Optimal first–passage time in gene regulatory networks

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Abstract— The inherent probabilistic nature of the biochemical reactions, and low copy number of species can lead to stochasticity in gene expression across identical cells. As a result, after induction of gene expression, the time at which a specific protein count is reached is stochastic as well. Therefore events taking place at a critical protein level will see stochasticity in their timing. First-passage time (FPT), the time at which a stochastic process hits a critical threshold, provides a framework to model such events. Here, we investigate stochasticity in FPT. Particularly, we consider events for which controlling stochasticity is advantageous. As a possible regulatory mechanism, we also investigate effect of auto-regulation, where the transcription rate of gene depends on protein count, on stochasticity of FPT. Specifically, we investigate for an optimal auto-regulation which minimizes stochasticity in FPT, given fixed mean FPT and threshold.

For this purpose, we model the gene expression at a single cell level. We find analytic formulas for statistical moments of the FPT in terms of model parameters. Moreover, we examine the gene expression model with auto-regulation. Interestingly, our results show that the stochasticity in FPT, for a fixed mean, is minimized when the transcription rate is independent of protein count. Further, we discuss the results in context of lysis time of an *E. coli* cell infected by a λ phage virus. An optimal lysis time provides evolutionary advantage to the λ phage, suggesting a possible regulation to minimize its stochasticity. Our results indicate that there is no auto-regulation of the protein responsible for lysis. Moreover, congruent to experimental evidences, our analysis predicts that the expression of the lysis protein should have a small burst size.

I. INTRODUCTION

Gene expression is the process of *transcription* of genetic information to mRNAs, and *translation* of each mRNA to proteins. As the copy number of species involved in the process is small, the probabilistic nature of biochemical reactions reflects as stochasticity in gene expression [1]–[6].

Stochasticity in gene expression has an important role in several cellular functions. For example, it can lead genetically identical cells to different cell-fates [7]–[12]. This helps the cells in responding to the ever-changing environment [13]–[16]. On the other hand, stochasticity in expression of housekeeping genes can lead to diseased states [17]–[19], and needs to be minimized [20], [21]. Accordingly, different regulatory mechanisms are employed to control stochastic fluctuations [22]–[29]. Auto–regulation wherein transcription rate is a function of protein count is an example of one such mechanism. Its effect on stochasticity in gene expression has been a subject of several studies [27]–[29].

After onset of gene expression, its stochasticity consequently manifests into stochasticity in the time at which a certain protein level is reached. This implies that the timing of a cellular event which triggers at a critical protein level is stochastic in nature [30], [31]. For instance, lysis time for an *E. coli* cell infected by a λ phage virus is stochastic. Lysis of the cell takes place when holin, the protein responsible for lysis, reaches a critical threshold [32]–[34].

Further, it has been suggested that optimality in lysis time provides evolutionary advantage to λ phage virus [35]–[39]. This indicates that there could be some regulation of gene expression to ensure lysis at the optimal time, with minimum stochastic fluctuations. In this work, we study stochasticity in first–passage time (FPT), the time it takes for the protein count to reach a fixed threshold for the first time [40], at a single–cell level. We investigate the effect of auto-regulation of transcription on stochasticity of FPT. In particular we seek answer to the question: given the mean FPT (corresponding to optimal lysis time, for example), what auto-regulatory feedback will lead to minimum stochasticity in the FPT?

We first formulate an unregulated gene expression model assuming transcription, translation, and mRNA degradation while considering proteins to be stable. Along the lines of [34], we find expressions for statistical moments of FPT for this model, and discuss their implications with respect to minimizing variance in FPT for given mean FPT. Next, we introduce auto-regulation in the above model and derive the moments for FPT. Then, we deduce the expression for optimal feedback function that minimizes the variance in FPT for a given mean. We show that a negative or positive feedback always results into higher variance in first passage time for a given mean than the case when there is no feedback. The results are validated by carrying out simulations. Also, various notations used in this work are tabulated in Table I.

II. FIRST–PASSAGE TIME FOR GENE EXPRESSION MODEL WITHOUT REGULATION

In this section, we formulate a stochastic gene expression model (as shown in Fig. 1). Then, we define the FPT for this model and derive expressions for its statistical moments. We also discuss the implications of these expressions in context of minimizing variance of FPT, for fixed mean and threshold.

