Stochastic chemical kinetics and the quasi-steady-state assumption: Application to the Gillespie algorithm

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Biochemical dynamics are often determined by series of single molecule events such as gene expression and reactions involving protein concentrations at nanomolar concentrations. Molecular fluctuations, consequently, may be of biological significance. For example, heterogeneity in clonal populations is believed to arise from molecular fluctuations in gene expression. A realistic description, therefore, requires a probabilistic description of the biochemical dynamics as deterministic descriptions cannot capture the inherent molecular fluctuations. The Gillespie algorithm [D. T. Gillespie, J. Phys. Chem. **81**, 2350 (1977)] is a stochastic procedure for simulating chemical systems at low concentrations. A limitation of stochastic kinetic models is that they require detailed information about the chemical kinetics often unavailable in biological systems. Furthermore, the Gillespie algorithm is computationally intensive when there are many molecules and reaction events. In this article, we explore one approximation technique, well known in deterministic kinetics, for simplifying the stochastic model: the quasi-steady-state assumption (QSSA). We illustrate how the QSSA can be applied to the Gillespie algorithm. Using the QSSA, we derive stochastic Michaelis–Menten rate expressions for simple enzymatic reactions and illustrate how the QSSA is applied when modeling and simulating a simple genetic circuit. © *2003 American Institute of Physics.* [DOI: 10.1063/1.1545446]

I. INTRODUCTION

At the level of the cell, the chemical dynamics are often determined by the action of only a few molecules and, consequently, molecular fluctuations may dominate the dynamics. These molecular fluctuations appear to have many important consequences in biology.[1,2] Gene expression, for example, involves a series of single molecule events. Fluctuations in gene expression may lead to a divergence of fate and, consequently, to nongenetic population heterogeneity.[3,4] Likewise, fluctuations in gene expression and protein concentrations have also been implicated in phenotypic variation in clonal populations.[5–7] Deterministic models, consequently, do not always accurately describe the chemical dynamics for such systems, as statistical averages do not account for molecular fluctuations, and these fluctuations may have a profound effect on the physiology of the cell. To model molecular fluctuations, a probabilistic model of the chemical dynamics is often necessary.

A defining attribute of probabilistic kinetic models is that they account for each molecule and every reaction event. For complex processes involving many species and reactions, this fine detail poses many modeling and computational barriers. Consider, for example, a dimerization reaction at steady state. Unlike a deterministic formulation, steady state in a stochastic formulation does not imply the system has converged to a static ratio of monomer and dimer molecules. Rather, only the likelihood of a particular ratio has converged to a static distribution. If the reactions events are fast, then simulating even a short time interval of the dimerization reaction at steady state is computationally intensive. From a modeling perspective, information regarding the association and dissociation rates is rarely available in a biological system. Often, the only information available is a dissociation constant, something that cannot be directly translated into a stochastic model. As we are often not interested in fast fluctuations, but rather integrated biochemical reaction networks involving many different molecular species and reactions, we seek to reduce both the model and computational complexity.

In this article, we consider the Gillespie algorithm for simulating stochastic chemical kinetics.[8,9] While the Gillespie algorithm is a simple procedure for exactly simulating stochastic kinetics, the algorithm is slow. Gibson and Bruck[10] have recently proposed a streamlined version of the Gillespie algorithm. However, the core algorithm is same: each molecule and reaction event is accounted for. If one wants to reduce the complexity, then one needs to look for approximations to the model. One strategy is to consider the dynamics at asymptotic limits. For example, as the number of molecules increase, one can approximate the molecular fluctuations as a realization of Brownian motion.[11,12] The discrete model then becomes a continuous model in the form

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of a stochastic differential equation or Langevin equation. Alternatively, one can consider a time-scale separation, where a subset of species is asymptotically at steady state on the time scale of interest. This approximation is known as the quasi-steady-state assumption (QSSA). The QSSA reduces the model complexity and, consequently, the computational complexity by effectively reducing the number of molecular species and reactions. Hence, it eliminates the fast dynamics that contribute most to the computational cost and are rarely of interest. Model reduction is particularly useful when we are unable to ascertain certain information from experiments, such as association and dissociation rates. The strength of the QSSA is that it uses our physical intuition of the system. Verifying its validity, therefore, is often straightforward. Time scale separation by adiabatic elimination has previously been applied to the chemical master equation.[13–16] The goal of this article is to extend these results to stochastic simulation and the Gillespie algorithm, and to apply the QSSA to some problems common in cell biology. To the best of our knowledge, these results are novel.

II. THE QUASI-STEADY-STATE ASSUMPTION

When the intermediate species in a reaction network are transitory and highly reactive, one commonly assumes in deterministic kinetics that the *net* rate of formation is approximately equal to zero. Examples of transitory intermediate species include enzyme-substrate complexes and surface species. We refer to this assumption as the quasi-steady-state assumption (QSSA), though it is also referred to as Bodenstein–Semenov kinetics or the pseudo-steady-state assumption. One can establish the validity of the QSSA using singular perturbation theory for differential equations.[17] The utility of the QSSA is that it allows us to reduce the dimension of the model by eliminating the intermediate species from the model. The intermediate species are implicitly accounted for by assuming that they are in quasi-steady state with the primary species. By quasi-steady state, we mean that on the time scale of interest the instantaneous rates of change of the intermediate species are approximately equal to zero. As we demonstrate, we can also use the QSSA to reduce the problem dimension when we consider a stochastic description of the chemical kinetics.

Consider a homogeneous mixture of n chemical species that undergo m reactions in a closed vessel of fixed volume and constant temperature. Let the n-dimensonal vector x denote the number of molecules of each species. For each reaction, let the function $a_k(x)$ denote the propensity of the kth reaction. In other words, the probability that the kth reaction with occur in the time interval dt is $a_k(x)dt+o(dt)$, where $o(x)$ satisfies the condition $\lim_{x\to 0} o(x)/x=0$. Let the n-dimensional vector v_k denote the stoichiometry associated with the kth reaction. The probability $P(x;t)$ of x species at time t is given by the master equation

$$\frac{dP(x;t)}{dt}=\sum_{k=0}^{m}\ a_k(x-v_k)P(x-v_k;t)-a_k(x)P(x;t)] \tag{1}$$

subject to the initial condition $P(x_0;0)$. The reader is directed to Ref. 9 for a discussion of the assumptions underlying stochastic kinetics.

When one applies the QSSA in deterministic kinetics, one eliminates the differential equations describing the intermediate species by setting them equal to zero. In stochastic kinetics, a single equation describes the probability of a given state rather than a given chemical species. Consequently we need to separate the primary species from the intermediate species in order to apply the QSSA. We separate the species by partitioning the species vector x into the set of species y and z where $x\triangleq(y,z)$. We let the vector y denote primary species and the vector z denote the intermediate, or ephemeral, species. Substituting into Eq. (1), the chemical master equation for the partitioned species vector is

$$\frac{dP(y,z;t)}{dt}=\sum_{k=0}^{m}\ [a_k(y-v_k^y,z-v_k^z)P(y-v_k^y,z-v_k^z;t) - a_k(y,z)P(y,z;t)], \tag{2}$$

where v_k^y and v_k^z denote the associated partition of v_k. Using the definition of conditional probabilities, we can represent the joint probability as

$$P(y,z;t)=P(z|y;t)P(y;t). \tag{3}$$

Using the chain rule of differentiation, the master equation becomes

$$P(y;t)\frac{dP(z|y;t)}{dt}+P(z|y;t)\frac{dP(y;t)}{dt} =\sum_{k=0}^{m}\ [a_k(y,z-v_k^z)P(z-v_k^z|y-v_k^y;t) \times P(y-v_k^j;t)-a_k(y,z)P(z|y;t)P(y;t)], \tag{4}$$

