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Stochastic Analysis of Retroactivity in Transcriptional Networks through Singular Perturbation

Massachusetts Institute of Technology

Stochastic Analysis of Retroactivity in Transcriptional Networks through Singular Perturbation

Reza Ghaemi and Domitilla Del Vecchio

Abstract— The input/output dynamic behavior of a biomolecular system is affected by interconnection to other downstream systems through impedance-like effects called retroactivity. In this paper, we study the effects of retroactivity at the interconnection between two transcriptional modules, focusing on stochastic behavior. In particular, we describe the system through the Master equation and develop a singular perturbation theory to obtain a reduced Master equation. We prove that the solution of the original Master equation converges fast to an ϵ neighbor of the solution of the reduced Master equation, in which ϵ is the singular perturbation parameter. Our analysis shows that the upstream system and the downstream one are statistically independent at the steady state. However, the interconnection slows down the dynamics of both the expectation and the variance of the output of the upstream transcriptional module.

I. INTRODUCTION

Modularity is a fundamental property that guarantees that the input/output behavior of a system does not change upon interconnection with other systems. This property is often employed in biomolecular systems to predict the behavior of a complex network from the behavior of the composing modules characterized in isolation [1]. It has been recently shown that the dynamic behavior of a biomolecular system, such as a transcriptional network, is affected by the interconnection with other systems through a phenomenon, called retroactivity, which is similar to loading and impedance in engineering systems [2–4]. All these works characterizing retroactivity have assumed deterministic ordinary differential equation (ODE) models. Biological networks, however, exhibit stochastic behavior and fluctuations [5], which can be studied through stochastic models. These models describe the behavior of the number of molecules and, as opposed to ODE models, hold even for very low molecule numbers [6–9, 16]. Hence, to have a complete and general characterization of retroactivity, we need to perform stochastic analysis. Initial results in this direction have appeared in [12, 13], in which approximated stochastic models, such as the Langevin equation and/or linear noise approximation are employed.

In this paper, we employ an approach different from those of [12, 13]. Specifically, we analytically study the chemical Master equation [23] for a transcriptional component both when isolated and when connected to its downstream systems, by exploiting the natural time scale separation characterizing the constituent processes. In fact, transcriptional networks are characterized by large time scale separation between protein production/decay processes and the binding

of protein with DNA, which provides the physical means by which transcriptional modules are connected with each other [24]. For ODE models, singular perturbation theory provides tools for analyzing systems with time scale separation [14, 15], which have been extensively applied to biochemical systems [10, 11]. By contrast, for stochastic models such as the Master equation, singular perturbation theory has been rarely addressed. Pioneering work in this area has recently appeared, which allows to reduce the dimension of large stochastic models, hence lightening the computational cost of simulation [9, 16–18]. In particular, in [16] a singular perturbation method is introduced that is based on approximating an infinite dimensional stochastic model with a finite one using a projection algorithm [19] in conjunction with singular perturbation. In [17], power expansion of the probability distribution in terms of a perturbation parameter is employed to derive the reduced model of the slow time-scale process of the system. However, even though the finite dimensional system can approximate the infinite dimensional one, the reduced model approximation of the finite-dimensional system that is achieved using singular perturbation analysis is not generally an approximation of the infinite-dimensional system. As described in [22] page 160, with ϵ being the singular perturbation parameter and N being the dimension of the finite dimensional system that approximated the infinite-dimensional one, the limits $N \to \infty$ and $\epsilon \to 0$ are not in general interchangeable. Consequently, it is inferred that singular perturbation analysis applied on the finite-dimensional systems does not provide an approximation of the infinite-dimensional system.

In this paper, we introduce singular perturbation analysis for the Master equation modeling the cascade of two transcriptional components, to analytically study the effects of retroactivity from the downstream component on the upstream one. Specifically, our analysis provides two Master equations with different time scales, slow and fast, which together accurately approximate the joint probability distribution of the species of the whole system with quantified accuracy over a finite interval of time. To reach this result, we employ singular perturbation theory for continuous Markov chains [22] and adapt it to the system under study. We analyze both the transient and stationary stochastic behavior of the upstream transcriptional component as a function of retroactivity from the downstream system employing the reduced Master equation. We show that the mean and variance of the number of protein molecules in the upstream transcriptional component converge to their steady states slower when the system is connected. Specifically, we quantify the amount

Reza Ghaemi and Domitilla Del Vecchio are with the department of Mechanical Engineering at MIT.

by which this transient behavior is slowed down as a function of the biomolecular characteristics of the interconnection. By contrast, we show that the stationary values of the mean and variance do not depend on the downstream component.

