

Noise Enhanced Persistence in a Biochemical Regulatory Network with Feedback Control

Michael Assaf and Baruch Meerson

Racah Institute of Physics, Hebrew University of Jerusalem, Jerusalem 91904, Israel

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We find that discrete noise of inhibiting (signal) molecules can greatly delay the extinction of plasmids in a plasmid replication system: a prototypical biochemical regulatory network. We calculate the probability distribution of the metastable state of the plasmids and show in this example that the reaction rate equations may fail in predicting the average number of regulated molecules even when this number is large, and the time is much shorter than the mean extinction time.

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Many molecular species that control genetic regulatory networks are present in low concentrations. The resulting fluctuations in reaction rates may cause large random variations in the instantaneous intracellular concentrations of molecular species which, in their turn, may have important consequences in biological functioning. This and related topics have attracted much recent interest from the biology and physics communities [1–8]. Intracellular processes are often regulated via negative feedback by signal molecules. It was assumed in the past that noise in the signal component would randomize control of the regulated component. More recently, it has been shown that this noise may actually *enhance* the robustness of the regulated component, bringing the variation of its probability distribution below the Poissonian limit [4]. Here we report a previously unexplored dramatic impact the noise can have on the *persistence* of the regulated component in systems with negative feedback control. Following Paulsson *et al.* [4], we will consider a minimal two-component copy number control (CNC) model that, on the one hand, includes standard intracellular processes and, on the other hand, provides an adequate description to CNC of bacterial plasmids. Plasmids are extra-chromosomal DNA molecules (typically, circular and double-stranded) that are capable of autonomous replication. They undergo intracellular dynamics of the birth-death type with decay (mostly dilution by cell division) and autocatalytic production inhibited by signal molecules. If there is no penetration of new plasmids into the cell, a rare sequence of multiple decay events will ultimately drive the plasmid population to extinction. It may even cause the death of the cell if the plasmid contains a vital gene. We will combine analytical and numerical approaches to show that noise in the number of signal molecules can greatly delay the plasmid extinction. We will also calculate analytically the probability distribution function (PDF) of the metastable state of the regulated (plasmid) molecules and show that widely used deterministic reaction rate equations (RRE) may fail in predicting the average number of plasmids even when this number is large. These remarkable effects do not require an unusual molecular distribution

and occur due to rare events when the number of signal molecules is very small.

Model.—Consider a double negative-positive feedback loop with plasmids denoted by X and signal molecules denoted by S . The plasmids promote the production of the signal molecules, whereas the signal molecules inhibit the autocatalytic production of the plasmids. The RRE for the average concentrations of the two species are [4]

$$\dot{X} = X\Psi(S/A) - X, \quad \dot{S} = \alpha X - \beta S, \quad (1)$$

where $\Psi(S/A)$ is a nonlinear and monotonic decreasing function of S , $\Psi(0) > 1$, the parameter A specifies the inhibition strength of the signal molecules S , and time and the rates are rescaled by the decay rate of the plasmids. Equations (1) have an attracting fixed point (\bar{X}, \bar{S}) [where $\bar{X} = \bar{S}/\eta$, $\Psi(\bar{S}/A) = 1$, and $\eta = \alpha/\beta$] and an unstable fixed point $(X_0, S_0) = (0, 0)$. According to the RRE, the system would stay in the (\bar{X}, \bar{S}) state forever. The underlying *stochastic* process, however, behaves quite differently. A large enough fluctuation ultimately depletes the plasmid population. The state with no plasmids is an absorbing state, as the probability of escape from it is zero. Therefore, the (X_0, S_0) state is actually stable, whereas the stable fixed point (\bar{X}, \bar{S}) of the deterministic model is *metastable*. The mean extinction time (MET), the mean time it takes this stochastic process to reach the absorbing state, is expected to be exponentially long in the (presumably large) average number of plasmids in the metastable state; see, e.g., Refs. [9–11].