In order to apply the QSSA, we first need to assume that z conditional on y is Markovian. In other words, for fixed y the conditional probability distribution of the intermediate species $P(z|y;t)$ approximately satisfies the master equation

$$\frac{dP(z|y;t)}{dt}\approx\sum_{k=0}^{m}\ [a_k(y-v_k^y,z-v_k^z)P(z-v_k^z|y;t) - a_k(y,z)P(z|y;t)] \tag{5}$$

on the time scales of interest. The QSSA in stochastic kinetics then assumes that the *net* rate of change for the conditional probability distribution of the intermediate species $P(z|y;t)$ is approximately equal to zero:

$$\frac{dP(z|y;t)}{dt}\approx 0. \tag{6}$$

An immediate consequence of the second assumption (6) is that the conditional probability function $P(z|y;t)$ is time invariant: $P(z|y;t)\approx P(z|y)$. If we substitute Eq. (6) into (5), then $P(z|y)$ satisfies the approximate steady-state master equation

$$\sum_{k=0}^{m} [a_k(y-v_k^y, z-v_k^z)P(z-v_k^z|y;t) - a_k(y,z)P(z|y;t)] \approx 0. \quad (7)$$

It is important to note that we make two assumptions in order to apply the QSSA to stochastic kinetics.

If we apply the QSSSA, the approximate chemical master equation for the subset of species y is

$$P(z|y)\frac{dP(y;t)}{dt} \approx \sum_{k=0}^{m} [a_k(y-v_k^y, z-v_k^z) \times P(z-v_k^z|y-v_k^y)P(y-v_k;t) - a_k(y,z)P(z|y)P(y;t)] \quad (8)$$

As $\Sigma_z P(z|y;t)=1$, we can obtain the following approximate master equation for the marginal distribution $P(y;t)$:

$$\frac{dP(y;t)}{dt} = \sum_z P(z|y)\frac{dP(y;t)}{dt} \approx \sum_z \sum_{k=0}^{m} [a_k(y-v_k^y, z-v_k^z)P(z-v_k^z|y-v_k^y) \times P(y-v_k;t) - a_k(y,z)P(z|y)P(y;t)]. \quad (9)$$

The significance of the above equation is that we can eliminate the intermediate species z from the chemical master equation (8) by summing over the states of z. This elimination also implies that y is separately Markovian—a limiting assumption of QSSA.[16] Simplifying the notation of Eq. (9), we obtain the following approximate master equation solely in terms of primary species y:

$$\frac{dP(y;t)}{dt} = \sum_{k=0}^{m} [b_k(y-v_k^y)P(y-v_k^y;t) - b_k(y)P(y;t)], \quad (10)$$

where

$$b_k(y) \triangleq \sum_z a_k(y,z)P(z|y). \quad (11)$$

The functional $b_k(\cdot)$ is the conditional expectation of the functional $a_k(\cdot)$.

When we apply the QSSA, we implicitly assume that we can expand the conditional probability function $P(z|y;t)$ in some parameter ϵ such that

$$P(z|y;t) = P(z|y) + o(\epsilon).$$

The QSSA is exact when $\epsilon=0$ and the errors associated with the QSSA are roughly proportional to magnitude of ϵ. One obtains the parameter ϵ by scaling parameters in the model.[18] As we demonstrate with examples in Secs. IV and V, the Markovian assumption can also arise from the same scaling arguments.

III. AN ALGORITHM FOR APPROXIMATE STOCHASTIC SIMULATION

The chemical master equation provides a complete description for the chemical kinetics. Even though the chemical master equation is linear, we are usually unable to solve it either analytically or numerically as the dimension explodes with the number of molecules and reactions. For example, if we consider a reaction

$$A \rightleftharpoons B \rightleftharpoons C,$$

then the order of the chemical master equation is equal the number of possible molecular combinations. For 200 molecules, there are one million different molecular combinations. While for most applications it either impractical or infeasible to solve the chemical master equation, we can readily generate realizations of the stochastic process described by the chemical master equation. These realizations are usually sufficient to address our questions. If necessary, one can obtain the moments of the chemical master equation using Monte Carlo strategies.

In some cases one can algebraically reduce the chemical master equation as discussed in Secs. IV and V. One can then apply the Gillespie algorithm to the reduced system described by the stoichiometric matrix v^y and propensity functions $b_k(\cdot)$. When algebraic expressions do not exist for $b_k(\cdot)$, which often is the case, then one needs to employ a modified Gillespie algorithm. We assume that one possesses expressions for the conditional probability function $P(z|y)$. The limitation of this approach is that exact expressions for $P(z|y)$ are computationally unwieldy as illustrated in Sec. VI. One may circumvent this problem either by replacing the conditional expectation $b_k(\cdot)$ of the function $a_k(\cdot)$ with the function of the conditional expectation $a_k(E[z|y),y)$ or by approximating the conditional probability $P(z|y)$ as a Gaussian. Both of these alternative are discussed in Sec. VI.

A. Modified Gillespie algorithm

Data: Partitioned stoichiometric matrix $[v^y;v^z]$, the set of propensity functions $a_k(\cdot)$ such that $v_k^y \neq 0$, the stationary conditional probability density $P(z|y)$, and the initial number of primary species $y(0)$.

Initialization: Set $t=0$.

Step 1: Generate the conditional random variable $z(t)$ from the stationary distribution $P(z(t)|y(t))$.

Step 2: Compute reaction probabilities

$$p_k = a_k(y(t),z(t)) \quad \text{for} \quad k=1,...,m.$$

Step 3: Generate two uniformly distributed pseudo-random variables r_1 and r_2 on (0,1). Set

$$\tau = -\frac{\log(r_1)}{\Sigma_{k=0}^{p} p_k}$$

and choose j such that

$$\sum_{k=0}^{j-1} p_k < r_2 \sum_{k=0}^{p} p_k \leqslant \sum_{k=0}^{j} p_k.$$

Step 4: Update the number of species

$$y(t+\tau) = y(t) + v_j^y$$

and let $t \leftarrow t+\tau$. Go to Step 1.

It is important to note that the modified Gillespie algorithm described above does not generate exact realizations of the

master equation, even when the assumptions underlying the QSSA are strictly true as it does not employ the function $b_k(\cdot)$. However, it provides a simple procedure for separating time scales in stochastic models, and our experience indicates the approximation is often valid.

As we have mentioned in the Introduction, Gibson and Bruck[10] have provided streamlined version of the Gillespie algorithm. Their techniques are also applicable when one applies the quasi-steady-state assumption. The interested reader is directed to their article.

