Time Sharing for Transcription

Diego Leonardo Castelo España
Facultat de Física, Universitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain.

Advisor: Marta Ibañes Miguez

Abstract: In this work, we study the partitioning called Time-sharing. Specifically, it is studied the case of the competition for RNA Polymerase (RNAP) by the transcription sigma factors in the bacteria *Bacillus subtilis*. Each sigma factor associates with RNAP, and the dynamics of the complex association is randomly pulsatile but with time-sharing. Thanks to the algorithm programmed by us, we could replicate the competition for RNAP and we conclude that sigma factors do not pulse at the same time, verifying we got time-sharing in our simulations.

I. INTRODUCTION

The study of the dynamics in biological systems is essential in order to understand specific behaviors and responses. It has been found that many biological systems show dynamics such as oscillations or random transient activations. The spectrum of pulsatile dynamics covers a large range of temporal scales, as is reviewed in [1]. ATP and AMP production occurs periodically, with periods of 2 min and 5 min, respectively. Circadian cycle has pulses with periods of 24h. Oscillations, called Mayer Waves, in cardiovascular system rhythms are another example. They have periods around 10s [2]. The tumor suppressor p53 oscillates too: the γ-radiation activates a pulsing p53 activity whose pulse number depends on radiation dose (reviewed in [3]).

But how do we understand pulsing? And what do these dynamics and examples have in common? A pulsing dynamics are defined as an evolution of the system with repeated events over time. Pulsatile dynamics can be very different, from irregular and stochastic to more uniform and periodic [3]. Common features are the existence of delayed negative feedbacks and fluctuations [3]; a feedback could be a complex process to create a protein or substance that, afterwards, interacts with the original reactors, increasing the primary reaction (positive feedback) or negatively altering it (negative feedback) [1]. The delay could be provoked by a physical restriction, like the minimal time to execute the transcription, by the interactions with multiple reactions, like a set of negative feedbacks, or by a positive feedback [1]. Fluctuations are intrinsic features since chemical reactions are stochastic systems originating from random collisions between finite number of particles at a specific temperature, and, indeed, the examples above are chemical reactions.

Oscillations and some pulses can be considered as a structure described in the phase-space [4]. Here we can separate the oscillatory from the excitable regime. Pulses, in the excitable regime, can be understood like the excursion in phase space afterwards the system was subjected to a perturbation and moved out of balance [4] (see Fig. 1). Instead, oscillations in the oscillatory regime are a closed trajectory in phase space, due to the existence of a unique and unstable fixed point. Oscillations are continually formed because it is not possible to achieve balance. During the journey, in both regimes, the system is not able to start a new pulse; this time interval is called refractive time. The period of the oscillation is determined by the refractive time [4].

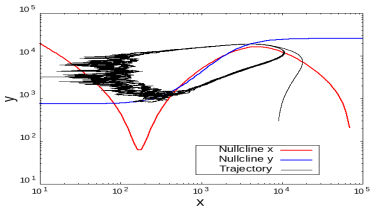

FIG. 1: Phase space for two variables, x and y, in the excitable regime. Nullclines (red and blue) and trajectory (black) of the system described by Eqs.[(8),(9)] are shown. The trajectory corresponds to the dynamics in the bottom panel of Fig. 3. Each nullcline corresponds to the null time derivative of the corresponding variable.

Another interesting behavior related to pulsing is Time-Sharing. Let's introduce it with the next example, the growth rate of two colonies of bacteria in limited nutrients conditions interacting and competing for nutrients between each other [5]. Each colony's growth exhibits an oscillatory dynamics. Common sense suggests an equal partitioning of the nutrients among the two colonies. However, it has been found that resources are divided with turns on time, what is called, Time-Sharing (Fig. 2) [5]. This consumption of nutrients on turns translates into a faster growth rate compared with the growth at higher concentrations of nutrients [5].

