables n_s is equal to the nullity of W). Here we use the fact that the intrinsic variables describe the numbers of certain molecules, and "conservation laws" for the fast reactions are always linear in the number of molecules.

The equations for $\mathbf{I}_s$ in the slow time scale $T=\epsilon t$ read

$$\frac{d\mathbf{I}_s}{dT} = \hat{\mathbf{h}}_s(\mathbf{E})\cdot\mathbf{F}_s(\mathbf{f}_s(\mathbf{I}_s,\mathbf{E}),\mathbf{E}), \tag{33}$$

where $\mathbf{I}=\mathbf{f}_s(\mathbf{I}_s,\mathbf{E})$ is the unique solution for the enlarged set of algebraic equations

$$\mathbf{F}_f(\mathbf{I},\mathbf{E})=0, \quad \hat{\mathbf{h}}_s(\mathbf{E})^T\cdot\mathbf{I}=\mathbf{I}_s, \tag{34}$$

and $\hat{\mathbf{h}}_s(\mathbf{E})$ is the $n_i\times n_s$ matrix with rows $\mathbf{h}_s^i(\mathbf{E})^T$.

This reduction can offer a significant advantage in the efficiency of simulations since the fast reactions usually consume most of the computational resources. In the case of Eq. (33), not only the number of independent variables is reduced, but the time step for numerical integration can be dramatically increased without a significant loss of accuracy.

As an illustration of adiabatic elimination of fast reactions, let us consider a very simple example of single gene production of a protein X with its subsequent dimerization and degradation,

$$\overset{\alpha}{D\to D+X}, \quad \overset{k_2}{X+X\to X_2}, \quad \overset{k_{-2}}{X_2\to X+X},$$

$$\overset{\gamma}{X\to\varnothing}, \quad \overset{\gamma_2}{X_2\to\varnothing}.$$

We assume that production and degradation rates α, γ, $\gamma_2=O(\epsilon)$ are small, and the dimerization rates k_2, k_{-2} are large. We also ignore the extrinsic fluctuations. The mass-action reactions for this system read

$$\dot{x} = \alpha - 2k_2x^2 + 2k_{-2}x_2 - \gamma x, \tag{35}$$

$$\dot{x_2} = k_2x^2 - k_{-2}x_2 - \gamma_2x_2. \tag{36}$$

The Wronskian of the fast subsystem with respect to x,

$$W = \begin{vmatrix} -2k_2x^2+2k_{-2}x_2 & k_2x^2-k_{-2}x_2 \\ -4k_2x & 2k_2x \end{vmatrix},$$

is evidently zero. The corresponding Wronskian matrix has nullity 1 and the null eigenvector $(1,2)^T$, i.e., there is one slow variable of this system, namely, the total number of monomers $x_s=x+2x_2$. The equation for the slow variable x_s reads

$$\dot{x}_s = \alpha - \gamma x(x_s) - 2\gamma_2K_2x^2(x_s), \tag{37}$$

where $K_2=k_2/k_{-2}$, and $x(x_s)$ is found from the quadratic equation $x_s=x+2K_2x^2$. In terms of the original variable (number of monomers) x, the reduced equation reads

$$(1+4K_2x)\dot{x} = \alpha - \gamma x - 2\gamma_2K_2x^2. \tag{38}$$

In the particular case of one intrinsic variable I and one dichotomic extrinsic variable $E \in \{0,1\}$ controlled by a Markovian jump process, it is possible to find the exact stationary pdf for an arbitrary relation between the correlation times of the intrinsic and extrinsic processes[18] [see Eq. (48)].

