Stochastic hybrid systems for studying biochemical processes

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Many protein and mRNA species occur at low molecular counts within cells, and hence are subject to large stochastic fluctuations in copy numbers over time. Development of computationally tractable frameworks for modeling stochastic fluctuations in population counts is essential to understand how noise at the cellular level affects biological function and phenotype. We show that stochastic hybrid systems provide a convenient framework for modeling the time evolution of population counts of different chemical species involved in a set of biochemical reactions. We illustrate recently developed techniques that allow fast computations of the statistical moments of the population count, without having to run computationally expensive Monte Carlo simulations of the biochemical reactions. Finally, we review different examples from the literature that illustrate the benefits of using stochastic hybrid systems for modeling biochemical processes.

Keywords: stochastic hybrid systems; stochastic chemical kinetics; moment dynamics; moment closure; gene regulatory networks; gene autoregulation; negative feedback

1. Introduction

Deterministic hybrid systems that integrate continuous dynamics with discrete events have been used to model a wide array of biological processes that exhibit switching behavior (Belta et al. 2004, Lincoln & Tiwari 2004, Tanaka et al. 2008, Ghosh & Tomlin 2004). However, deterministic frameworks often fail to capture biochemical processes within living cells where low population counts of mRNAs and proteins can set the stage for significant stochastic effects (Raj & van Oudenaarden 2008). The inherent stochastic nature of cellular processes has motivated the use of Stochastic Hybrid Systems (SHS) for modeling biological phenomenon at the single cell level (Julius et al. 2008, Lygeros et al. 2008, Hu et al., 2004). SHSs (formally defined in Section 2), combine the generality of hybrid systems with stochastic processes.

Biochemical reactions inside cells are often modeled using a stochastic formulation, which takes into account the inherent randomness of thermal molecular motion (Gillespie 1976). In the stochastic formulation of chemical kinetics, reactions are treated as probabilistic events that change the population counts of individual chemical species based on the stoichiometry of the reactions. We show in Section 3 that the time evolution of the number of molecules of different chemical species involved in a set of chemical reactions can be modeled using a SHS. We use this SHS formalism to compute the time-derivatives of the lower-order statistical moments (for example means, standard deviations, correlation, etc.) of the population count. It turns out that the differential equations that describes the time evolution of the lower order statistical moments, are generally "not closed", in

the sense that the right-hand side of these equations depend on higher order moments. In Section 4, we illustrate recently developed techniques that "closes" these moment equations by expressing high order moments as *nonlinear* functions of lower order moments. The resulting closed differential equations provide quick and efficient computations of the statistical moments for a given chemical reaction network, without having to use Monte Carlo simulations that come at a significant computational cost.

Finally, in Section 5, we review different examples from literature that illustrate how stochastic hybrid systems have been used to model uncertainty in different biochemical processes. These examples include: (i) modeling random gene activation/inactivation in gene regulatory networks; (ii) modeling the effects of perturbations on stochastic chemical kinetics and (iii) modeling noise-induced transitions between different steady-states of a multi-stable biological network.

2. Stochastic Hybrid Systems

The state-space of a Stochastic Hybrid System (SHS) is composed of a *continuous component* $\mathbf{x}$ that takes values in Euclidean space $\mathbb{R}^n$, and a *discrete component* $\mathbf{q}$ that takes values in a finite set $\{q_1,\ldots,q_N\}$. The continuous state evolves according to the Ordinary Differential Equation (ODE)

$$\dot{\mathbf{x}} = f(\mathbf{q},\mathbf{x},t) \tag{2.1}$$

where the vector field f depends on the discrete state of the SHS. During the evolution of the above ODE, stochastic transitions or jumps may occur which change both the discrete state and the continuous state of the SHS. More specifically, these random transitions are characterized by a family of *k reset maps*

$$(\mathbf{q},\mathbf{x}) \mapsto \varphi_i(\mathbf{q},\mathbf{x},t), \quad \forall i \in \{1,\ldots,k\}, \tag{2.2}$$

and a corresponding family of *k transition intensities*

$$\lambda_i(\mathbf{q},\mathbf{x},t), \quad \forall i \in \{1,\ldots,k\}. \tag{2.3}$$

In essence, between stochastic transitions, the discrete state remains constant whereas the continuous state flows according to (2.1). At transition times, the continuous and discrete states are reset according to (2.2). The frequency with which different transitions occur is determined by the transition intensities (2.3). In particular, the probability that a particular transition will occur in an "elementary interval" $(t,t+dt]$, and therefore the corresponding reset map will be "activated" is given by $\lambda_i(\mathbf{q}(t),\mathbf{x}(t),t)dt$. Equations (2.1)-(2.3) define a SHS, which is often conveniently represented by a directed graph as in Figure 1. We refer interested readers to Hespanha (2005) for a mathematically precise characterization of a SHS and an algorithm to run Monte Carlo simulations of its trajectories.

Since the time evolution of the continuous state between stochastic transitions is deterministic, these SHSs have often been referred to in literature as Piecewise Deterministic Markov Processes (Davis 1993). However, equation (2.1) can be modified to allow the continuous state $\mathbf{x}$ to evolve according to a Stochastic Differential Equation (SDE) rather than an ODE (Hespanha & Singh 2005). Another straightforward extension of the above SHS is to include deterministic transitions between discrete states, where a transition is triggered when a certain "'guard condition" is satisfied (Hespanha 2005). We show next that SHSs are convenient for modeling the temporal dynamics of population counts of different species involved in a set of chemical reactions.

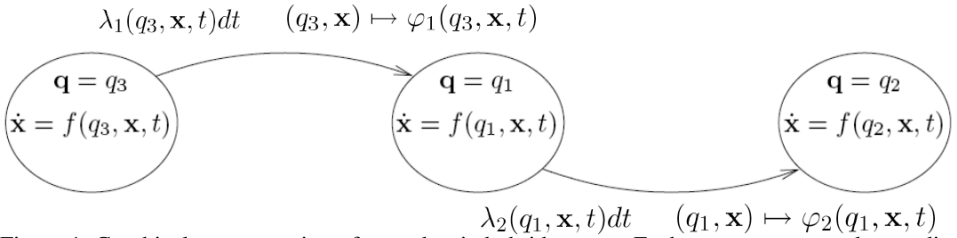

Figure 1. Graphical representation of a stochastic hybrid system. Each vertex corresponds to a discrete mode and each edge to a transition between discrete modes. The vertices are labeled with the corresponding discrete mode and the vector fields that determine the evolution of the continuous state in that particular mode. The edges are labeled with the transition intensities and their corresponding reset maps.

3. Stochastic modeling of chemical reactions

We begin this section by reviewing the stochastic formulation of chemical kinetics.

