Time delays in a genetic positive-feedback circuit

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Abstract—Many principles of feedback control can be found implemented in complex biological networks. Dealing with transcription networks, positive feedbacks have been shown to frequently occur, providing biological toggle switches eventually leading a cell to its correct fate according to the proper stimulation. This note investigates the effects of delays related to the positive feedback of a basic transcription network. Motivation stems from the fact that, in spite of its toy-model features, the chosen transcription network is exploited to model the Tat feedback circuit that drives the HIV infected cells fate from active viral replication to latency. The delay is modeled by means of a cascade of transformations required to activate the transcription factor deputed to control: similar expedients are known to be exploited in cellular activities to schedule different biological functions at different timings. Our investigation is carried out by means of the stochastic approach, shown to be unavoidable to catch the noise-induced bimodality fashion of the circuit: by properly tuning the stochastic delay parameters, the regulatory circuit loses bimodality, and the transcription factor probability distribution converges to a Poisson distribution.

Index Terms—Chemical Master Equations, Positive feedback, Stochastic Systems, Systems Biology

I. Introduction

SYSTEMS Biology investigates the emergent properties of natural networks. To this end, system and control theory provide mathematical tools to tackle the extreme complexity of the biological systems by means of simplifying paradigms. Among these, the concept of feedback seems to be ubiquitous at every level of cellular investigation and, nowadays, there can be found different and diverse biological functions explained in terms of feedback loops, see e.g. [1], [25], [3] and references therein. Within this framework, similarly to the control-theoretic framework, positive feedbacks are known to produce bistability and biological toggle switches, eventually leading a cell to its correct fate according to the proper stimulation (see, e.g. [19], [9], [30], [12]).

This note investigates the effects of a delay in the positive feedback of a basic transcription network consisting of a gene whose expression is positively regulated by its own transcript. This kind of feedback occurs in the Tat protein circuit that regulates the switch between viral replication or latency in HIV infected cells. In more detail, after the HIV infection of a host cell and the consequent integration of the HIV RNA into the cellular DNA, the HIV enters a quiescent or an active replication state. Both cases are well known and clinically relevant (see, e.g. [20], [18] and references therein). The aforementioned cell fate is triggered by the Tat protein circuit: low Tat levels mean latency, whilst high Tat levels mean viral replication. Previous works have shown that such bimodal behavior can be explained only according to a stochastic approach since the deterministic model lacks bistability for physiologically relevant parameter regimes (see [27], [29], [28], [24], [24], [21]). Within the literature dealing with the analysis of the effects of noise in stochastic biological circuits, one can refer e.g. to [2], [5], [13], [15], [14] and references therein.

The novelty of the present work is to extend the modeling assumptions by assuming the existence of a delay in the positive feedback loop. In more detail, we assume that the Tat protein needs to be activated before exerting its feedback control role, and that such activation occurs at the end of a cascade of chemical modifications. Examples of cascades of activations are given by multisite phosphorylations, known to be involved in many cellular functions, e.g. whenever a specific timing is required for a sequence of scheduled biological activities such as the ones that regulate cell cycle (see, e.g. [17], [6] and also [14] as an example of cascade activation model with a priori unknown length). With regards to the delay, the use of a cascade characterizes the delay average value and coefficient of variation by means of independent parameters. This fact may be useful within a Synthetic Biology framework, whenever the goal is to investigate how to design synthetic circuits performing specific biological tasks (see, e.g. [8], [7] or the recent review [11] and references therein). Within this framework, different wirings such as a longer/shorter cascade or the presence/absence of a feedback loop may provide a different noise propagation eventually affecting the fluctuations of the average circuit outcomes, and a correct stochastic investigation may suggest which model parameters need to be varied (and how) in order to reduce the impact of noise [16], [4].

Similarly to the delay-free case discussed in [24], [21], also in the present framework bimodality may arise (consistently with experimental observations) and is a noise-induced phenomenon because the deterministic Ordinary Differential Equations (ODE) associated to the average value dynamics of the stochastic Chemical Master Equations (CME) exploited to model the transcription network under investigation fail to catch any bistable behaviors. CMEs are written for the general case but, as usual, they cannot be exploited to achieve the explicit solutions because of the *curse of dimensionality* [26]. Therefore, general results are provided by means of the Gillespie Stochastic Simulation Algorithm (SSA) [10], the numerical golden standard to solve CMEs by means of Monte Carlo simulations. The whole campaign of SSA simulations

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TABLE I
CHEMICAL REACTIONS

Event	Reset	Propensity
S_0 production, j bursts, $j = 1, 2, \ldots$	$n_0 \mapsto n_0 + j,$	$f(n_p)\mathbb{P}(\beta = j)$
S_i transformation, $i = 0, 1, \ldots, p-1$	$n_i \mapsto n_i - 1$ $n_{i+1} \mapsto n_{i+1} + 1$	$\lambda_i n_i$
S_p degradation	$n_p \mapsto n_p - 1$	γn_p

is carried out by varying the cascade parameters and results show that the delay plays a crucial role in determining whether bimodality arises or not: by increasing the delay, bimodality is definitively lost and the transcription factor stationary distribution converges to a Poisson distribution. Although being achieved by simulations, these results are supported by a theoretical analysis carried out on a reduced-order model obtained by fixing the length of the cascade to the smallest possible value.