IV. BINARY GATING OF CONSTITUTIVE GENE EXPRESSION

We now consider constitutive gene expression affected by a dichotomous stochastic extrinsic process (gating). The state of the system is described by two variables: the binary $s=\{0,1\}$ for the state of the gate and the integer m for the number of proteins produced. We characterize both transcription and translation by the lumped transcription/translation rate α_s, which depends on the gate state s (we choose $\alpha_1 > \alpha_0$ for definiteness), and the protein degradation rate δ is assumed independent of s. The transition rates of the gating process are Ck_0 for the transition $0 \to 1$ and Ck_1 for $1 \to 0$ (we choose C so $k_0+k_1=1$). The intrinsic biochemical reactions can be written as

$$m \overset{\alpha_s}{\to} m+1, \quad m \overset{\delta_m}{\to} m-1, \tag{39}$$

and the only extrinsic reaction is

$$s \overset{Ck_s}{\to} \hat{s}, \tag{40}$$

where $\hat{s}=1-s$. This extrinsic process is Markovian, with exponentially distributed autocorrelation function $C(t) \equiv \overline{s(t)s(t')}=k_0k_1 \exp(-Ct)$. We first apply the linear noise approximation method described in the previous section. The components of the intrinsic propensity matrix are $W_1=\alpha_1 s + \alpha_0(1-s)$ and $W_2=\delta m$. The corresponding stoichiometric coefficients are $a_{I1}=1$, $a_{I2}=-1$. The extrinsic propensity matrices are $W_1^E=Ck_1 s$ and $W_2^E=Ck_2(1-s)$, and the corresponding stoichiometric coefficients are $a_{E1}=-1$, $a_{E2}=1$. From this we immediately obtain the elements of the Jacobian

$$k_{II}=-\delta, \quad k_{IE}=\alpha_1-\alpha_0, \quad k_{EE}=-C, \tag{41}$$

and the diffusion coefficients $D_E=Ck_1s_0+Ck_2(1-s_0)$, $D_I=\alpha_1 s+\alpha_0(1-s)+\delta m_0$. Here $s_0=k_2$ and $m_0=\delta^{-1}(k_1\alpha_0+k_2\alpha_1)$ are fixed points of the deterministic rate equations

$$\begin{aligned} \dot{s} &= -Ck_1 s + Ck_2(1-s), \\ \dot{m} &= \alpha_1 s + \alpha_0(1-s) - \delta m. \end{aligned} \tag{42}$$

Substituting these constants into Eqs. (27), we obtain

$$\begin{aligned} \Sigma^{EE} &= k_1k_2, \quad \Sigma^{EI} = k_1k_2\frac{\alpha_1-\alpha_0}{C+\delta}, \\ \Sigma^{II} &= \frac{k_1\alpha_0+k_2\alpha_1}{\delta} + k_1k_2\left(\frac{\alpha_1-\alpha_0}{\delta}\right)^2\frac{\delta}{C+\delta}. \end{aligned} \tag{43}$$

A more general description of Sec. III C, which does not involve the linear noise approximation, can be deduced from the chemical master equation for the probability $p_m^s(t)$ to have the gate in state s and m proteins at time t:

$$\begin{aligned} \frac{dp_m^s}{dt} &= \alpha_s(p_{m-1}^s - p_m^s) + \delta[(m+1)p_{m+1}^s - mp_m^s] \\ &\quad + C(k_{\hat{s}}p_m^{\hat{s}} - k_s p_m^s). \end{aligned} \tag{44}$$

Note that in this formulation the problem is equivalent to the description of a single gene with operator fluctuations considered in Ref. 3, in which case the fluctuations were intrinsic. Furthermore, since the reactions (39) and (40) are zero and first order, the exact expressions for all moments can be derived directly from (44), and the exact expression for the intrinsic noise variance coincides with (43) (see Ref. 3).

When the number of proteins m is large, we can take the continuum limit and arrive at a system of two coupled Fokker-Planck equations for $p^s(m,t)$:

$$\begin{aligned} \partial_t p^s(m,t) &= -\partial_m[(\alpha_s-\delta m)p^s(m,t)] \\ &\quad + 1/2\partial_m^2[(\alpha_s+\delta m)p^s(m,t)] \\ &\quad + C[k_{\hat{s}}p^{\hat{s}}(m,t) - k_s p^s(m,t)]. \end{aligned} \tag{45}$$

It can be shown that this set of equations corresponds to the Langevin equation

$$\frac{dm}{dt} = \alpha_s - \delta m + \sqrt{\alpha_s+\delta m}\,\xi(t), \tag{46}$$

coupled with the Markovian stochastic process (40). Here $\xi(t)$ is a white Gaussian noise term with unit variance. Since s has a finite correlation time, it is permissible to multiply $\xi(t)$ by $\sqrt{\alpha_s+\delta m}$.