(*a*) *Stochastic formulation of chemical kinetics*

Consider a spatially uniform mixture of n chemical species $\mathrm{X}_1, \mathrm{X}_2, \ldots, \mathrm{X}_n$ in a fixed volume V involved in a system of k reactions $\mathrm{R}_1, \mathrm{R}_2, \ldots, \mathrm{R}_k$ of the form

$$\begin{aligned}
\mathrm{R}_1: &\quad u_{11}\mathrm{X}_1 + u_{12}\mathrm{X}_2 + \ldots + u_{1n}\mathrm{X}_n \xrightarrow{c_1} v_{11}\mathrm{X}_1 + v_{12}\mathrm{X}_2 + \ldots + v_{1n}\mathrm{X}_n \\
\mathrm{R}_2: &\quad u_{21}\mathrm{X}_1 + u_{22}\mathrm{X}_2 + \ldots + u_{2n}\mathrm{X}_n \xrightarrow{c_2} v_{21}\mathrm{X}_1 + v_{22}\mathrm{X}_2 + \ldots + v_{2n}\mathrm{X}_n \\
&\qquad\qquad\qquad\qquad \vdots \\
\mathrm{R}_k: &\quad u_{k1}\mathrm{X}_1 + u_{k2}\mathrm{X}_2 + \ldots + u_{kn}\mathrm{X}_n \xrightarrow{c_k} v_{k1}\mathrm{X}_1 + v_{k2}\mathrm{X}_2 + \ldots + v_{kn}\mathrm{X}_n,
\end{aligned}$$

where u_{ij} is the stoichiometry associated with the j^{th} reactant of the i^{th} reaction and v_{ij} is the stoichiometry associated with the j^{th} product of the i^{th} reaction. In the sequel, we denote by $\mathbf{x}_i(t)$ as the number of molecules of the species $\mathrm{X_i}$ at time t.

At high population counts, the time evolution of $\mathbf{x} = [\mathbf{x}_1, \ldots, \mathbf{x}_n]^T$ can be treated as a continuous and deterministic process governed by an ordinary differential equation, often referred to in literature as *chemical rate equations* (Wilkinson 1980). However, this deterministic framework fails within single cells where many species occur at very low molecular counts and change by discrete integer amounts whenever a reaction occurs inside the cell. The time evolution of such low-copy bio-chemical species is more accurately represented by a stochastic formulation of chemical kinetics which treats $\mathbf{x}(t)$ as a stochastic process (Gillespie 1976).

In the stochastic formulation of chemical kinetics, each reaction is a probabilistic event, and is assigned a probability that it will occur in the next "infinitesimal" time interval $(t, t+dt]$. This probability is given by the propensity function of the reaction, which is a product of the following two terms:

1. the number $h_i(\mathbf{x})$ of distinct molecular reactant combinations for the reaction $\mathrm{R_i}$ present in the volume V at time t,

2. the probability $c_i dt$ that a particular reactant combination of R_i will actually react on $(t, t+dt]$. The constant c_i for each chemical reaction depends on the physical prop-

Table 1. *Propensity functions for different reaction types.*

Reaction R_i	Probability reaction will occur in interval $(t, t+dt]$
$* \rightarrow$ reaction products	$c_i dt$
$X_i \rightarrow$ reaction products	$c_i \mathbf{x}_i dt$
$X_i + X_j \rightarrow$ reaction products, $(i \neq j)$	$c_i \mathbf{x}_i \mathbf{x}_j dt$
$2X_i \rightarrow$ reaction products	$\frac{c_i}{2} \mathbf{x}_i (\mathbf{x}_i - 1) dt$

erties of the reacting molecules and the temperature of the system, and is typically experimentally determined.

Table 1 shows the form of the propensity function for different reaction types (Gillespie 1976). In summary, the stochastic formulation treats reactions as a set of stochastic channels, and whenever a particular channel "fires" the molecular counts changes based on the stoichiometry of that reaction. Moreover, the frequencies at which these channels "fire" is determined by the propensity functions of the individual reactions.

(*b*) *Representing chemical reactions as a Stochastic Hybrid System*

The evolution of the number of molecules $\mathbf{x}_1, \mathbf{x}_2, \ldots, \mathbf{x}_n$ can be generated by a SHS. Since the number of molecules take values in the discrete set on integers, they can be regarded as either part of the discrete or the continuous state of the SHS. However, it will turn out to be more convenient to view them as part of a continuous state. In this case, the SHS has a single discrete mode, which we omit for simplicity. The continuous state of the SHS consists of the vector $\mathbf{x} = [\mathbf{x}_1, \ldots, \mathbf{x}_n]^T$ and has trivial dynamics, i.e.,

$$\dot{\mathbf{x}} = 0. \tag{3.1}$$

Each of the reactions is represented using a reset map defined by the stoichiometry

$$\mathbf{x} \mapsto \varphi_i(\mathbf{x}) = \begin{bmatrix} \mathbf{x}_1 - u_{i1} + v_{i1} \\ \mathbf{x}_2 - u_{i2} + v_{i2} \\ \vdots \\ \mathbf{x}_n - u_{in} + v_{in} \end{bmatrix}, \tag{3.2}$$

and a corresponding transition intensity

$$\lambda_i(\mathbf{x}) = c_i h_i(\mathbf{x}) \tag{3.3}$$

given by the propensity function of the reaction. Thus, between reactions, the population count remains constant and whenever the i^{th} reaction "fires", the state $\mathbf{x}$ is reset according to (3.2), furthermore, the probability of the activation taking place in an "infinitesimal" time interval $(t, t+dt]$ is given by $\lambda_i(\mathbf{x})dt$.

In many cases bio-chemical reactions can be divided into subsystems of fast and slow reactions. For example, the binding and unbinding of a transcription factor to a promoter typically occurs at much faster time-scales than the process of transcription, which create mRNAs from DNA. When such differences in time-scale exist between reactions, slow reactions or reactions containing low-copy molecular species should be modeled using

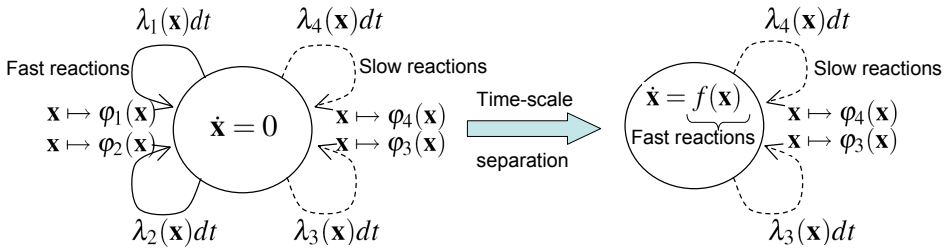

Figure 2. SHS representation of chemical reactions, where reactions are modeled as stochastic transitions that reset the population count $\mathbf{x}$ based on the stoichiometry of the reaction (left figure). Note that this SHS representation of the chemical reaction network is a special case of the SHS given by equations (2.1)-(2.3) as it has a only a single discrete mode, which we omit for simplicity. To reduce the computational costs of simulating these SHSs, fast reactions or reactions containing high copy molecular species (solid arrows) are often modeled using differential equations (right figure).

stochastic transitions and resets, as in the stochastic formulation of chemical kinetics. However, fast reactions or reactions containing high copy molecular species can be modeled using ODEs (or in some cases SDEs) resulting in a reduced approximate SHS where the dynamics of the continuous state is no longer trivial (Figure 2). These reduced SHSs provide much faster simulation times than the original SHS, with only a marginal decrease in accuracy (Neogi 2004, Salis & Kaznessis 2005, Chen et al. 2009) and have been used to model a wide array of biological processes ranging from lactose regulation in Escherichia coli (Julius et al. 2008), HIV transactivation network (Griffith et al. 2006) and synthetic gene networks (Bortolussi & Policriti 2008).