II. MODEL SETTING

Consider protein S_0 undergoing a cascade of p functional modifications, ultimately allowing its final activation as S_p, Fig. 1. According to the stochastic approach, the state of the system is given by the copy number of the different protein modifications (namely, n_0, ..., n_p). S_p controls in positive feedback S_0 production, which occurs in noisy bursts [22], where the size burst β (i.e. the burst copy number) is a geometric random variable with probability distribution:

$$\mathbb{P}(\beta = j) = \alpha(1-\alpha)^{j-1}, \qquad \alpha \in (0,1), \quad j = 1, \ldots \quad (1)$$

The positive feedback is modeled by the Hill function:

$$f(n_p) = k_m \frac{b + cn_p^H}{1 + cn_p^H} \qquad \text{with} \quad 0 < b < 1. \quad (2)$$

Production, degradation and modifications resets are described in Table I.
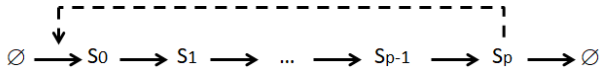

Fig. 1. Cascade of biochemical reactions

The mathematical model here reported is exploited to describe the Tat feedback circuit, where Tat is a protein essential for HIV replication, since it controls in positive feedback the expression of the gene deputed to its production. Experimental evidence has shown that the Tat feedback circuit has a unitary Hill coefficient [29], [28], [24], i.e. $H = 1$ in (2). Such a model has been investigated according to both the deterministic and stochastic approach in [21], where no cascade was considered (i.e. $p = 0$). In that case, for $H = 1$, the ODE model shows a unique stable equilibrium point regardless of the other parameter values, whilst the stochastic approach provides a stationary unimodal or bimodal distribution (the latter case with one of the two modes in zero) according to different parameter values. Therefore, bimodality is a noise-induced phenomenon [24].

Here we generalize the framework according to the scheme of Fig. 1, and investigate the role of the delay in terms of the length of the cascade (parameter p) and of the strength of the protein modifications (parameters λ_i). Aiming to exploit the model to investigate the Tat protein circuit, we assume $H = 1$ in the positive feedback. With respect to the stochastic delay, we suppose that $\lambda_i = p\lambda$ for any $i = 0, 1, \ldots, p-1$. This way, we constrain the time delay associated to any copy number that is transformed from S_i into S_{i+1} to the length of the cascade. Indeed, according to the CME stochastic approach [26], the waiting time for species S_i, $i < p$, to transit from n_i to $n_i - 1$ copy numbers because of the intermediate transformation $S_i \mapsto S_{i+1}$ (regardless of any other possible reactions) is an exponential random variable with average value $1/(\lambda_i n_i) = 1/(p\lambda n_i)$. Therefore, dealing with a specific molecule of the n_i copies of S_i, its waiting time before being transformed into a molecule of S_{i+1} will be n_i-times longer, i.e. an exponential random variable with average value $1/(p\lambda)$. As a matter of fact, the time delay associated to the cascade of protein transformations ultimately leading a molecule of S_0 to be activated into S_p is the sum of p exponentials, each of average value $1/(p\lambda)$, i.e. a Gamma distribution $\Gamma(p, \theta)$ with $\theta = 1/(p\lambda)$, average value $1/\lambda$ and Coefficient of Variation $CV^2 = 1/p$. This way, we can vary independently the average time delay (by means of the rate λ) and its CV (by means of the length of the cascade p).

III. DETERMINISTIC ODE MODEL

This section studies the qualitative behavior of the ODE model associated to the positive feedback scheme under investigation, in particular with respect to bistability.

Obtained by the first-order approximation of the average value dynamics, the ODE model is below reported, with the average copy numbers denoted by overbars:

$$\dot{\overline{n}}_0(t) = -p\lambda\overline{n}_0(t) + \frac{1}{\alpha}f(\overline{n}_p)$$
$$\dot{\overline{n}}_i(t) = -p\lambda\overline{n}_i(t) + p\lambda\overline{n}_{i-1}, \qquad i = 1, 2, \ldots, p-1 \quad (3)$$
$$\dot{\overline{n}}_p(t) = p\lambda\overline{n}_{p-1}(t) - \gamma\overline{n}_p$$

In case of $H = 1$ in (2), the ODE system (3) is monostable, since it admits a unique equilibrium point, regardless of the other parameter values. Indeed, by setting equal to zero the ODE dynamics in (3), one gets

$$\overline{n}_0 = \cdots = \overline{n}_{p-1} = \frac{1}{p\alpha\lambda}f(\overline{n}_p), \qquad \overline{n}_p = \frac{1}{\alpha\gamma}f(\overline{n}_p). \quad (4)$$

By construction, the Hill function in the right-hand-side of (4) starts with a positive value in zero (i.e. $f(n_p = 0) = k_m b > 0$), it is monotonically increasing (because $f'(n_p) > 0$ for $n_p > 0$), and it asymptotically reaches a plateau for $n_p \mapsto +\infty$ (since $\lim_{n_p \mapsto +\infty} f(n_p) = k_m$). Moreover, for $H = 1$, the Hill function does not change its convexity (its second derivative is negative for $n_p > 0$), therefore it admits a unique intersection with the straight line at the left-hand-side of (4).

