Stochastic simulation of enzymatic reactions under transcriptional feedback regulation

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***Abstract*— The interaction between gene expression and metabolism is a form of feedback control that allows cells to up- or downregulate specific reactions according to the environmental conditions. Although gene expression is an inherently stochastic process, the effect of genetic feedback on the propagation of noise to the metabolic layer remains largely unexplored. These systems operate in two timescales, and a major challenge is to devise stochastic simulation techniques that can cope with this stiffness in reasonable computational time. We propose a simulation technique, based on the slow-scale Stochastic Simulation Algorithm, to rapidly compute realizations of the Markov process associated to an enzymatic reaction under genetic feedback regulation. We show that in the case of constant substrate, the enzyme-substrate complexes have a binomial stationary distribution. With this result we can avoid the explicit simulation of the binding/dissociation of the enzyme and substrate, leading to a significant improvement in computational speed. We discuss the extension of the algorithm to networks of enzymatic reactions. The proposed method can be used to systematically compute the stationary distributions of the species for different combinations of model parameters, thus opening the way for the identification of the cellular processes that can modulate the amplification or attenuation of genetic noise in enzymatic reactions.**

I. Introduction

Metabolism and gene expression are key players in enabling the self-regulation of living cells. Although metabolism has been traditionally seen as a static chemical workhorse of cells [1], recent studies have uncovered how dynamic behaviours can emerge from the interplay between gene expression and metabolic activity [2], and how this interaction is key to cellular functions [3], [4], [5].

The metabolites needed for cellular functions are synthesized through cascades of biochemical reactions catalyzed by specific enzymes. The catalytic enzymes are produced by the gene expression machinery, which in turn can be modulated by some of the metabolites. This interaction leads to a feedback interconnection between a metabolic pathway and its associated gene regulatory network (see Fig. 1a). This type of feedback systems appears in natural systems such as the tryptophan operon [6], [7] (see Fig. 1b) and amino acid biosynthesis [8] in the *E. coli* bacterium. Genetic feedback circuits have also been designed to control the dynamics of metabolic pathways [9], [10].

Biochemical reactions depend on specific binding events between molecules, and therefore they are inherently stochastic. Studies on metabolism usually neglect the effect of stochasticity on the dynamics of the metabolites. This simplification is based on the fact that metabolites are typically present in the order of thousands of molecules per cell, and therefore the high molecule count will generally average out any stochastic effects. In the case of genetic circuits, however, transcriptional initiation depends on molecules (such as transcription factors, or σ factors) that may appear in less than 10 molecules per cell [11], [12], [13].

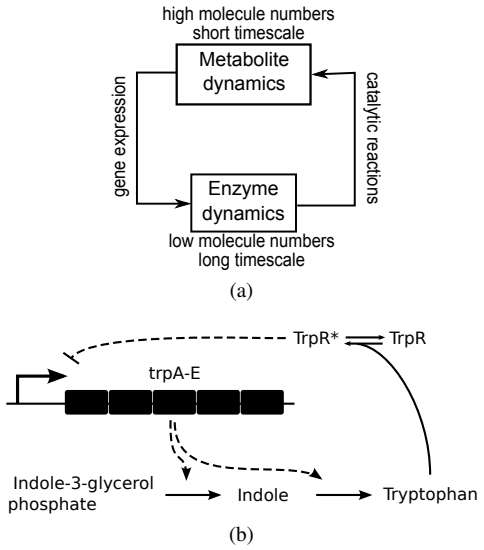

Fig. 1: Metabolic networks under transcriptional regulation. **(a)** Feedback interconnection between a metabolic network (i.e. the "plant") and enzyme expression dynamics (i.e. the controller). **(b)** Schematic of the tryptophan operon [6], [7] in *E. coli*, whereby tryptophan interacts with a transcription factor (TrpR), repressing the expression of the enzyme responsible for synthesis of tryptophan.

In genetic-metabolic systems, noisy enzyme concentrations drive the dynamics of the metabolites, which in turn are back-fed to control enzyme expression. From classic feedback control theory, we know that feedback systems may amplify or reduce noise depending on the feedback architecture and gain. It is therefore unclear whether the feedback interconnection between gene expression and metabolism amplifies or reduces the noise levels. This calls for a rigorous analysis of the propagation of noise in genetic-metabolic networks. Our goal in this paper is to present a statistically accurate simulation framework for genetic-metabolic networks. The algorithm runs in reasonable computational time, and thus allows to systematically explore the effect of noise propagation between the genetic and metabolic layers.