4. Computing moment dynamics of a chemically reacting system

The stochastic formulation of chemical reactions permit the computation of the probability density function of the population count $\mathbf{x}(t)$, which is often done through various Monte Carlo techniques at a significant computational cost (Gillespie 1976, 2001). Since one is often interested in computing only the first and second order moments for the number of molecules of the different species involved, much time and effort can be saved by applying approximate methods to directly compute these low-order moments (for example, Van Kampen's linear noise approximation (Kampen01)), without actually having to solve for the probability density function. In this section, we describe a procedure to compute the time evolution of the statistical moments of $\mathbf{x}(t)$ for an arbitrary set of chemical reactions.

Given a vector $\{m_1, m_2, \ldots, m_n\}$ of n non-negative integers, we define the *uncentered* moment of $\mathbf{x} = [\mathbf{x}_1, \ldots, \mathbf{x}_n]^T$ to be

$$\mathbf{E}\left[\mathbf{x}_1^{m_1}\mathbf{x}_2^{m_2}\cdots\mathbf{x}_n^{m_n}\right] \tag{4.1}$$

where $\mathbf{E}$ stands for the expected value. We refer to the sum $\sum_{j=1}^{n} m_j$ as the *order of the moment*. For example, consider a system of reactions with two species with population counts $\mathbf{x}_1$ and $\mathbf{x}_2$. Then, the first order moments are given by

$$\mathbf{E}[\mathbf{x}_1], \;\; \mathbf{E}[\mathbf{x}_2], \tag{4.2}$$

the second order moments are given by

$$\mathbf{E}[\mathbf{x}_1^2], \;\; \mathbf{E}[\mathbf{x}_2^2], \;\; \mathbf{E}[\mathbf{x}_1\mathbf{x}_2], \tag{4.3}$$

and so on. The SHS formalism for chemical reactions introduced in the previous section allows a straightforward derivation of the moment dynamics using the Dynkin's equation (Davis 1993). Applying the Dynkins equation to the SHS given by equations (3.1)-(3.3), gives the following time derivative of an uncentered moment of the population count:

$$\frac{d\mathbf{E}\left[\mathbf{x}_1^{m_1}\mathbf{x}_2^{m_2}\cdots\mathbf{x}_n^{m_n}\right]}{dt} = \mathbf{E}\left[\sum_{i=1}^{k} c_i h_i(\mathbf{x})\left\{\left[\prod_{j=1}^{n}(\mathbf{x}_j - u_{ij} + v_{ij})^{m_j}\right] - \mathbf{x}_1^{m_1}\mathbf{x}_2^{m_2}\cdots\mathbf{x}_n^{m_n}\right\}\right] \quad (4.4)$$

(Singh & Hespanha 2006). The right-hand-side of the above equation can be interpreted as the expected value of the product of the change in the monomial $\mathbf{x}_1^{m_1}\mathbf{x}_2^{m_2}\cdots\mathbf{x}_n^{m_n}$ whenever a reaction occurs and the frequency with which reaction occurs, summed up over all chemical reactions. Since the propensity functions $c_i h_i(\mathbf{x})$ are polynomials in the population count $\mathbf{x}$ (see Table 1) , it follow from (4.4) that the time derivative of an uncentered moment $\mathbf{E}\left[\mathbf{x}_1^{m_1}\mathbf{x}_2^{m_2}\cdots\mathbf{x}_n^{m_n}\right]$ will be a linear combination of uncentered moments of $\mathbf{x}(t)$. Note that this result will also hold for SHSs where the continuous dynamics is non trivial, i.e., $\dot{\mathbf{x}} = f(\mathbf{x})$ (as in Figure 2), as long as the vector field f is a polynomial in $\mathbf{x}$ (see Hespanha & Singh 2005). Thus for chemically reacting systems, uncentered moments of the population count evolve according to a linear system of equations. However, these moment equations may not always be "closed" in the sense that the time derivative of a m^{th} order moment may depend on moments of order higher than m. We discuss techniques used for solving such system of equations below, but we first consider a class of chemical reactions where the moment dynamics is always "closed".

(*a*) *Linear system of chemical reactions*

Consider a system of chemical reactions where all reactions have linear propensity functions $c_i h_i(\mathbf{x})$. This implies from Table 1 that we only have reactions of the form $* \rightarrow \mathrm{X_i}$ or $\mathrm{X_i} \rightarrow \mathrm{X_j}$. In this case, it follows from (4.4) that the time derivative of a m^{th} order moment $\mathbf{E}\left[\mathbf{x}_1^{m_1}\mathbf{x}_2^{m_2}\cdots\mathbf{x}_n^{m_n}\right]$ is a linear combination of moments of $\mathbf{x}$ of order up to m. Hence, if we construct a vector μ consisting of the first $\mathbf{M}$ order moments of $\mathbf{x}$, then its time evolution is given by

$$\dot{\mu} = \hat{\mathbf{a}} + A\mu \quad (4.5)$$

for some appropriate constant vector $\hat{\mathbf{a}}$ and constant matrix A. Thus for a chemically reacting system with linear propensity functions, the moments of the population count can always be computed by solving equation (4.5). We illustrate this point with a stochastic model of gene expression, which is given by the following set of chemical reactions

$$* \xrightarrow{c_1} \mathrm{mRNA},\ \mathrm{mRNA} \xrightarrow{c_2} *,\ \mathrm{mRNA} \xrightarrow{c_3} \mathrm{mRNA} + \mathrm{Protein},\ \mathrm{Protein} \xrightarrow{c_4} * \quad (4.6)$$