Remark 1: It is apparent from the steady-state equation (4) that, although intermediate stationary states depend on the delay introduced by the cascade, the final product accumulation does not depend on the delay (either in terms of the cascade

length p, or in terms of the rate λ). In other words, the delay does not affect the qualitative behavior of the deterministic ODE model (that lacks bistability for $H = 1$), nor it affects the average value of the accumulation of the final product $\bar{n}_p$.

IV. Stochastic CME model

This section is devoted to studying the stationary distribution associated to the marginal probability of the final product S_p, investigating whether bimodality arises or not despite the lack of bistability in the ODE model.

Let $P_{0,\cdots,p}(n_0, \ldots, n_p)$ denote the joint probability distribution associated to the CME. For the sake of a more compact notation, in the following formulas, we explicitly report entry i, for $i = 0, \ldots, p$, in $P_{0,\cdots,p}$ only if different than n_i. Then, the CME associated to a generic point $(n_0, \ldots, n_p)$ is:

$$
\begin{aligned}
\frac{dP_{0,1,\cdots,p}}{dt} &= \gamma(n_p + 1)P_{0,\cdots,p}(\ldots, n_p + 1) - \gamma n_p P_{0,\cdots,p} \\
&+ p\lambda(n_0 + 1)P_{0,\cdots,p}(n_0 + 1, n_1 - 1, \ldots) + \cdots \\
&+ p\lambda(n_{p-1} + 1)P_{0,\cdots,p}(\ldots, n_{p-1} + 1, n_p - 1) \\
&- p(\lambda n_0 + \cdots + \lambda n_{p-1})P_{0,\cdots,p} \\
&+ \sum_{j=1}^{n_0}(1 - \alpha)^{j-1}\alpha f(n_p)P_{0,\cdots,p}(n_0 - j, \ldots) \\
&- \sum_{j=1}^{+\infty}(1 - \alpha)^{j-1}\alpha f(n_p)P_{0,\cdots,p}
\end{aligned}
$$

(5)

Since we are interested in the final product stationary distribution, we need to set the CME equal to zero and compute the stationary marginal distribution $P_{ss,p}(n_p)$ by properly summing the joint distribution with respect to all other entries $n_0, n_1, \ldots, n_{p-1}$. After computations, we obtain the following constraint:

$$
\begin{aligned}
&\gamma(n_p + 1)P_{ss,p}(n_p + 1) - \gamma n_p P_{ss,p}(n_p) \\
&= p\lambda \sum_{n_{p-1}=1}^{+\infty} n_{p-1}\big(P_{ss,p-1,p}(n_{p-1}, n_p) \\
&\hspace{4cm} - P_{ss,p-1,p}(n_{p-1}, n_p - 1)\big)
\end{aligned}
$$

(6)

where $P_{ss,p-1,p}$ denotes the stationary joint distribution with respect to the pair (n_{p-1}, n_p). The presence of such joint probability prevents the possibility to carry out exact computation without moment closure techniques. Therefore, in order to provide most general results, the investigation is carried out according to a large campaign of Monte Carlo simulations achieved by means of the Gillespie SSA [10], the numerical approach usually exploited for approximating the CME solution, since it provides accurate estimates of the probability distributions associated to the reaction network, and is useful even when the average copy number becomes high and explicit CME computations [31] are unfeasible.

Except for the cascade parameters p and λ (supposed to vary), the other parameter values have been fixed to the ones reported in Table II, complying with the constraints provided in [21] that ensure bimodality for the delay-free case. The following simulation results, obtained via SSA exploiting the

TABLE II
Model parameters

Parameter	b	H	c	k_m	γ	α
Value	0.05	1	0.6162	0.15	0.075	0.0375

apparent ergodicity of the stochastic model (see Table I and reference [31] for further details), are obtained:

- the n_p stationary marginal distribution definitively loses its bimodal fashion for sufficiently small values of λ (i.e. large values of average delay), as shown in Fig. 2, where it seems to approach the same (Poisson) distribution, independently of the length of the cascade (hence, for any $p \geq 1$). This fact is coherent with the results achieved in Section III, where it is shown that the stationary average value of n_p does not depend of the delay parameters, since for a Poisson distribution the average value unequivocally determines the shape of the distribution;
- for a fixed length of the cascade p, computations of moments for λn_i, $i < p$, highlight that $\langle \lambda n_i \rangle = \lambda \langle n_i \rangle$ is approximately constant with respect to λ, and the variance of λn_i goes to zero when λ decreases. As a consequence, for $\lambda \mapsto 0$, the random variable λn_i tends to a finite deterministic value $\lambda \langle n_i \rangle$, although $\langle n_i \rangle$ becomes larger and larger. Moreover, from the ODE model (4) at the equilibrium, one further obtains that $\lambda \langle n_i \rangle \simeq \gamma \langle n_p \rangle$;
- with regards to the case $p = 1$, the marginal Poisson distribution achieved for $\lambda \mapsto 0$ characterizes a 1D continuous-time Markov process with one-step linear clearance γn_1 and one-step constant birth propensity equal to $\gamma \langle n_1 \rangle$, which approximately holds for small λ (as discussed above). Fig. 3 shows the stationary marginal distributions for $p = 1$ getting closer to the Poisson distribution as long as λ decreases.
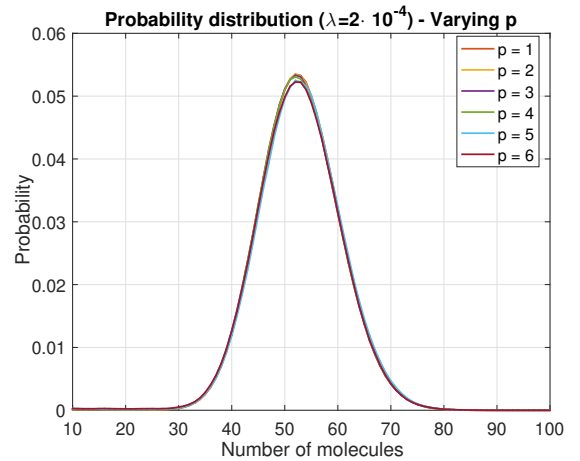

Fig. 2. Stationary distribution for small λ ($= 2 \cdot 10^{-4}$) and varying p.

Since Fig. 2 seems to imply that mono-modality holds for sufficiently small λ, independently of p, it is of interest to study (via SSA) the minimum λ to recover the bi-modal behavior, which we will call $\lambda_{\min}^{bimod}(p)$. This time, the results seem to depend on p and are summarized in Table III.

Number of stages p	$\lambda_{\min}^{bimod}(p)$
1	0.042
2	0.040
3	0.036
4	0.033

TABLE III
MINIMUM VALUE OF λ INDUCING A BIMODAL BEHAVIOR, AS A FUNCTION
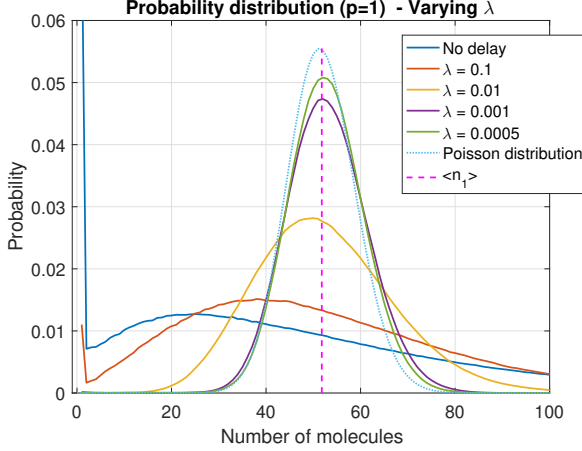OF THE NUMBER OF CASCADE STAGES p

Fig. 3. Stationary distribution of n_1 for varying λ.

V. STATIONARY MARGINAL DISTRIBUTION FOR $p = 1$

The aim of the Section is to theoretically support the previous numerical results in the simplified case of $p = 1$.

For $p = 1$, then the CME (5) reduces to:

$$\frac{dP_{0,1}}{dt} = \gamma(n_1 + 1)P_{0,1}(n_0, n_1 + 1) - \gamma n_1 P_{0,1}(n_0, n_1)$$
$$+ \lambda(n_0 + 1)P_{0,1}(n_0 + 1, n_1 - 1) - \lambda n_0 P_{0,1}(n_0, n_1)$$
$$+ \sum_{j=1}^{n_0}(1 - \alpha)^{j-1}\alpha f(n_1)P_{0,1}(n_0 - j, n_1)$$
$$- \sum_{j=1}^{+\infty}(1 - \alpha)^{j-1}\alpha f(n_1)P_{0,1}(n_0, n_1)$$

(7)

and the constraint in (6) reduces to:

$$\gamma(n_1 + 1)P_{ss,1}(n_1 + 1) - \gamma n_1 P_{ss,1}(n_1)$$
$$= \lambda \sum_{n_0=1}^{+\infty} n_0 \big(P_{ss,0,1}(n_0, n_1) - P_{ss,0,1}(n_0, n_1 - 1) \big).$$

(8)

Before stating the main result of the Section, we introduce the following notation for a class of moments associated to the joint distribution

$$\Xi^k(n_1) = \sum_{n_0=1}^{+\infty} n_0^k P_{0,1}(n_0, n_1)$$
$$= \left(\sum_{n_0=1}^{+\infty} n_0^k P_{0|1}(n_0|n_1) \right) P_1(n_1) = \langle n_0^k | n_1 \rangle P_1(n_1),$$

(9)

where the brackets in (9) shortly denote the conditional expectation. Then, the following preliminary result is provided.