A. Model Formulation

In the model under consideration transcription of mRNAs from the gene occurs at a rate k_m , translation of proteins from each mRNA occurs at a rate k_p , and each mRNA degrades at a rate γ_m . The time interval between two transcription

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TABLE I Description of notations used in this work

k_m	Transcription rate for unregulated gene expression model.			
k_p	Translation rate for both unregulated, and regulated gene			
	expression. models			
γ_m	mRNA degradation rate for both unregulated, and regu-			
	lated gene. expression models			
B_i	Burst size after <i>i</i> th transcriptional event.			
μ	Parameter of geometric distribution corresponding to.			
	protein bursts			
b	Mean of protein burst size.			
P(t)	Protein count at time t.			
P_i	Protein count after <i>i</i> th burst.			
$k_m(P_i)$	Transcription rate for auto-regulated gene expression			
	model after <i>i</i> th transcription event.			
X	Threshold for protein count.			
Ν	Minimum number of transcription events for protein			
	count to reach the threshold X.			
T_i	Waiting time for <i>i</i> th transcription event.			
$Y \sim \exp(\alpha)$	Y is an Exponential random variable with parameter α .			
	The probability density function of <i>Y</i> is given by $f_Y(y) =$			
	$\alpha e^{-\alpha y}, y \ge 0.$			
$f_N(n)$	Probability mass function for minimum number of tran-			
	scription events to reach the threshold X.			
$f_{P_i}(j)$	Probability mass function for protein count after <i>i</i> tran-			
	scription events.			
$\langle . \rangle$	Expectation operator.			
Var	Variance.			
k _{max}	Maximum possible transcription rate in model with feed-			
	back implemented using Hill function .			
r	Fraction of transcription rate k_{max} that corresponds to			
	minimum transcription rate in model with feedback im-			
	plemented using Hill function.			
Н	Hill coefficient.			
с	Coefficient proportional to binding efficiency; decides			
	when half rate concentration is reached.			

events is exponentially distributed. We assume proteins to be stable as the lysis protein in λ phage, i.e. holin, is stable [41]. To further simplify the model, we assume each mRNA molecule degrades instantaneously after producing a burst of random number of protein molecules [42]–[45]. Consistent with experimental, and theoretical evidences; we assume that protein burst follows a geometric distribution, and the mean burst size is given by $b = k_p/\gamma_m$ [46], [47]. Thus, the simplified model considers gene expression wherein each burst event (equivalent to transcription event) occurs at an exponentially distributed time with parameter k_m , and size of burst follows a geometric distribution with mean b.

Let us denote the size of i^{th} burst by random variable B_i and the parameter of its distribution by μ . The probability mass function, therefore, can be written as [48]:

$$\Pr(B_i = k) = \mu (1 - \mu)^k, \, \mu \in (0, 1], \, k \in \{0, 1, 2..\}.$$
(1)

The mean burst size, b, can be expressed as [48]:

$$\langle B_i \rangle = b = \frac{1-\mu}{\mu}.$$
 (2)

Further, let protein count after *n* transcription events be denoted as P_n . It can be expressed as a sum of random variables B_i :

$$P_n = \sum_{i=1}^n B_i. \tag{3}$$



Fig. 1. Model for gene expression without regulation: The figure shows expression of a gene where mRNAs are transcribed from the gene at a rate k_m and proteins are translated from each mRNA at a rate k_p . Proteins are assumed to be stable while each mRNA degrades with a rate γ_m .

Being sum of independent and identically distributed geometric random variables, P_n has a negative binomial distribution with parameters n and μ [49]. The probability mass function of P_n , denoted as $f_{P_n}(j)$, can be expressed as [49]:

$$f_{P_n}(j) = \Pr\left(\sum_{i=1}^n B_i = j\right) = \binom{n+j-1}{n-1} \mu^n \left(1-\mu\right)^j.$$
(4)

Also, the cumulative distribution function is given by [50]:

$$\Pr\left(\sum_{i=1}^{n} B_{i} \le j\right) = 1 - I_{1-\mu}(j+1,n),$$
 (5)

where $I_{1-\mu}(j+1,n)$ is regularized incomplete beta function:

$$I_{1-\mu}(j+1,n) = \sum_{l=j+1}^{n+j} \binom{n+j}{l} (1-\mu)^l \mu^{j+n-l}, \quad (6)$$

and satisfies the following property:

$$I_{1-\mu}(j+1,n) = 1 - I_{\mu}(n,j+1).$$
(7)

We have determined the distribution for protein population. Next, we defined the first–passage time (FPT) for the protein count to reach a certain threshold.