The first two reactions represent mRNA transcription from DNA at a rate c_1 and mRNA degradation at a constant rate c_2. The last two reactions correspond to protein translation from the mRNA and protein degradation at rates c_3 and c_4, respectively. Let $\mathbf{x}_1$ and $\mathbf{x}_2$ denote the population count of the mRNA and the protein, respectively. Then, the time evolution of $\mathbf{x} = [\mathbf{x}_1, \mathbf{x}_2]^T$ can be represented by a SHS with trivial continuous dynamics,

four reset maps

$$\mathbf{x} \mapsto \varphi_1(\mathbf{x}) = \begin{bmatrix} \mathbf{x}_1 + 1 \\ \mathbf{x}_2 \end{bmatrix}, \quad \mathbf{x} \mapsto \varphi_2(\mathbf{x}) = \begin{bmatrix} \mathbf{x}_1 - 1 \\ \mathbf{x}_2 \end{bmatrix}, \tag{4.7a}$$

$$\mathbf{x} \mapsto \varphi_3(\mathbf{x}) = \begin{bmatrix} \mathbf{x}_1 \\ \mathbf{x}_2 + 1 \end{bmatrix}, \quad \mathbf{x} \mapsto \varphi_4(\mathbf{x}) = \begin{bmatrix} \mathbf{x}_1 \\ \mathbf{x}_2 - 1 \end{bmatrix}. \tag{4.7b}$$

and corresponding transition intensities $\lambda_1(\mathbf{x}) = c_1$, $\lambda_2(\mathbf{x}) = c_2\mathbf{x}_1$, $\lambda_3(\mathbf{x}) = c_3\mathbf{x}_1$ and $\lambda_4(\mathbf{x}) = c_4\mathbf{x}_2$. From (4.4) the time evolution of the first and second order moments of the mRNA and the protein count are given by the following system of linear equations

$$\begin{gathered} \tfrac{d\mathbf{E}[\mathbf{x}_1]}{dt} = c_1 - c_2\mathbf{E}[\mathbf{x}_1], \ \ \tfrac{d\mathbf{E}[\mathbf{x}_1^2]}{dt} = c_1 + 2c_1\mathbf{E}[\mathbf{x}_1] + c_2\mathbf{E}[\mathbf{x}_1] - 2c_2\mathbf{E}[\mathbf{x}_1^2] \\ \tfrac{d\mathbf{E}[\mathbf{x}_2]}{dt} = c_3\mathbf{E}[\mathbf{x}_1] - c_4\mathbf{E}[\mathbf{x}_2], \ \ \tfrac{d\mathbf{E}[\mathbf{x}_2^2]}{dt} = c_3\mathbf{E}[\mathbf{x}_1] + c_4\mathbf{E}[\mathbf{x}_2] + 2c_3\mathbf{E}[\mathbf{x}_1\mathbf{x}_2] - 2c_4\mathbf{E}[\mathbf{x}_2^2] \\ \tfrac{d\mathbf{E}[\mathbf{x}_1\mathbf{x}_2]}{dt} = c_1\mathbf{E}[\mathbf{x}_2] + c_3\mathbf{E}[\mathbf{x}_1^2] - 2c_2\mathbf{E}[\mathbf{x}_1\mathbf{x}_2] - c_4\mathbf{E}[\mathbf{x}_1\mathbf{x}_2]. \end{gathered} \tag{4.8}$$

A steady-state analysis of the above moment dynamics shows that the steady-state variance σ^2 of protein levels is given by $\sigma^2 = \left(\frac{c_3}{c_2+c_4} + 1\right)\overline{\mathbf{E}[\mathbf{x}_1]}$, and scales linearly with the steady-state value of the mean protein level $\overline{\mathbf{E}[\mathbf{x}_1]}$. This linear scaling of variance with the mean protein levels obtained from the stochastic model is consistent with experimental measurements of gene expression noise in both eukaryotes (Bar-Even et al. 2006, Newman et al. 2006) and prokaryotes (Ozbudak et al. 2002)

(*b*) *Nonlinear system of chemical reactions*

We next consider the scenario where the system of chemical reactions contains at least one reaction with a *nonlinear* propensity function. Then, the time derivative of the vector μ consisting of the first $\mathbf{M}$ order moments of $\mathbf{x}$ is given by

$$\dot{\mu} = \hat{\mathbf{a}} + A\mu + B\bar{\mu}, \tag{4.9}$$

for an appropriate constant vector $\hat{\mathbf{a}}$, constant matrices A and B, and a (time-varying) vector $\bar{\mu}$ containing moments of order $\mathbf{M}+1$ and higher. For example, consider the following reactions

$$* \xrightarrow{c_1} \mathrm{X}, \ \ 2\mathrm{X} \xrightarrow{c_2} \mathrm{Y}, \ \ \mathrm{Y} \xrightarrow{c_3} * \tag{4.10}$$

where species X is produced as a monomer at a constant c_1 and dimerizes to form Y. The dimer Y then decays at a constant rate c_3. Let $\mathbf{x}_1$ and $\mathbf{x}_2$ denote the population count of species X and Y, respectively. Then, the time evolution of $\mathbf{x} = [\mathbf{x}_1, \mathbf{x}_2]^T$ can be represented by a SHS with trivial continuous dynamics, three reset maps

$$\mathbf{x} \mapsto \varphi_1(\mathbf{x}) = \begin{bmatrix} \mathbf{x}_1 + 1 \\ \mathbf{x}_2 \end{bmatrix}, \ \mathbf{x} \mapsto \varphi_2(\mathbf{x}) = \begin{bmatrix} \mathbf{x}_1 - 2 \\ \mathbf{x}_2 + 1 \end{bmatrix}, \ \mathbf{x} \mapsto \varphi_3(\mathbf{x}) = \begin{bmatrix} \mathbf{x}_1 \\ \mathbf{x}_2 - 1 \end{bmatrix} \tag{4.11}$$

and corresponding transition intensities $\lambda_1(\mathbf{x}) = c_1$, $\lambda_2(\mathbf{x}) = \frac{c_2}{2}\mathbf{x}_1(\mathbf{x}_1 - 1)$ and $\lambda_3(\mathbf{x}) = c_3\mathbf{x}_2$. Using the Dynkin's equation for the above SHS, the time evolution of the first and second order moments of the population count is given by (4.9) where

$$\bar{\mu} = \left[\mathbf{E}[\mathbf{x}_1^3], \ \mathbf{E}[\mathbf{x}_1^2\mathbf{x}_2]\right]^T \tag{4.12}$$

Table 2. *Moment closure approximation for third order moments*

Third order moment	Moment closure approximation
$\mathbf{E}[\mathbf{x}_1^3]$	$\left(\frac{\mathbf{E}[\mathbf{x}_1^2]}{\mathbf{E}[\mathbf{x}_1]}\right)^3$
$\mathbf{E}[\mathbf{x}_1^2\mathbf{x}_2]$	$\left(\frac{\mathbf{E}[\mathbf{x}_1^2]}{\mathbf{E}[\mathbf{x}_2]}\right)\left(\frac{\mathbf{E}[\mathbf{x}_1\mathbf{x}_2]}{\mathbf{E}[\mathbf{x}_1]}\right)^2$
$\mathbf{E}[\mathbf{x}_1\mathbf{x}_2\mathbf{x}_3]$	$\frac{\mathbf{E}[\mathbf{x}_1\mathbf{x}_2]\mathbf{E}[\mathbf{x}_2\mathbf{x}_3]\mathbf{E}[\mathbf{x}_1\mathbf{x}_3]}{\mathbf{E}[\mathbf{x}_1]\mathbf{E}[\mathbf{x}_2]\mathbf{E}[\mathbf{x}_3]}$

and is dependent on the third order moments of the population count. This example illustrates the general principle that nonlinear propensity functions result in a "non-closed" system of moment equations, where the dynamics of the lower order moments depends on higher order moments. For analysis purposes, the time evolution of the vector μ is often made to be closed by approximating the higher order moments $\bar{\mu}$ as nonlinear functions of lower order moments in μ, as in $\bar{\mu} \approx \varphi(\mu)$. This procedure is referred to in literature as *moment closure*, (Nasell 2003, Gillespie 2009) and results in a nonlinear approximated moment dynamics given by

$$\dot{\nu} = \hat{\mathbf{a}} + A\nu + B\varphi(\nu), \tag{4.13}$$

where the state of this closed system $\nu(t)$ can be viewed as an approximation for $\mu(t)$.