Proposition 1: The stationary marginal distribution $P_{ss,1}(n_1)$ satisfies the following constraints

$$P_{ss,1}(n_1+1) = \frac{\lambda}{\gamma(n_1 + 1)}\Xi_{ss}^1(n_1), \qquad n_1 = 0, 1, \ldots, \quad (10)$$

where $\Xi_{ss}^1(n_1)$ is the stationary value of the first-order moment defined in (9) and fulfills the following constraints:

$$\gamma\Big((n_1 + 1)\Xi_{ss}^1(n_1 + 1) - n_1\Xi_{ss}^1(n_1)\Big)$$
$$+ \lambda\Big(\Xi_{ss}^2(n_1 - 1) - \Xi_{ss}^2(n_1) - \Xi_{ss}^1(n_1 - 1)\Big) \qquad (11)$$
$$+ \frac{f(n_1)}{\alpha}P_{ss,1}(n_1), \qquad n_1 > 0$$

with the initial condition

$$\gamma\Xi_{ss}^1(1) - \lambda\Xi_{ss}^2(0) + \frac{f(0)}{\alpha}P_{ss,1}(0). \qquad (12)$$

Proof. Because of (9), the constraint in (8) becomes

$$\gamma(n_1 + 1)P_{ss,1}(n_1 + 1) - \gamma n_1 P_{ss,1}(n_1)$$
$$= \lambda\big(\Xi_{ss}^1(n_1) - \Xi_{ss}^1(n_1 - 1)\big) \qquad (13)$$

for $n_1 > 0$, and reduces to

$$\gamma P_{ss,1}(1) = \lambda\Xi_{ss}^1(0) \qquad (14)$$

for $n_1 = 0$. According to iterative substitutions, we have that (14) can be generalized into (10). In order to derive a recursive equation for $\Xi_{ss}^1(n_1)$, and couple it to (10), we compute the dynamics of $\Xi^1(n_1)$ from the CME (7):

$$\frac{d\Xi^1(n_1)}{dt} = \sum_{n_0=1}^{+\infty} n_0 \frac{dP_{0,1}(n_0, n_1)}{dt}$$
$$= \gamma \sum_{n_0=1}^{+\infty} n_0(n_1 + 1)P_{0,1}(n_0, n_1 + 1)$$
$$- \gamma \sum_{n_0=1}^{+\infty} n_0 n_1 P_{0,1}(n_0, n_1)$$
$$+ \lambda \sum_{n_0=1}^{+\infty} n_0(n_0 + 1)P_{0,1}(n_0 + 1, n_1 - 1)$$
$$- \lambda \sum_{n_0=1}^{+\infty} n_0^2 P_{0,1}(n_0, n_1)$$
$$+ \sum_{n_0=1}^{+\infty}\sum_{j=1}^{n_0}(1 - \alpha)^{j-1}\alpha n_0 f(n_1)P_{0,1}(n_0 - j, n_1)$$
$$- \sum_{n_0=1}^{+\infty}\sum_{j=1}^{+\infty}(1 - \alpha)^{j-1}\alpha n_0 f(n_1)P_{0,1}(n_0, n_1).$$

(15)

Notice that by changing the order of the sums in the second-to-last terms in (15) we have

$$
\sum_{j=1}^{+\infty} \sum_{n_0=j}^{+\infty} (1-\alpha)^{j-1}\alpha n_0 f(n_1) P_{0,1}(n_0-j,n_1)
$$
$$
= \sum_{j=1}^{+\infty} \sum_{n_0=0}^{+\infty} (1-\alpha)^{j-1}\alpha(n_0+j) f(n_1) P_{0,1}(n_0,n_1)
$$
$$
= \sum_{n_0=1}^{+\infty} \sum_{j=1}^{+\infty} (1-\alpha)^{j-1}\alpha n_0 f(n_1) P_{0,1}(n_0,n_1)
$$
$$
+ \frac{f(n_1)}{\alpha} P_1(n_1) \tag{16}
$$

so that the sum of the last two terms in (15) simplifies into $\frac{f(n_1)}{\alpha}P_1(n_1)$. In summary, after further computations, we obtain the stationary solution provided by (11)-(12). ∎