B. Expression for First Passage Time

For a random process corresponding to protein count, P(t), with P(0) = 0, the first passage time (FPT), for a threshold *X* is defined as:

$$FPT := \inf\{t : P(t) \ge X\}, \quad X \in \{1, 2, 3, ...\}.$$
(8)

Because in our model, the protein count changes only when a burst occurs (or equivalently, a transcription event occurs); we can calculate the minimum number of transcription events, N, it takes for the protein count to reach the threshold X and define the FPT as sum of inter–burst arrival times. This has been depicted in Fig. 2.

Let the time between $i - 1^{th}$ and i^{th} bursts be denoted by random variable T_i , then:

$$FPT = \sum_{i=1}^{N} T_i, \tag{9}$$



Fig. 2. First-passage time for gene expression in burst limit: The gene expresses in bursts which arrive at time intervals T_i , i = 1, 2, ... The protein count after i^{th} burst is denoted by P_i . Protein count at time t is denoted by P(t), and is equal to P_i , where i is number of bursts until time t. The first-passage time can be expressed as the sum of inter-burst arrival times till P_i crosses the threshold X for the first time.

where N is given by the following equation:

$$N = \inf(n : P_n \ge X), \quad n \in \{1, 2, ...\}, \ X \ge 1.$$
(10)

Note that in Eq. (9), T_i are independent, and identically distributed exponential random variables with parameter k_m . We denote this by $T_i \sim \exp(k_m)$. Also, each of T_i is independent of N.

Using standard results from probability theory, one may write [51]:

$$\langle FPT \rangle = \langle N \rangle \langle T_i \rangle,$$
 (11a)

$$\operatorname{Var}(FPT) = \langle N \rangle \operatorname{Var}(T_i) + \operatorname{Var}(N) \langle T_i \rangle^2.$$
 (11b)

It can be noted that to determine statistical moments of FPT in Eq. (11a)–(11b), we need to derive expressions for first two moments of T_i , and N.

1) First Two Moments of N: The cumulative distribution function for N defined in Eq. (10) can be written as:

$$\Pr(N \le n) = \Pr\left(P_n \ge X\right),\tag{12a}$$

$$= 1 - \Pr(P_n \le X - 1).$$
 (12b)

Since P_n is a negative binomial distribution, we have:

$$\Pr(N \le n) = 1 - (1 - I_{1-\mu}(X, n)), \quad (13a)$$

$$=I_{1-\mu}(X,n).$$
 (13b)

Using the property of incomplete beta function mentioned in Eq. (7), we get:

$$\Pr(N \le n) = 1 - I_{\mu}(n, X).$$
(14)

Comparing with Eq. (4) and Eq. (5), the probability mass function corresponding to Eq. (14) can be written as:

$$f_N(n) = \binom{n+X-2}{n-1} (1-\mu)^{n-1} \mu^X, \ n \in \{1, 2, ...\}, \ X \ge 1.$$
 (15)

First two statistical moments of the distribution in Eq. (15) are given by [49]:

$$\langle N \rangle = \frac{\mu X}{1 - \mu} + 1 = \frac{X}{b} + 1,$$
 (16a)

$$\operatorname{Var}(N) = \langle N^2 \rangle - \langle N \rangle^2 = \frac{\mu X}{(1-\mu)^2} = \frac{X}{b} \frac{1+b}{b}.$$
 (16b)

2) First Two Moments of T_i : Since $T_i \sim \exp(k_m)$, its statistical moments are given by:

$$\langle T_i \rangle = \frac{1}{k_m},\tag{17a}$$

$$\operatorname{Var}(T_i) = \left\langle T_i^2 \right\rangle - \left\langle T_i \right\rangle^2 = \frac{1}{k_m^2} = \left\langle T_i \right\rangle^2.$$
(17b)