IV. EXAMPLE: ENZYME KINETICS AND THE MICHAELIS–MENTEN ASSUMPTION

To illustrate the QSSA, consider the simple enzymatic reaction:

$$\mathrm{E}+\mathrm{S}\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}\mathrm{ES} \tag{12a}$$

$$\mathrm{ES}\xrightarrow{k_2}\mathrm{Product}+\mathrm{E} \tag{12b}$$

where E denotes the enzyme and S denotes the substrate. If we use a deterministic description (mass action) of the chemical dynamics, then we obtain the following set of differential equations

$$\frac{d[\mathrm{S}]}{dt}=-k_1[\mathrm{S}]([\mathrm{E}]_0-[\mathrm{ES}])+k_{-1}[\mathrm{ES}], \tag{13a}$$

$$\frac{d[\mathrm{ES}]}{dt}=-(k_{-1}+k_2)[\mathrm{ES}]+k_1[\mathrm{S}]([\mathrm{E}]_0-[\mathrm{ES}]), \tag{13b}$$

where [X] denotes the concentration of species X and $[\mathrm{E}]_0\triangleq[\mathrm{E}]+[\mathrm{ES}]$). If we use a stochastic description of the chemical dynamics, then the chemical master equation is

$$\begin{aligned}\frac{dP(s,es;t)}{dt}=&-[k_1s(e_0-es)+(k_{-1}+k_2)es]P(s,es;t)\\&+k_1(s+1)(e_0-es+1)P(s+1,es-1;t)\\&+k_{-1}(es+1)P(s-1,es+1;t)\\&+k_2(es+1)P(s,es+1;t),\end{aligned} \tag{14}$$

subject to the appropriate boundary conditions, here s, es, and e_0 are the number of substrate molecules S, number of enzyme complexes ES, and total number of enzymes E, respectively.

Numerous articles have been written on enzyme kinetics using a stochastic framework.[19–24] These articles aimed to solve the chemical master equation for the isolated enzymatic reaction (12) either by obtaining approximate expressions for first two moments (mean and variance) of the chemical master equation, characterizing the equilibrium probability by assuming the reaction (12b) is reversible, or by characterizing the initial velocity phase. Our derivation differs from the above references, because we employ the QSSA and scaling arguments to reduce the dimension of the chemical master equation. We do not attempt to solve the chemical master equation for this isolated equation.

When the concentration of substrate is much larger than the enzyme concentration, one may use the quasi-steady-state approximation for the enzyme-substrate complex ES to derive the rate law for the enzymatic reaction (12). If we assume $[\mathrm{E}]_0/[\mathrm{S}]_0\approx 0$, where $[\mathrm{S}]_0$ denotes the initial or average concentration of S, then $d[\mathrm{ES}]/dt\approx 0$ and one obtains the approximation

$$\frac{d[\mathrm{S}]}{dt}=-\frac{V_{\max}[\mathrm{S}]}{K_m+[\mathrm{S}]},$$

where $V_{\max}=k_2[\mathrm{E}]_0$, and $K_m=(k_{-1}+k_2)/k_1$. The reader is directed to the literature[18,25] for a detailed deviation.

We can equivalently apply the quasi-steady-state approximation to the chemical master equation. Our discussion is limited to the case when there are a fixed number of enzyme molecules. One can tacitly assume that the QSSA is equivalent to assuming that the propensity function for the reaction

$$\mathrm{S}\rightarrow\mathrm{Product}$$

is given by

$$a(s)=\frac{V_{\max}s}{K_m+s},$$

where $V_{\max}=k_2e_0$ and K_m is given above. The resulting chemical master equation is then

$$\frac{dP(s;t)}{dt}=a(s+1)P(s+1;t)-a(s)P(s;t),$$

subject to appropriate boundary conditions. Our goal is to provide a mechanistic derivation.

In order to derive the quasi-steady-state solution to the stochastic formulation, it is convenient to recast the chemical master equation in terms of the total amount of substrate, free and bound. Let $s_T=s+es$ denote the total amount of substrate present. In this example the primary specie y is the substrate s_T and the intermediate species z is the enzyme complex es. We can rewrite the chemical master equation (14) as

$$\begin{aligned}\frac{dP(es,s_T;t)}{dt}=&-[k_1(s_T-es)(e_0-es)+(k_{-1}+k_2)es]\\&\times P(es,s_T;t)+k_1(s_T-es+1)\\&\times(e_0-es+1)P(es-1,s_T;t)\\&+k_{-1}(es+1)P(es+1,s_T;t)+k_2(es+1)\\&\times P(es+1,s_T+1;t)\end{aligned} \tag{15}$$

To obtain a scaling solution, we nondimensionalize the following variables:

$$\bar{s}\triangleq\frac{s_T}{s_0},\quad \bar{e}\triangleq\frac{es}{e_0},\quad \epsilon\triangleq\frac{e_0}{s_0}$$

As the variables are not longer integers, we introduce the incremental variable $d\triangleq 1/e_0$. We also rescale time as $\tau=e_0^2t$. If we make the substitutions in (15), we obtain the master equation

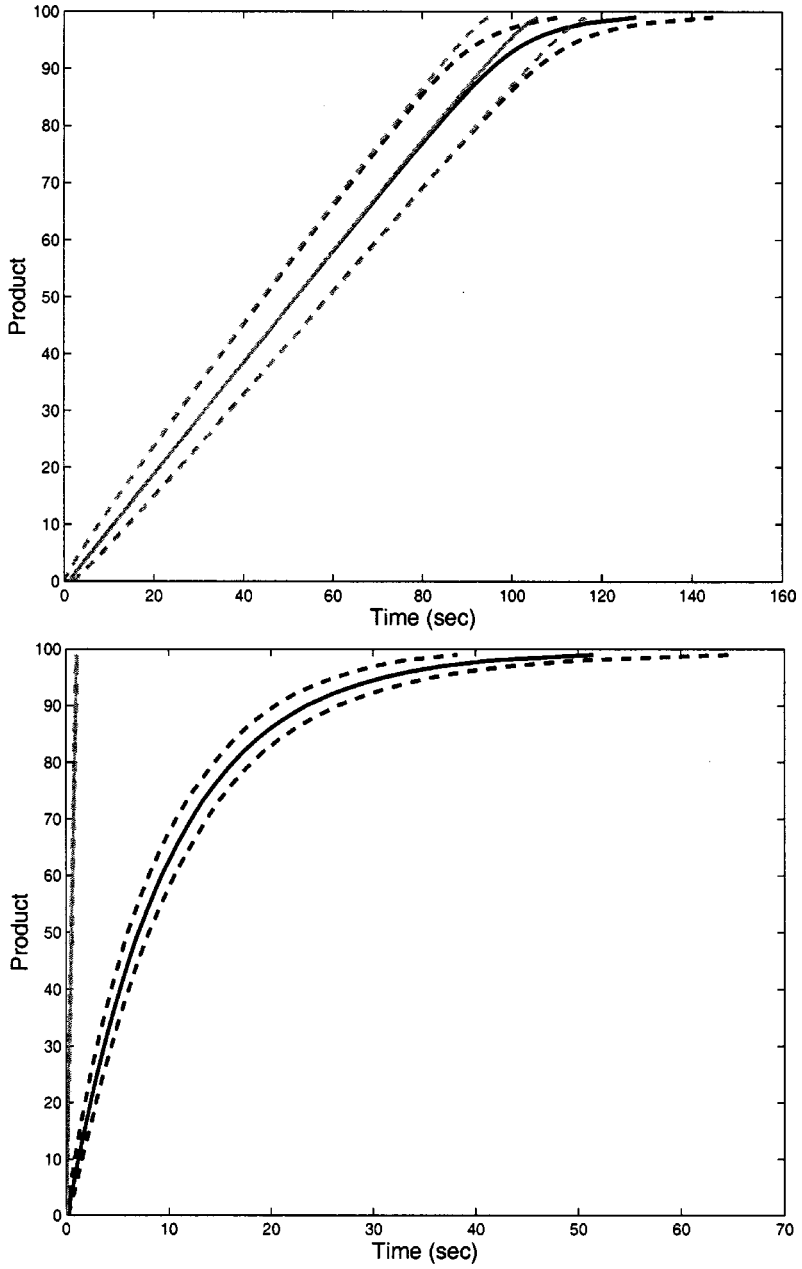

FIG. 1. A comparison of the exact solution and the Michaelis–Menten approximation. Both figures show the production of product as a function of time. The black lines denote the exact solution and the gray lines denote the Michaelis–Menten approximation. The solid line denotes the mean and the dashed lines denote one standard deviation away from the mean. The mean and standard deviation were evaluated from 50 000 realizations of the system. The upper plot shows the results for 10 enzyme and 100 substrate molecules and the lower plot shows the results for 1000 enzyme and 100 substrate molecules. Note that when enzyme is in excess (lower plot), the QSSA predicts the substrate is consumed within 1 s whereas the exact model predicts nearly 50 s. Both examples used the kinetic parameters: $k_1=1$, $k_{-1}=1$, and $k_2=0.1$.