In a typical enzymatic reaction, the enzyme e reversibly

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binds the substrate s to form a complex c, which in turn is converted into a product p, thereby freeing the enzyme:

$$e + s \underset{2}{\overset{1}{\rightleftharpoons}} c \xrightarrow{3} p + e \tag{1}$$

The classic tool for simulating stochastic chemical systems is known as the Stochastic Simulation Algorithm (SSA) [14]. The SSA is exact and requires the simulation of every reaction event. However, in enzymatic reactions the binding and unbinding events (numbered 1 and 2 in (1)) are orders of magnitude more likely to occur than the product formation event (numbered 3 in (1)). As a consequence, the SSA requires the computation of thousands of binding/unbinding events per each product formation event, leading to impractically long simulation times.

The slow-scale Stochastic Simulation Algorithm (ss-SSA) was proposed as a solution to this stiffness problem [15]. The algorithm is based on a form of timescale separation, increasing the computational speed while obtaining statistically accurate realizations for the enzymes and metabolites. In this paper we use an extension of the ss-SSA that accounts for feedback regulation of the enzyme birth process. We first describe a stochastic model for a single metabolic reaction under transcriptional repression (Section II). We then show how the ss-SSA algorithm proposed in [15] can be adapted to include transcriptional regulation, and compare its performance with the exact SSA and deterministic simulations (Section III). We illustrate our approach by quantifying the noise levels in a metabolic reaction as a function of the repression strength (Section IV). We conclude with a generalized version of the algorithm that can include unbranched pathways of arbitrary length (Section V) and finally give directions of future research (Section VI).

II. Stochastic model for a metabolic reaction under transcriptional repression

We consider an enzymatic reaction subject to transcriptional repression from the product, Fig. 2a. The system consists of six reactions, Fig. 2b, and we denote as e_1, c_1 and s_2 the number of molecules of enzyme, substrate-enzyme complex and product, respectively. We assume that the number of substrate molecules, s_1^0, is constant. This assumption accounts for scenarios in which the substrate is an extracellular nutrient pool consumed by a low density cell population. Note that by this assumption we exclude the case in which the metabolic reaction eventually depletes the substrate (thus reaching a nil equilibrium).

The binding and dissociation of the enzyme and substrate is represented by reactions 1 and 2 in Fig. 2b, whereas the product formation is reaction 3. Each molecule of complex is transformed into one s_2 product molecule and one e_1 enzyme molecule through reaction 3. Reactions 4 and 5 represent the birth and death of enzyme molecules, and reaction 6 describes the natural degradation and/or consumption of product by the remaining cellular processes.

In stochastic chemical kinetics, reactions are described in terms of their propensities. A reaction has a propensity a if

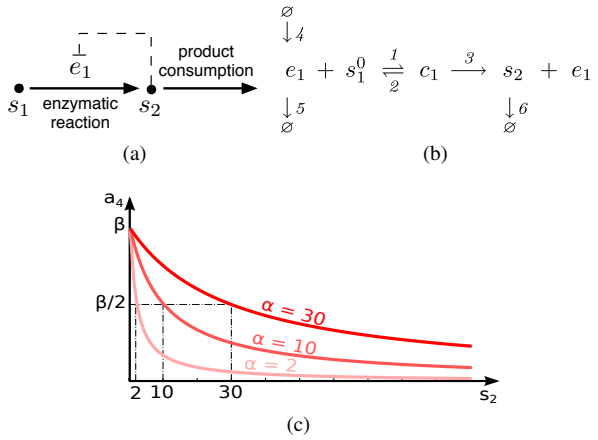

Fig. 2: **(a)** Schematic of a single enzymatic reaction under transcriptional repression from the product. **(b)** Corresponding reaction network. **(c)** Propensity function of reaction 4 (enzyme birth) for various values of α.

the probability that it occurs in the next infinitesimal time dt is $a \times dt$. For the system in Fig. 2b, if we define $\underline{S} = (e_1, c_1, s_2)$ as the vector of species counts, the propensities $a_i(\underline{S})$ are:

TABLE I: propensity functions of the system in Fig. 2b

$$a_1 = m_1 e_1 s_1^0, \qquad a_2 = m_2 c_1,$$
$$a_3 = m_3 c_1, \qquad a_4 = \frac{\beta}{1 + (s_2/\alpha)},$$
$$a_5 = m_5 e_1, \qquad a_6 = m_6 s_2.$$

The propensity a_4 represents the feedback effect of the product on the production of enzyme molecules. Transcriptional regulation depends on the interaction between the product and regulatory molecules (such as transcription factors), but for simplicity we have opted for a propensity a_4 that is a lumped description of the regulatory effect. We thus model the feedback repression by a decreasing Michaelis-Menten function. The feedback interaction does not consume product molecules. In the definition of a_4, the parameter β is the maximal enzyme birth propensity, whereas α is the number of product molecules required to reduce the birth propensity by 50% (i.e. the repression threshold), see Fig. 2c.

Assuming that the molecular binding events are Markovian, the probability density function of the species molecule numbers, $P(\underline{S})$, satisfies the Chemical Master Equation (CME)

$$\frac{\partial P(\underline{S},t)}{\partial t} = \sum_{i=1}^{6} a_i(\underline{S} - R_i) P(\underline{S} - R_i, t) - \sum_{i=1}^{6} a_i(\underline{S}) P(\underline{S}, t), \tag{2}$$

where R_i is the i^{th} column of the reaction matrix

$$R = \begin{bmatrix} -1 & 1 & 1 & 1 & -1 & 0 \\ 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & -1 \end{bmatrix}$$

The CME is a deterministic differential equation for the time evolution of the probability density function $P(\underline{S})$. To

quantify the levels of noise, we focus on the properties of the stationary distribution, i.e. the time-independent distribution $P(\underline{S})$ such that $\partial P/\partial t = 0$ in the CME. For a given distribution with mean μ and variance σ^2, the molecular noise is typically defined as the coefficient of variation $\eta = \sigma/\mu$ or the Fano factor $F = \sigma^2/\mu$ (note that neither of these quantities are good proxies for the the noise intensity when species display heavily skewed or multimodal stationary distributions). From the CME, an analytic solution for $P(\underline{S})$ can be obtained only in special cases, see e.g. [16]. An numerical alternative is to approximate $P(\underline{S})$ by computing histograms of sufficiently long sample paths of the associated Markov process. The sample paths can be computed with the Stochastic Simulation Algorithm (SSA) [14], which provides statistically exact realizations of the stochastic process.

A key difficulty in using the SSA for metabolic systems under genetic feedback is that catalytic reactions are numerically stiff. The binding and dissociation processes in enzyme kinetics (reactions 1 and 2 in Fig. 2b) are much more probable events than the conversion of the substrate-enzyme complex into product (reaction 3 in Fig. 2b). This leads to impractically long simulation times, as the SSA requires the simulation of every reaction event and this typically involves thousands of binding and dissociation events per each product-forming event. Moreover, this numerical stiffness is aggravated when one also considers the production of enzyme molecules by gene expression, as this occurs on a much slower timescale than the enzymatic reactions. In the next section we show how this obstacle can be overcome using an adapted version of the slow-scale SSA introduced in [15].

For further reference, the deterministic model for the system in Fig. 2b is

$$\frac{d[e_1]}{dt} = \frac{\beta_c}{1+([s_2]/\alpha_c)} + (m_2+m_3)[c_1] \tag{3}$$

$$- (m_5 + m_{1c}[s_1]^0)[e_1], \tag{4}$$

$$\frac{d[c_1]}{dt} = m_{1c}[s_1]^0[e_1] - (m_2+m_3)[c_1], \tag{5}$$

$$\frac{d[s_2]}{dt} = m_3[c_1] - m_6[s_2], \tag{6}$$

where $[x] = x/(N_\mathrm{a}V)$ is the concentration of species x, N_a is the Avogadro constant, and V is the cell volume ($10^{-15}L$). Note that some of the parameters differ from those found in the propensity functions a_{1-6} in Table I. $\beta_c = \beta/(N_\mathrm{a}V), \alpha_c = \alpha/(N_\mathrm{a}V)$ and $m_{1c} = m_1 N_\mathrm{a} V$ need to be scaled for the deterministic model; the remaining ones are unchanged.