We now have expressions for first two moments of T_i , and N. The expressions for first two moments of FPT in terms of model parameters can, therefore, be written as:

$$\langle FPT \rangle = \left(\frac{X}{b} + 1\right) \frac{1}{k_m} \approx \frac{X}{bk_m},$$
 (18)

$$\operatorname{Var}(FPT) = \frac{X(2b+1) + b^2}{b^2 k_m^2} \approx \frac{X}{b^2 k_m^2} (1+2b), \qquad (19)$$

where the approximations are valid when $X \gg b$. It can be observed a smaller mean burst size *b* would result in smaller variance of FPT. The mean FPT can be kept fixed by a commensurate change in the transcription rate, k_m . Therefore, the variance can independently be reduced by a lower mean burst size $b = k_p/\gamma_m$. This means adopting a high transcription rate k_m , and a low translation rate k_p (and/or having a higher degradation rate γ_m for the mRNAs) results in a lower variance in FPT without affecting its mean.

Further, we note that by using $\operatorname{Var}(T_i) = \langle T_i \rangle^2$ from Eq. (17b), we can deduce the following relationship between $\langle FTP \rangle$ and $\langle FPT^2 \rangle$ from Eq. (11a) and Eq. (11b):

$$\langle FPT^2 \rangle = \frac{\langle FPT \rangle^2}{\langle N \rangle^2} \langle N^2 \rangle + \frac{\langle FPT \rangle^2}{\langle N \rangle}.$$
 (20)

We shall use above relationship in the later part of the paper while deriving expression of the auto-regulation function that minimizes variance in FPT, for given mean FPT.

Next, we introduce auto-regulation of transcription rate by the protein count to investigate how the expressions for statistical moments of FPT change.

III. INTRODUCING AUTO-REGULATION IN GENE EXPRESSION MODEL

To investigate the effect of auto-regulation on statistical moments of FPT, we assume that transcription rate is a function of protein count, i.e., it changes after each transcription event. We denote the transcription rate after arrival of i^{th} burst as $k_m(P_i)$. Similar to previous section, we need to derive expression for moments of inter–burst arrival times T_i , and

minimum number of transcription events N in order to derive the expression for FPT moments defined in Eq. (9).

We note that the translation burst size is independent of the transcription rate. Therefore, distribution of N to reach a certain threshold X is same as gene expression model without any regulation discussed in previous section. However, distribution of each T_i is different and depends upon corresponding rate of transcription.

We derive expressions for first two moments of each T_i to find analytical forms of first two moments of FPT.

A. Inter-burst arrival time for auto-regulatory gene expression model

It may be noted that if protein count after any burst event is known, arrival time for the next burst will be exponentially distributed. Therefore, the distribution of each T_i can be modelled as a conditional exponential distribution. More specifically, we can write:

$$T_i \sim \exp\left(k_m(P_{i-1})|P_{i-1}\right),\tag{21}$$

where T_i , and P_{i-1} respectively denote the arrival time for i^{th} burst and protein count after the $i - 1^{th}$ burst.

The expressions for mean and variance of T_i can be calculated as follows.

1) Mean: Before arrival of the first burst, there are no protein molecules, i.e., $P_{i-1} = 0$ for i = 1. Therefore, we can write the mean for arrival time for the first burst as:

$$\langle T_1 \rangle = \frac{1}{k_m(0)}.$$
(22)

For $i \in \{2, 3, 4...\}$, the corresponding arrival times would be conditionally exponential, implying:

$$\langle T_i | P_{i-1} = j \rangle = \frac{1}{k_m(j)},\tag{23a}$$

$$\implies \langle T_i \rangle = \sum_{j=0}^{\infty} \frac{1}{k_m(j)} \Pr\left(P_{i-1} = j\right), \qquad (23b)$$

$$=\sum_{j=0}^{\infty}\frac{1}{k_m(j)}f_{P_{i-1}}(j).$$
 (23c)

2) Second Order Moments: Adopting similar approach as above, we derive the expressions for second order moments of T_i . For i = 1, we have:

$$T_1^2 \rangle = \frac{2}{k_m^2(0)}.$$
 (24a)

For $i \in \{2, 3, 4...\}$:

$$\langle T_i^2 | P_{i-1} = j \rangle = \frac{2}{k_m^2(j)},$$
 (24b)

$$\implies \langle T_i^2 \rangle = \sum_{j=0}^{\infty} \frac{2}{k_m^2(j)} \Pr\left(P_{i-1}=j\right), \qquad (24c)$$

$$=\sum_{j=0}^{\infty} \frac{2}{k_m^2(j)} f_{P_{i-1}}(j).$$
(24d)