Recent work has proposed a novel moment closure technique based on derivative-matching, where the moment closure is done by matching time derivatives of the exact (not closed) moment equations with that of the approximate (closed) moment equations at some initial time t_0 and set of initial conditions (Hespanha & Singh 2005). In particular, this derivative matching approach attempt to determine nonlinear functions φ for which

$$\frac{d^i\mu(t)}{dt^i}\Big|_{t=t_0} = \frac{d^i\nu(t)}{dt^i}\Big|_{t=t_0} \tag{4.14}$$

holds for deterministic initial conditions $\mathbf{x}(t_0) = \mathbf{x}_0$ with probability one. The main rationale for doing so is that, if a sufficiently large number of derivatives of $\mu(t)$ and $\nu(t)$ match point-wise at an initial time t_0, then from a Taylor series argument the trajectories of $\mu(t)$ and $\nu(t)$ will remain close at least locally in time. Singh & Hespanha (2006, 2007) provide explicit formulas to construct a class of functions φ for which equation (4.14) holds approximately for all $i \geq 1$, i.e., all time derivatives of $\nu(t)$ and $\mu(t)$ match at $t = t_0$ with small errors. Table 2 provides the nonlinear approximations for all possible third order moments as a function of the first and second order moments based on the above derivative matching moment closure technique. We close the dynamics of the first and second order moments for the reaction set (4.10) by using the corresponding nonlinear approximations for the third order moments $\mathbf{E}[\mathbf{x}_1^3]$ and $\mathbf{E}[\mathbf{x}_1^2\mathbf{x}_2]$. Numerical solutions of the resulting approximated moment dynamics is shown in Figure 3. The procedure described here to generate approximated moment dynamics can be fully automated. The software StochDynTools (Hespanha 2006) is available to compute the approximated moment dynamics starting from a simple ASCII description of the chemical reactions involved.

A striking feature of the above moment closure technique is that its accuracy can be arbitrarily increased by reducing the error in matching the time derivative between the

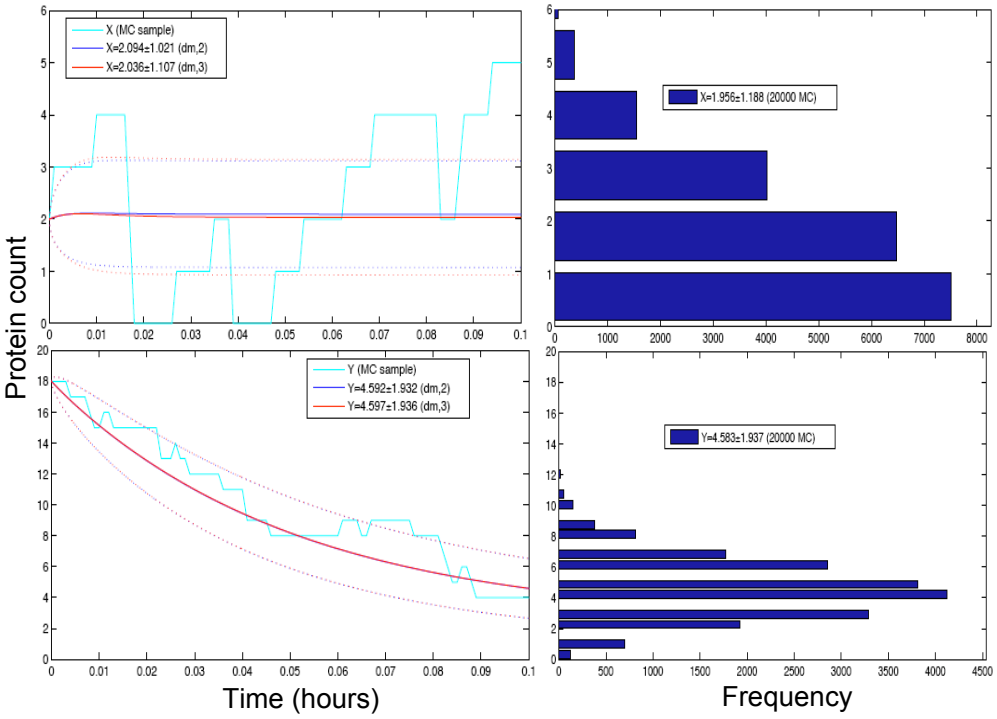

Figure 3. The left plots show the time evolution of the means (solid line)±one standard deviations (dashed lines) obtained from the approximated moment dynamics (4.13) corresponding to $\mathbf{M} = 2$ (dm,2) and $\mathbf{M} = 3$ (dm,3) for the reaction set (4.10). The legends shows the values of the mean±one standard deviation at the final time. The distributions, means and standard deviations shown on the right correspond to 20,000 Monte Carlo simulations done using the stochastic simulation algorithm (Gillespie 1976). The left plots include a typical Monte Carlo run. Reaction parameters were taken as $c_1 = 100$ *molecules hour*$^{-1}$, $c_2 = 30$ *molecules*$^{-1}$ *hour*$^{-1}$ and $c_3 = 20$ *hour*$^{-1}$. As one increases $\mathbf{M}$ there is an improvement in the moment estimates for species X. However, there is no significant change in the moment estimates of Y, and the moment trajectories corresponding to $\mathbf{M} = 2$ and $\mathbf{M} = 3$ lie on top of each other.

exact and the approximate moment dynamics. More specifically, if we close the dynamics of the first $\mathbf{M}$ order moments, then the derivative matching error given by

$$\left\| \frac{\frac{d^i \mu(t)}{dt^i}\Big|_{t=t_0} - \frac{d^i \nu(t)}{dt^i}\Big|_{t=t_0}}{\frac{d^i \mu(t)}{dt^i}\Big|_{t=t_0}} \right\| \tag{4.15}$$

scales as $||\mathbf{x}_0||^{-\mathbf{M}}$ (Singh & Hespanha 2006). Thus by increasing $\mathbf{M}$, which corresponds to including higher order moments in the vector μ, the approximated moment dynamics (4.13) provides more accurate approximations to the exact moment dynamics (4.9), as long as the elements of $\mathbf{x}_0$ are larger than one.