$$\epsilon\frac{dP(\bar{\bar{e}},\bar{\bar{s}};\tau)}{d\tau}=-\left[k_1(\bar{\bar{s}}-\epsilon\bar{\bar{e}})(1-\bar{\bar{e}})+\frac{(k_{-1}+k_2)}{s_0}\bar{\bar{e}}\right]\times P(\bar{\bar{e}},\bar{\bar{s}};\tau)+k_1(\bar{\bar{s}}-\epsilon\bar{\bar{e}}+\epsilon d)(1-\bar{\bar{e}}+d)\times P(\bar{\bar{e}}-d,\bar{\bar{s}};\tau)+\frac{k_{-1}}{s_0}(\bar{\bar{e}}+d)P(\bar{\bar{e}}+d,\bar{\bar{s}};\tau)+\frac{k_2}{s_0}(\bar{\bar{e}}+d)P(\bar{\bar{e}}+d,\bar{\bar{s}}+\epsilon d;\tau). \tag{16}$$

If we set $\epsilon=0$, we obtain the algebraic relation

$$\left[k_1\bar{\bar{s}}(1-\bar{\bar{e}})+\frac{(k_{-1}+k_2)}{s_0}\bar{\bar{e}}\right]P(\bar{\bar{e}},\bar{\bar{s}};\tau)=k_1\bar{\bar{s}}(1-\bar{\bar{e}}+d)P(\bar{\bar{e}}-d,\bar{\bar{s}};\tau)+\frac{(k_{-1}+k_2)}{s_0}(\bar{\bar{e}}+d)P(\bar{\bar{e}}+d,\bar{\bar{s}};\tau). \tag{17}$$

Implicit in the approximation above is the assumption that the probability $P(\bar{\bar{e}},\bar{\bar{s}};\tau)$ is analytic in ϵ. The relation (17) is approximate and $o(\epsilon)$. We suspect that it is possible to derive higher-order approximation, though they likely lead to non-Markovian (or age-dependent) master equations.

As s_T is fixed, we can interpret the relation (17) as the steady-state solution to the following master equation (in unscaled variables):

$$\frac{dP(es,s_T;t)}{dt}=-[k_1s_T(e_0-es)+(k_{-1}+k_2)es]\times P(es|s_T;t)P(s_T;t)+k_1s_T(e_0-es+1)\times P(es-1|s_T;t)P(s_T;t)+(k_{-1}+k_2)(es+1)\times P(es+1|s_T;t)P(s_T;t).$$

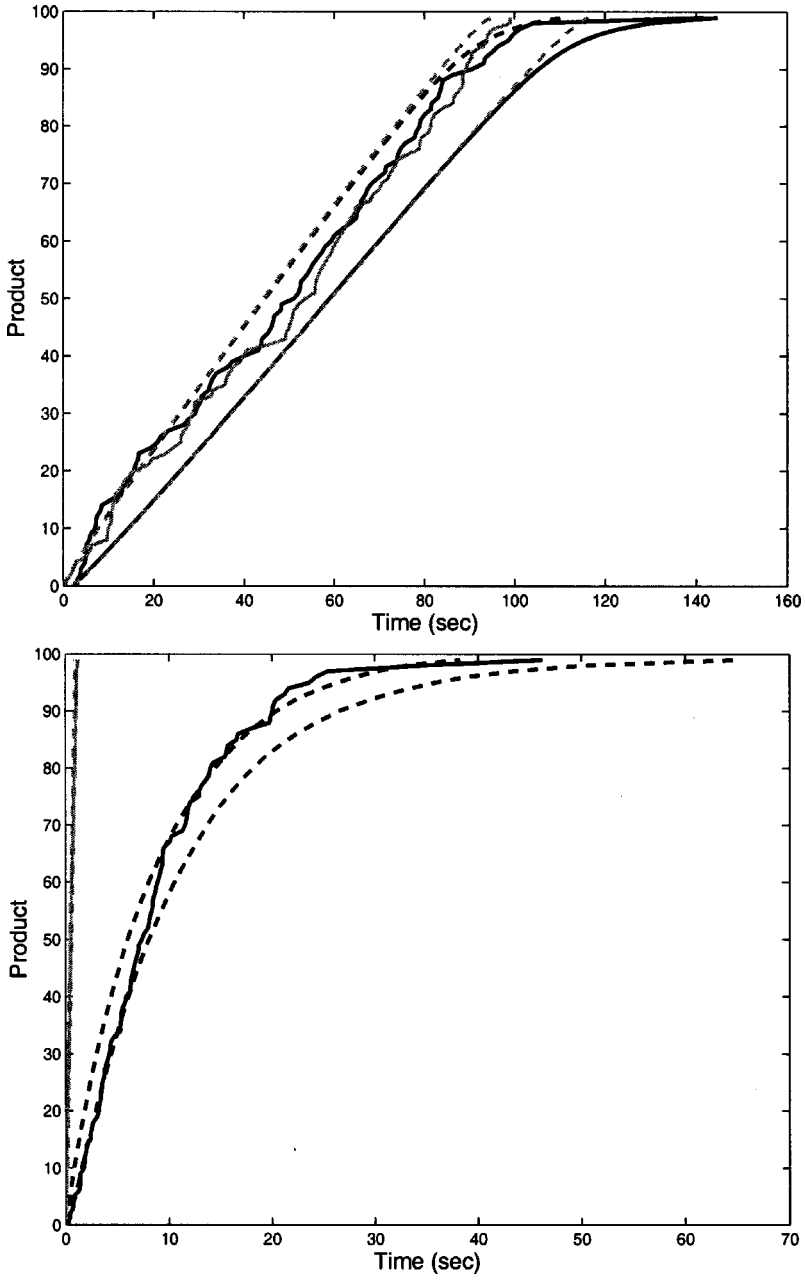

FIG. 2. A comparison of a single realization of the exact solution and the Michaelis–Menten approximation. The black lines denote the exact solution and the gray line denotes the Michaelis Menten approximation. The solid line denotes a single realization and the dashed lines denote one standard deviation away from the mean. The parameters are the same as in Fig. 1. Note again that when enzyme is in excess (lower plot), the QSSA predicts the substrate is consumed within 1 s.