If we ignore the intrinsic fluctuations altogether (the condition for this will be obtained below), we obtain the following stochastic equation for the number of proteins:

$$\frac{dm}{dt} = A(m,s), \tag{47}$$

with $A(m,s)=\alpha_s-\delta m$. It is possible to find the exact stationary probability distribution for the solution of Eq. (47) for an arbitrary correlation time of the dichotomous extrinsic process (39),[18]

$$\begin{aligned} p(m) &= P_0\frac{A(m,1)-A(m,0)}{A(m,1)A(m,0)} \\ &\quad \times \exp\left[-C\int^x \frac{k_1A(z,0)+k_0A(z,1)}{A(z,1)A(z,0)}dz\right]. \end{aligned} \tag{48}$$

Here P_0 is determined by the normalization condition $\int p(x)dx=1$.

For the particular case of $A(m,s)=\alpha(s)-\delta m$, integration leads to the reduction of Eq. (48) to

$$p(m) = C_1|\alpha_0-\delta m|^{Ck_1/\delta-1}|\alpha_1-\delta m|^{Ck_0/\delta-1} \tag{49}$$

for $\alpha_0\delta^{-1} < x < \alpha_1\delta^{-1}$, and zero outside. Here $\alpha_0=\alpha(0)$ and $\alpha_1=\alpha(1)$. Note that despite the fact that the controlling process is binary and the intrinsic noise has been neglected, the distribution of protein fluctuations (49) is continuous.

For a symmetric gating process ($k_0=k_1=1/2$), the mean and the variance of m can be found analytically,

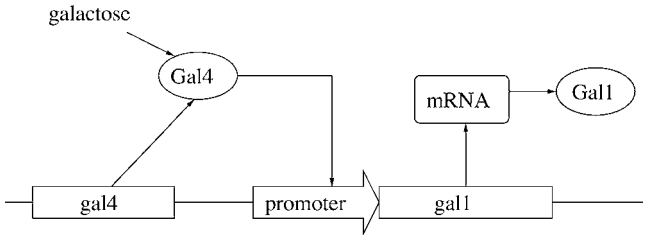

FIG. 3. Simplified model of the galactose utilization circuit.

$$M = \frac{\alpha}{\delta}, \tag{50}$$

$$V = \frac{\Delta\alpha^2}{4\delta(C+\delta)}, \tag{51}$$

where $\alpha=(k_1\alpha_0+k_0\alpha_1)/2$, $\Delta=\alpha_1-\alpha_0$. This expression agrees with (43), as it should.

V. EXTRINSIC FLUCTUATIONS IN GAL1 EXPRESSION

In this section we will consider fluctuations in a simplified model of the galactose utilization pathway in yeast (cf. Ref. 19). In the absence of glucose, galactose is transported into the cell by a permease and binds to the GAL3 protein. The galactose-GAL3 complex then binds and suppresses the ability of GAL80 to bind to the global activator GAL4. When free GAL80 binds to GAL4, it inhibits the ability of GAL4 to activate the promoters of many of the members of the GAL family (GAL1, GAL2, GAL3, GAL80). In our simplified version of the system[9] (see Fig. 3), we assume that galactose controls the degradation of constitutively produced GAL4, which in turn activates transcription of GAL1 and other galactose genes. The only difference between this model and the one presented in Ref. 9 is that we ignore cellular growth and division.