Therefore the expression for variance of T_1 :

$$\operatorname{Var}(T_1) = \frac{1}{k_m^2(0)} = \langle T_1 \rangle^2.$$
 (25)

For $i \in \{2, 3, 4, ...\}$, the expression for $Var(T_i)$ will be

$$\operatorname{Var}(T_{i}) = \sum_{j=0}^{\infty} \frac{2}{k_{m}^{2}(j)} f_{P_{i-1}}(j) - \left[\sum_{j=0}^{\infty} \frac{1}{k_{m}(j)} f_{P_{i-1}}(j)\right]^{2}.$$
 (26)

Moreover, we have following relationship first two moments of the random variable $1/k_m(P_{i-1})$:

$$\sum_{j=0}^{\infty} \frac{1}{k_m^2(j)} f_{P_{i-1}}(j) \ge \left[\sum_{j=0}^{\infty} \frac{1}{k_m(j)} f_{P_{i-1}}(j)\right]^2, \quad (27)$$

which alongwith Eq. (26), and (25) yields:

$$\operatorname{Var}(T_i) \ge \langle T_i \rangle^2 \,. \tag{28}$$

We note that the equality above holds for i = 1. We will use it in later part of the paper while deducing the expression for optimal auto-regulation that leads to minimum variance in the FPT for fixed mean.

Having derived the expressions for moments of interbursts arrival times, we see how the introduction of autoregulation influences the expressions for FPT moments.

B. FPT for auto-regulatory gene expression model

We present the expressions for statistical moments of FPT in theorem–proof format. In developing the proofs, we make use of the fact that each T_i will be independent of N. Also, T_i are independent of each other. However, they are not identically distributed like the unregulated gene expression case discussed in previous section.

Theorem 1 (Mean of First Passage Time): For the FPT defined in Eq. (9), the mean FPT is given by following expression:

$$\langle FPT \rangle = \sum_{n=1}^{\infty} \sum_{i=1}^{n} \langle T_i \rangle f_N(n),$$
 (29)

where $f_N(n)$ is defined in Eq. (15), $\langle T_i \rangle$ is given by Eq. (22), (23c) and $\langle N \rangle$ is given by Eq. (16a).

Proof: To prove the result, we first find conditional expectation given N = n then we have:

$$\langle FPT|N=n\rangle = \left\langle \sum_{i=1}^{n} T_i \right\rangle,$$
 (30a)

$$=\sum_{i=1}^{n} \langle T_i \rangle.$$
 (30b)

Unconditioning above expression with respect to N:

$$\langle FPT \rangle = \sum_{n=1}^{\infty} \sum_{i=1}^{n} \langle T_i \rangle Pr(N=n),$$
 (30c)

$$=\sum_{n=1}^{\infty}\sum_{i=1}^{n}\left\langle T_{i}\right\rangle f_{N}(n). \tag{30d}$$

This completes the proof.

Theorem 2 (Variance of First Passage Time): For the FPT defined in Eq. (9), the variance of FPT is given by the following expression:

$$\sum_{n=1}^{\infty} \left(\sum_{i=1}^{n} \operatorname{Var}\left(T_{i}\right) + \left(\sum_{i=1}^{n} \left\langle T_{i} \right\rangle\right)^{2} \right) f_{N}(n) - \left(\sum_{n=1}^{\infty} \sum_{i=1}^{n} \left\langle T_{i} \right\rangle f_{N}(n) \right)^{2},$$
(31)

where $f_N(n)$ is defined in Eq. (15), $\langle N \rangle$ is given by Eq. (16a), $\langle N^2 \rangle$ can be deduced from Eq. (16b), $\langle T_i \rangle$ is given by Eq. (22), (23c) and Var (T_i) is given by Eq. (25), (26).

Proof: Since expression for $\langle FPT \rangle$ is known and given by Eq. (29), we need to find expression for $\langle FPT^2 \rangle$, in order to find expression for variance of FPT.