Here, we focus on another scenario. In response to external environmental changes, the gene expression in a cell can be altered through the gene transcription process. In the bacteria *Bacillus subtilis*, these alterations are controlled by the association of the protein sigma factor and the transcribing enzyme of the DNA, the RNA Polymerase (RNAP) [6]. Here, we examine another instance of time-sharing behavior and pulsing dynamics. We examine the competition for RNAP among multiple sigma factors in individual bacteria (*Bacillus subtilis*) with low RNAP concentration [7]. There are 17 proteins that are known as sigma-factors and each one has

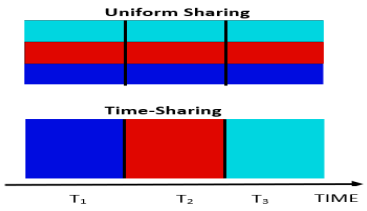

FIG. 2: Time-sharing visual guidance. Each color is the use of the resource by one consumer. The arrow illustrates an increase in time ($T_3 > T_2 > T_1$); T_i is a point in time. According to uniform sharing, the resource is constantly and equally divided over time. Time-sharing, instead, shows a use of the resource with turns on time among the consumers.

a group of elements that describes its dynamics. This information is crucial to visualize the variety of interactions and mechanisms that contribute to the competition. In other words, the complexity of the dynamics is enormous. Here, we use the mathematical model presented in [7] to describe the sigma factor pulsing dynamics and we compare it to other pulsing dynamics in order to better understand the nature of the system. We, then, look closely at the case of multiple sigma factors and reproduce part of the computational results in [7] competing all for RNAP. We focus on a few number of sigma-factors to comprehend their role in the overall pulsing dynamics.

II. RESULTS

A. The Model for Sigma Factors

Each sigma-factor pulses and its evolution is described by a set of equations proposed in [7]. The following components and associations hold true for all sigma factor types, but let's focus on just one sigma factor dynamics. The main characters are the next elements: the sigma-factor, the anti-sigma factor, the free and accessible RNAP, and the protein called Ligand. The sigma-factor can bind to the RNAP. This union produces through transcription free sigma-factor and its own inhibitor, the anti-sigma factor. The anti-sigma factor can bind to the sigma factor. The sigma-antisigma factor cannot bind the RNAP. Thus, the anti-sigma factor prevents the formation of union of RNAP-sigma factor. The ligand binds to the anti-sigma factor. By doing so, it prevents the anti-sigma factor from biding its sigma factor. The concentration of Ligand acts as an input of the system, playing the role of the perturbing fluctuation. In the equations of the system [(1)-(6)], the free sigma factor is S_i, the free anti-sigma factor is A_i, the free ligand is L_i, the union RNAP-sigma factor is RS_i; the association sigma factor-anti sigma factor is SA_i; and the free RNAP is R. The brackets mean concentration. The subindex i denotes the specific sigma factor, anti-sigma factor and ligand. Notice that each sigma factor has its own specific anti-sigma factor and this latter has its own specific ligand. The structure of equations is as follows: the rate of change of concentration over time is due to a complex association, a complex dissociation and a degradation (excluding Eq. (6)). Eqs. (1) and (2) have additional terms (transcription and regulation). The constant transcription term is α. The regulation term is actually a positive auto-regulation for the sigma factor Eq. (1) ($\beta_s[RS]_i$) and a positive regulation for the anti-sigma factor Eq. (2) ($\beta_a[RS]_i$) where β is the transcription rate. The parameters k are the binding or unbinding rates whenever there is an association or dissociation reaction. Finally the decay term corresponds to the degradation rate, δ, multiplied by its own protein [7]. The free RNAP is the total amount minus all the already associated to sigma factors. The parameter values are the ones used in [7] (set A).

$$\frac{d[S]_i}{dt} = \alpha_s + \beta_s[RS]_i + k_{rs-}[RS]_i + k_{sa-}[SA]_i - k_{rs+}[R][S]_i - k_{sa+}[S]_i[A]_i - \delta_s[S]_i, \quad (1)$$

$$\frac{d[A]_i}{dt} = \alpha_a + \beta_a[RS]_i + k_{sa-}[SA]_i + k_{al-}[AL]_i - k_{sa+}[S]_i[A]_i - k_{al+}[A]_i[L]_i - \delta_a[A]_i, \quad (2)$$

$$\frac{d[RS]_i}{dt} = k_{rs+}[R][S]_i - k_{rs-}[RS]_i - \delta_{rs}[RS]_i, \quad (3)$$

$$\frac{d[SA]_i}{dt} = k_{sa+}[S]_i[A]_i - k_{sa-}[SA]_i - \delta_{sa}[SA]_i, \quad (4)$$

$$\frac{d[L]_i}{dt} = k_{al-}[AL]_i - k_{al+}[A]_i[L]_i - \delta_l[L]_i, \quad (5)$$

$$[R] = R_{TOT} - \sum_i [RS]_i, \quad (6)$$

One sigma factor is distinct from the others. This is the housekeeping sigma factor. It has the highest affinity for RNAP and has no anti-sigma factor. Its behavior is described by the above equations, without the anti-sigma and Ligand connections. Hence it doesn't have a negative feedback and it is not able to pulse by itself.

