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Out-of-equilibrium fluctuations drive correlations between enzyme and metabolic product levels

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Abstract— **Enzyme-driven catalysis of a substrate into a product forms the fundamental backbone of cellular metabolic pathways. In the deterministic formulation of such a reaction scheme, the equilibrium level of the metabolic product is independent of the steady-state enzyme, so that any perturbation in enzyme levels causes a transient change in metabolic product levels that perfectly adapts to the original enzyme-independent steady state. In this work, we consider a stochastic formulation of the problem, where enzyme levels constantly fluctuate due to the inherently noisy gene expression process as well as to the extrinsic noise in substrate availability. Our results show that such out-of-equilibrium fluctuations can result in positive (or negative) enzyme-product and substrate-product correlations, whose behavior qualitatively and quantitatively changes in different scenarios characterized by perturbations of nominal parameters and variable noise levels.**

Index Terms— **Metabolic Pathways, Enzymatic Reactions, Systems Biology, Chemical Master Equations**

I. Introduction

One of the most challenging fields in Life Science research involves deep understanding of how complex cellular functions (like metabolism, growth, cycle, etc.) arise from the interactions of molecules in a biochemical network. To this end, mathematical and computational methods in systems biology (including recent results on large-scale gene regulatory networks [1], [2]) are fundamental to study the complex molecular interactions within biological systems and to accelerate discoveries.

In this note we investigate a modified version of the classical enzymatic reaction scheme, where a product P accumulates by means of the catalytic action on an enzyme E on a substrate S. Enzyme activity is ubiquitous in many and diverse cellular machinery, including metabolism control in both transcriptional and post-translational way with interesting applications in the designing of synthetic circuits to improve the productivities of engineered metabolic pathways [3]. Besides, these studies are gaining an even growing interest because of the recent possibility to measure enzyme activity at a single-cell level [4]. Within these single-cell frameworks, the intrinsic noise affecting the stochasticity of biochemical interaction of particles in gene expression processes, as well as the extrinsic noise accounting for other kind of fluctuations, mostly provided by the environment, cannot be neglected: examples can be found in metabolic pathways affected by stochastic fluctuations [5], [6], [7], [8], [9], dealing with single-cell metabolite distributions [10] or with metabolic heterogeneity emerging from fluctuations in enzyme expression and catalysis [11], as well as in other cellular functions, such as in sequestration mechanisms [12].

The aim of the work is to investigate product-enzyme correlations according to a stochastic formulation of the enzymatic reaction network where two noise sources are considered, one acting on the enzyme production level, the other acting as an extrinsic noise in substrate availability. The following Section is devoted to introducing the model in details. We will show in Section III that dealing with just first-order moments (that means, according to a mere deterministic approach) any relationships in enzyme-product levels may be lost or, at least, underestimated, since perturbations in enzyme levels cause a transient change in metabolic product levels that perfectly adapts to the original enzyme-independent steady-state level. On the other hand, by explicitly accounting for the noisy fluctuations (the explicit computations of the second-order moments are reported in Section IV), non-trivial correlations emerge: these results are reported in Section V, according to a preliminary investigation across different parameter regimes.

II. Model formulation

The scheme of the metabolic network under investigation is reported in Figure 1. Besides E we consider a second enzyme, Z, acting to catalyze the substrate production. The red arrows denote the control action of E on product production and of Z on substrate production. In the following, we will refer to n_z, n_e, n_s, n_p to denote the copy numbers of the four molecular players.

The rate of P production, namely $k_2\varphi(n_s, n_e)$, is modeled by the general Michaelis-Menten formalism where φ is a saturating function with respect to the substrate S and is linear with respect to the enzyme E:

$$\varphi(n_s, n_e) = \frac{n_s}{n_s + M_s} \cdot n_e \tag{1}$$

One first source of noise in our model is provided by the enzyme production, supposed to happen in bursts [13], [14]:

$$n_e \to n_e + \eta, \qquad a_{1\eta} = c_1 \cdot \mathbb{P}(\xi_1 = \eta), \qquad \eta = 1, 2, \dots \tag{2}$$

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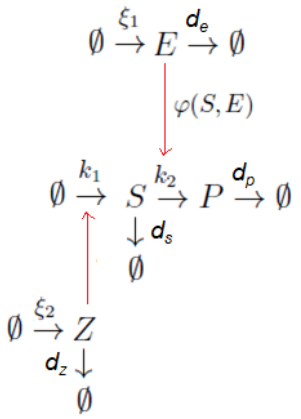

Fig. 1. Molecular scheme of the reaction network under investigation

where ξ_1 is a discrete random variable with a given probability distribution. In the following, we will denote with $\bar{\xi}_1$ and σ_1^2 the mean value and variance of ξ_1, respectively.