II. DEFINITIONS AND PRELIMINARIES

Consider a stochastic process M with finite or countably infinite state space $\mathcal{M}$. Let $M(t)$ denote the state of the system at time t. If for any realization of M, there exist sequences $\{\tau_k\}$ and $\{m_k\}$ such that

$$
M(t) = \begin{cases}
m_0, & 0 \leq t < \tau_1 \\
m_1, & \tau_1 \leq t < \tau_2 \\
m_2, & \tau_2 \leq t < \tau_3 \\
\vdots &
\end{cases}
$$

then the process M is a jump process. If $\lim_{k \to \infty} \tau_k = \infty$, almost surely, i.e., $P(\lim_{k \to \infty} \tau_k = \infty) = 1$, then the process is a pure jump process. In other words, the process is pure if it does not jump infinitely in a finite interval of time.

Let $P(m; t, m_0)$ denote the probability that a process starting in state m_0 at time zero will be in state m at time t, called transition function. A pure jump process satisfies the stationary Markov property if for $0 \leq t_1 \leq \cdots \leq t_n \leq t' \leq t$, and $m_1, \cdots, m_n, m', m \in \mathbb{N}$, we have that

$$
P(M(t) = m \mid M(t_1) = m_1, \cdots, M(t_n) = m_n, M(t') = m')
$$
$$
= P(m; t - t', m'). \tag{1}
$$

A pure jump process that satisfies the stationary Markov property is a Markov pure jump process. The infinitesimal parameters of the process are defined as

$$
w(m, m_0) := \frac{d}{dt} P(m; t, m_0) \mid_{t=0} . \tag{2}
$$

The function π is a *stationary distribution* if $\pi(m)$, $m \in \mathcal{M}$ are nonnegative numbers summing up to one and if $\sum_{\bar{m} \in \mathcal{M}} \pi(\bar{m}) P(m; t, \bar{m}) = \pi(m)$ for all $m \in \mathcal{M}$ and all $t > 0$.

A Markov pure jump process is *irreducible* if, starting from a state m_1, it reaches to state m_2 in finite time with positive probability for all choices of m_1 and m_2.

For a Markov pure jump process, the transition function $P(m; t, m_0)$ satisfies the following differential equation, called the forward equation or Master equation [21, 23]:

$$
\frac{d}{dt} P(m; t, m_0) = \sum_{\bar{m} \in \mathcal{M}} w(m, \bar{m}) P(\bar{m}; t, m_0). \tag{3}
$$

Proposition 2.1: A function $\pi : \mathcal{M} \to [0, 1] \subset \mathbb{R}$ is a *stationary distribution* if and only if $\sum_{\bar{m} \in \mathcal{M}} w(m, \bar{m}) \pi(\bar{m}) = \pi(m)$.

According to Proposition 2.1, π is a *stationary distribution* if and only if it is the steady state solution of the Master equation.

Proposition 2.2: Let M be an *irreducible* Markov pure jump process that has a *stationary distribution* π such that

$\pi(m) > 0$ for all $m \in \mathcal{M}$. Then π is the unique *stationary distribution* for M. Moreover,

$$
\lim_{t \to \infty} P(m; t, m_0) = \pi(m) \text{ for all } m, m_0 \in \mathcal{M} \tag{4}
$$
$$
\lim_{t \to \infty} P(M(t) = m) = \pi(m). \tag{5}
$$

Note that (4) states that the conditional probability $P(M(t) = m \mid M(0) = m_0)$ converges to the *stationary distribution* $\pi(m)$ independent of initial state m_0. Additionally, (5) states that the probability of being at state m converges to the *stationary distribution* $\pi(m)$ as time goes to infinity (at steady state). l_1 denotes the space of all sequences equipped with $l_1 - norm$.