Another striking feature of the above moment closure technique is that the nonlinear functions φ, that express high order moments as functions of lower order moments, are consistent with *lognormal distributions*. Singh & Hespanha (2010) perform a systematic comparison of the derivative matching moment closure technique to an alternative procedure, where moment closure is performed by setting the third and higher order cumulants

of **x** equal to zero (Goutsias 2007, Gomez-Uribe and Verghese 2007, Lee et al. 2009). As for a gaussian distribution all cumulants of order three and higher are equal to zero, the zero-cumulant moment closure is consistent with gaussian distributions. Singh & Hespanha (2010) show that at low population counts the derivative matching moment closure technique provides more accurate estimates of the lower order moments than both the zero-cumulant moment closure. Intuitively, this occurs because at low population regimes molecular counts have highly skewed distributions that are much closer to a lognormal distribution than to a gaussian distribution. In fact, using the zero cumulant moment closure technique for low population species often results in unstable approximated moment dynamics with unbounded solutions (Nasell 2003).

In summary, moment closure allows one to approximate the moment dynamics of a chemically reacting system by an ordinary differential equation, which provides fast and efficient computation of stochasticity in a given reaction network.

5. Modeling gene regulatory networks and other biological processes

In this section we briefly review how SHSs have been used to model uncertainties arising from different sources in biochemical processes.

(*a*) *Modeling Gene regulatory networks*

Gene regulatory networks consists of a collection of genes that regulate the transcriptional activity of each other through their expressed proteins. SHSs have frequently been used to model the uncertainties associated with activation/inactivation of a gene in response to binding/unbinding of protein complexes to its promoter. We illustrate this with the simplest possible network: an autoregulatory gene network where a protein inhibits or activates its own gene expression (Figure 4a). Zeiser et al. (2009) models autoregulatory gene networks as a SHS with two discrete states, which represent a gene in an "ON" or "OFF" state (Figure 4b). The protein count represents the continuous state of the SHS and evolves according to a linear differential equation with production and degradation in the "ON" state, and only degradation in the "OFF" state. For simplicity, the authors combine transcription and translation into a single protein production term, and do not explicitly consider the mRNA dynamics. Random gene activation/inactivation is modeled through stochastic transitions between the discrete states with frequencies that are dependent on the protein count. In this SHS model, noise in protein levels only comes from stochastic promoter transitions between different transcriptional states, which is likely to be true when these transitions occur at a much slower time scales than those of the protein production and decay.

Mathematical models of autoregulatory gene networks have been extensively used to study negative feedback regulation, where a protein inhibits its own expression. Such negative feedback loops occur commonly in many cellular genes (Alon 2007), and have been hypothesized as a mechanism to reduce stochastic fluctuations in protein levels (Becskei & Serrano 2000). A negative feedback can be easily implement in the above SHS model by assuming that the promoter is more likely to transition to the OFF state if the protein count increases within the cell. Analysis of these models not only predict conditions under which feedback will provide the best suppression of gene expression noise but also determine the fundamental limits of noise suppression possible through negative autoregulation (Singh & Hespanha 2009a, 2009b). Counter intuitively, these models also show, that in

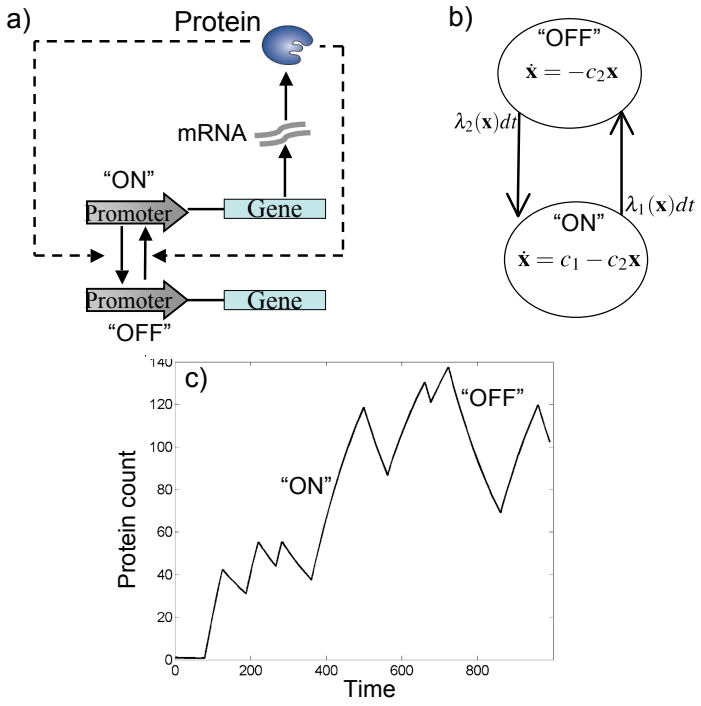

Figure 4. a) An autoregulatory gene network where a protein regulates its own expression; b) A SHS model of the autoregulatory gene network with two discrete states representing gene "ON" and "OFF" states. The protein count $\mathbf{x}$ exponentially increases to c_1/c_2 in the gene "ON" state and decrease exponentially to zero in the gene "OFF" state. Transitions between transcriptionally "ON" and "OFF" states occurs with transition intensities that are dependent on the protein count. As there are no changes in the protein population when the gene turns "ON" or "OFF", the rest maps for this SHS are the identity map. c) A singe realization of the protein count which shows increase and decrease in protein levels as the SHS transitions between "ON" and "OFF" states.

some cases, introducing negative feedback may actually increase gene expression noise rather than decreasing it (Zeiser et al. 2009, Stekel & Jenkins 2008).

The autoregulatory gene network model presented above can be easily extended to a network of N genes. Assuming each gene can be either "ON" or "OFF", then the SHS will have 2^N discrete states with each discrete state corresponding to some set of genes being in the "ON" state and others being in the "OFF" state. As in Figure 4b, the protein population either exponentially grows or decays depending on the transcriptional status of its gene. SHS models of gene networks, in which genes stochastically transition between transcriptional states and protein counts evolve according to linear differential equations, have proven to be very useful for both parameter identification and modeling of subtilin production in *Bacillus subtilis* (Cinquemani et al. 2008a) and nutrient stress response in *Escherichia coli* (Cinquemani et al. 2008b). An important caveat of using these models is that many mRNA species occur at very low molecular counts within cells (Bar-Even et al. 2006). Thus by modeling transcription as a completely deterministic process, one may fail to capture the stochasticity in protein levels due to thermal fluctuations in the corresponding mRNA counts. However, this limitation can be obviated by incorporating mRNA production and degradation as stochastic events, while still modeling protein translation and degradation as ordinary differential equations.