Another source of noise is on the substrate production rate, defined by $k_1\psi(n_z)$, with $\psi(n_z)$ provided by the following saturating function

$$\psi(n_z) = \frac{n_z}{n_z + M_z} \tag{3}$$

Z is also supposed to be produced in bursts, that is:

$$n_z \to n_z + \eta, \qquad a_{2\eta} = c_2 \cdot \mathbb{P}(\xi_2 = \eta), \qquad \eta = 1, 2, \dots \tag{4}$$

where ξ_2 is a discrete random variable with a given probability distribution. In the following, we will denote with $\bar{\xi}_2$ and σ_2^2 the mean value and variance of ξ_2, respectively. Notice that there is no consumption for Z when S is produced: Z acts as an enzyme catalyzing S production.

Furthermore, linear clearance rates are supposed for the four molecular players, denoted by

$$S \xrightarrow{d_s} \emptyset, \qquad E \xrightarrow{d_e} \emptyset, \qquad Z \xrightarrow{d_z} \emptyset, \qquad P \xrightarrow{d_p} \emptyset$$

The modeling choice adopted to deal with the proposed enzymatic reaction scheme is that of a Stochastic Hybrid Systems (SHS) [15], where the state variables n_z, n_e, n_s, n_p evolve continuously according to the following Ordinary Differential Equation (ODE) system

$$\begin{aligned} \dot{n}_z &= -d_z n_z \\ \dot{n}_e &= -d_e n_e \\ \dot{n}_s &= k_1\psi(n_z) - k_2\varphi(n_s, n_e) - d_s n_s \\ \dot{n}_p &= k_2\varphi(n_s, n_e) - d_p n_p \end{aligned} \tag{5}$$

between any two stochastic events provided by the noisy enzymes productions (2)-(4), with n_e and n_z updating according to the related Continuous Time Markov Chain.

III. First-order moments

Let $X = (n_z, n_e, n_s, n_p)^T$, and consider a generic nonlinear function $\chi(X)$. In the following we will denote average values with $\langle\cdot\rangle$. The general formula providing the dynamics of the average value $\langle\chi(X)\rangle$ is [15]:

$$\begin{aligned} \frac{d}{dt}\langle\chi(X)\rangle = &\left\langle \frac{d\chi}{dX} h(X) \right\rangle \\ &+ \sum_{\eta=1}^{\infty} \left\langle \big(\chi(X+\delta_{1\eta}) - \chi(X)\big) a_{1\eta} \right\rangle \\ &+ \sum_{\eta=1}^{\infty} \left\langle \big(\chi(X+\delta_{2\eta}) - \chi(X)\big) a_{2\eta} \right\rangle \end{aligned} \tag{6}$$

where $h(\cdot)$ is the nonlinear function describing the ODE part (continuous flow) of the SHS in (5), and $\delta_{1\eta} = (\eta, 0, 0, 0)^T$, $\delta_{2\eta} = (0, \eta, 0, 0)^T$ are the reset provided by the stochastic part (discrete jump) of the SHS. By properly setting $\chi(\cdot)$ we can write the dynamic equations for any order moments. However, because of the nonlinearities in $h(\cdot)$, moment equations are not available in closed form, not even for the first-order case, therefore we need to resort to approximation techniques, like moment closure techniques [16], or Linear Noise Approximations (LNA) [17], the latter based on the linearization of the nonlinear functions of the ODE.