III. TRANSCRIPTIONAL SYSTEM

To analyze the effect of retroactivity, we consider a transcriptional component connected to a downstream one (Figure 1). The upstream transcriptional component takes a transcription factor (protein) as an input and produces a transcription factor Z as an output. The rate of expression of protein Z is k and its decay (degradation or dilution) rate is identified by δ. Interconnection of this transcriptional component to a downstream one occurs through a reversible binding reaction between Z (output of upstream transcriptional component) and DNA binding sites $\mathcal{P}$ (present on the promoter controlling the production of the downstream transcriptional component protein).
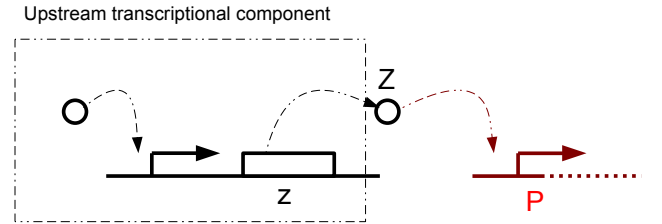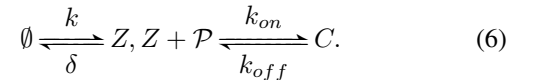

Fig. 1. Interconnected transcriptional components.

In this paper, we analyze the stochastic model of the connected transcriptional components to study the effect of retroactivity on the behavior of the upstream system. The reactions of the interconnected system are given by

$$
\emptyset \underset{\delta}{\overset{k}{\rightleftharpoons}} Z, \; Z + \mathcal{P} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} C. \tag{6}
$$

Note that the total amount of DNA is conserved, i.e., $\mathcal{P} + C = p_T$. It is important to understand the effect of the downstream system $\mathcal{P}$, or load, on the steady state and transient behavior of the transcription component. Specifically, it is important to know the effect of the downstream system on the concentration of the transcription factor Z. To analyze the behavior of this system, a deterministic approach is adopted in [2] which analyzes the steady state as well as transient behavior of the concentration of Z. However, to be reliable, the deterministic approach requires the number of molecules

to be large. Moreover, the deterministic approach does not provide an insight on how the load affects the intrinsic noise that is present in the system, specifically the noise on the concentration of Z. In addition, stochastic analysis allows calculation of the mean and the variance of the number of molecules Z over time, which describes the dynamic behavior of the concentration of Z as well as the noise accompanying this concentration.

IV. STOCHASTIC MODELING

The number of molecules of C, Z, and $\mathcal{P}$ are stochastic processes. That is at each time instant the number of molecules is a random variable. Assume the system starts at time 0 with number of molecules $(C(0), Z(0), \mathcal{P}(0)) = (c_0, z_0, p_0)$. The system remains in this state until some positive time τ_1, at which the system jumps to a new state $(C(\tau_1), Z(\tau_1), \mathcal{P}(\tau_1)) = (c_1, z_1, p_1)$ and the system continues in the way that it remains in state (c_k, z_k, p_k) until time τ_{k+1} at which it jumps to state $(c_{k+1}, z_{k+1}, p_{k+1})$. Hence, the stochastic process $M := (C, Z, \mathcal{P})$ is a jump process. In system (6), only a finite number of jumps occurs in finite time. That is if $\{\tau_k\}$ is the sequence of time instants at which a jump occurs, $\lim_{k \to \infty} \tau_n = \infty$. Therefore the stochastic process M is a pure jump process.

Let $P(c, z, p; t, m_0)$, or equivalently $P(m; t, m_0)$, denote the probability that the process starting in state $m_0 = (c_0, z_0, p_0)$ at time zero, is in state $m = (c, z, p)$ at time t, i.e., $P(m; t, m_0) := P(M(t) = m \mid M(0) = m_0)$. It is assumed, as elaborated upon in [23], that the pure jump process M has the Markov property and therefore it is a Markov pure jump process.

The infinitesimal parameters for the Markov pure jump process M, according to (6) and [23, p. 172], are given by

$$
w((c, z+1, p), (c, z, p)) = \Omega k, \ w((c, z-1, p), (c, z, p))
$$
$$
= \Omega \delta, \ w((c+1, z-1, p-1), (c, z, p)) = k_{on} z \frac{(p)}{\Omega},
$$
$$
w((c-1, z+1, p+1), (c, z, p)) = k_{off} c
$$
$$
w((c, z, p), (c, z, p))
$$
$$
= -(w((c, z+1, p), (c, z, p)) + w((c, z-1, p), (c, z, p))
$$
$$
+ w((c+1, z-1, p-1), (c, z, p))
$$
$$
+ w((c-1, z+1, p+1), (c, z, p))),
$$
$$
\tag{7}
$$

where Ω denotes the volume of the system. $w(m, m_0)$ can be interpreted as transition probability per unit time from state m_0 to state m, once $m \neq m_0$. The infinitesimal parameters for all other transitions are zero.