Unfortunately, eq. (11) cannot be straightforwardly exploited, since it involves the second order conditional moments $\Xi_{ss}^2(n_1)$. This is a typical drawback arising in CMEs, dealing with non-closed moment equations, whenever nonlinear propensities are exploited. Different approximations can be found in the literature in order to close the moment equations: the approach we follow somewhat resembles the one that allows to write first-order moment equations by substituting higher-order moments as nonlinear functions of first-order moments [23], [26]. In fact, according to the definition in (9), one has:

$$
\Xi^2(n_1) = \langle n_0^2 | n_1 \rangle P_1(n_1). \tag{17}
$$

If we expand with Taylor n_0^2 around its conditional expectation value $\langle n_0|n_1\rangle = \sum_{n_0=1}^{+\infty} n_0 P_{0|1}(n_0|n_1)$, and approximate it up to the linear terms, we have

$$
n_0^2 \simeq \left(\langle n_0|n_1\rangle\right)^2 + 2\langle n_0|n_1\rangle \left(n_0 - \langle n_0|n_1\rangle\right). \tag{18}
$$

By substituting (18) into $\langle n_0^2|n_1\rangle$, we obtain:

$$
\langle n_0^2|n_1\rangle \simeq \left(\langle n_0|n_1\rangle\right)^2 \tag{19}
$$

and so

$$
\Xi^2(n_1) \simeq \left(\langle n_0|n_1\rangle\right)^2 P_1(n_1) = \left(\Xi^1(n_1)\right)^2/P_1(n_1). \tag{20}
$$

Remark 2: It is worth noticing that the approximation (19) is closer and closer to reality for conditional probability distributions of $n_0|n_1$ with a smaller and smaller variance. This information could be *a-posteriori* exploited by Monte Carlo simulations (SSA) to assess the validity of (19), which approximately holds in our case for the chosen set of parameters and $p=1$.

Plug the approximation (20) into the stationary equation coming from (11). Then, after computations, one gets:

$$
\gamma\Big((n_1+1)\Xi(n_1+1) - n_1\Xi(n_1)\Big) + \frac{f(n_1)}{\alpha}P_1(n_1)
$$
$$
+\lambda\left(\frac{\left(\Xi(n_1-1)\right)^2}{P_1(n_1-1)} - \frac{\left(\Xi(n_1)\right)^2}{P_1(n_1)} - \Xi(n_1-1)\right) = 0 \tag{21}
$$

where $\Xi_{ss}^1(\cdot)$ and $P_{ss,1}(\cdot)$ have been substituted by $\Xi(\cdot)$ and $P_1(\cdot)$ for short. Then, by further substituting (10) into (21)

we obtain the following recursive equation for the stationary marginal distribution P_1:

$$
\frac{\gamma^2}{\lambda}n_1\Big((n_1+1)P_1(n_1+1) - (n_1-1)P_1(n_1)\Big)
$$
$$
+ \frac{f(n_1-1)}{\alpha}P_1(n_1-1)
$$
$$
+\gamma\left(\frac{\gamma(n_1-1)^2}{\lambda}\frac{P_1^2(n_1-1)}{P_1(n_1-2)} - \frac{\gamma n_1^2}{\lambda}\frac{P_1^2(n_1)}{P_1(n_1-1)}\right)
$$
$$
- (n_1-1)P_1(n_1-1)\Big) = 0 \tag{22}
$$

recursively providing $P_1(n_1+1)$ as a function of $P_1(n_1)$, $P_1(n_1-1)$ and $P_1(n_1-2)$. Initialization derives by substituting (14) and (20) into (12), providing the constraint:

$$
\frac{2\gamma^2}{\lambda}P_1(2) - \frac{\gamma^2}{\lambda}\frac{P_1^2(1)}{P_1(0)} + \frac{f(0)}{\alpha}P_1(0) = 0. \tag{23}
$$

It clearly comes from (22)-(23) that the whole marginal distribution $P_1(n_1)$ may be written with respect to the unknown probability pair $P_1(0)$, $P_1(1)$, that requires to be identified by means of the usual normalization constraint

$$
\sum_{n_1=0}^{+\infty} P_1(n_1) = 1. \tag{24}
$$

Positive feedback circuits with extremely long delays

When substituting $\lambda = 0$ in the reset maps of Table I, transformations from any S_0 into S_1 are forbidden, the marginal distribution of n_1 is a flat zero distribution for $n_1 > 0$ with $P_{ss,1}(n_1 = 0) = 1$, since only degradations are admitted for S_1, and n_0 definitively increases without reaching a stationary distribution since only n_0 productions are admitted. Such a behavior is lost when making the limit for $\lambda \mapsto 0$ in the CME solution. Numerical simulations reported in the previous section suggest that the stationary probability distribution $P_{ss,p}(n_p)$ converges to a Poisson distribution for any value of $p > 0$ (see Fig. 2). As a matter of fact, bimodality is lost by sufficiently increasing the average delay $1/\lambda$. Below, a theorem is reported that proves the correctness of this conjecture for the approximated marginal distribution achieved for $p=1$.