Therefore, accounting for the linear approximations of the involved nonlinear functions

$$\langle\psi(n_z)\rangle \simeq \psi(\langle n_z\rangle), \quad \langle\varphi(n_s, n_e)\rangle \simeq \varphi(\langle n_s\rangle, \langle n_e\rangle) \tag{7}$$

the following approximated first-order moment equations are obtained from (6), by setting $\chi(X) = n_j$, for $j \in \{z, e, s, p\}$:

$$\begin{aligned} \frac{d\langle n_z\rangle}{dt} &= c_2\bar{\xi}_2 - d_z\langle n_z\rangle \\ \frac{d\langle n_e\rangle}{dt} &= c_1\bar{\xi}_1 - d_e\langle n_e\rangle \\ \frac{d\langle n_s\rangle}{dt} &= k_1\psi(\langle n_z\rangle) - k_2\varphi(\langle n_s\rangle, \langle n_e\rangle) - d_s\langle n_s\rangle \\ \frac{d\langle n_p\rangle}{dt} &= k_2\varphi(\langle n_s\rangle, \langle n_e\rangle) - d_p\langle n_p\rangle \end{aligned} \tag{8}$$

According to (8), by exploiting the notation

$$\lim_{t\mapsto+\infty}\langle n_j\rangle = \overline{n_j}, \qquad j \in \{z, e, s, p\} \tag{9}$$

for the average stationary solutions, they satisfy the following algebraic system

$$\begin{aligned} \overline{n_z} &= \frac{c_2\bar{\xi}_2}{d_z} & \overline{n_e} &= \frac{c_1\bar{\xi}_1}{d_e} \\ \overline{n_p} &= \frac{k_1}{d_p}\overline{\psi} - \frac{d_s}{d_p}\overline{n_s} & k_2\overline{\varphi} &= d_p\overline{n_p} \end{aligned} \tag{10}$$

where

$$\overline{\varphi} = \varphi(\overline{n_s}, \overline{n_e}), \qquad \overline{\psi} = \psi(\overline{n_z}) \tag{11}$$

for short.

Remark 1: The average ODE model in (8) results in the so-called deterministic formulation of the problem, detailing the average values of the involved molecular players without accounting for the fluctuations of internal/external noises. According to the stationary values obeying the constraints reported in (10), we find that the steady-state level of the metabolic product may be made independent of the steady-state enzyme level: for instance by suitably tuning the model

parameters in order to keep fixed $\overline{n_p}$ according to different sets of parameter values. In these cases, according to such a deterministic formulation, any perturbation in enzyme levels causes a transient change in metabolic product levels that perfectly adapts to the original stationary level. It will be shown how such illusory independence falls apart according to the stochastic approach, pointing out the inadequacy of a purely deterministic framework.

The following Lemmas address systematically the qualitative behavior of the deterministic formulation.

Lemma 2: According to (10), there exists a unique positive solution for the average stationary values $\overline{n_s}$, $\overline{n_p}$, and is the following

$$\overline{n_s} = \frac{k_1\overline{\psi} - k_2\overline{n_e} - d_sM_s + \sqrt{\Delta}}{2d_s} \tag{12}$$

$$\overline{n_p} = \frac{k_1\overline{\psi} + k_2\overline{n_e} + d_sM_s - \sqrt{\Delta}}{2d_p} \tag{13}$$

with

$$\Delta = \left(d_sM_s + k_2\overline{n_e} - k_1\overline{\psi}\right)^2 + 4d_sk_1M_s\overline{\psi}. \tag{14}$$

Proof. By substituting $\overline{n_e}$ and $\overline{n_p}$ (the latter as a function of $\overline{n_s}$) in the last of (10) we have the following second-order equation for $\overline{n_s}$:

$$d_s\overline{n_s}^2 + \left(d_sM_s + k_2\overline{n_e} - k_1\overline{\psi}\right)\overline{n_s} - k_1M_s\overline{\psi} = 0 \tag{15}$$

whose real roots are readily computed by

$$\overline{n_s} = \frac{k_1\overline{\psi} - k_2\overline{n_e} - d_sM_s \pm \sqrt{\Delta}}{2d_s} \tag{16}$$

with $\Delta > 0$ given by (14). It readily comes from the Routh criterion that the two real roots have opposite sign; therefore there exists a unique positive root for $\overline{n_s}$, provided by (12). By substituting (12) into $\overline{n_p}$ equation in (10), we have (13). Besides, $\overline{n_p}$ is positive, since, the following inequality holds:

$$\Delta < \left(k_1\overline{\psi} + k_2\overline{n_e} + d_sM_s\right)^2. \tag{17}$$

■

Lemma 3: The positive solution provided by Lemma 2 is locally asymptotically stable.