The stochastic biochemical reactions describing this system are as follows. There are two extrinsic reactions driving the production of GAL4:

$$g_4 \xrightarrow{k_4} g_4+1, \quad g_4 \xrightarrow{\gamma_4(\mathrm{gal})g_4} g_4-1, \tag{52}$$

and four intrinsic reactions—GAL1 transcription, mRNA degradation, mRNA translation, and protein degradation:

$$\begin{aligned} r &\xrightarrow{k_r H(g_4)} r+1, \quad r \xrightarrow{\gamma_r r} r-1, \\ g &\xrightarrow{k_g r} g+1, \quad g \xrightarrow{\gamma_g} g-1, \end{aligned} \tag{53}$$

where $H(g_4)=Kg_4^z/(1+Kg_4^z)$ is the Hill function for the activation process, and the galactose dependence enters via the degradation rate $\gamma_4=c\,\mathrm{gal}^{-1}$. The extrinsic 1×2 stoichiometric matrix $\mathbf{A}_E=(1,-1)$, and the propensity function $\mathbf{W}^E=(k_4,\gamma_4 g_4)^T$. Thus, the scalar extrinsic Jacobian $K^{EE}=-\gamma_4$ and the diffusion coefficient, $D_E=k_4+\gamma_4 g_4$.

The intrinsic 2×4 stoichiometric matrix,

$$\mathbf{A} = \begin{pmatrix} 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 \end{pmatrix}, \tag{54}$$

and the propensity function $\mathbf{W}^I=(k_r H(g_4), \gamma_r r, k_g r, \gamma_g g)^T$ correspond to the Jacobians

$$\mathbf{K}^{II} = \begin{pmatrix} -\gamma_r & 0 \\ k_g & -\gamma_g \end{pmatrix} \quad \mathbf{K}^{IE} = \begin{pmatrix} k_r H' \\ 0 \end{pmatrix} \tag{55}$$

and the diagonal diffusion matrix

$$\mathbf{D}^I = \begin{pmatrix} k_r H(g_4)+\gamma_r & 0 \\ 0 & k_g r+\gamma_g g \end{pmatrix}. \tag{56}$$

Here $H' \equiv H'(g_4^*)$, where $g_4^*=k_4\gamma_4^{-1}$ is the mean GAL4 concentration. Using these expressions in Eqs. (23)–(25), we obtain

$$\begin{aligned} \Sigma^{EE} &\equiv \langle g_4^2\rangle - \langle g_4\rangle^2 = k_4/\gamma_4, \\ \Sigma_1^{EI} &\equiv \langle g_4 r\rangle - \langle g_4\rangle\langle r\rangle = k_4 k_r H'[\gamma_4(\gamma_r+\gamma_4)]^{-1}, \\ \Sigma_2^{EI} &\equiv \langle g_4 g\rangle - \langle g_4\rangle\langle g\rangle = k_4 k_r k_g H'[\gamma_4(\gamma_r+\gamma_4)(\gamma_g+\gamma_4)]^{-1}, \\ \Sigma_{11}^{II} &\equiv \langle r^2\rangle - \langle r\rangle^2 = [k_r H'\Sigma_1^{EI} + D_1^I/2]/\gamma_r, \\ \Sigma_{12}^{II} &\equiv \langle rg\rangle - \langle r\rangle\langle g\rangle = [k_g\Sigma_{11}^{II} + k_r H'\Sigma_2^{EI}]/(\gamma_r+\gamma_g), \\ \Sigma_{22}^{II} &\equiv \langle g^2\rangle - \langle g\rangle^2 = [k_g\Sigma_{12}^{II} + D_2^I/2]/\gamma_g. \end{aligned} \tag{57}$$

Neglecting the intrinsic fluctuations and following the algorithm described in Sec. III C, we arrive at the following set of stochastically driven mass-action equations for the number of mRNA and protein molecules:

$$\dot{r} = k_r H(g_4) - \gamma_r r, \quad \dot{g} = k_g r - \gamma_g g. \tag{58}$$

The only source of noise here is the stochastic fluctuations of g_4, which are produced by the birth-death reactions given by Eq. (52).

We simulated the set of biochemical reactions (52) and (53) using the direct Gillespie algorithm,[20] along with the mass-action equations (58) driven by extrinsic noise (52), and compared the results with the analytical linear noise approximation formulas (57). First, we consider the case where $H(g_4)=Kg_4$, which implies no cooperativity in transcriptional activation. For this case we obtain very good agreement between direct simulations, driven mass-action equations, and the linear noise approximation. Figure 4 shows the probability distributions functions of the number of proteins GAL4, mRNA, and GAL1 obtained using direct Gillespie simulations and the Langevin approach for the parameters of the circuit, $k_4=8$, $k_r=800$, $k_g=40$, $K=0.05$, $\gamma_4=0.5\ \mathrm{gal}^{-1}$, $\gamma_r=4$, $\gamma_g=1$, which are scaled by the GAL1 degradation rate.