III. Stochastic simulation algorithm for stiff biochemical systems

We base our approach on the slow-scale Stochastic Simulation Algorithm (ss-SSA), which was proposed as a means to accelerate the simulation of stiff reactions [15]. The ss-SSA exploits a separation between fast and slow reactions to avoid the simulation of the former ones.

The basic principle behind the ss-SSA is to assume that the fast processes reach a stable stationary state in a much shorter timespan than the expected time between two consecutive slow reactions. If this assumption holds, then instead of simulating the individual fast reactions, the algorithm sets the molecule counts for the species reacting in fast reactions as the mean of their stationary distribution (for a detailed description of the algorithm, see [15]). The validity of the assumption can be numerically checked by comparing the fluctuation time [17] of the fast processes with the expected time to the next slow reaction (which can be deduced from the propensities of the slow reactions). The original formulation of the ss-SSA considered a constant enzyme pool as in (1) and it was shown in [15] that the stationary distribution of the complex, $P_s(c_1)$, can be approximated as a Gaussian. The ss-SSA for a reaction with constant enzyme can be summarized as:

Step 0: initialisation
Initialize the number of molecules, reaction constants, and random number generators.
The loop:
Step 1: Monte Carlo step
Generate random numbers to determine the time interval to the next slow reaction (reaction 3 in (1)).
Step 2: Update
Increase the time step by the randomly generated time in Step 1, and apply the slow reaction (reaction 3 in (1)) to molecule numbers. Compute the stationary distribution of c_1, and set its molecule count to the mean.
Step 3: Iterate
Go back to Step 1 unless the number of reactants is zero or the simulation time has been exceeded.

The availability of a closed-form expression for the stationary distribution of the complex is a major advantage, as it allows to rapidly compute its mean and speed up the calculations dramatically.

As pointed out in [15], the algorithm can be extended to account for systems with more slow reactions by adding a randomized selection step to choose which slow reaction is executed in each step. In the case of the system of Fig. 2, we effectively have four slow reactions (labelled as 3, 4, 5 and 6 in Fig. 2b), and therefore we included the following selection step (analogous to selection of the next reaction in the classic Stochastic Simulation Algorithm)

Step 1*: Selection step
Generate random numbers to determine the next slow reaction to occur (among reactions 3, 4, 5, and 6 in Fig. 2b) as well as the time interval to the next reaction.

To obtain a closed-form expression for the stationary distribution of the complex c_1, we use the method of "stepping functions" described in [17]. The stepping functions describe the propensity that a random variable either increases or decreases by 1. The stepping functions for the number of complex molecules are $W_- = a_2(c_1)$ and $W_+ = a_1(e_1, s_1^0)$,

where the propensities a_1 and a_2 are defined in Section II. Because the total amount of free/bound enzymes $x_t = e_1 + c_1$ and free/bound substrate $(s_1^0 + c_1)$ are constant in between two slow reactions (reactions 3 to 6 in Fig. 2), we can write $W_+ = W_+(c_1)$. The distribution $P_s(c_1)$ for $c_1 = 0, 1, \ldots, x_t$, can be obtained by iterating the recursion relation $P_s(c_1) = P_s(c_1 - 1)W_+(c_1 - 1)/W_-(c_1)$, which is given by

$$P_s(c_1) = \frac{m_1 s_1^0 (x_t - c_1 + 1)}{m_2 c_1} P_s(c_1 - 1). \tag{7}$$

Iterating the expression in (7) we get

$$\begin{aligned} P_s(c_1) &= P_s(0) \prod_{j=1}^{c_1} \frac{m_1 s_1^0 (x_t - j + 1)}{m_2 j}, \\ &= P_s(0) \binom{x_t}{c_1} \left(\frac{m_1 s_1^0}{m_2}\right)^{c_1}. \end{aligned} \tag{8}$$

Since $P_s(c_1)$ must satisfy $\sum_{c_1=0}^{x_t} P_s(c_1) = 1$, substituting in (8) and solving for $P_s(0)$ we obtain

$$\begin{aligned} P_s(0) &= \left(1 + \sum_{c_1=1}^{x_t} \binom{x_t}{c_1} \left(\frac{m_1 s_1^0}{m_2}\right)^{c_1}\right)^{-1}, \\ &= \left(\sum_{c_1=0}^{x_t} \binom{x_t}{c_1} \left(\frac{m_1 s_1^0}{m_2}\right)^{c_1}\right)^{-1}, \\ &= \left(1 + \frac{m_1 s_1^0}{m_2}\right)^{-x_t}. \end{aligned} \tag{9}$$