Proof. The proof readily comes from the computation of the Jacobian matrix from (8). Indeed,

$$J = \begin{bmatrix} -d_z & 0 & 0 & 0 \\ 0 & -d_e & 0 & 0 \\ k_1\overline{\psi_z} & -k_2\overline{\varphi_e} & -d_s - k_2\overline{\varphi_s} & 0 \\ 0 & k_2\overline{\varphi_e} & k_2\overline{\varphi_s} & -d_p \end{bmatrix} \tag{18}$$

with

$$\overline{\psi_z} = \psi_z(\overline{n_z}) = \left.\frac{d\psi}{dn_z}\right|_{\overline{n_z}} = \frac{M_z}{(\overline{n_z} + M_z)^2}, \tag{19}$$

and

$$\overline{\varphi_e} = \varphi_e(\overline{n_s}) = \left.\frac{\partial\varphi}{\partial n_e}\right|_{(\overline{n_s},\overline{n_e})} = \frac{\overline{n_s}}{\overline{n_s} + M_s}, \tag{20}$$

$$\overline{\varphi_s} = \varphi_s(\overline{n_s},\overline{n_e}) = \left.\frac{\partial\varphi}{\partial n_s}\right|_{(\overline{n_s},\overline{n_e})} = \frac{M_s\overline{n_e}}{(\overline{n_s} + M_s)^2} \tag{21}$$

The eigenvalues are clearly given by the diagonal elements, which are all strictly negative since φ_s is a positive function for positive entries.

■

Besides the general case, there can be found different simplified frameworks according to the following hypotheses.

A. The case of $n_s \ll M_s$

In case of $n_s \ll M_s$, we have scarce copy numbers of substrate, so that

$$\varphi(n_s, n_e) \simeq \frac{n_s}{M_s} \cdot n_e \tag{22}$$

and the stationary solutions for $\overline{n_s}$ and $\overline{n_p}$ in (12)-(13) simplify into

$$\overline{n_s} = \frac{k_1M_s\overline{\psi}}{k_2\overline{n_e} + d_sM_s}, \qquad \overline{n_p} = \frac{k_1k_2\overline{\psi}\overline{n_e}}{d_p(k_2\overline{n_e} + d_sM_s)} \tag{23}$$

B. The case of $n_s \gg M_s$

In case of $n_s \gg M_s$, we have abundance of substrate, so that

$$\varphi(n_s, n_e) \simeq n_e \tag{24}$$

In this case the stationary solutions for $\overline{n_s}$ and $\overline{n_p}$ in (12)-(13) simplify into

$$\overline{n_s} = \frac{k_1\overline{\psi} - k_2\overline{n_e}}{d_s}, \qquad \overline{n_p} = \frac{k_2\overline{n_e}}{d_p} \tag{25}$$

provided that

$$k_2\overline{n_e} < k_1\overline{\psi} \tag{26}$$

in order to ensure positiveness of the solution. Indeed, by looking at (12) as coming from Lemma 2 and treating the case of $n_s \gg M_s$ as a limit for $M_s \to 0$, then $\overline{n_s}$ converges to (25), provided that condition (26) is true; otherwise, $\overline{n_s}$ would converge to 0, a not admissible solution according to (24).

C. The case of $n_z \gg M_z$

In case of $n_z \gg M_z$, i.e. we have abundance of Z, so that

$$\psi(n_z) \simeq 1 \tag{27}$$

that means the substrate production does not depend of Z explicitly any more. From a deterministic viewpoint, we just have to replace (27) into Lemmas 2–3.

IV. Second-order moments

In order to investigate correlations in enzyme-product levels, we need to compute the second-order moments, by properly setting $\chi(X)$ in (6) as any quadratic function of X:

$$\chi(X) = n_z^{j_z}n_e^{j_e}n_s^{j_s}n_p^{j_p}, \qquad j_x \in \{0,1,2\}, \quad x \in \{z,e,s,p\} \tag{28}$$

with $j_z + j_e + j_s + j_p = 2$.