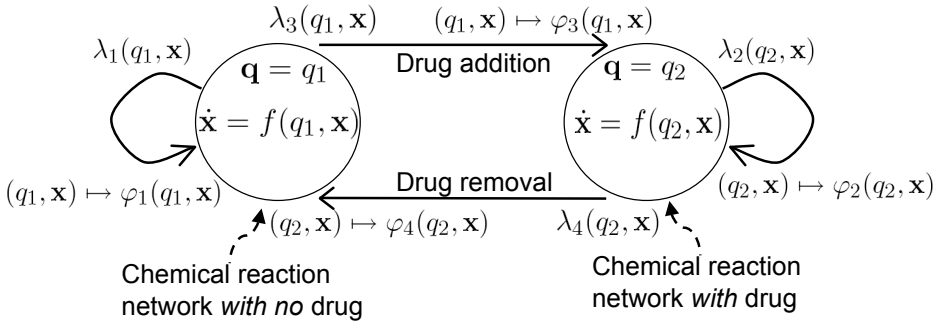

Figure 5. Modeling perturbations of chemical kinetics using stochastic hybrid systems. The discrete mode q_1 represents a set of chemical reactions where some reactions are modeled deterministically as differential equations while others are modeled stochastically through transitional intensities and reset maps. The discrete mode q_2 represents the same set of reactions under a perturbation (such as additions of a drug) which can alter the vector field f and the transition intensities/reset maps. Drug addition and removal are modeled via stochastic transitions between the discrete modes which are governed by the transition intensities $\lambda_3(q_1, \mathbf{x})$ and $\lambda_4(q_2, \mathbf{x})$ and their corresponding reset maps. SHSs such as the one illustrated here have recently been used to study disease progression under intermittent drug treatment (see Riley et al. 2009).

(*b*) *Modeling perturbations of stochastic chemical kinetics*

The SHS framework for chemical reacting systems introduced above provides a convenient model to investigate perturbations to the system caused by discrete events (Figure 5). This point is best illustrated by the recent work of Riley et al. 2009 that investigates the effect of drug treatment on the sugar cataract development process. This process can be expressed as a set of biochemical reactions, and hence modeled as a SHS with a single discrete state (Figure 2). To study the effects of drug treatment, the authors introduce a new discrete state, which models the time evolution of the biochemical reactions in the presence of the drug. The transitions between the two discrete states of the SHS are determined by the criterion used to add or remove the drug from the system. Using stochastic reachability analysis methods for SHSs the authors were able to compute the probability that a patient will develop a cataract with no drug treatment and with an intermittent drug treatment.

(*c*) *Modeling complex dynamics with multistability*

SHSs have also been used to model random transitions between different stable-states of a multi-stable biological network. These transitions occur due to noise in protein levels, which causes the system to move from the region of attraction of one stable-state to another. Noise-induced transitions between alternative stable states play a key role in mediating cell fate decisions in stem cells (Losick & Desplan 2008) and viruses (Singh & Weinberger 2009). A well-known example of this is the lactose regulation system in *Escherichia coli*, where in response to external lactose, a colony of identical cells bifurcate into two distinct populations: either fully induced with high lactose metabolizing enzyme levels or uninduced, with no enzymes (Novick & Weiner 1957). This bifurcation at the population level occurs due to an underlying bistability in the lactose metabolic network, with stochastic fluctuations in regulatory molecules causing single cells to converge to either one of the two stable steady-states. Starting from a full SHS model of the lactose metabolic network, Julius et al. (2008) builds a reduced model that can quickly predict the

fraction of induced and uninduced cells in response to a given concentration of lactose. This reduced model allowed the design of control feedback laws that can robustly steer a colony of cells to a desired fraction of induced cell, using external lactose as a control input. Perhaps, one day, similar reduced SHS models of multi-stable networks underlying cell fate decisions in stem cells can be controlled for possible therapeutic benefit.

6. Conclusions

Intracellular processes are driven by reactant molecules randomly diffusing and colliding within the cell and are thus inherently stochastic. We showed that the time evolution of the number of molecules of different species involved in an arbitrary system of biochemical reactions can be modeled as a SHS. The SHS framework is useful as it provides the flexibility of modeling fast reactions deterministically, using differential equations, while slow reactions can evolve stochastically using stochastic transitions that reset the population count based on the stoichiometry of the reaction (Figure 2).

The SHS framework also provides a method to compute the moment dynamics for a given set of chemical reactions. We illustrated a novel moment closure scheme based on derivative matching, which allows one to approximate the time evolution of the statistical moments by a nonlinear ordinary differential equation. These equations not only provide quick computations of the means, standard deviation, correlation etc., but can also be used for investigating how stochasticity in molecular counts is affected by the parameters of the reaction network. Comparisons with alternative techniques showed that this moment closure scheme performs best at low population counts, where stochastic effects are most likely to be manifested.

Given the recent evidence that proteins involved in various cellular pathways exhibit stochastic fluctuations in their copy numbers (Bar-Even et al., 2006), SHSs will likely find increased use to answer fundamental questions in noise biology. For example, what mechanisms ensure that, in spite of noise in protein levels, signal transmission and information processing occurs with sufficiently high fidelity inside cells? How is noise at the cellular level exploited to create variability at the population level for dealing with environmental uncertainties? Finally, SHS models will also likely play an important role in parameter identification as has been illustrated by Cinquemani et al. 2008b. Models that take into account the inherent stochastic nature of biochemical reactions will likely provide better parameter estimates from biological data, than purely deterministic models that consider all variation as measurement noise.

7. Acknowledgment

JPH acknowledges funding from the Institute for Collaborative Biotechnologies through grant DAAD19-03-D-0004 from the U.S. Army Research Office and by the National Science Foundation under Grant numbers ECCS-0725485 and ECCS-0835847. AS acknowledges support from Leor Weinberger throughout this work.

References

Alon, U. 2007. Network motifs: theory and experimental approaches. *Nature Reviews Genetics*, **8**, 450–461.

Bar-Even, A., Paulsson, J., Maheshri, N., Carmi, M., O'Shea, E., Pilpel, Y. & Barkai, N. 2006. Noise in protein expression scales with natural protein abundance. *Nature Genetics*, **38**, 636–643.

Becskei, A. & Serrano, L. 2000. Engineering stability in gene networks by autoregulation. *Nature*, **405**, 590–593.

Belta, C., Finin, P., Habets, L.C.G.J.M., Halasz, A., Imielinski, M., Kumar, V. & Rubin, H. 2004. Understanding the bacterial stringent response using reachability analysis of hybrid systems. In *Hybrid Systems: Computation and Control, HSCC 2004, LNCS 2993*, pp. 111–126, Springer Berlin, Heidelberg.

Bortolussi, L. & Policriti, A. 2008. Hybrid Systems and Biology: Continuous and Discrete Modeling for Systems Biology . In *Formal Methods for Computational Systems Biology, LNCS 5016*, pp. 424–448, Springer Berlin, Heidelberg.

Chen, L., Wang, R. & Aihara, K. 2009. Stochastic Hybrid System for Chemical Master Equation. *In Proc. of the Third International Symposium on Optimization and Systems Biology, China*, 475–481.

Cinquemani, E., Milias, A., Summers, S. & Lygeros, J. 2008a. Stochastic dynamics of genetic networks: modelling and parameter identification. *Bioinformatics*, **24**, 2748–2754.

Cinquemani, E., Porreca, R., Ferrari-Trecate, G. & Lygeros, J. 2008b. Subtilin Production by Bacillus Subtilis: Stochastic Hybrid Models and Parameter Identification. *IEEE Transactions on Automatic Control*, **53**, 38–50.

Davis, M. H. A. 1993. *Markov models and Optimization*, Chapman and Hall, London.