Figure 5 depicts the mean, variance, and the coefficient of variation for g_4, r, and g_4. As expected, the driven mass-action calculation slightly underestimates the coefficient of variation because it ignores the intrinsic component of noise, however, the linear noise approximation formulas are in excellent agreement with the direct Gillespie simulations.

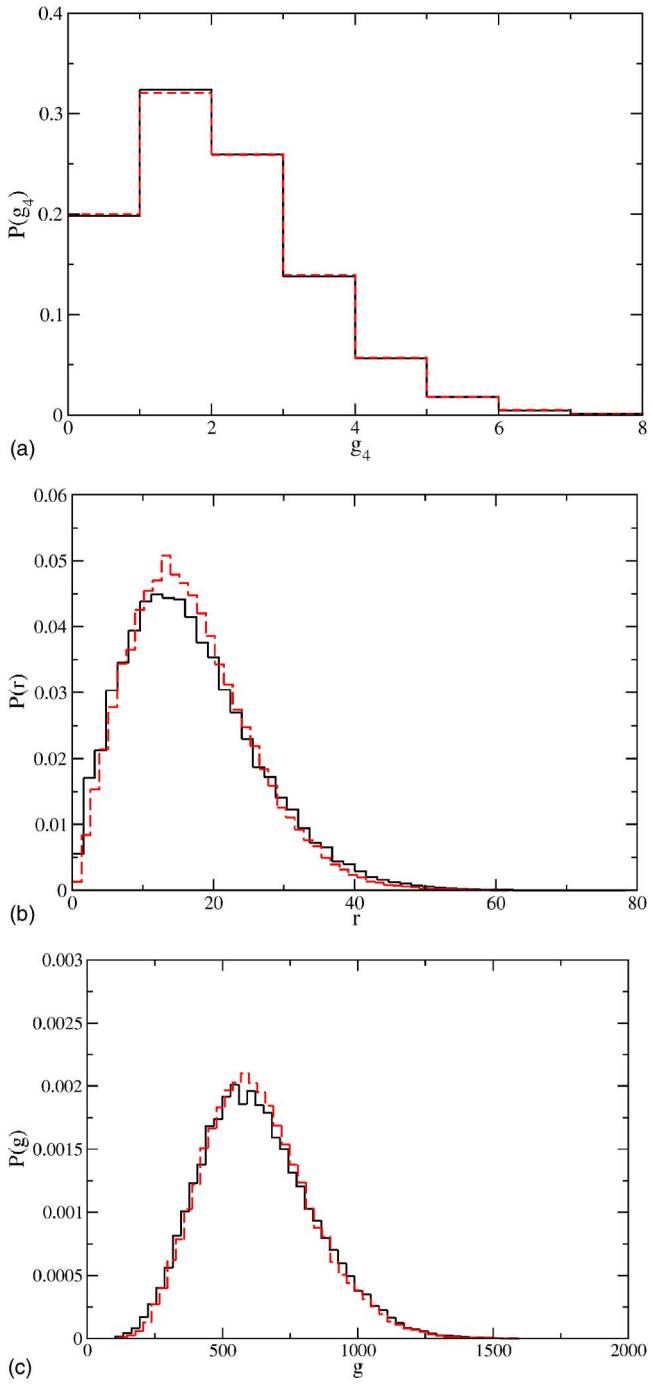

FIG. 4. Probability distributions of the number of molecules of GAL4 (a), mRNA (b), and GAL1 (c) for gal=0.1% according to the direct Gillespie simulations (solid lines) and stochastically driven mass-action equations for the intrinsic subsystem (dashed lines).