The scaling arguments therefore justify the assumptions that we can write a separate master equation for *es* conditional on s_T and apply to QSSA to *es*. In other words,

$$\frac{dP(es|s_T;t)}{dt} \approx 0$$

and

$$\frac{dP(es,s_T;t)}{dt} \approx P(es|s_T;t)\frac{dP(s_T;t)}{dt}.$$

If we substitute the algebraic relation (17) into the master equation (15), we obtain

$$\begin{aligned}\frac{dP(es,s_T;t)}{dt} &= -k_2(es+1)P(es+1|s_T)P(s_T;t) \\ &\quad + k_2(es+1)P(es+1|s_T+1)P(s_T+1;t) \\ &\quad - k_1 es(e_0-es)P(es,s_T;t) + k_1(es-1) \\ &\quad \times(e_0-es+1)P(es-1,s_T;t). \qquad (18)\end{aligned}$$

If we take the marginal density, then we obtain the following approximation to the chemical master equation

$$\begin{aligned}\frac{dP(s_T;t)}{dt} &= -k_2 E[es|s_T]P(s_T;t) \\ &\quad + k_2 E[es|s_T+1)P(s_T+1;t). \qquad (19)\end{aligned}$$

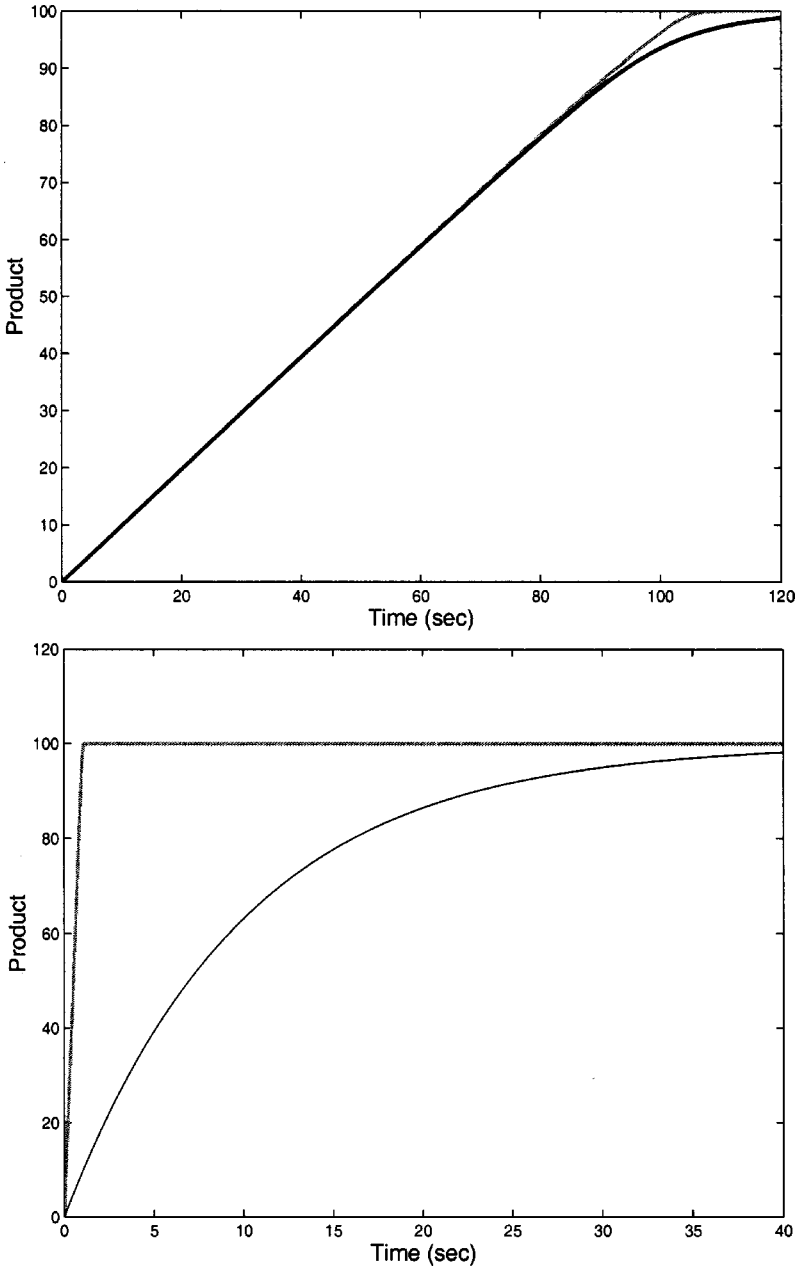

FIG. 3. A comparison of the exact solution and the Michaelis–Menten approximation for the deterministic case. Both figures show the production of product as a function of time. The black lines denote the exact solution and the gray lines denote the Michaelis–Menten approximation. The upper plot shows the results for 10 enzyme and 100 substrate molecules. Both examples used the kinetic parameters: $k_1=1$, $k_{-1}=1$, and $k_2=0.1$. Note again that when enzyme is in excess (lower plot), the QSSA predicts the substrate is consumed within 1 s.

Note that technically summing over the states of *es* in (18) does not define the expectation $E[es|s_T]$ in (19) as we sum over the product $(es+1)P(es+1|s_T)$. However, for this problem, summing over the states in (18) does yield an equivalent expression to the expectation. The conditional expectation is given by the expression

$$E(es|s_T)=\frac{e_0 s_T}{K_m+s_T}.$$

We then obtain an approximate chemical master equation with the Michaelis–Menten form:

$$\frac{dP(s_T t)}{dt}=-\frac{V_{\max}s_T}{K_m+s_T}P(s_T;t)+\frac{V_{\max}(s_T+1)}{K_m+(s_T+1)}\times P(s_T+1;t).$$

A. Numerical comparison

To investigate the accuracy and efficiency of the Michaelis–Menten approximation (and the QSSA), we compared the exact solution to the Michaelis–Menten approximation. The results are shown in Figs. 1 and 2. The upper plot shows the comparison when $e_0/s_0=0.1$ and the lower plot shows the comparison when $e_0/s_0=10.0$. When substrate is in excess of the enzyme, the solution match well as predicted by the theory. However, when the enzyme is in excess of the substrate, the solutions diverge significantly; the Michaelis–Menten approximation greatly overestimates the rate of production. This error is expected as the enzyme is in excess of substrate. The speed of the reaction is no longer limited by how fast the enzymes work, but rather by the rate of association of the substrate to the enzyme. This case clearly illustrates the limits of the Michaelis–Menten

approximation. For comparison, the associated deterministic solution is shown in Fig. 3. Note that the deterministic solutions also diverge when $e_0/s_0=10.0$, thus illustrating the limits of the Michaelis–Menten approximation and the QSSA in deterministic models. For the details of the simulations, the reader is directed to Figs. 1–3.

One of the goals of the QSSA is to reduce the complexity of the system. The best measure of complexity for this example is the number of reactions required to consume all of the substrate. The efficiency of the Michaelis–Menten assumption is realized by assuming that the enzyme-substrate complex is in quasi-steady state. As expected, the Michaelis–Menten approximation requires fewer reactions to consume the substrate: the number of reactions equals the number of substrate molecules (100). The number of the reactions for the exact solution was a function of the kinetic parameters and number of enzyme molecules. In the scenarios we investigated, approximately 2100 reactions were required to consume the substrate in both cases: a 95% reduction in computation. As the parameter k_2 decreased in magnitude relative to k_1 and k_{-1}, the number of reactions increased. And, vice versa, increasing k_2 decreased the number of reactions.