Theorem 1: Let $p = 1$. Then, the marginal distribution $P_1(n_1)$ written according to approximation (20) converges to a Poisson distribution for $\lambda \mapsto 0$.

Proof. According to approximation (20), $P_1(n_1)$ satisfies the constraint in (22). By taking the limit for $\lambda \mapsto 0$, terms that do not have λ at the denominator in (22) are negligible with respect to the others, therefore such a recursive equation simplifies into:

$$
n_1\Big((n_1+1)P_1(n_1+1) - (n_1-1)P_1(n_1)\Big)
$$
$$
+ (n_1-1)^2\frac{P_1^2(n_1-1)}{P_1(n_1-2)} - n_1^2\frac{P_1^2(n_1)}{P_1(n_1-1)} = 0. \tag{25}
$$

Analogously, (23) converges to the following constraint:

$$
2P_1(2) - \frac{P_1^2(1)}{P_1(0)} = 0. \tag{26}
$$

Given the pair of initial conditions $P_1(0)$, $P_1(1)$, the pair of recursive equations (25)-(26) clearly provides a unique solution. Such solution writes as:

$$P_1(n_1) = \frac{1}{n!} \frac{P_1^{n_1}(1)}{P_1^{n_1-1}(0)}, \qquad n_1 = 2, 3, \ldots \qquad (27)$$

The proof that (27) satisfies constraints (25)-(26) is readily achieved by mathematical induction. By further considering the normalization constraint (24), we have:

$$P_1(0) + P_1(1) + \sum_{n_1=2}^{+\infty} P_1(n_1)$$
$$= \sum_{n_1=0}^{+\infty} \frac{P_1(0)}{n!} \left(\frac{P_1(1)}{P_1(0)}\right)^{n_1} = P_1(0) e^{\frac{P_1(1)}{P_1(0)}} = 1 \qquad (28)$$

so that $P_1(0)$ and $P_1(1)$ are required to satisfy the constraint

$$P_1(1) = -P_1(0) \ln\big(P_1(0)\big). \qquad (29)$$

By substituting (29) in (27) we have

$$P_1(n_1) = \frac{1}{n!} P_1(0) \left(\ln\left(\frac{1}{P_1(0)}\right)\right)^{n_1}, \qquad n_1 = 2, 3, \ldots \qquad (30)$$

which is, actually, a Poisson distribution with parameter $\rho = -\ln\big(P_1(0)\big)$. ∎

VI. Conclusions

This work has investigated the emergence of noise-induced bimodality in a positive feedback circuit. From the application viewpoint, this circuit represents the Tat-feedback circuit encoded by HIV, which has been shown to control the outcome of a cellular infection between virus active replication and latency, where the virus becomes dormant inside the cell. Interestingly, while the Tat-feedback circuit is monostable (i.e. it lacks bistability), it shows a bimodal distribution of Tat, with high (respectively, low) Tat levels corresponding to virus replication (respectively, latency). Here we have investigated the presence of delays in the Tat-feedback circuit and have shown that a delay can convert a bimodal distribution into a Poisson distribution where Tat levels are always high. This result has important implications for therapy – introducing delays through decoy-binding sites or small molecule drugs can prevent low Tat levels, and hence prevent cells from becoming latent. Latency is currently considered the biggest obstacle for purging the virus from the patient, as latently infected cells cannot be targeted by antiretroviral drug therapy or by the host's immune response [24], [18].

References

[1] U. Alon, *An Introduction to Systems Biology: Design Principles of Biological Circuits*, Chapman and Hall/CRC, 2006.

[2] R. Bahar, C.H. Hartmann, K.A. Rodriguez, A.D. Denny, R.A. Busuttil, M.E. Dollé, R.B. Calder, G.B. Chisholm, B.H. Pollock, C.A. Klein and J. Vijg, Increased cell-to-cell variation in gene expression in ageing mouse heart, *Nature*, 441, 1011-1014, 2006.

[3] F. Blanchini, H. El-Samad, G. Giordano, E.D. Sontag, Control-theoretic methods for biological networks, *Proc. IEEE Conf. Dec. Control*, pp.466-483, 2018.

[4] A. Borri, P. Palumbo, A. Singh, Impact of negative feedback in metabolic noise propagation, *IET Systems Biology*, Vol. 10 (5), pp. 179-186, 2016.

[5] F.J. Bruggeman, N. Blüthgen, H.V. Westerhoff, Noise management by molecular networks, *PLoS Computational Biology*, 5(9), 1-11. 2009.

[6] A. Brümmer, C. Salazar, V. Zinzalla, L. Alberghina, and T. Höfer, Mathematical modelling of DNA replication reveals a trade-off between coherence of origin activation and robustness against rereplication, *PLoS Comput. Biol.*, 6(5), 2010, Art. no. e1000783.

[7] D. Del Vecchio, E.D. Sontag, Engineering principles in biomolecular systems: from retroactivity to modularity, *European Journal of Control*, 3-4, 389-397, 2009.