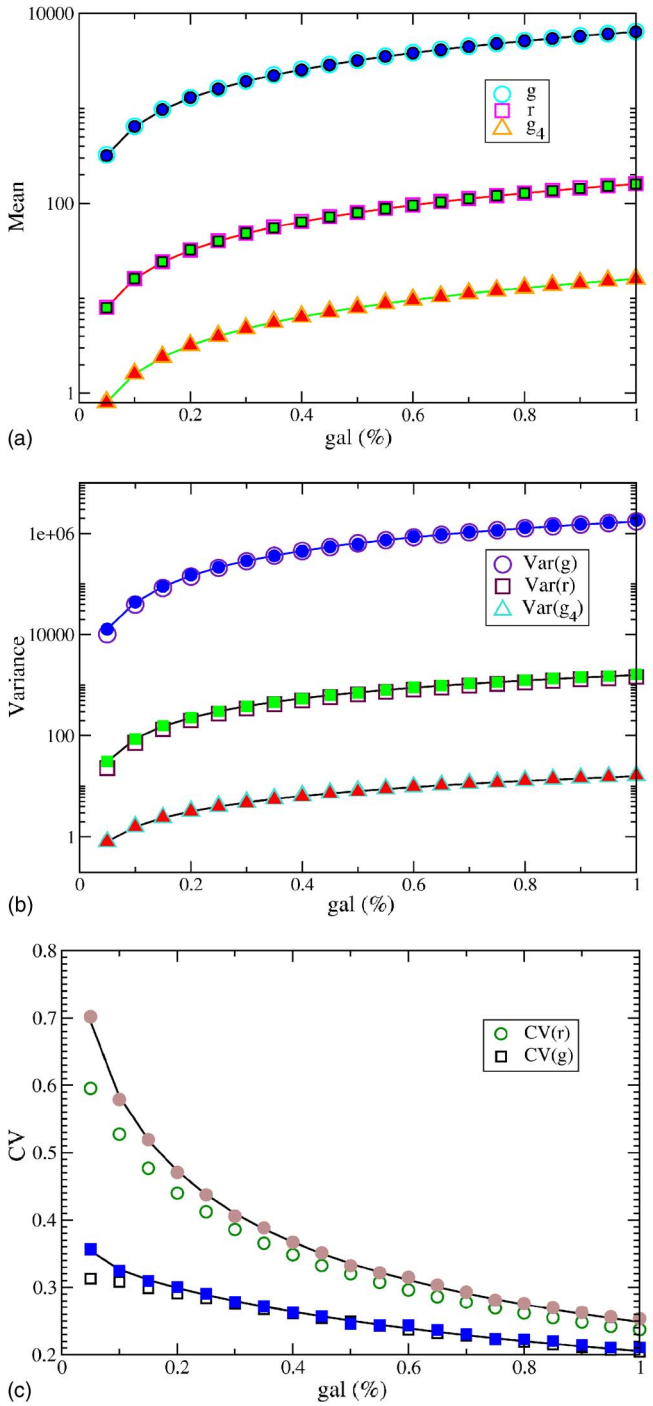

FIG. 5. Mean level (a), variance (b), and coefficient of variation (c) as a function of galactose concentration gal obtained with direct Gillespie method (solid symbols), stochastically driven mass-action equations for the intrinsic subsystem (open symbols), and the LNA approximation (lines).

Figure 6 compares the probability distributions for the case of high cooperativity, $H(g_4)=Kg_4^z/(1+Kg_4^z)$ with $z=8$, which corresponds to the binding of four GAL4 dimers to the GAL1 promoter.

Figure 7 shows the comparison between the mean and the variance of the GAL1 concentration according to (57), from solving the mass-action model (58) numerically with the fluctuating concentration g_4 according to the stochastic model (58), and the full Gillespie simulations. These dependencies are also in a qualitative agreement with experiments.[9] An even better quantitative agreement with the data can be obtained if growth and division of cells is taken into consideration (see Ref. 9).