Dealing with the two enzymes E and Z, it is easy to write their variances (σ_e^2 and σ_z^2, respectively) as functions of the two sources of noise ξ_1 and ξ_2. Indeed, by setting

$\chi(X) = n_e^2$ and $\chi(X) = n_z^2$ in (6) and, then, looking for stationary solutions, we obtain:

$$\sigma_e^2 = \overline{\langle n_e^2 \rangle} - \overline{n_e}^2 = \frac{c_1}{2d_e}\left(\sigma_1^2 + \bar{\xi}_1^2\right) \tag{29}$$

$$\sigma_z^2 = \overline{\langle n_z^2 \rangle} - \overline{n_z}^2 = \frac{c_2}{2d_z}\left(\sigma_2^2 + \bar{\xi}_2^2\right) \tag{30}$$

A further result that straightforwardly comes from the reaction network is that the two enzymes are uncorrelated. Indeed, by setting $\chi(X) = n_z n_e$ in (6), it readily comes that

$$\sigma_{ze} = \overline{\langle n_z n_e \rangle} - \overline{n_z}\,\overline{n_e} = 0 \tag{31}$$

Dealing with the other covariances involving Z and E, we have:

$$\sigma_{zs} = \frac{k_1 \overline{\psi_z}}{d_z + d_s + k_2\overline{\varphi_s}} \cdot \sigma_z^2 \tag{32}$$

$$\sigma_{zp} = \frac{k_1 k_2 \overline{\psi_z}\,\overline{\varphi_s}}{(d_z + d_p)(d_z + d_s + k_2\overline{\varphi_s})} \cdot \sigma_z^2 \tag{33}$$

$$\sigma_{es} = -\frac{k_2 \overline{\varphi_e}}{d_e + d_s + k_2\overline{\varphi_s}} \cdot \sigma_e^2 \tag{34}$$

$$\sigma_{ep} = \frac{k_2(d_e + d_s)\overline{\varphi_e}}{(d_e + d_p)(d_e + d_s + k_2\overline{\varphi_s})} \cdot \sigma_e^2 \tag{35}$$

Regards to the noises involved, as expected, σ_{zs} and σ_{zp} involve only Z fluctuations, whilst σ_{es} and σ_{ep} involve only E fluctuations.

Finally, dealing with S, P fluctuations and their covariance, we have:

$$\sigma_s^2 = \frac{k_1^2 \overline{\psi_z}^2}{(d_s + k_2\overline{\varphi_s})(d_z + d_s + k_2\overline{\varphi_s})} \cdot \sigma_z^2 + \frac{k_2^2 \overline{\varphi_e}^2}{(d_s + k_2\overline{\varphi_s})(d_e + d_s + k_2\overline{\varphi_s})} \cdot \sigma_e^2 \tag{36}$$

$$\sigma_{sp} = \frac{k_1^2 k_2 \overline{\psi_z}^2 \overline{\varphi_s}(d_s + d_z + d_p + k_2\overline{\varphi_s})}{(d_z + d_p)(d_s + k_2\overline{\varphi_s})(d_z + d_s + k_2\overline{\varphi_s})(d_s + d_p + k_2\overline{\varphi_s})} \cdot \sigma_z^2 - \frac{k_2^2 \overline{\varphi_e}^2\left((d_e + d_s)(d_s + k_2\overline{\varphi_s}) + d_s(d_e + d_p)\right)}{(d_e + d_p)(d_s + k_2\overline{\varphi_s})(d_e + d_s + k_2\overline{\varphi_s})(d_s + d_p + k_2\overline{\varphi_s})} \cdot \sigma_e^2 \tag{37}$$

$$\sigma_p^2 = \frac{k_1^2 k_2^2 \overline{\psi_z}^2 \overline{\varphi_s}^2(d_s + d_z + d_p + k_2\overline{\varphi_s})}{d_p(d_z + d_p)(d_s + k_2\overline{\varphi_s})(d_z + d_s + k_2\overline{\varphi_s})(d_s + d_p + k_2\overline{\varphi_s})} \cdot \sigma_z^2 + \frac{k_2^2 \overline{\varphi_e}^2\left(d_s(d_e + d_s)(d_s + d_p) + k_2\overline{\varphi_s}(d_s^2 + d_e d_p)\right)}{d_p(d_e + d_p)(d_s + k_2\overline{\varphi_s})(d_e + d_s + k_2\overline{\varphi_s})(d_s + d_p + k_2\overline{\varphi_s})} \cdot \sigma_e^2 \tag{38}$$

V. Simulations

Numerical Monte Carlo simulations have been performed in MATLAB® to support and validate analytical results achieved according to linearizations (7). In our simulations, achieved according to a sufficiently long run of the Gillespie Stochastic Simulation Algorithm (SSA) [18] applied to the SHS (ergodic approach), we consider approximate steady-state sampled first-order and second-order moments for the model, obtained when a numerical fixed point is reached (our stopping criterion consists in the Euclidean distance between two consecutively computed sampled moments, collected in vector form, being lower than 10^{-6}).