V. EXAMPLE: COMPETITIVE INHIBITION

As a further example of the QSSA, we considered the simple enzymatic reaction with competitive inhibition:

$$\mathrm{E}+\mathrm{S}\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}\mathrm{ES}, \tag{20a}$$

$$\mathrm{E}+\mathrm{I}\xrightarrow{k_2}\mathrm{Product}+\mathrm{E}, \tag{20b}$$

$$\mathrm{E}+\mathrm{I}\underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}}\mathrm{EI}. \tag{20c}$$

If we use a deterministic description (mass action) of the chemical kinetics, then we obtain the following set of differential equations:

$$\frac{d[\mathrm{S}]}{dt}=-k_1[\mathrm{S}]([\mathrm{E}]_0-[\mathrm{ES}]-[\mathrm{EI}])-k_{-1}[\mathrm{ES}], \tag{21a}$$

$$\frac{d[\mathrm{ES}]}{dt}=k_1[\mathrm{S}]([\mathrm{E}]_0-[\mathrm{ES}]-[\mathrm{EI}])-(k_{-1}+k_3)[\mathrm{ES}], \tag{21b}$$

$$\frac{d[\mathrm{EI}]}{dt}=-k_3[\mathrm{I}]([\mathrm{E}]_0-[\mathrm{ES}]-[\mathrm{EI}])-k_{-3}[\mathrm{EI}], \tag{21c}$$

If we assume $[\mathrm{E}]_0/[\mathrm{S}]_0\approx 0$ and $[\mathrm{E}]_0/[\mathrm{I}]_0\approx 0$ where $[\mathrm{I}]_0$ denotes the initial or average concentration of I, then we obtain the approximation

$$\frac{d[\mathrm{S}]}{dt}=\frac{v_{\max}[\mathrm{S}]}{K_m+[\mathrm{S}]+K_m[\mathrm{I}]/K_b}$$

where $K_b=k_{-3}/k_3$.[26]

If we use a stochastic description, then we obtain the following chemical master equation:

$$\begin{aligned}\frac{dP(es,ei,s_T,i_T;t)}{dt}=&-[(k_1(s_T-es)+k_3(i_T-ei))(e_0-es-ei)+(k_{-1}+k_2)es+k_{-3}ei]P(es,ei,s_T,i_T;t)\\&+k_1(s_T-es+1)(e_0-es-ei+1)P(es-1,ei,s_T,i_T;t)+k_{-1}(es+1)P(es+1,ei,s_T,i_T;t)\\&+k_3(i_T-ei+1)(e_0-es-ei+1)P(es,ei-1,s_T,i_T;t)+k_{-3}(ei-1)P(es,ei-1,s_T,i_T;t)\\&\times k_2(es+1)P(es+1,ei,s_T+1,i_T;t),\end{aligned} \tag{22}$$

subject to the appropriate boundary conditions. In this example, the primary species are the total substrate s_T and inhibitor i_T, and the intermediate species are the enzyme complexes *es* and *ei*. Proceeding in the same manner as before, we nondimensionalize the following variables

$$\bar{s}=\frac{s_T}{s_0},\quad \bar{i}=\frac{i_T}{i_0},\quad \bar{x}=\frac{es}{s_T},\quad \bar{y}=\frac{ei}{i_0},\quad \epsilon\triangleq\frac{e_0}{s_T},\quad \eta\triangleq\frac{e_0}{i_T},$$

and consider the transformation $d\triangleq 1/e_0$ and $\tau=e_0^3t$. If we make the substitutions in (22), we obtain the master equation

$$\begin{aligned}\epsilon\eta\frac{dP(\bar{x},\bar{y},\bar{s},\bar{i};\tau)}{d\tau}=&-\left[\left(\frac{k_1}{i_0}(\bar{s}-\epsilon\bar{x})+\frac{k_3}{s_0}(\bar{i}-\eta\bar{y})\right)(1-\bar{x}-\bar{y})\right.\\&\left.+\frac{(k_{-1}+k_2)}{i_0s_0}\bar{x}+\frac{k_{-3}}{s_0i_0}\bar{y}\right]P(\bar{x},\bar{y},\bar{s},\bar{i};\tau)+\frac{k_1}{i_0}(\bar{s}-\epsilon\bar{x}+\epsilon d)(1-\bar{x}-\bar{y}+d)P(\bar{x}-d,\bar{y},\bar{s},\bar{i};\tau)\\&+\frac{k_{-1}}{s_0i_0}(\bar{x}+d)P(\bar{x}+d,\bar{y},\bar{s},\bar{i};\tau)+\frac{k_3}{s_0}(\bar{i}-\eta\bar{y}+\eta d)(1-\bar{x}-\bar{y}+d)P(\bar{x},\bar{y}-d,\bar{s},\bar{i};\tau)\\&+\frac{k_{-3}}{s_0i_0}(\bar{y}-d)P(\bar{x},\bar{y}-d,\bar{s},\bar{i};\tau)+\frac{k_2}{s_0i_0}(\bar{x}+d)P(\bar{x}+d,\bar{y},\bar{s}+\epsilon d,\bar{i};\tau).\end{aligned} \tag{23}$$

If we set $\epsilon=0$ and $\eta=0$, then we obtain the equality

$$\left[\left(\frac{k_1}{i_0}\overline{s}+\frac{k_3}{s_0}\overline{i}\right)(1-\overline{x}-\overline{y})+\frac{(k_{-1}+k_2)}{i_0 s_0}\overline{x}+\frac{k_{-3}}{s_0 i_0}\overline{y}\right]P(\overline{x,y,s,i};\tau)$$
$$=\frac{k_1}{i_0}\overline{s}(1-\overline{x}-\overline{y}+d)P(\overline{x}+d,\overline{y,s,i};\tau)+\frac{k_{-1}}{s_0 i_0}(\overline{x}+d)P(\overline{x}+d,\overline{y,s,i};\tau)$$
$$+\frac{k_3}{s_0}\overline{i}(1-\overline{x}-\overline{y}+d)P(\overline{x,y}-d,\overline{s,i};\tau)+\frac{k_{-3}}{s_0 i_0}(\overline{y}-d)P(\overline{x}+d,\overline{y}-d,\overline{s,i};\tau)+\frac{k_2}{s_0 i_0}(\overline{x}+d)p(\overline{x}+d,\overline{y,s,i};\tau).$$

We can interpret the above equality as the steady-state solution to the conditional chemical master equation for the condition probability density $P(es,ei|s_T,i_T;t)$.

In a similar manner as before, if we substitute the above algebraic relation into the master equation (22) and take the marginal density, then we obtain the following approximation to the chemical master equation:

$$\frac{dP(s_T,i_T;t)}{dt}=-k_2E[es,ei|s_T,i_T]P(s_T,i_T;t)$$
$$+k_2E[es,ei|s_T+1,i_T]P(s_T+1,i_T;t).$$

As i_T is fixed, we can factor out the term $P(i_T;t)$. In application, $P(i_T;t)$ is a function of other reactions in the network. If we evaluate the expectations, we obtain the approximate chemical master equation in the familiar Michaelis–Menten form:

$$\frac{dP(s_T|i_T;t)}{dt}=-\frac{V_{\max}s_T}{k_m^{\text{app}}+s_T}P(s_T|i_T;t)$$
$$+\frac{V_{\max}(s_T+1)}{K_m^{\text{app}}+(s_T+1)}P(s_T+1|i_T;t),$$

where

$$K_m^{\text{app}}=K_m(1+i_T/K_b).$$

The goal in the preceding two sections was to demonstrate that Michaelis–Menten-type rate laws may be derived in a stochastic formulation using scaling arguments and the quasi-steady-state assumption. Likewise, we expect one may derive many common biological rate laws for stochastic kinetics such as the Hill equation and the Monod–Wyman–Changeux and Koshalnd–Nemethy–Filmer models of allostery and cooperativity.[26] One may also use similar arguments to derive stochastic analogs to the Langmuir–Hinselwood and Hougen–Watson rate laws in heterogeneous catalysis.[27]

VI. EXAMPLE: GENE EXPRESSION

To illustrate the quasi-steady-state assumption in conjunction with the Gillespie algorithm, we examined a simplified model of the P_R promoter in bacteriophage λ. The P_R promoter is an integral component of the genetic circuit controlling the lysis/lysogeny decision in the Lambda infection lifecycle in the Escherichia coli.[28] A stochastic model of the genetic circuit controlling the lysis/lysogeny decision was proposed by Arkin and co-workers;[29] the reader is directed to their article for the details of the model. We focus solely on the P_R promoter in conjunction with the protein Cro to illustrate the application and validity of the QSSA. A diagram and brief description of the P_R promoter and the protein Cro are given in Fig. 4. The reactions and their associated parameters are given in Table I.