Combining equations (8) and (9) we get a Binomial distribution for c_1 with probability $m_1 s_1^0/(m_1 s_1^0 + m_2)$ and number of trials x_t:

$$\begin{aligned} P_s(c_1) &= \left(\frac{m_2}{m_1 s_1^0 + m_2}\right)^{x_t} \binom{x_t}{c_1} \left(\frac{m_1 s_1^0}{m_2}\right)^{c_1}, \\ &= \binom{x_t}{c_1} \left(\frac{m_1 s_1^0}{m_1 s_1^0 + m_2}\right)^{c_1} \left(\frac{m_2}{m_1 s_1^0 + m_2}\right)^{x_t - c_1}. \end{aligned} \tag{10}$$

Note that the distribution of e_1 can be inferred from the relation $e_1 = x_t - c_1$. This procedure is independent of the slow reactions, and therefore as long as the two timescales remain well separated we can use the expression for $P_s(c_1)$ to systematically study the effect of the transcriptional parameters on the noise levels.

We ran our implementation of the ss-SSA for a set of parameters that lead to physiologically realistic molecule numbers. The results are shown in Fig. 3, where we verify that the mean molecule numbers match the steady state of the deterministic model in (3)–(6). In Fig. 3b-c we compare the ss-SSA against the results given by the exact SSA. The stationary distributions are statistically indistinguishable from each other, and moreover, an exact SSA simulation that took ∼20 hours on 6 CPU cores was reduced by the ss-SSA method to 9 minutes on a single core.

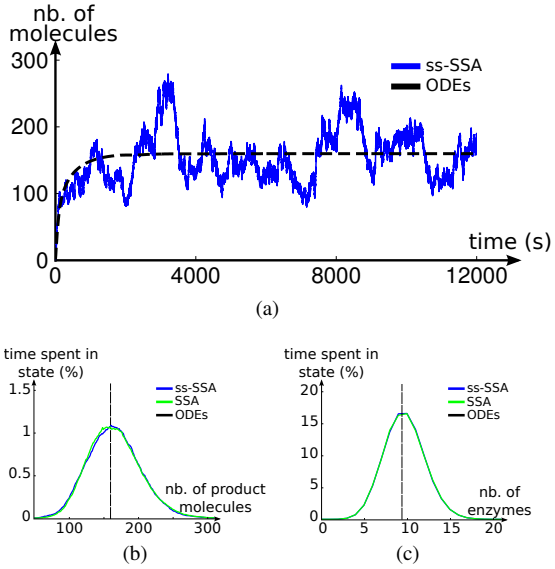

Fig. 3: Performance of the ss-SSA for a catalytic reaction with transcriptional feedback. **(a)** One realization of the product molecules obtained with the ss-SSA in blue, and the deterministic result for the same system in black. The stationary distributions of the product **(b)** and enzyme **(c)** molecule counts computed from a long realization (final time $= 8 \times 10^5$ s), for the ss-SSA and exact SSA, and the corresponding deterministic results. The parameter values are in Table II, and the repression threshold was set to $\alpha = 10$.

IV. Effect of transcriptional feedback on the propagation of noise

We explored the effect of transcriptional feedback on the properties of enzymatic noise in the catalytic reaction of Fig. 2. We computed the stationary distributions of the enzyme numbers for a range of values of the repression threshold α spanning four orders of magnitude, and then computed their mean μ and coefficient of variation η. The results are presented in Fig. 4. They indicate two key aspects:

- A strong repression (or equivalently, a low repression threshold α) decreases the probability of enzyme births, leading to a lower mean and standard deviation of enzyme molecules.
- A strong repression can dramatically increase the noise level (up to five-fold for tested range of α), suggesting that feedback repression enhances the fluctuation of the enzyme numbers around its mean value. In contrast, for a large repression threshold α, the noise level is equal to its open-loop value, i.e. the level of noise when there is no feedback from the product and the propensity of enzyme birth is set to its maximal value β. This is consistent with the fact that

$$\lim_{\alpha \to \infty} a_4(s_2) = \beta, \tag{11}$$

thus indicating that the feedback disappears for sufficiently large repression threshold.