TABLE I
Nominal model parameters.

Parameter	Description	Value
k_1	Substrate production rate	60
k_2	Maximal product production rate	0.8
d_s	Substrate clearance rate	0.1
d_p	Product clearance rate	0.025
d_e	Enzyme clearance rate	0.3
d_z	Z clearance rate	0.2
M_s	S value of half-maximal P production	300
M_z	Z value of half-maximal S production	300
c_1	Enzyme production propensity	6
c_2	Z production propensity	10
$\bar{\xi}_1$	Average E burst size	4
$\bar{\xi}_2$	Average Z burst size	2

The nominal values of the parameters are reported in Table I and are partially varied according to the different scenarios described in the following subsections. Regards to the random variables ξ_1 and ξ_2 related to the two noise sources, we adopted a geometric probability distribution according to past literature [6], [19]:

$$\mathbb{P}(\xi_i = \eta) = \alpha_i(1 - \alpha_i)^{\eta - 1}, \qquad \alpha_i \in (0, 1), \quad \eta = 1, \dots \tag{39}$$

for $i = 1, 2$, with α_i set in order to have average burst sizes $\bar{\xi}_i = 1/\alpha_i$ equal to 4 and 2 for the nominal values of $\bar{\xi}_1$ and $\bar{\xi}_2$ in Table I, respectively.

According to the values in Table I we have the following nominal values for the stationary values:

$$\overline{n_z} = 100, \quad \overline{n_e} = 80, \quad \overline{n_s} = 53.36, \quad \overline{n_p} = 386.57. \tag{40}$$

The next subsections report on the investigated scenarios.

A. Variable average burst size $\bar{\xi}_1$ for the enzyme E

Scenario A accounts for varying values of the average burst size $\bar{\xi}_1$ of the enzyme E (which straightforwardly leads to variations of the average stationary value of E, see the second equation in (10)), keeping unchanged all other values in Table I.

By varying $\bar{\xi}_1$, $\overline{n_e}$ varies as well and, because of the last constraints in (10), also $\overline{n_s}$ and $\overline{n_p}$ vary. On the other hand, it would be interesting to investigate what happens by keeping fixed $\overline{n_p}$ in spite of varying $\overline{n_e}$, in order to understand whether there exists a correlation in enzyme-product (and in substrate-product) levels, and whether such correlations vary with varying $\overline{n_e}$. Therefore, in order to get unchanged stationary values of $\overline{n_s}$ and $\overline{n_p}$ with varying $\bar{\xi}_1$, we jointly vary M_s. More in details, by varying $\bar{\xi}_1$, $\overline{n_e}$ varies from (10) and we solve (15) with respect to M_s, by fixing $\overline{n_s}$ at the nominal values in (40). This way, only $\overline{n_e}$ varies, see Fig. 2.

Fig. 3 shows correlations between P and S and between P and E, drawn according to the analytical formulas written in Section IV for the second-order moments, and validated by the Gillespie algorithm applied to the SHS. They apparently show a non-trivial stationary correlation (P correlates positively with E and negatively with S), that does not seem to vary by increasing the average burst size for enzyme E production.

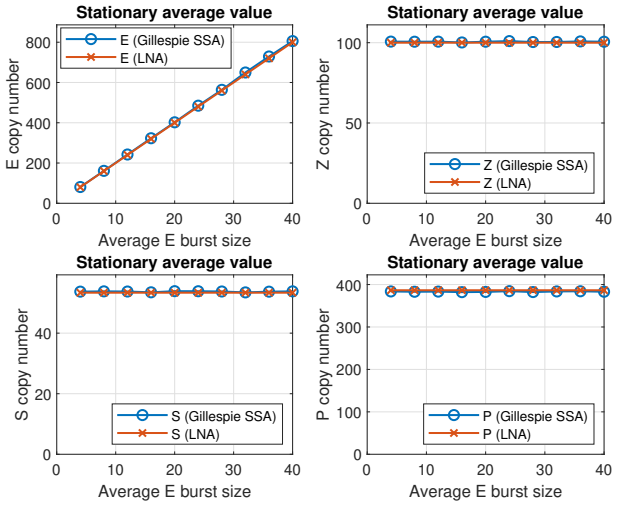

Fig. 2. Average stationary values for varying average burst size $\bar{\xi}_1$ of the enzyme E with respect to its nominal value, and M_s (substrate value of half-maximal product production rate) changed to keep constant stationary values for S and P.