Using the definition of first passage time in Eq. (9), we have:

$$\langle FPT^2|N=n\rangle = \left\langle \sum_{i=1}^n \sum_{j=1}^n T_i T_j \right\rangle,$$
 (32a)

$$= \left\langle \sum_{i=1}^{n} T_i^2 + \sum_{i=1}^{n} \sum_{j=1 \neq i}^{n} T_i T_j \right\rangle$$
(32b)

Since T_i^2 are independent of each other, and T_j are independent of T_i for each $j \neq i$; we can write:

$$\left\langle FPT^2 | N = n \right\rangle = \sum_{i=1}^n \left\langle T_i^2 \right\rangle + \sum_{i=1}^n \sum_{j=1 \neq i}^n \left\langle T_i T_j \right\rangle, \qquad (32c)$$

$$=\sum_{i=1}^{n} \langle T_i^2 \rangle + \sum_{i=1}^{n} \sum_{j=1 \neq i}^{n} \langle T_i \rangle \langle T_j \rangle.$$
(32d)

Using $\operatorname{Var}(T_i) = \langle T_i^2 \rangle - \langle T_i \rangle^2$, we have:

$$\langle FPT^2 | N = n \rangle = \sum_{i=1}^n \operatorname{Var}(T_i) + \left(\sum_{i=1}^n \langle T_i \rangle\right)^2.$$
 (32e)

Unconditioning with respect to *N*, expression for $\langle FPT^2 \rangle$ becomes:

$$\langle FPT^2 \rangle = \sum_{n=1}^{\infty} \left(\sum_{i=1}^n \operatorname{Var}(T_i) + \left(\sum_{i=1}^n \langle T_i \rangle \right)^2 \right) f_N(n).$$
 (33)

Therefore, using Eq. (29), and Eq. (33); expression for Var(FPT) becomes:

$$\sum_{n=1}^{\infty} \left(\sum_{i=1}^{n} \operatorname{Var}\left(T_{i}\right) + \left(\sum_{i=1}^{n} \left\langle T_{i} \right\rangle \right)^{2} \right) f_{N}(n) - \left(\sum_{n=1}^{\infty} \sum_{i=1}^{n} \left\langle T_{i} \right\rangle f_{N}(n) \right)^{2}.$$
(34)

This completes the proof.

So far we have developed analytical expressions for mean and variance of FPT when there is an auto-regulatory feedback to transcription rate from protein count. In the next section, we make use of these expressions to deduce the optimal auto-regulation function to minimize the variance of FPT assuming fixed mean FPT.

IV. MINIMIZING VARIANCE IN FIRST PASSAGE TIME FOR GIVEN MEAN

In this section, we find expression for the auto-regulatory feedback function, $k_m(P_{i-1})$, $i \in \{1, 2, 3, ...\}$ that gives minimum variance in FPT, given the mean FPT and event threshold are fixed. The result is presented in form of a theorem.

Theorem 3 (Optimal feedback for minimum variance): Let the first passage time be defined as Eq. (9), and its mean and variance, respectively, given by Eq. (29) and Eq. (31). Then, the optimal function to minimize the variance of FPT for a given mean of FPT will be constant, given by following expression:

$$k_m(P_{i-1}) = \frac{\langle N \rangle}{\langle FPT \rangle}, \quad \forall i \in \{1, 2, 3, ...\},$$
(35)

where $\langle N \rangle$ denotes the minimum number of transcription events required to reach the FPT threshold, and is given by Eq. (16a).

Proof: We assume that each burst event adds a perturbation to transcription rate, i.e., $1/k_m(P_{i-1})$ can be written as:

$$\frac{1}{k_m(P_{i-1})} := \frac{\langle FPT \rangle}{\langle N \rangle} + \delta_i, \tag{36}$$

where δ_i is perturbation corresponding to transcription rate after $i - 1^{th}$ burst. To prove the result, we shall prove that the variance of FPT for given mean will minimize when $\delta_i = 0$.