The first assumption typically made when modeling gene expression is to use the QSSA to obtain an expression for the promoter activity. Shea and Ackers[30] proposed the following deterministic model for activity of the P_R promoter:

$$P(\text{RNAP}_c-\text{DNA})=\frac{[\text{RNAP}]\exp^{-\Delta G_5/RT}}{1+2[\text{Cro}]\exp^{-\Delta G_{2,3}/RT}+[\text{Cro}_2]^2\exp^{-\Delta G_4/RT}+[\text{RNAP}]\exp^{-\Delta G_5/RT}},$$

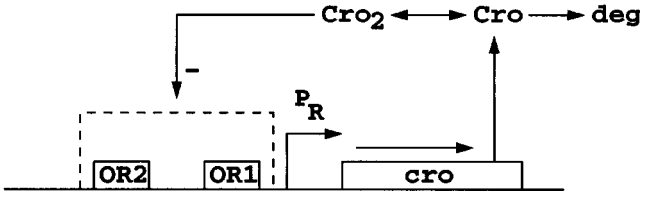

FIG. 4. The P_R promoter: The promoter P_R controls the expression of the 201 nucleotide (nt) gene *cro*. The protein Cro dimerizes and is subject to proteolytic degradation. The cro dimer binds to one of two operator sties O_R1 and O_R2 and inhibits transcription by occluding the P_R promoter.

where $[\cdot]$ denotes the concentration. Activity is defined here as the probability that the RNA polymerase (RNAP) is bound to the promoter. In this model, Shea and Ackers applied the QSSA to the closed RNAP-DNA complex (RNAP_c-DNA) and the Cro dimer/operator complex (Cro_2-O_R and Cro_2-O_R1). The parameters for the model are given in Table II. The Shea–Ackers promoter model is equivalent in many ways to the Langmuir–Hinselwood and Hougen–Watson rate equations in heterogeneous catalysis.[27]

In the context of stochastic kinetics, we can view the Shea–Ackers promotor model as a special case of the Michaelis–Mentenkinetics with competitive inhibition. In

TABLE I. Parameters for transcription, translation, and housekeeping reactions.

No.	Reaction	k_f	k_b
1	$\mathrm{Cro}\rightarrow(\ )$	0.0025 s^{-1}	
2	$\mathrm{Cro}\leftrightarrow\mathrm{Cro}_2$	0.05 $M^{-1}\,s^{-1}$	0.5 s^{-1}
3	$\mathrm{Cro}_2+O_R1\leftrightarrow\mathrm{Cro}_2-O_R1$	(Table II)	
4	$\mathrm{Cro}_2+O_R2\leftrightarrow\mathrm{Cro}_2-O_R2$	(Table II)	
5	$\mathrm{RNAP}+\mathrm{DNA}\leftrightarrow\mathrm{RNAP}_c-\mathrm{DNA}$	(Table II)	
6	$\mathrm{RNAP}_c-\mathrm{DNA}\rightarrow\mathrm{RNAP}-\mathrm{DNA}_0$	0.014 s^{-1}	
7	$\mathrm{RNAP}-\mathrm{DNA}_n\rightarrow\mathrm{RNAP}-\mathrm{DNA}_{n+1}$	30 nt s^{-1}	
8	$\mathrm{Ribosome}+\mathrm{RNA}_{\mathrm{RBS}}\rightarrow\mathrm{Ribosome}-\mathrm{RNA}_{\mathrm{RBS}}$	0.002 $M^{-1}\,s^{-1}$	
9	$\mathrm{Ribosome}-\mathrm{RNA}_n\rightarrow\mathrm{Ribosom\text{-}RNA}_{n+1}$	100 nt s^{-1}	
10	$\mathrm{RNA}_{\mathrm{RBS}}\rightarrow()$	0.2 s^{-1}	

this model, there is a single enzyme and two sites for inhibition (O_R1 and O_R2), where RNAP is the substrate and the Cro dimer is the inhibitor. The Shea–Ackers promotor model, therefore, is valid to a first approximation when the number of RNAP and Crop dimer molecules are in excess of one. From a modeling standpoint, only equilibrium data is available for the P_R promotor. As the binding and dissociation rates are difficult to determine, the QSSA is a convenient simplification. In this example, the QSSA and the associated scaling arguments validate the approximation even though the information necessary for a more detailed model is unavailable.

One can simplify the model of the P_R promotor further by applying the QSSA and the Cro dimer. In their deterministic model of the P_R promoter, Shea and Ackers applied the QSSA to the dimer. Consider the dimerization reaction

$$A+A\underset{k_B}{\overset{k_f}{\rightleftharpoons}}A_2.$$

If we assume there are a total of N molecules ($N=A+2A_2$), the stationary distribution is given by

$$P(A_2=j|N)\propto\frac{k_f^j k_b^{(N/2-j)}N!}{(N-2j)!j!2^j}, \tag{24}$$

when N is even, and

$$P(A_2=j|N)\propto\frac{k_f^j k_b^{(N/2-j-1)}N!}{(N-2j)!j!2^j}, \tag{25}$$

when N is odd. Generating random variables from this distribution is relatively difficult as one needs to recalculate the probabilities each time the total amount of Cro changes. One alternative is to use the conditional expectation of Cro_2 in the model of P_R promoter activity. Here, one approximates the mean promoter activity [e.g., $b_k(\cdot)$] with the activity for the mean Cro_2 concentration. Even then, we still do not possess an algebraic expression for the mean Cro_2 concentration. We can instead use the deterministic equilibrium value

$$\mathrm{Cro}_2^d=\frac{(4\mathrm{Cro}_{\mathrm{tot}}+K_D)-\sqrt{(4\mathrm{Cro}_{\mathrm{tot}}+K_D)^2-\mathrm{Cro}_{4\mathrm{tot}}}}{16}$$

as an approximation for the mean, where $\mathrm{Cro}_{\mathrm{tot}}$ denotes the total amount of the protein Cro. As the amount of Cro increases, we expect that the conditional expectation converges to the deterministic equilibrium value almost surely. A second alternative is to approximate the stationary distribution (24) and (25) with a Gaussian distribution. We do not possess specific asymptotic results concerning the stationary distributions (24) and (25), though one would assume that the distribution is approximately Gaussian with mean Cro_2^d and a variance that is inversely proportional to total amount of Cro. Numerical results indicate that a Gaussian approximates the stationary distributions (24) and (25) reasonably well when the variance is $1/4Cro_{tot}$. A similar result was reported by Kepler and Elston.[31]

Figure 5 shows the time course of the mean Cro dimer concentration using the modified Gillespie algorithm. The exact solution required an average of 152 000 reactions; the approximate solution applying the QSSA to the Cro dimer using the two methods described above required an average of 58 600 and 79 700 reactions, respectively: at least a 50% reduction in computation. In both examples, we used the modified Gillespie algorithm for the Shea–Ackers model, rather than marginalizing the distribution as we did in the Michaelis–Menten examples. If we increase the rate of Cro dimerization (0.5 $M^{-1}\,s^{-1}$) and dissociation (5 s^{-1}) by a factor of 10, then the average number of reactions increases to 397 600 while the number of reactions for the QSSA model does not change. We note that these kinetic rates are unknown and only the equilibrium dissociation constant K_D is known. As evident from Fig. 5, the application of the QSSA to the Cro dimer does not affect the accuracy of the solution. This result was expected as over half the reactions involved either Cro dimerization or dissociation.