As mentioned before, the total number of molecules of type C and $\mathcal{P}$ are constant. Hence, for all $t \geq 0$, $C(t) + \mathcal{P}(t) = p_T = C(0) + \mathcal{P}(0) = c_0 + p_0$ where p_T is the initial total number of molecules. For system (6) the forward equation or the Master equation that characterizes the evolution of the joint probability distribution of number of molecules of C and Z over time is as follows:

$$
\dot{P}_{C,Z}(c, z; t, m_0)
$$
$$
= \Omega[k P_{C,Z}(c, z-1; t, m_0) + \delta \frac{(z+1)}{\Omega} P_{C,Z}(c, z+1; t, m_0)
$$
$$
+ k_{on} \frac{(z+1)}{\Omega} \frac{(p_T - c + 1)}{\Omega} P_{C,Z}(c-1, z+1; t, m_0)
$$
$$
+ k_{off} \frac{(c+1)}{\Omega} P_{C,Z}(c+1, z-1; t, m_0)
$$
$$
- \left(k + \delta \frac{z}{\Omega} + k_{on} \frac{z(p_T - c)}{\Omega^2} + k_{off} \frac{c}{\Omega} \right) P_{C,Z}(c, z; t, m_0)],
$$
$$
\tag{8}
$$

where $P_{C,Z}(c, z; t, m_0)$ denotes the conditional probability that at the time t, $Z = z$ and $C = c$. We first investigate the steady state behavior of Master equation (8). Then, we study the transient behavior of the system, under the assumptions that binding(unbinding) of transcription factor Z to(from) the promoter sites $\mathcal{P}$ is sizably faster than the rate of expression of protein Z [24]. The transient time analysis includes singular perturbation analysis and analysis of the convergence of the probability distribution to the *stationary distribution*. Based on these analyses, we are able to achieve a reduced model for the joint probability distribution of the number of molecules that makes it possible to study the transient behavior of expectation and variance of Z. Consequently, we can analyze the concentration of Z and the effect of noise over time for the connected system, namely when the downstream system is present.

V. STATIONARY ANALYSIS

In this section, we show existence and uniqueness of the *stationary distribution* for stochastic processes (C, Z), $\pi_{C,Z}(c, z)$. Consequently, we show that regardless of the initial distribution of the process, the distribution of $(C(t), Z(t))$ converges to the *stationary distribution* π.

Theorem 5.1: The process (C, Z) has a unique *stationary distribution* $\pi_{C,Z}(c, z)$, which is the product of *stationary distribution* of the random process Z, $\pi_Z(z)$, and *stationary distribution* of the random process C, $\pi_C(c)$, i.e.,

$$
\pi_{C,Z}(c, z) = \pi_C(c) \pi_Z(z). \tag{9}
$$

Furthermore, the random process C has binomial *stationary distribution* as follows

$$
\pi_C(c) = \frac{p_T!}{c!(p_T - c)!(k_d k_z)^c} (1 + \frac{1}{k_d k_z})^{-p_T},
$$
$$
k_d := \frac{k_{off}}{k_{on}}, \ k_z := \frac{\delta}{k},
$$
$$
\tag{10}
$$

and the random process Z has Poisson *stationary distribution* given by

$$
\pi_Z(z) = \frac{\Omega_z^z}{z!} e^{-\Omega_z}, \ \Omega_z := \frac{\Omega}{k_z}. \tag{11}
$$

Moreover, $P((C(t), Z(t)) = (c, z)) \to \pi_{C,Z}(c, z)$ as $t \to \infty$.

Proof: The chemical reaction network (6) has a deficiency of zero and is weakly reversible as defined in [20]. Therefore, considering [20], the stationary distribution is is in

the form of sum of product of Poisson distributions for Z, C and P subject to the constraint $c + p = p_T$ which leads to the product of a binomial and Poisson distribution. ∎ According to (9) and (11), Z has a Poisson *stationary distribution* with expected value $E(Z) = \Omega_z = \frac{k}{\delta}\Omega$. This value is exactly the same as the expected value of the isolated system when the downstream system is not connected. Since the *stationary distribution* of Z is a Poisson distribution, the variance is the same as the mean, i.e., $Var(Z) = \Omega_z$ which is the same for the isolated system. Namely, stationary variance and mean do not depend on load.