The pulsatile concentration we have studied is the $[RS]_i$ (Fig. 3 top panel). Let's identify the essentials features to drive pulses in the above equations. The concentration of $[RS]_i$ increases with the accessible RNAP and the free sigma factor. As it is viewed in Eq. (1), the sigma factor has a positive production regulated by $[RS]_i$. So, at the end, $[RS]_i$ increases its own concentration by producing more sigma factor that can associate to RNAP. This process is the positive feedback. But, the anti-sigma factor has a production regulated by $[RS]_i$

(Eq. (2)). $[RS]_i$ produces anti-sigma factor that can bind to sigma factor, having less concentration of sigma factor that can associate to RNAP. This is the delayed negative feedback. The positive feedback causes an instantaneous increase of RNAP-sigma factor, making the pulse higher due to the sigma-factor production. The delayed negative feedback ends the pulse, and turns the system to the steady conditions.

We programmed the algorithm of Euler numerical method for ordinary differential equations in FORTRAN coding language and we were able to replicate the system. In this mathematical model, the external perturbation is to change the input ligand concentration. This is done by adding a random concentration of it, that follows an exponential distribution, in a randomly chosen time within a time interval T, for every successive time interval T. The time to change L_i has a uniform probability across T. Every time interval T, there is perturbation that starts the pulse. Perturbing L_i, we perturb the concentration of free anti-sigma factor and this perturbs the whole sigma factor dynamics.

B. Pulsing Dynamics

One goal of our project was to comprehend how a pulse is generated, and then, connect this knowledge with the sigma factor case. To this end, we studied two additional different models each of which drives very distinct pulsatile dynamics. These models are not related to sigma factors.

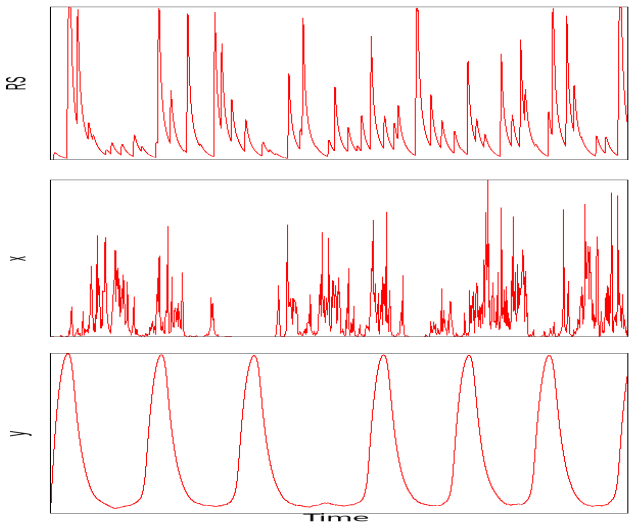

FIG. 3: Pulsing dynamics along time. The top panel is the dynamics of RNAP-sigma factor for only one sigma factor without any coupling. Dynamics from middle panel is described by Eq. (7). The bottom panel is the excitable regime for the variable y, described by Eqs. (8) and (9), and shown in phase space in Fig. 1. The figure has not any values in the axes because it is a visual comparison of their features.

The first dynamics (middle panel of Fig. 3), is highly stochastic. The properties of pulses are: variable intensities with random pulses on time, and with a variable width. Indeed, there isn't a refractive time, so the system can pulse again even if a pulse has already started. The model studied in this case involves only one mathematical variable [8]:

$$\frac{dx}{dt} = -x^3 + ax + x\sqrt{D}\xi(t), \tag{7}$$

Eq. (7) corresponds to the dissipative dynamics of the variable x in a potential that has two symmetrical minima. The parameters are $a = 0.1$ and $D = 1.0$. The pulses are caused due to the multiplicative noise that moves the system from the balance.