If we increase the rate of Cro dimerization (0.0005 $M^{-1}\,s^{-1}$) and dissociation (0.005 s^{-1}) by a factor of 100, then the QSSA is no longer valid as illustrated in Fig. 6. Because the rate of association and dissociation are not fast relative to the dynamics of gene expression, protein synthesis, and degradation, the Cro dimerization reaction never

TABLE II. Parameter for the Shea–Ackers model of the promoter P_R.

No.	O_R2	O_R1	$-\Delta G/RT$
1	⋯	⋯	0
2	⋯	Cro	17.5
3	Cro	⋯	17.5
4	Cro	Cro	35.1
5	RNAP		20.3

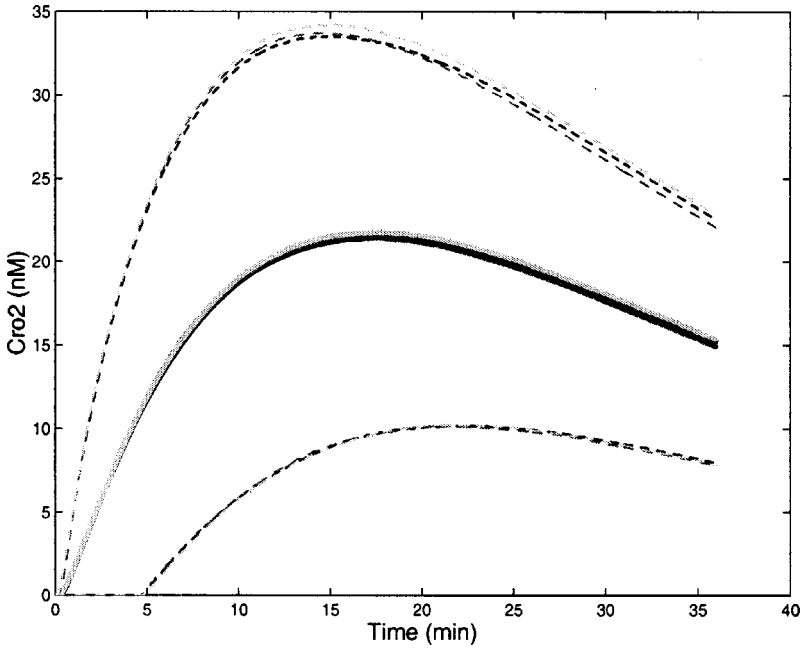

FIG. 5. The time course of Cro dimer concentration using the modified Gillespie algorithm for an average cell lifecycle. The solid lines show mean concentration of the Cro dimer and the dashed lines show one standard deviation away from the mean concentration. The mean and standard deviation were evaluated using 50 000 realizations of the stochastic model. The black lines denote the exact solution, while dark and light gray lines denote the solution when the QSSA is applied to the cro dimer. The dark gray lines show the solution of the deterministic equilibrium value for the Cro dimer Cro_2^d and the light gray lines show the solution when stationary distribution is taken as Gaussian with mean Cro_2^d and variance $1/4\mathrm{Cro}_{\mathrm{tot}}$. All three models employed the Shea–Ackers model.

reaches its steady-state value. The QSSA, consequently, overestimates the amount of Cro dimer. The example again demonstrates the limits of the QSSA.

VII. CONCLUSION

The QSSA is a powerful tool for simplifying the reaction kinetics, and it has been successfully applied to numerous problems in deterministic kinetics. We have demonstrated how the QSSA may be applied to stochastic kinetics. Our experience to date suggests that the conditions for the QSSA in stochastic kinetics are the same as for deterministic kinetics. We expect exceptions, though it is not clear whether these will be contrived. We emphasize that the same limitations of the QSSA in the deterministic case also hold in the stochastic framework.[32] In the Michaelis–Menten example, we assumed that the reactions occur in isolation and that the amount of enzyme is fixed. In most biological systems, these assumptions are violated. Most reactions occur in highly integrated networks. The amount of enzyme is not fixed; rather, the enzyme concentration is controlled by a number of regulatory and environmental factors. Furthermore, the enzyme is inevitable subject to degradation, due to, for example, proteolysis. However, on the time scales of interest, these issues are rarely of concern.

We have illustrated through example how the QSSA can significantly reduce the computationally complexity. While this speedup makes the QSSA important in its own right, the true strength of the QSSA, we believe, is as a tool for model

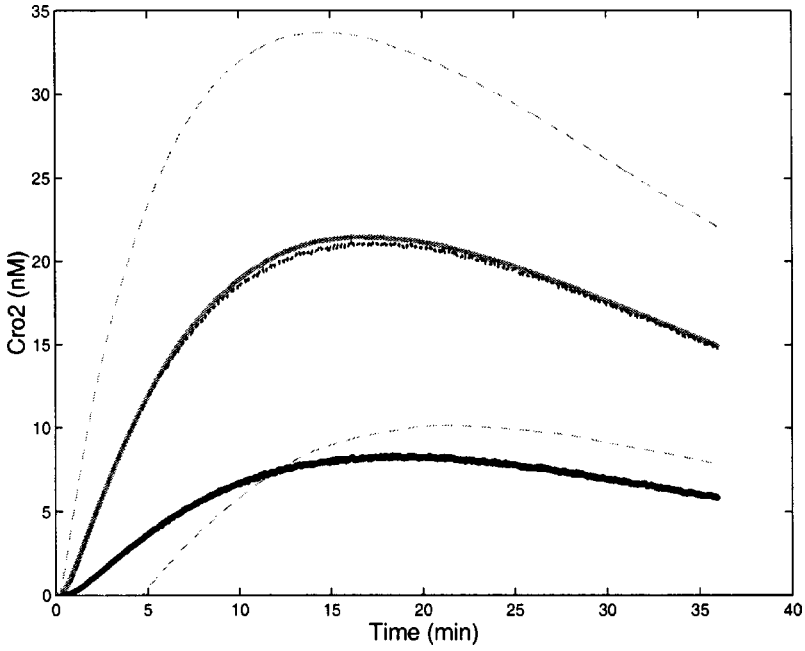

FIG. 6. The time course of Cro dimer concentration using the modified Gillespie algorithm for an average cell lifecycle when the rate Cro dimerization is 0.0005 $\mathrm{M}^{-1}\,\mathrm{s}^{-1}$ and dissociation is 0.005 s^{-1}. The solid lines show mean concentration of the Cro dimer and the dashed lines show one standard deviation away from the mean concentration. The mean and standard deviation were evaluated using 50 000 realizations of the stochastic model. The black lines denote the exact solution, while gray lines denote the solution when the QSSA is applied to the Cro dimer. One standard deviation less the mean of the exact solution is not shown as it is less than zero.

reduction. More often than not, we do not possess the information necessary to generate detailed kinetic models. For example, in enzyme kinetics, only the parameters k_2 and K_m are readily available from experiments. These constraints more often that not provide the rational for choosing a particular rate law. In this context, the QSSA provides a measure of whether these constraints actually limit the range and validity of the model. For example, the strength of the Shea–Ackers model is that it only requires equilibrium data. As we demonstrate using the QSSA, the use of the equilibrium data is often a valid modeling approximation.

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