Recalling the expression for $\langle FPT \rangle$ from Eq. (29):

$$\langle FPT \rangle = \sum_{n=1}^{\infty} \sum_{i=1}^{n} \langle T_i \rangle f_N(n).$$
 (37)

Using expressions in Eqs. (22), (23c), we can deduce the expressions for $\langle T_i \rangle$ as:

$$\langle T_i \rangle = \frac{\langle FPT \rangle}{\langle N \rangle} + \varepsilon_i,$$
 (38)

where ε_i is related with δ_i by following expression:

$$\varepsilon_i := \sum_{j=1}^{\infty} \delta_i f_{P_{i-1}}(j) = \langle \delta_i \rangle.$$
(39)

Substituting expression for $\langle T_i \rangle$ from Eq. (38), we have:

$$\langle FPT \rangle = \sum_{n=1}^{\infty} \sum_{i=1}^{n} \left(\frac{\langle FPT \rangle}{\langle N \rangle} + \varepsilon_i \right) f_N(n), \tag{40a}$$

$$=\sum_{n=1}^{\infty} \left(\frac{\langle FPT \rangle}{\langle N \rangle} n + \sum_{i=1}^{n} \varepsilon_i \right) f_N(n), \tag{40b}$$

$$= \frac{\langle FPT \rangle}{\langle N \rangle} \sum_{n=1}^{\infty} n f_N(n) + \sum_{n=1}^{\infty} \sum_{i=1}^{n} \varepsilon_i f_N(n).$$
(40c)

Since $\sum_{n=1}^{\infty} n f_N(n) = \langle N \rangle$, we have:

$$\sum_{n=1}^{\infty}\sum_{i=1}^{n}\varepsilon_{i}f_{N}(n)=0.$$
(41)

Note that for a fixed mean FPT, minimizing the variance of FPT and minimizing the second order moment $\langle FPT^2 \rangle$ are equivalent.

Now, we consider the expression for $\langle FPT^2 \rangle$, and use expression in Eq. (41) to deduce the desired optimal function. From Eq. (31), we have:

$$\langle FPT^2 \rangle = \sum_{n=1}^{\infty} \left(\sum_{i=1}^n \operatorname{Var}(T_i) + \left(\sum_{i=1}^n \langle T_i \rangle \right)^2 \right) f_N(n).$$
 (42)

 TABLE II

 MODEL PARAMETERS USED FOR SIMULATION OF POSITIVE, NEGATIVE, AND NO FEEDBACK CASES.

Parameter	Unit	Positive feedback	Negative feedback	No feedback
k _{max}	mRNA produced per minute	19.35	84	10
k_p	protein produced per mRNA per minute	2.65	2.65	2.65
Ϋ́m	per minute	0.3	0.3	0.3
X	molecules	5000	5000	5000
r	-	0.05	0.05	-
с	per molecule	0.002	0.002	-
Н	-	2	2	-

Substituting value of $\langle T_i \rangle$ from Eq. (36), we get following expression for $\langle FPT^2 \rangle$:

$$\left\langle FPT^{2}\right\rangle = \sum_{n=1}^{\infty} \left(\sum_{i=1}^{n} \operatorname{Var}\left(T_{i}\right) + \left(\sum_{i=1}^{n} \left(\frac{\left\langle FPT\right\rangle}{\left\langle N\right\rangle} + \varepsilon_{i}\right)\right)^{2}\right) f_{N}(n).$$
(43)

Further simplifying and using relation obtained in Eq. (41) yields:

$$\left\langle FPT^{2} \right\rangle = \frac{\left\langle FPT \right\rangle^{2}}{\left\langle N \right\rangle^{2}} \left\langle N^{2} \right\rangle + \sum_{n=1}^{\infty} \left(\sum_{i=1}^{n} \operatorname{Var}\left(T_{i}\right) + \left(\sum_{i=1}^{n} \varepsilon_{i} \right)^{2} \right) f_{N}(n).$$
(44)

Using Eq. (28) in Eq. (44):

$$\langle FPT^2 \rangle \geq \frac{\langle FPT \rangle^2}{\langle N \rangle^2} \langle N^2 \rangle$$

$$+ \sum_{n=1}^{\infty} \left(\sum_{i=1}^n \langle T_i \rangle^2 + \left(\sum_{i=1}^n \varepsilon_i \right)^2 \right) f_N(n), \quad (45a)$$

$$\Longrightarrow \langle FPT^2 \rangle \geq \frac{\langle FPT \rangle^2}{\langle N \rangle^2} \langle N^2 \rangle + \frac{\langle FPT \rangle^2}{\langle N \rangle}$$

$$+ \sum_{n=1}^{\infty} \left(\sum_{i=1}^n \varepsilon_i^2 + \left(\sum_{i=1}^n \varepsilon_i \right)^2 \right) f_N(n). \quad (45b)$$

Further, we note that in above expression if $\varepsilon_i = 0$ (or equivalently $\langle \delta_i \rangle = 0$), the expression minimizes and reduces to:

$$\langle FPT^2 \rangle \ge \frac{\langle FPT \rangle^2}{\langle N \rangle^2} \langle N^2 \rangle + \frac{\langle FPT \rangle^2}{\langle N \rangle}.$$
 (46)

Recalling Eq. (20), we observe that equality in above expression holds for unregulated gene expression case, which essentially means $\delta_i = 0$. This proves the desired result.