VI. SINGULAR PERTURBATION ANALYSIS OVER COMPACT INTERVAL OF TIME

In this section, we seek to characterize the transient behavior of mean and variance of Z and study how they are affected by retroactivity. In system (6), the binding and unbinding reactions between Z and $\mathcal{P}$ are much faster than protein production and decay processes. Exploiting this property, we now introduce a singular perturbation analysis for Master equation (8), which results in a reduced model. Using the reduced order model, we analysis the effect of the downstream system on the upstream system and the transient behavior of Z.

Let us define the process Y as $Y := C + Z$. Let $P_{C,Y}(c, y; t, m_0)$ denote the conditional probability that at the time t, $C = c$ and $Y = y$, starting from state m_0 at the time 0. Defining $\epsilon := \frac{\delta}{k_{off}}$, $k_d := \frac{k_{off}}{k_{on}}$, $\bar{k}_{on} := \frac{\delta}{k_d}$, and $\bar{k}_{off} := \delta$, the Master equation (8) can be written in the following form:

$$
\begin{aligned}
\dot{P}_{C,Y}(c, y; t, m_0) &= \Omega[k P_{C,Y}(c, y-1; t, m_0) \\
&+ \delta \frac{(y-c+1)}{\Omega} P_{C,Y}(c, y+1; t, m_0) \\
&+ \frac{1}{\epsilon}\bar{k}_{on} \frac{(y-c+1)}{\Omega} \frac{(p_T-c+1)}{\Omega} P_{C,Y}(c-1, y; t, m_0) \\
&+ \frac{1}{\epsilon}\bar{k}_{off} \frac{(c+1)}{\Omega} P_{C,Y}(c+1, y; t, m_0) \\
&- \left(k + \delta\frac{y-c}{\Omega} + \frac{1}{\epsilon}\bar{k}_{on}\frac{(y-c)(p_T-c)}{\Omega^2} \right. \\
&\left. + \frac{1}{\epsilon}\bar{k}_{off}\frac{c}{\Omega}\right) P_{C,Y}(c, y; t, m_0)].
\end{aligned} \tag{12}
$$

The infinitesimal parameters for the process (C, Y) are

$$\omega((c, y), (c_0, y_0)) := w((c, y-c, p_T-c), (c_0, y_0-c_0, p_T-c_0)). \tag{13}$$

We provide two probability distributions evolving according to two different differential equations (or Master equations). These two probability distributions approximate the joint probability distribution $P_{C,Y}$ accurately, considering the aforementioned two time scale property. In other words, the probability distribution $P_{C,Y}$ is split into a fast evolving part and a slow evolving part P_Y. Because the fast part vanishes, the slow part will be the approximation of the main joint probability distribution $P_{C,Y}$ introduced above. Hence, analysis of the evolution of the joint probability distribution

is reduced to the analysis of the single distribution P_Y. This allows mathematical analysis of the system.

To provide the singular perturbation analysis, we transform the joint probability distribution $P_{C,Y}$ into a one-variable probability distribution so that we can invoke the results for singular perturbation theory for continuous Markov chains [22]. In this section, we provide an $O(\epsilon)$ approximation of the solution to (12). Let $P_Y(y; t)$ be the marginal probability distribution at the time instant t, i.e., $P_Y(y; t) := \sum_{c=0}^{\min y, p_T} P_{C,Y}(c, y; t)$, $E^s(C|Y = y) := \sum_{c=0}^{\min y, p_T} c \pi_{C|Y}(c|y)$, $\pi_{C|Y}(c|y) := \frac{\pi_{C,Y}(c,y)}{\pi_Y(y)}$. Define $P_Y^s(y; t)$ as the solution of the following forward equation:

$$
\begin{aligned}
\dot{P}_Y^s(y; t) &= \Omega[k P_Y^s(y-1; t) \\
&+ \delta \frac{(y+1-E^s(C|Y=y+1))}{\Omega} P_Y^s(y+1; t) \\
&- (k + \delta \frac{y - E^s(C|Y=y)}{\Omega}) P_Y^s(y; t)],
\end{aligned} \tag{14}
$$

with initial distribution $P_Y^s(y; 0) = P_Y(y; 0)$. Let $P_{C,Y}^f(c, y; \tau)$ denote the solution to the following forward equation