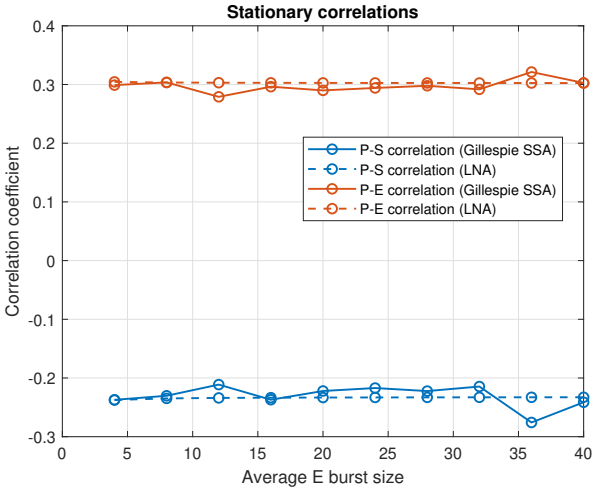

Fig. 3. Stationary correlations for varying average burst size $\bar{\xi}_1$ of the enzyme E with respect to its nominal value.

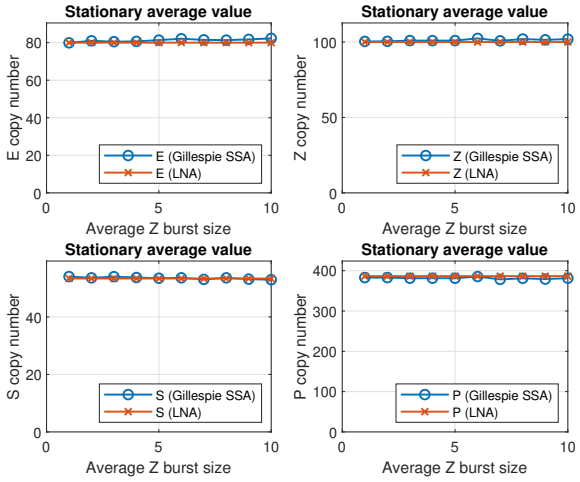

Fig. 4. Scenario B: average stationary values for varying average burst size $\bar{\xi}_2$ of species Z, and c_2 (Z production propensity) changed with respect to its nominal value to keep constant stationary values for all species.

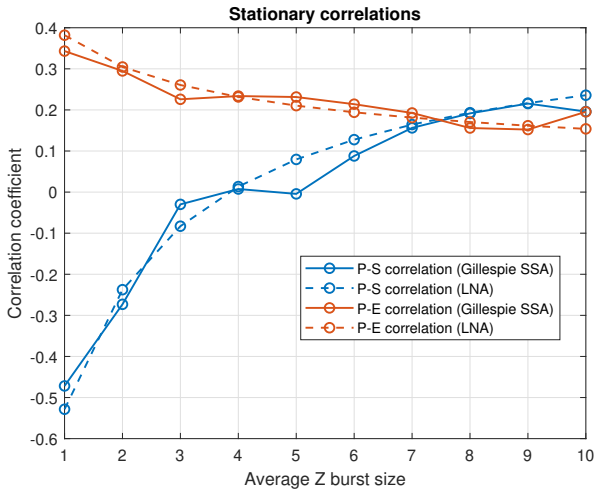

Fig. 5. Scenario B: stationary correlations for varying average burst size $\bar{\xi}_2$ of species Z, and c_2 (Z production propensity) changed with respect to its nominal value to keep constant stationary values for all species.

B. Variable average burst size $\bar{\xi}_2$ for enzyme Z

Scenario B accounts for varying values of the average burst size $\bar{\xi}_2$ for enzyme Z (which straightforwardly leads to variations of the average stationary value of Z, see the first equation in (10)), keeping unchanged all other values in Table I. Also in this case, by varying $\bar{\xi}_2$ the four stationary values vary (because $\overline{n_z}$ varies), unless the simplifying assumption of $n_z \gg M_z$ holds true, according to (27) in Section III.C. Instead, similarly to the investigations carried out in Section V.A, we aim at fixing the four stationary values and find out what happens because of the stochastic fluctuations. Therefore we jointly change c_2 in order to keep fixed all stationary values at their nominal values, see Fig. 4.