Ghosh, R. & Tomlin, C. J. 2004. Symbolic reachable set computation of piecewise affine hybrid automata and its application to biological modeling: Delta-Notch protein signaling. *IEE Transactions on Systems Biology*, **1**, 170–183.

Gillespie, C. S. 2009. Moment-closure approximations for mass-action models. *IET Syst Biology*, **3**, 52–58.

Gillespie, D. T. 1976. A general method for numerically simulating the stochastic time evolution of coupled chemical reactions. *J. of Computational Physics*, **22**, 403–434.

Gillespie, D. T. 2001. Approximate accelerated stochastic simulation of chemically reacting systems. *Journal of Chemical Physics*, **115**, 1716–1733.

Gomez-Uribe, C. A. & Verghese, G. C. 2007. Mass uctuation kinetics: Capturing stochastic effects in systems of chemical reactions through coupled mean-variance computations. *Journal of Chemical Physics*, **126**, 024109.

Goutsias, J. 2007. Classical versus stochastic kinetics modeling of biochemical reaction systems. *Biophysical Journal*, **92**, 2350—2365.

Griffith, M., Courtney, T., Peccoud, J. & Sanders W. H. 2006. Dynamic partitioning for hybrid simulation of the bistable HIV-1 transactivation network. *Bioinformatics*, **22**, 2782–2789.

Hespanha, J. P. & Singh, A. 2005. Stochastic models for chemically reacting systems using polynomial stochastic hybrid systems. *International Journal of Robust and Nonlinear Control*, **15**, 669–689.

Hespanha, J. P. 2005. A Model for Stochastic Hybrid Systems with Application to Communication Networks. *Nonlinear Analysis, Special Issue on Hybrid Systems*, **62**, 1353–1383.

Hespanha, J. P. 2006. StochDynTools a MATLAB toolbox to compute moment dynamics for reactions, available at http://www.ece.ucsb.edu/∼hespanha/software/stochdyntool.html.

Hu, J., Wu, W. & Sastry, S. 2004. Modeling Subtilin Production in Bacillus subtilis Using Stochastic Hybrid Systems . In *Hybrid Systems: Computation and Control, HSCC 2004, LNCS 2993*, pp. 163–166, Springer Berlin, Heidelberg.

Julius, A.A., Halasz, A., Sakar, M.S., Rubin, H., Kumar, V. & Pappas, G.J. 2008. Controlling biological systems: the lactose regulation system of Escherichia coli. *IEEE Transaction on Automatic Control*, **53**, 51–65.

Kampen, N. G. V. 2001. Stochastic Processes in Physics and Chemistry. Amsterdam, The Netherlands: Elsevier Science.

Lee, C. H., Kim, K. & Kim, P. 2009. A moment closure method for stochastic reaction networks. *Journal of Chemical Physics*, **130**, 134107.

Lincoln, P. & Tiwari, A. 2004. Symbolic systems biology: Hybrid modeling and analysis of biological networks. 2004. In *Hybrid Systems: Computation and Control, HSCC 2004, LNCS 2993*, pp. 660–672, Springer Berlin, Heidelberg.

Losick, R. & Desplan, C. 2008. Stochasticity and cell fate. *Science*, **320**, 65–68.

Lygeros, J., Koutroumpas, K., Dimopoulos, S., Legouras, I., Kouretas, P., Heichinger, C., Nurse, P. & Lygerou Z. 2008. Stochastic hybrid modeling of DNA replication across a complete genome. *PNAS*, **105**, 12295-12300.

McQuarrie, D. A. 1967. Stochastic approach to chemical kinetics. *Journal of Applied Probability*, **4**, 413–478.

Nasell, I. 2009. An extension of the moment closure method. *Theoretical Population Biology*, **64**, 233–439.

Neogi, N. A. 2004. Dynamic partitioning of large discrete event biological systems for hybrid simulation and analysis. In *Hybrid Systems: Computation and Control, HSCC 2004, LNCS 2993*, pp. 229–248, Springer Berlin, Heidelberg.

Newman, J. R., Ghaemmaghami, S., Ihmels, J., Breslow, D. K., Noble, M., DeRisi, J. L. & Weissman, J. S. 2006. Single-cell proteomic analysis of S. cerevisiae reveals the architecture of biological noise. *Nature*, **441**, 840–846.

Novick A. & Weiner M. 1957. Enzyme induction as an all-or-none phenomenon. *PNAS*, **43**, 553–566.

Ozbudak, E. M., Thattai, M., Kurtser, I., Grossman, A.D. & van Oudenaarden, A. 2002. Regulation of noise in the expression of a single gene. *Nature Genetics*, **31**, 69–73.

Raj, A. & van Oudenaarden, A. 2008. Nature, nurture, or chance: stochastic gene expression and its consequences. *Cell*, **135**, 216–226.

Riley, D., Koutsoukos, X. & Riley, K. 2009. Modelling and analysis of the sugar cataract development process using stochastic hybrid systems. *IET Systems Biology*, **3**, 137–154.

Salis, H. & Kaznessis, Y. 2005. Accurate hybrid stochastic simulation of a system of coupled chemical or biochemical reactions. *Journal of Chemical Physics*, **122**, 54103.

Singh, A. & Hespanha, J. P. 2006. Lognormal moment closures for bio-chemical reactions. *In Proc. of the 45th IEEE Conference on Decision and Control, San Diego, CA*, 2063–2068.

Singh, A. & Hespanha, J. P. 2007. A derivative-matching approach to moment closure for the stochastic logistic model. *Bulletin of Math Biology*, **69**, 1909–1925.

Singh, A. & Hespanha, J. P. 2009a. Optimal feedback strength for noise suppression in autoregulatory gene networks. *Biophysical Journal*, **96**, 4013–4023.

Singh, A. & Hespanha, J. P. 2009b. Evolution of autoregulation in the presence of noise. *IET Systems Biology*, **3**, 368–378.

Singh, A. & Hespanha, J. P. 2010. Approximate Moment Dynamics for Chemically Reacting Systems. *Submitted to IEEE Trans. Automatic Control*.

Singh, A. & Weinberger, L. S. 2009. Noise in viral gene expression as a molecular switch for viral latency. *Current Opinion in Microbiology*, **12**, 460–466.

Stekel, D. J. & Jenkins D. J. 2008. Strong negative self regulation of Prokaryotic transcription factors increases the intrinsic noise of protein expression. *BMC Systems Biology*, **2**, 6.

Tanaka, G., Tsumoto, K., Tsuji, S. & Aiharaa, K. 2008. Bifurcation analysis on a hybrid systems model of intermittent hormonal therapy for prostate cancer. *Physica D: Nonlinear Phenomena*, **237**, 2616–2627.

Wilkinson, F. 1980. Chemical Kinetics and Reaction Mechanisms. Van Nostrand Reinhold Co, New York.

Zeiser, S., Franz, U., Mller, J. & Liebscher, V. 2009. Hybrid modeling of noise reduction by a negatively autoregulated system. *Bulletin of Math Biology*, **71**, 1006–1024.