$$
\begin{aligned}
\frac{d}{dt} &P_{C,Y}^f(c, y; t) \\
&= \Omega[\bar{k}_{on} \frac{(y-c+1)}{\Omega} \frac{(p_T-c+1)}{\Omega} P_{C,Y}^f(c-1, y; t) \\
&+ \bar{k}_{off} \frac{(c+1)}{\Omega} P_{C,Y}^f(c+1, y; t) \\
&- (\bar{k}_{on} \frac{(y-c)(p_T-c)}{\Omega^2} + \bar{k}_{off}\frac{c}{\Omega}) P_{C,Y}^f(c, y; t)].
\end{aligned} \tag{15}
$$

with initial distribution $P_{C,Y}^f(c, y; 0) = P_{C,Y}(c, y; 0) - P_Y^s(y; 0)\pi_{C|Y}(c|y)$. The following Theorem provides an approximation of order ϵ of the solution to the forward equation (12).

Theorem 6.1: Let $P_Y^s(y; t)$ and $P_{C,Y}^f(c, y; t)$ be solutions to (14) and (15), respectively, with specified initial distributions and let $P_{C,Y}^e(c, y; t, \epsilon) := P_Y^s(y; t)\pi_{C|Y}(c|y) + P_{C,Y}^f(c, y; \frac{t}{\epsilon})$. Then for all $0 < T < \infty$,

$$
\begin{aligned}
&\sup_{t \in [0,T]} \|P_{C,Y}(\cdot, \cdot; t, \epsilon) - P_{C,Y}^e(\cdot, \cdot; t, \epsilon)\|_1 \\
&= \sup_{t \in [0,T]} \left\{ \sum_{y=0}^{\infty} \sum_{c=0}^{p_T} |P_{C,Y}(c, y; t, \epsilon) - P_{C,Y}^e(c, y; t, \epsilon)| \right\} \\
&= O(\epsilon).
\end{aligned} \tag{16}
$$

Moreover, there exists $\kappa > 0$ and $\alpha > 0$ such that $\|P_{C,Y}^f(\cdot, \cdot; \tau)\|_1 < \kappa e^{-\alpha\tau}$.

Proof: Let us first transform the joint probability distribution $P_{C,Y}(\cdot, \cdot; t) \in \mathbb{R}^{p_T} \times l_1$ to a single probability distribution $\tilde{P}(\cdot; t) \in l_1$ at each instant of time t. To this end, define $m_y := \min(y, p_T) + 1$ and $s_y := \sum_{i=0}^{y-1} m_i$ and

$$\tilde{P}(s_y + c; t) := P_{C,Y}(c, y; t). \tag{17}$$

Considering (12), the infinitesimal generator Q, [21], of the continuous Markov process $\tilde{P}$ is in the forms $Q = A/\epsilon + B$,

i.e.,

$$\frac{d}{dt}\tilde{P}(\cdot;t) = (A/\epsilon + B)\tilde{P}(\cdot;t), \tag{18}$$

where the generator A has the following diagonal form:

$$A = Diag\{A_0, \cdots, A_y, \cdots\}, \tag{19}$$

where A_y can be considered as the infinitesimal generator of an irreducible Markov chain with $V^y = \pi_{C,Y}(\cdot, y) \in \mathbb{R}^{\min(y,p_T)+1}$ being the stationary distribution and $V := Diag(\{V^y\})$. Considering asymptotic expansion method, [22], a set of two dynamics approximates $P(\cdot;t)$, a slow and a fast one. Let us define $\tilde{\mathbf{1}}$ with its ith column being

$$\tilde{\mathbf{1}}^i = [\overbrace{0, \cdots, 0}^{s_y}, \overbrace{1, \cdots, 1}^{m_y}, 0, \cdots]^T \tag{20}$$

and $\mathbf{v}(t) = \{v^0(t), \cdots, v^y(t), \cdots\}$ be the sequence in l^1 such that $\frac{d}{dt}\mathbf{v}(t) = \tilde{\mathbf{1}}^T B\tilde{P}_0(t)V\mathbf{v}(t)$ with $v^y(0) = P_Y(y, 0)$ and let $P_0(t) = \tilde{\mathbf{1}}\mathbf{v}(t)$. Moreover, let $\bar{P}_0(t)$ be the solution of the following differential equation

$$\frac{d}{d\tau}\bar{P}(\tau) = A\bar{P}(\tau), \tag{21}$$

with the initial condition $\bar{P}_0(0) = P(0) - P_0(0)$. It can be shown that if $\lambda(A_y)$ be the eigenvalue of A_y with the smallest non-zero absolute value, then $\inf_y \lambda(A_y) > 0$.