The features of the next case of pulsing dynamics (bottom panel in Fig. 3) are: constant pulse intensities with a refractive time. These pulses are born in an excitable regime, formed by a positive and negative feedback coordination. We can see this architecture in the next equations [4]. The parameter values are those on [4] circuit B Type I.

$$\frac{dx}{dt} = a_1 + \frac{x^n b_1}{(K_1)^n + x^n} - gxy - xd_1 + \sqrt{D}\xi(t), \tag{8}$$

$$\frac{dy}{dt} = a_2 + \frac{x^m b_2}{(K_2)^m + x^m} - yd_1 + \sqrt{D}\xi(t), \tag{9}$$

Eqs. (8) and (9) display a system with two regulators. Each variable is subjected to noise plus transcription and degradation terms. Variable x has an auto-regulation part and also regulates variable y. The part $-gxy$ corresponds to the elimination of x by y.

The top panel in Fig. 3, corresponds to the dynamics of one *Bacillus subtilis* sigma factor without the others (Eqs. (1)-(6) for $i = 1$). We can see pulses in random times with variable intensities, widths and there is no refractive time either. Therefore, the pulsatile dynamics of one sigma factor resembles those driven by Eq. (7) and not those of an excitable system.

C. Sigma Factor Competition and Time-Sharing

The results of our simulation with six sigma factors (housekeeping included) are the next ones. In Fig. 4, each sigma factor pulses randomly just once in each consecutive interval of 300 min. In other words, we change the Ligand of each sigma factor with a random uniform time in the interval of 300 min, every 300 min.

The results are in agreement with those in [7] and show that sigma factors do not pulse at the same time, suggesting time-sharing is present. RNAP is limiting since the housekeeping is sensitive to each pulse.

To understand better this dynamics, we wanted to get the same dynamics with less number of sigma factors:

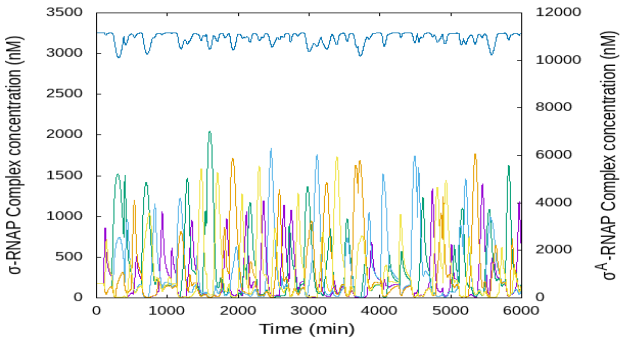

FIG. 4: Time sharing for six sigma factors (housekeeping included). Two axes y with different scales. Each color illustrates one sigma factor associated with RNAP, with concentration values in left y axis. Housekeeping sigma factor union with RNAP is the light blue color at the top, with concentration values in right y axis. Total RNAP = 12600 nM and perturbed with a period = 300 min for each sigma factor.

only two plus the housekeeping. In order to achieve this aim we reduced the time interval of pulsing to 100 min (Fig. 5).

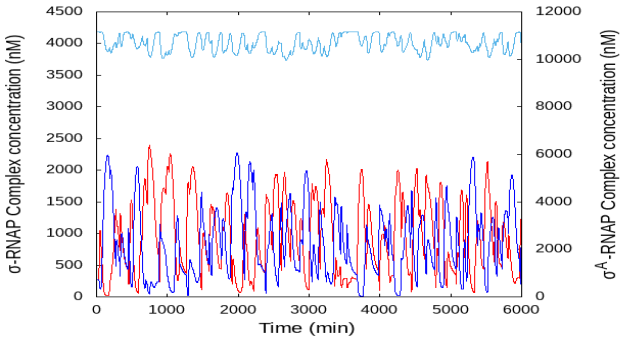

FIG. 5: Time sharing for three sigma factors (housekeeping included). Two axes y with different scales. Each color illustrates one sigma factor associated with RNAP, with concentration values in left y axis. Housekeeping sigma factor union with RNAP is the light blue color at the top, with concentration values in right y axis. Total RNAP = 12600 nM and perturbed with a period = 100 min for each sigma factor.

As we expected, the resource RNAP to share is more abundant in Fig. 5, hence we have pulses with higher intensities. The housekeeping as before is sensitive to each pulse. Sigma factors do not pulse at the same time suggesting time-sharing. To confirm it, we forced our system to both sigma factors pulse at the same time, but with different concentrations of L_i (Fig. 6). The results show that sigma factors do not pulse at the same time, hence time-sharing is clear. Fig. 5 and 6 have almost the same results, supporting that Time-sharing is also acting when each sigma factor is perturbed at its own time (Fig. 5). Time-sharing involves that pulses are inhibited due to the competition for RNAP.