It is interesting to observe that driven mass-action model describes stochastic fluctuations in the full system well at small levels of galactose, whereas the LNA approximation is valid for high galactose. It is easily explained by the fact that at small gal, the extrinsic noise dominates, however, the LNA does not work well because of the strong nonlinearity

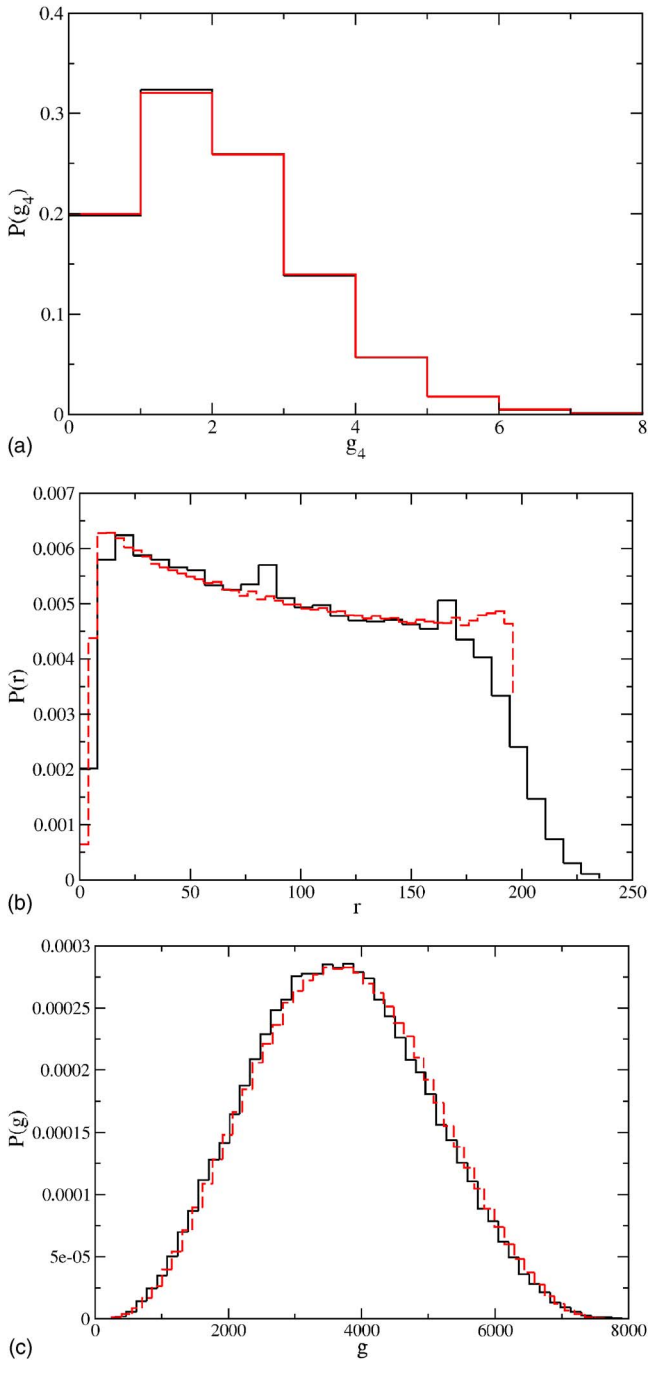

FIG. 6. Probability distributions of the number of molecules of GAL4 (a), mRNA (b), and GAL1 (c) for gal=0.1% for cooperative activation ($z=8$) according to the direct Gillespie simulations (solid lines) and stochastically driven mass-action equations for the intrinsic subsystem (dashed lines).