In this section, we proved that having no auto-regulation of transcription rate provides minimum stochasticity in the FPT, if mean FPT and event threshold are kept fixed. However, since our analysis simplified the gene expression model to burst–limit, we are interested in validating whether it is true if we don't make an approximation. In the next section, we discuss the computer simulations we carried out for this purpose.

V. SIMULATION RESULTS

In order to verify the result deduced in previous section, we carried out Monte Carlo simulations using Gillespie's algorithm [52]. We did not specifically assume that production of protein is in geometric bursts with parameter *b*. Instead, we assumed a non-zero half-life for mRNA thereby relaxing the burst approximation.

To simulate, we considered three separate cases: no feedback, negative feedback and positive feedback. The positive feedback is implemented using Hill function as follows:

$$k_m(j) = k_{\max}\left(r + (1-r)\frac{(jc)^H}{1 + (jc)^H}\right),$$
(47)

where k_{max} is maximum transcription rate, *r* represents minimum transcription rate as the fraction of k_{max} , *H* denotes the Hill coefficient while *c* is coefficient proportional to the binding affinity (when j = 1/c, $k_m(j) = k_{max}/2$).

Similarly, the negative feedback is implemented using following function:

$$k_m(j) = k_{\max}\left(r + (1 - r)\frac{1}{1 + (jc)^H}\right).$$
 (48)

We carried out the simulations for several sets of parameters assuming a fixed event threshold. Rest of the model parameters were chosen to keep the mean FPT approximately equal. In all of them, we found that no-feedback case has minimum variance in FPT.

In Table II, we present one set of such parameters. We assumed the event threshold X = 5000. Other parameters are chosen in a way that the mean FPT ≈ 60 minutes.

Simulation results for 10000 realizations are shown in Fig. 3. We note that the variance is minimum in no–feedback case, validating our theoretical claims for this set of parameter values.

VI. DISCUSSION

In this work, we studied stochasticity in event timing at a single cell level. We considered a standard gene expression model without protein degradation. Next, we formulated the FPT problem for this model and derived the formulas for statistical moments of FPT. Further, we introduced autoregulation in the gene expression wherein the transcription rate is a function of protein count. We derived the formulas for moments of FPT in this case as well, and demonstrated that for a given mean of FPT, the variance in FPT is minimized when there is no auto-regulation of gene expression. The result was verified with simulations as well.

The result can be connected to the λ phage lysis time. Due to existence of optimal lysis time [35], [36], the phage would possibly like to kill the cell at that time with as much



Fig. 3. No protein-feedback regulation of transcription rate results in minimum stochasticity in FPT for a given mean and threshold. In each figure, the dashed line in red represents the FPT threshold (assumed to be 5000 protein molecules here); the trajectories in the lower part depict the time evolution of protein population (10 sample trajectories); the histogram of on top represents distribution of FPT (10000 simulations); the parameters have been chosen to keep the mean FPT ≈ 60 min.

precision as possible. Thus, it should resort to a strategy that would minimize the lysis time variance and hence have no protein-dependent feedback regulation of transcription rate in the expression of holin. In expression from late promoter in λ phage, which produces holin, has no evidence of a regulation [53], [54].

Recalling that in no auto-regulation case too, the variance of FPT can be independently decreased by lowering the mean burst size *b*. Other studies also reveal that in case of λ phage, the burst size is indeed small [33], [35]. Also, antiholin, another protein expressed from the same promoter that expresses holin, binds to holin to decrease the effective burst size [34], [55].

In this paper, there is an underlying assumption of protein being stable. In future work, we plan to use a gene expression model with protein degradation, and carry out a similar analysis. This can be further extended to more generalized gene expression models wherein the promoter can also switch between *on* and *off* states [12], [43].

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