To account for the stochastic effects, consider a chemical master equation (CME) that describes the evolution of the probability $P_{m,n}(t)$ of having, at time t , m plasmids and n S molecules. For $m, n \geq 1$ the CME is [4]

$$\begin{aligned} \dot{P}_{m,n} = & (E_m^{-1} - 1)g_{m,n}P_{m,n} + (E_m^1 - 1)mP_{m,n} \\ & + \alpha m(E_n^{-1} - 1)P_{m,n} + \beta(E_n^1 - 1)nP_{m,n}, \quad (2) \end{aligned}$$

where $E_n^j f(n) = f(n+j)$ and $g_{m,n} = m\Psi(n/A)$ [12]. Let us denote by $P_{n|m}$ the probability of having, at time t , n S molecules conditioned on having m plasmids, and by π_m the probability of having m plasmids regardless of the

number of S molecules. We substitute the identity $P_{m,n} = P_{n|m}\pi_m$ into Eq. (2) and sum over all n . The result is

$$\dot{\pi}_m = (E_m^{-1} - 1)g_m\pi_m + (E_m^1 - 1)m\pi_m, \quad (3)$$

where $g_m = \sum_{n=0}^{\infty} P_{n|m}(t)g_{m,n}$ is the production rate of the plasmids averaged over the (yet unknown) conditional distribution $P_{n|m}(t)$.

Further analytical progress is only possible in some limits. Following Paulsson *et al.* [4], we assume that the S dynamics is much faster than the X dynamics. At the level of the RRE, S adjusts rapidly to the current value of X . Then $S(t) \simeq \eta X(t)$ holds, while $X(t)$ and $S(t)$ flow relatively slowly towards the fixed point (\bar{X}, \bar{S}) according to the reduced equation $\dot{X} \simeq X\Psi(\eta X/A) - X$. We will perform further calculations in two particular examples: exponential and hyperbolic inhibition.

Exponential inhibition.—Here $\Psi(S/A) = k\exp(-S/A)$, $k > 1$ (we assume that k is not too close to 1), and $(\bar{X}, \bar{S}) = (A \ln k / \eta, A \ln k)$. The time scale of the fast dynamics is $\sim 1/\beta$, and the time scale of the slow dynamics is $\sim 1/\ln k$ [see Fig. 1], so the time scale separation occurs when $\ln k \ll \beta$.

At the level of the CME we can perform adiabatic elimination of the fast dynamics in the variable $P_{n|m}(t)$ by assuming that the S population rapidly adjusts to a Poisson distribution $P_{n|m}^{(P)} = e^{-\eta m}(\eta m)^n/n!$ about the current value of the mean, $\eta m(t)$. Now the effective stochastic rate g_m in Eq. (3) can be easily calculated [4]:

$$g_m = \sum_{n=0}^{\infty} P_{n|m}^{(P)} g_{m,n} = k m e^{-r\eta m}, \quad r = 1 - e^{-1/A}. \quad (4)$$

This procedure reduces the two-species problem to an effective one-species problem: a single-step birth-death process with the birth rate g_m and death rate $\mu_m = m$. In the most interesting case of $\langle m \rangle \gg 1$, there are two widely different time scales in this process. The first short time scale is the relaxation time to the metastable state. The second, exponentially long time scale is the life time of the metastable state, or the extinction time, see below. At intermediate times one observes a *quasistationary* distribution (QSD) $q_m(t) = \pi_m/[1 - \pi_0(t)]$: the PDF of having, at time t , m plasmids conditioned on their nonextinction;

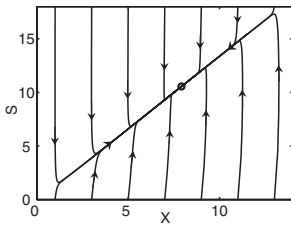


FIG. 1. The phase plane X, S of the reaction rate Eqs. (1) for $\Psi(S/A) = k\exp(-S/A)$, $\beta = 150$, $\alpha = 200$, $A = 4$, and $k = 14$. The \bullet denotes the fixed point (\bar{X}, \bar{S}) .

see, e.g., [13]. When $g_1/\mu_1 = ke^{-r\eta} \gg 1$, that is $\ln k \gg r\eta$, the probability flux to the zero state $m = 0$ is negligible, and $q_m(t)$ can be approximated by putting, in Eq. (3), $\dot{\pi}_m(t) = 0$ for all m and assuming $\mu_1 = 0$ [14]. In this way one obtains a recursion relation for q_m [10,15] which yields the QSD:

$$\frac{q_m}{q_1} \simeq \frac{g_1 g_2 \cdots g_{m-1}}{\mu_2 \mu_3 \cdots \mu_m} = \frac{e^{-(1/2)r\eta m(m-1)} k^{m-1}}{m}, \quad (5)$$

while q_1 can be found from the normalization $\sum_{m=1}^{\infty} q_m = 1$. Assuming $r\eta \ll 1$, we can replace the normalization sum by an integral [16] and, by the saddle point method, obtain

$$q_1^{-1} = \tau \simeq \frac{\sqrt{2\pi r\eta}}{\sqrt{k} \ln k} e^{(\ln k)^2/(2r\eta)}. \quad (6)$$