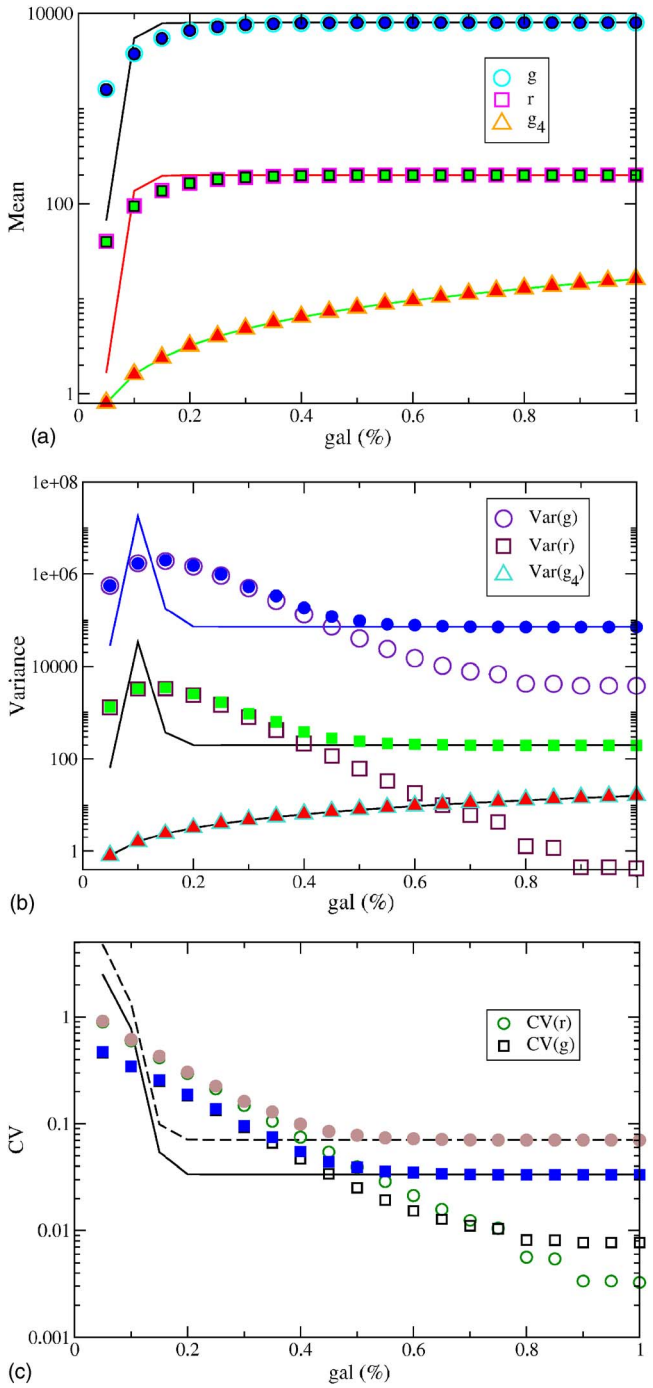

FIG. 7. Mean level (a), variance (b), and coefficient of variation (c) as a function of galactose concentration gal for cooperative activation ($z=8$) obtained with direct Gillespie method (solid symbols), stochastically driven mass-action equations for the intrinsic subsystem (open symbols), and the LNA approximation (lines).

of the function $H(g_4)$. On the other hand, at high galactose, the function $H(g_4)$ saturates at $H=1$, and therefore the extrinsic fluctuations become unimportant. In the meantime, the intrinsic fluctuations are linear, and hence they are well described by the LNA.

VI. CONCLUSIONS

In this paper we presented a short overview of the recent theoretical work on modeling stochastic dynamics of gene regulatory networks influenced by both intrinsic and extrinsic noise. As we discussed in Sec. II, according to recently published experimental data many gene regulatory circuits are dominated by extrinsic noise. Thus, we focused on situations when extrinsic noise dominates, and presented a number of approximate techniques to study the effects of extrinsic noise. In particular, we described two methods of treating the extrinsic noise in small genetic networks: linear noise approximation and stochastically driven mass-action equations. It is difficult to give *a priori* criteria of validity of

various approximations to the solution of the full CME, however, in certain cases it can be done *a posteriori*. For example, the LNA is valid and both intrinsic and extrinsic noise levels are small compared to the means. Similarly, we can roughly estimate *a posteriori* the level of intrinsic noise as a square root of the mean concentrations of corresponding proteins, and compare it with the level of extrinsic noise computed using stochastically driven mass-action equations. Unlike the LNA, which works well when the system is close to linear and/or the level of fluctuations is small, the driven mass-action method works well even in the case of a strongly nonlinear system provided that the intrinsic fluctuations are small. We presented the general theory pertinent to both of these methods and illustrated them by two simple examples: a linear constitutively transcribed gene controlled by a binary gating process, and the simplified model of galactose utilization pathway. In the first example, both methods agree very well with the results of direct Gillespie simulations, whereas in the second example, both methods work in different parameter regimes.

ACKNOWLEDGMENT

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