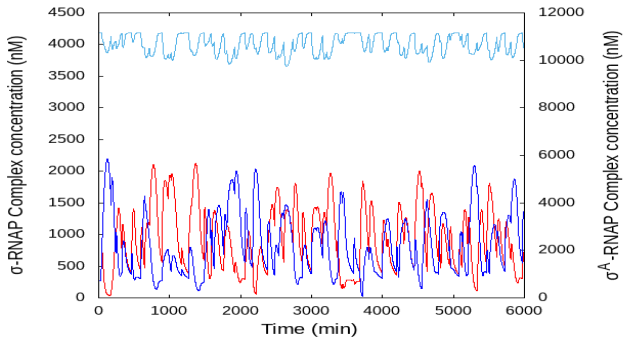

FIG. 6: Time sharing for three sigma factors (housekeeping included) as in Fig. 5 but forced to pulse all at the same time. Total RNAP = 12600 nM and perturbed with a period = 100 min for each sigma factor.

D. Time-Sharing and Cross-Correlation

The cross-correlations are calculated to prove mathematically the time-sharing and to understand better their behavior, in a quantitative point of view.

$$\rho_{ij}(\tau) = \sum_{g}^{P} \frac{1}{P} \sum_{k}^{N} \frac{([RS(t_{kg})]_i - <[RS]_i>)([RS(t_{kg}+\tau)]_j - <[RS]_j>)}{N}, \tag{10}$$

Where $< [RS]_i >= \sum_g^P \frac{1}{P} \sum_k^M \frac{[RS(t_{kg})]_i}{M}$, P is the number of seeds, M is the amount of iterations in our numerical method in each seed and N is the total of times selected in each seed to calculate the correlation. Each seed is a different sequence of random numbers. It means that the system has been simulated with a different sequence of random numbers for each seed. Index $(i, j = 1, 2)$ are the sigma factors which has anti-sigma factors in its dynamics. $[RS(t_{kg})]$ is the concentration of RNAP-sigma factor of the time k $(k = 1, 2, ..., N)$ of the seed g $(g = 1, 2, ..., P)$.

The results in Fig. 7 denote that cross-correlation is negative. So, they prove that sigma factors do not pulse at the same time. The cross-correlation is positive for times $\tau \sim period$. One sigma factor and another sigma factor pulse with a time gap with scale values such as the period. Self-correlation and cross-correlation tend to zero at long time τ as expected.

E. Unlimited Resources

Increasing the total amount of RNAP reduces the cross-correlation (Fig. 7), as expected [7]. At higher RNAP concentration we find a constant uniform sharing, being all sigma factors highly bound to RNAP at all times (Fig. 8). There are pulses of increased binding that show time-sharing. Results of Fig. 7, at higher RNAP than R_{TOT_I}, support results of Fig. 8 by a not zero value of cross-correlation. For higher amount of RNAP

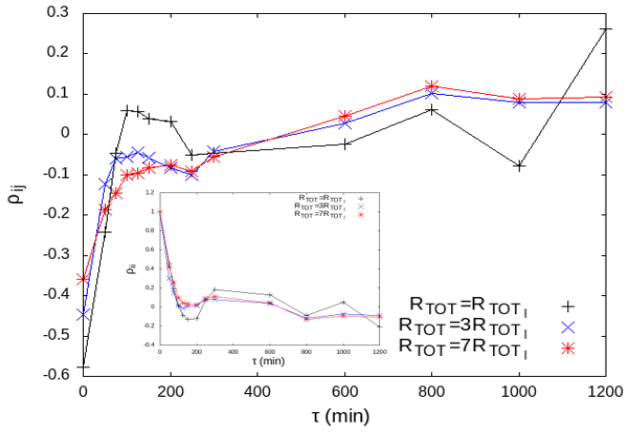

FIG. 7: Cross-correlation of one sigma factor with the other as a function of time interval τ, for different conditions of total RNAP and two sigma factor system. Lines are a visual guidance. R_{TOT_I} = 12600 nM. Self-correlation of one sigma factor as an inset. Computed for 3 seeds.

we expect only uniform sharing [7]. So, in terms of cross-correlation, we expect a zero value.