Therefore, employing Theorem 6.29 in [22], $\|\tilde{P}(t) - P_0(t) - \bar{P}_0(t/\epsilon)\|_1 = O(\epsilon)$ in $t \in [0, T]$. It can be shown that the dynamics of $v^y(t)$ is the same as (14). Hence, If we transform this expression to the space of joint probability distribution $\mathbb{R}^{p_T} \times l_1$, the Theorem is proved. ∎

VII. ANALYSIS OF TRANSIENT BEHAVIOR

In this section, we study the effect retroactivity on the dynamics of the expectation and variance of the output of the upstream component, i.e., Z. In the previous section, it is shown that if ϵ is sufficiently small, $P^e_{C,Y}(c, y; t, \epsilon)$ is an accurate approximation of the probability distribution $P_{C,Y}(c, y; t, \epsilon)$. Moreover, since $\|P^f_{C,Y}(c, y; t/\epsilon)\| < \kappa e^{-\alpha\frac{t}{\epsilon}}$, after a short interval of time $\bar{t}$, $P^s_Y(y; 0)\pi_{C|Y}(c|y)$ is an order ϵ approximation of the probability distribution $P_{C,Y}(c, y; t, \epsilon)$ over the time interval $[\bar{t}, T]$. Therefore, to study the transient behavior of the main Master equation (12), it is sufficient to study the dynamics of the reduced system (14). To derive the dynamics of $E(Z)$ and $Var(Z)$, we need to study the reduced Master equation (14) and therefore we need to characterize $E^s(C|Y = y)$ which appears as a nonlinear coefficient. According to (9) we have

$$\pi_{C|Y}(c|y) = \frac{\frac{1}{c!(p_T-c)!(\Omega k_d)^c(y-c)!}}{\sum_{c=0}^{\min(p_T,\Omega)} \frac{1}{c!(p_T-c)!(\Omega k_d)^c(y-c)!}}. \tag{22}$$

Therefore, as defined in the previous section, $E^s(C|Y = y)$ can be written as follows

$$E^s(C|Y = y) = \sum_{c=0}^{\min y, p_T} c\pi_{C|Y}(c|y)$$
$$= \frac{\sum_{c=0}^{\min(p_T,\Omega)} \frac{c}{c!(p_T-c)!(\Omega k_d)^c(y-c)!}}{\sum_{c=0}^{\min(p_T,\Omega)} \frac{1}{c!(p_T-c)!(\Omega k_d)^c(y-c)!}}. \tag{23}$$

Analyzing the transient behavior of the reduced system (14) requires good understanding of $E^s(C|Y = y)$ as a function of y. Such an understanding is hard to achieve from (23). Therefore, we provide an explicit function of y that approximates $E^s(C|Y = y)$ and show that this function is indeed a satisfactory approximation. Consequently, we use this approximation to analyze the transient behavior of the reduced system (14).

The following lemma provides a recursive expression for $E^s(C|Y = y)$ as a function of $E^s(C|Y = y-1)$.

Lemma 7.1:

$$E^s(C|Y = y) = \frac{[p_T - E^s(C|Y = y-1)]y}{p_T + k_d\Omega - E^s(C|Y = y-1)} \tag{24}$$
$$=: \Upsilon(E^s(C|Y = y-1), y),$$

with $E^s(C|Y = 0) = 0$.

It can be shown that the function $\hat{f}$ that is the fixed point of the map Υ at y, i.e., $\hat{f}(y) = \Upsilon(\hat{f}(y), y)$, is a good approximation of $E^s(C|Y = y)$. According to (24), $\hat{f}(y) = \frac{[p_T-\hat{f}(y)]y}{p_T+k_d\Omega-\hat{f}(y)}$ which can be converted to a second order algebraic equation with $\hat{f}$ being the solution in the following form

$$\hat{f}(y) = \frac{y + p_T + k_d\Omega - \sqrt{(y + p_T + k_d\Omega)^2 - 4yp_T}}{2}$$
$$\approx E^s(C|Y = y). \tag{25}$$