The stationary correlations are reported in Fig. 5 and show non-trivial correlations between P and S and between P and E. In this case, despite all molecular players have fixed stationary values, these correlations strongly vary with $\bar{\xi}_2$.

C. Variable clearance rate d_p for product P.

Scenario C accounts for varying values of the product clearance rate d_p (leading to variations of the average stationary value of P, see the last equation in (10)), keeping unchanged all other values in Table I. The average stationary values of the other species remain fixed, according to (12) in Lemma 2, see Fig. 6.

The stationary correlations are reported in Fig. 7 and show negative correlation between P and S and positive correlation between P and E; besides, it is apparent how P-E correlation has a definite positive trend, with respect to

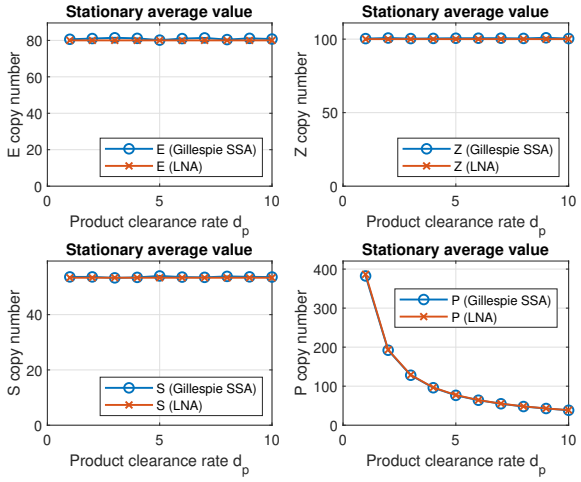

Fig. 6. Scenario C: average stationary values for varying clearance rate d_p of species P.

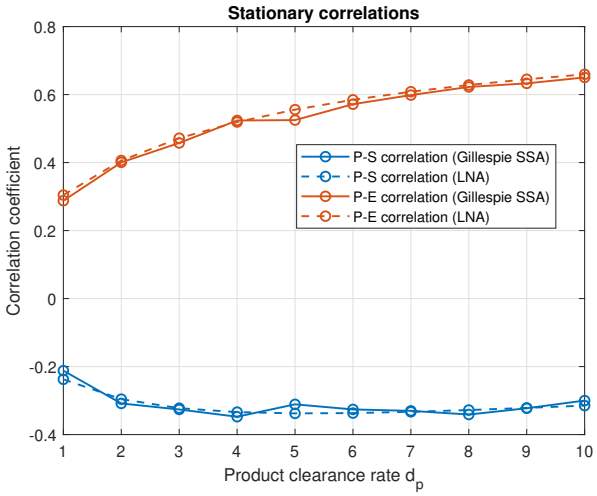

Fig. 7. Scenario C: stationary correlations for varying clearance rate d_p of species P.

increasing values of d_p, differently from P-S correlation that does not seem to vary as much with d_p.

VI. Conclusions

In this manuscript, we analyze a nonlinear dynamical system inspired by the fundamental process of enzyme-mediated conversion of a substrate into a product. We considered a stochastic formulation of the model where noise is incorporated in two different ways – the expression of the enzyme E occurs in stochastic bursts consistent with data on single-cell gene expression across cell types. Stochasticity is also introduced in the substrate import rate captured by its dependence on another enzyme Z that is also synthesized in bursts. All other processes were modeled using an ordinary differential equation leading to an overall SHS model formalism. Given the inherent nonlinearity that occurs due to the Michaelis–Menten kinetics of enzymatic reactions, we analyzed the system using the well-known LNA method that quantifies the magnitude of copy-number fluctuations in the small-noise regime.

A key contribution of this paper is the analytical derivation of closed-form formulas quantifying these fluctuations that we specifically use to study the correlation between the levels of the product and enzyme, and between the product and substrate levels. These formulas were rigorously validated with the exact simulation of the underlying SHS under a variety of parameter regimes.

Future work will extend this analysis to feedback between the product and enzyme expression, or the substrate import process, and also consider longer chains of metabolic pathways with multiple enzyme-catalyzed steps.

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