[8] D. Del Vecchio, E.D. Sontag, Synthetic biology: A systems engineering perspective, *Control Theory and Systems Biology*, 101–124, 2009.

[9] J.E. Ferrell Jr, S.H. Ha, Ultrasensitivity part III: cascades, bistable switches, and oscillators, *Trends Biochem Sci.*, 9:612-618, 2014.

[10] D. T. Gillespie, Exact Stochastic Simulation of Coupled Chemical Reactions, *The Journal of Physical Chemistry* 81(25), 23402361, 1977.

[11] V. Hsiao, A. Swaminathan, R.M. Murray, Control Theory for Synthetic Biology, *IEEE Control Systems Magazine*, pp.32-62, June 2018.

[12] Q.A. Justman, Z. Serber, J.E. Ferrell Jr., H. El-Samad, K.M. Shokat, Tuning the Activation Threshold of a Kinase Network by Nested Feedback Loops, *Science*, 324:509–512, 2009.

[13] D.J. Kiviet, P. Nghe, N. Walker, S. Boulineau, V. Sunderlikova, S.J. Tans, Stochasticity of metabolism and growth at the single-cell level. *Nature*, 514, 376–379, 2014.

[14] G. Neuert, B. Munsky, R. Zhen Tan, L. Teytelman, M. Khammash, A. van Oudenaarden, Systematic Identification of Signal-Activated Stochastic Gene Regulation, *Science*, 2013.

[15] T.M. Norman, N.D. Lord, J. Paulsson, R. Losick, Stochastic switching of cell fate in microbes,, *Annual Review of Microbiology*, 69, pp.381-403, 2015.

[16] D.A. Oyarzun, J.-B. Lugagne, G.-B.V. Stan, Noise propagation in synthetic gene circuits for metabolic control, *ACS Synthetic Biology*, 2014.

[17] P. Palumbo, M. Vanoni, C. Manes, V. Cusimano, S. Busti, F. Marano, L. Alberghina, Whi5 phosphorylation embedded in the G1/S network dynamically controls critical cell size and cell fate, *Nat. Commun.*, 7, 2016, 11272.

[18] D.D. Richman, D.M. Margolis, M. Delaney, W.C. Greene, D. Hazuda, R.J. Pomerantz, The challenge of finding a cure for HIV infection, *Science*, 323, pp. 1304–1307, 2009.

[19] M.A. Savageau, Comparison of classical and autogenous systems of regulation in inducible operons, *Nature*, 252, pp. 546–549, 1974.

[20] N. Seth, D. Kaufmann, T. Lahey, E.S. Rosenberg, K.W. Wucherpfennig, Expansion and contraction of HIV-specific CD4 T cells with short bursts of viremia, but physical loss of the majority of these cells with sustained viral replication, *J. of Immunol.*, 175, pp. 6948–6958, 2005.

[21] A. Singh, Stochastic analysis of genetic feedback circuit controlling HIV cell-fate decision, *Proc. 51st IEEE Conf. Dec. & Control*, 4918-4923, 2012.

[22] A. Singh, J.P. Hespanha, Optimal feedback strength for noise suppression in autoregulatory gene networks, *Biophysical Journal*, 96, pp. 4013-4023, 2009.

[23] A. Singh, J.P. Hespanha, Approximate moment dynamics for chemically reacting systems, *IEEE Transactions on Automatic Control,* 56(2), 414-418, 2011.

[24] A. Singh, L.S. Weinberger, Stochastic gene expression as a molecular switch for viral latency, *Current Opinion in Microbiology* 12, 460-466, 2009.

[25] E.D. Sontag, Molecular systems biology and control, *European J. Control*, 11(45):396–435, 2005.

[26] N.G. van Kampen, *Stochastic Processes in Physics and Chemistry*, North Holland, third edition, 2007.

[27] L.S. Weinberger, J. Burnett, J. Toettcher, A. Arkin, D. Schaffer, Stochastic gene expression in a lentiviral positive-feedback loop: HIV-1 tat fluctuations drive phenotypic diversity, *Cell*, 122, 169-182, 2005.

[28] L.S. Weinberger, R.D. Dar, M.L. Simpson, Transient-mediated fate determination in a transcriptional circuit of HIV, *Nature Genetics*, 40, 466-470, 2008.

[29] L.S. Weinberger, T. Shek, An HIV feedback resistor: autoregulatory circuit deactivator and noise buffer, *PLoS Biology*, 5, 2007.

[30] W. Xiong, J.E. Ferrell Jr., Positive-feedback-based bistable memory module that governs a cell fate decision, *Nature*, 426:460–465, 2003.

[31] A. Borri, F. Carravetta, G. Mavelli, P. Palumbo, Block-tridiagonal state-space realization of Chemical Master Equations: A tool to compute explicit solutions, *Journal of Computational and Applied Mathematics*, Vol. 296, 410–426, 2016.