Assuming that k_d is sufficiently large compared to $\frac{p_T}{\Omega}$ and $\frac{y}{\Omega}$, which is often a reasonable assumption, we have

$$\hat{f}(y) = \frac{2yp_T}{y + p_T + k_d\Omega + \sqrt{(y + p_T + k_d\Omega)^2 - 4yp_T}}$$
$$\approx \frac{p_T}{p_T + k_d\Omega}y. \tag{26}$$

Defining $\beta := \frac{k_d\Omega}{k_d\Omega+p_T}$, we have that

$$E^s(C|Y = y) \approx (1-\beta)y. \tag{27}$$

From forward equation (14) and (27), according to [23], the following differential equation characterizes $E^s(Y; t)$

$$\frac{d}{dt}E^s(Y; t) = -\delta\beta E^s(Y; t) + k\Omega. \tag{28}$$

Moreover, according to [23], the second moment of the random variable Y evolves according to the following differential equation

$$\frac{d}{dt}E^s(Y^2; t) = E^s(2Y(k\Omega - \delta\beta Y); t) + E^s(\delta\beta Y + k\Omega; t)$$
$$- 2\delta\beta E^s(Y^2; t) + (2k\Omega + \delta\beta)E^s(Y; t) + k\Omega. \tag{29}$$

The time constant corresponding to the dynamics of the second moment of Y, i.e., $E^s(Y^2; t)$ is $\frac{1}{2\delta\beta}$, which is the time constant of the variance as well. The time constant corresponding to the dynamics of the mean of Y, i.e., $E^s(Y; t)$ is $\frac{1}{\delta\beta}$. These would be the time constants corresponding to the isolated system dynamics if we set $\beta = 1$. We see that

the dynamics of variance and mean of Y slow down when the system is connected, compared to the isolated system.

Having dynamics of mean and variance of Y as in (28) and (29), we now analyze dynamics of mean and variance of Z. From (27), we have

$$
\begin{aligned}
E^s(Z;t) &= E^s(Y-C;t) = E^s(E^s(Y-C|Y);t) \\
&= E^s(Y-E^s(C|Y);t) = E^s(Y-(1-\beta)Y;t) \\
&= \beta E^s(Y;t).
\end{aligned}
\tag{30}
$$

The second moment of Z can be written in the following form

$$
E^s(Z^2|Y=y) = E^s(Z(Y-C)|Y=y) = E^s(ZY|Y=y) \\
- E^s(ZC|Y=y).
$$

Considering the Lemma last lemma, it can be shown that $E^s((Y-C)C|Y=y) = E^s(C|Y=y-1)E^s(Y-C|Y=y)$. Hence, we have

$$
E^s(Z^2|Y=y) \\
= yE^s(Z|Y=y) - E^s(C|Y=y-1)E^s(Y-C|Y=y) \\
yE^s(Z|Y=y) - E^s(C|Y=y-1)E^s(Z|Y=y).
$$

Using approximation (27), we have

$$
E^s(Z^2|Y=y) \approx \beta y^2 - (1-\beta)\beta y(y-1) = \beta^2 y^2 + \beta(1-\beta)y.
$$

Therefore,

$$
E^s(Z^2;t) \approx \beta^2 E^s(Y^2;t) + \beta(1-\beta)E^s(Y;t).
\tag{31}
$$

Since the fastest eigenvalue corresponding to which $E^s(Y^2;t)$ and $E^s(Y;t)$ evolve over time is $2\delta\beta$, we conclude that the dynamics of variance and the mean of Z slow down when the system is connected ($\beta < 1$), compared to the isolated system ($\beta = 1$).

VIII. CONCLUSION

In this paper, we studied the stochastic effects of retroactivity in a transcriptional module connected to downstream systems. Exploiting the natural time scale separation of the processes that constitute the system, we developed singular perturbation analysis for the Master equation. Specifically, we provided a reduced Master equation describing the slow processes and demonstrated that the solution of the original Master equation fast approaches a neighbor of the solution of the reduced Master equation. Employing the reduced model, we mathematically analyzed how retroactivity impacts both transient and stationary behavior of the system. We observed that the upstream system and the downstream one are statistically independent at the steady state. However, the interconnection slows down the dynamics of both the expectation and the variance of the output of the upstream transcriptional module. These results are in contrast with the results obtained in [12, 13], in which approximated techniques, such as the Langevin equation and linear noise approximation, led to conclude statistical dependence between the upstream and downstream systems at steady state.

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