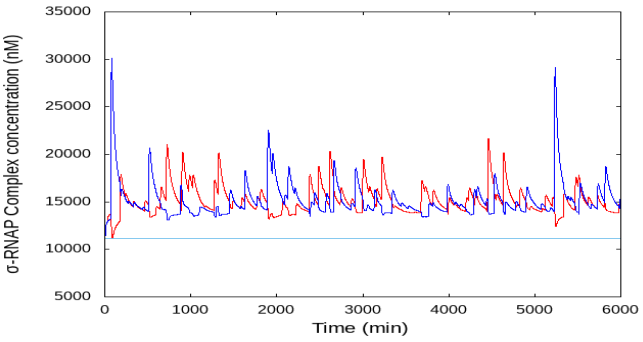

FIG. 8: System with two sigma factors. Each color illustrates one sigma factor associated with RNAP. Housekeeping sigma factor union with RNAP is the light blue color. $R_{TOT} = 7R_{TOT_I}$ and perturbed with a period = 100 min for each sigma factor. Notice the vertical axis initiates at 500 nM.

III. CONCLUSIONS

We learnt that oscillatory and pulsing dynamics are common and spontaneous in biological systems. We focused on the pulsatile dynamics of sigma-factors in *Bacillus subtilis* cells and on the competition of these factors for RNAP [7]. We studied and compared different pulsing dynamics. Moreover, we could replicate results in [7], simplify them to two sigma factors and change the input values and conditions. At the end, we calculated the correlations of our replicated systems. We conclude that there are different mechanisms of generating pulses and that these can lead to pulses with very different features. Specifically, each sigma factor does not pulsate with the characteristics of an excitable dynamics, but does it as a more random dynamics.

Each sigma factor has two types of production, a constant production and a regulated production which depends on total RNAP and on the amount of the other competing sigma factors. On the contrary, the sigma factor housekeeping is sensitive to pulses but it is not to the total RNAP. We showed that for small concentration of RNAP, the pulses of the sigma factors are inhibited by the other sigma factors and as a result the sigma factors do not pulse at the same time, reproducing [7]. This is known as time-sharing [7]. At high RNAP concentrations, the results show that each sigma factor has a constant concentration associated to RNAP (uniform sharing) and a part which displays time-sharing and pulsing dynamics. For higher RNAP concentrations, uniform sharing is only expected [7].

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IV. APPENDIX

TABLE I: Reaction, parameter symbol and data for the time-sharing in *Bacillus subtilis* cells. Parameter values from [7] (set A) used for Eqs. [(1)-(6)].

Reaction	Parameter	Value
Basal Transcription	α_s	1.5 nM/min
	α_{sA}	180 nM/min
	α_a	2.3 nM/min
Up-Regulation	β_s	0.06 min^{-1}
	β_{sA}	6×10^{-4} min^{-1}
	β_a	0.09 min^{-1}
Association	k_{rs+}	0.03 $\text{nM}^{-1}\times\text{min}^{-1}$
	k_{rsA+}	0.3 $\text{nM}^{-1}\times\text{min}^{-1}$
	k_{sa+}	0.024 $\text{nM}^{-1}\times\text{min}^{-1}$
	k_{al+}	0.018 $\text{nM}^{-1}\times\text{min}^{-1}$
Dissociation	k_{rs-}	0.3 min^{-1}
	k_{rsA-}	0.3 min^{-1}
	k_{sa-}	0.06 min^{-1}
	k_{al-}	0.03 min^{-1}
Degradation	δ_s	0.0167 min^{-1}
	δ_{sA}	0.0167 min^{-1}
	δ_a	0.0167 min^{-1}
	δ_{rs}	0.0167 min^{-1}
	δ_{rsA}	0.0167 min^{-1}
	δ_{sa}	0.0167 min^{-1}
	δ_{al}	0.0167 min^{-1}
	δ_l	0.0167 min^{-1}
Total RNAP	R_{TOT}	12600 nM

TABLE II: Parameter values for equation (8) and (9). Used in Fig. 1 and Fig. 3 bottom panel. Values from [4] circuit B Type I.

Parameter	Value
a_1	0.0085 nM/s
a_2	0.075 nM/s
b_1	7.5 nM/s
b_2	2.5 nM/s
d_1	0.0001 s^{-1}
d_2	0.0001 s^{-1}
g	$4x10^{-8}$ $(\text{nM}\times\text{s})^{-1}$
K_1	5000 nM
K_2	2500 nM
n	2
m	2
ξ	Random Gaussian number