It is important to emphasize that the curves in Fig. 4 can be obtained in reasonable computational time (∼10–20 min per data point on a single CPU machine). This task would

TABLE II: Parameter values for the stochastic simulations. Note that m_1 was set to 1 since the simulations only depend on the ratio m_2/m_1.

Parameter	Description	Value
m_1	substrate-enzyme binding constant	1 molecule$^{-1}.s^{-1}$
m_2	complex dissociation	28300 s^{-1}
m_3	product formation	3.2 s^{-1}
m_5	enzyme degradation	10^{-3} s^{-1}
m_6	product consumption	$2 \times 10{-}2$ s^{-1}
β	maximal enzyme birth	0.16 molecule.s^{-1}
s_1^0	number of substrate molecules	3000 molecule

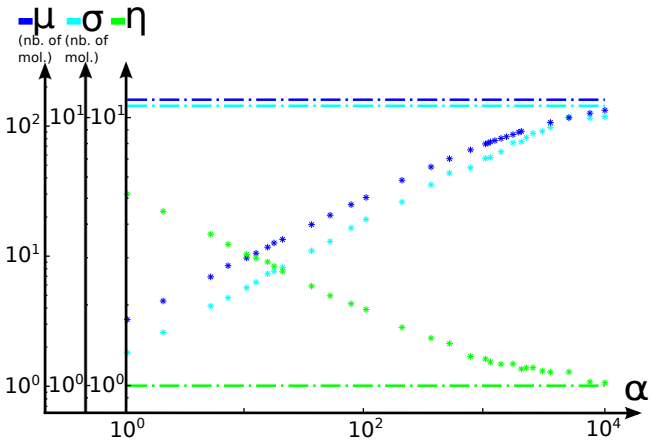

Fig. 4: Mean (μ, blue), standard deviation (σ, cyan) and noise level (η, green) of the enzyme molecule counts in the reaction network of Fig. 2 for different repression thresholds α. The noise level η is defined as coefficient of variation, and its values were normalized to the open-loop level (i.e. the coefficient of variation obtained when $a_4 = \beta$, shown in dashed line). The parameters values were set to the values in Table II.

have been impractical with other simulation techniques. For example, when using the exact SSA each point in Fig. 4 would take ∼20 hours to compute (recall the exact SSA results in Fig. 3).

V. Extension to metabolic pathways under transcriptional feedback

In our aim to scale up the proposed simulation technique to pathways with complex stoichiometries, as a first step we extended the algorithm to unbranched pathways of any length.

To automate the input of pathway data, the algorithm takes a Systems Biology Markup Language (SBML) description of the network. This prevents typical error-prone steps such as defining the reaction matrix (typically of tens of reactions affecting tens of species). SBML is the standard format for storing, processing and exchanging of models in systems biology, and there exist a wide range of software tools to produce SBML descriptions of complex networks.

The most important challenge when dealing with whole metabolic pathways is the presence of several fast processes. We first need to identify the fast processes, compute their corresponding stationary distributions, and make sure we update them only when necessary (i.e. only when a slow reaction that changes their stationary state occurs).

The current version of the algorithm singles out the fast processes from the list of fast reactions specified by the user, and identifies the list of slow reactions that can influence them. The algorithm then matches each fast process with one of the stationary distributions we have available. So far we only have the distributions for an enzymatic reaction with variable and constant substrates (i.e. the Gaussian distribution derived in [15] and the Binomial distribution derived in this paper, see (10)). These distributions are sufficient for simulating unbranched pathways of monomolecular reactions, but more complex networks, such as branched pathways or multi-enzyme catalytic reactions, need further investigation.

After these first steps of initialisation and pre-processing of the data in the SBML file, the algorithm remains the same as we previously described in Section III. The only difference now is that we update fast processes only if their stationary state is affected by the reaction that was just fired:

Step 2*: Update
Increase the time step by the randomly generated time in Step 1, and apply the slow reaction to molecule numbers. Compute the new stationary distribution only for the fast processes that were affected by the slow reaction in Step 1.

Updating the fast processes only when necessary has two advantages: it greatly reduces the amount of computation at each step, and it helps with the numerical validation of the timescale separation. Because all fast processes are affected by different slow reactions, we need to check the validity of the assumption on timescale separation for the fast processes with respect to only the slow reactions that can affect them.

In Fig. 5 we show the algorithm output for a cascade of three enzymatic reactions. The simulation of longer pathways takes longer time than single reactions, but the computation time is still reasonable for our purposes (∼30min per realization).

VI. Conclusions

In this paper we have presented a stochastic simulation framework for enzymatic reactions under genetic feedback regulation. The algorithm exploits the separation of timescales between the formation/dissociation of the enzyme-substrate complex and the dynamics of enzyme expression. This allows to obtain sample paths of the stochastic processes in reasonable computational time with a marginal loss of accuracy with respect to the exact Gillespie algorithm.

Gene expression is an inherently stochastic process, and therefore catalytic reactions are driven by noisy signals that back-propagate via the genetic feedback. Although cells must dampen noise to execute their functions accurately, some cell decisions are stochastically-driven [18], whereas in other cases cells exploit noise to enhance their phenotypic diversity and improve the robustness of their population [19]. A major challenge is therefore to understand how cells use the feedback architecture and strength as a mechanism to

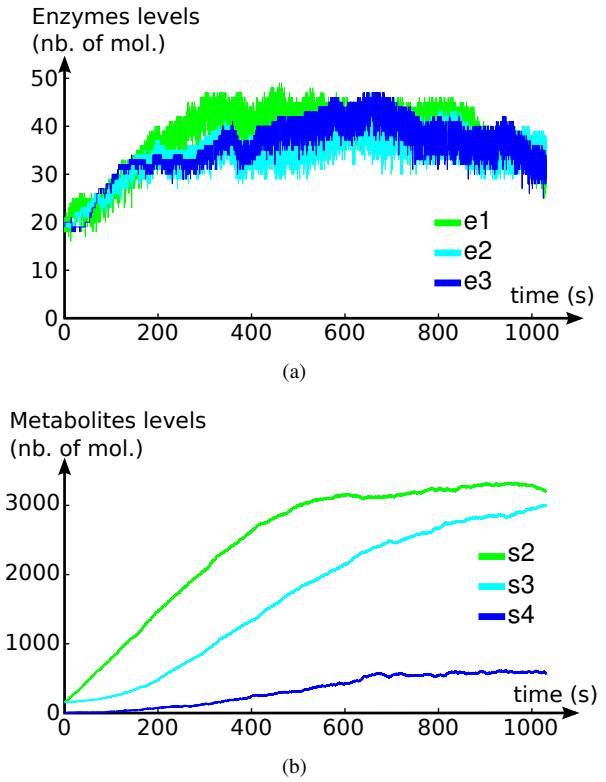

Fig. 5: Time course of a pathway of 3 enzymatic reactions, with a one-to-all transcriptional feedback loop from the product to the three enzymes. (a) Levels of enzymes (b) Levels of metabolites. All three enzymatic reactions in the cascade have the same parameters as in Table II, with only the first one based on the constant substrate model we developped. Second and third are based on non-constant substrate models (see [15]). The feedback loop parameters remain unchanged, and $\alpha = 100$.

amplify or attenuate the propagation of genetic noise to the metabolic layer.

The algorithm we have proposed provides a means to systematically explore the effect of different feedback configuration and parameters on the resulting noise levels of the molecular species. We have highlighted this application in Section IV, as part of our preliminary studies on the effect of transcriptional repression on the propagation of genetic noise. These results indicate that transcriptional repression can dramatically increase the noise levels of the enzyme molecule numbers (as measured by the coefficient of variation of their stationary distribution). This observation is consistent with recent findings [20] and can be understood as the outcome of two complementary phenomena: firstly, the mean enzyme count decreases with the feedback strength, and secondly, this reduction is more drastic than the change observed in standard deviation. The resulting coefficient of variation therefore tends to increase for increasing feedback strength.

We are currently working on a number of extensions of this work, including a systematic study of noise propagation in different feedback topologies and the extension of the algorithm to whole pathways of enzymatic reactions. As pointed out in Section V, we have developed an algorithm that can automatically perform such analysis for unbranched pathways described under the form of SBML files. In the case of more complex stoichiometries, the main challenge is to obtain analytic expression for the stationary distributions of the enzyme-substrate complexes, which is one of our subjects of active research.

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