Now, q_1^{-1} is nothing but the MET τ [13], and Eq. (6) yields an accurate approximation for it. The same result follows from an exact expression for the MET [17].

Let us calculate for comparison the MET for the “semi-deterministic” (SD) case, when $S = \eta X$ is a prescribed deterministic quantity. The SD rate $g_m^{\text{SD}} = k m e^{-\eta m/A}$ is obtained by putting $n = \eta m$. A similar calculation, for $\eta/A \ll 1$, yields

$$\tau^{\text{SD}} \simeq \frac{\sqrt{2\pi\eta}}{\sqrt{kA} \ln k} e^{A(\ln k)^2/(2\eta)}. \quad (7)$$

How do the fully stochastic (6) and SD (7) results for the METs compare? Consider their ratio

$$R \equiv \frac{\tau}{\tau^{\text{SD}}} = \sqrt{rA} \exp\left[\frac{(\ln k)^2}{2r\eta}(1 - rA)\right]. \quad (8)$$

The strongest effect is observed for $(\ln k)^2 \gg \eta$. In this case, and for $A \gg \eta$, we obtain $R \gg 1$: the discrete noise of the S molecules greatly (exponentially) delays the plasmid extinction. Note that in this parameter regime R is a monotone decreasing function of A . However, even for $A \rightarrow \infty$ the effect is strong, as $R \rightarrow e^{(\ln k)^2/(4\eta)} \gg 1$.

Using Eq. (6) for τ , we can determine the extinction probability $\pi_0(t)$: the probability that extinction occurs until time t , see, e.g., Ref. [11]. Also, by conservation of probability, we can restore the exponentially slow time dependence of the PDF of the metastable state, $\pi_{m>0}(t) \simeq q_m \exp(-t/\tau)$ [where τ is given by Eq. (6)]. We obtain

$$\begin{aligned} \pi_0(t) &\simeq 1 - e^{-t/\tau}, \\ \pi_{m>0}(t) &\simeq \frac{k^{m-1/2} \ln k}{m\sqrt{2\pi r\eta}} e^{-(\ln k)^2/(2r\eta) - r\eta m(m-1)/2 - t/\tau}. \end{aligned} \quad (9)$$

Using the PDF (9), we can calculate the (slowly decaying in time) average number of the plasmids:

$$\langle m(t) \rangle = \sum_{m=0}^{\infty} m\pi_m(t) \simeq \left(\frac{\ln k}{r\eta} + \frac{1}{2}\right) e^{-t/\tau}, \quad (10)$$

where we have again assumed $r\eta \ll 1$. For $A \lesssim 1$, $\langle m(t) \rangle$ strongly deviates from the RRE prediction $\bar{X} = A \ln k / \eta$, even at $t \ll \tau$, as was previously observed numerically [4]. Note that non-Gaussianity of the PDF (9) appears only in the preexponent.

To test our analytical results, we solved numerically a truncated CME (2). The numerically found PDF of the plasmids $\pi_m(t)$ exhibits a slow decay of the metastable state and a simultaneous growth of the extinction probability in time, see Fig. 2. Figure 3 compares our analytical and numerical results for $\pi_m(t)$. In addition, we compare there the analytical result (6) for the MET with the numerical result $\tau^{\text{num}} = -t / \ln[1 - \sum_{n=0}^N P_{0,n}(t)]$ (that approaches a constant after a transient), and also τ^{SD} from Eq. (7) with the result of a numerical solution of Eq. (3) with the SD rate g_m . Very good agreement is observed for all quantities [18].

Hyperbolic inhibition.—Our second example employs the widely used hyperbolic, or Michaelis-Menten, inhibition model [19]. Here $\Psi(S/A) = k/(1 + S/A)$, $k > 1$ (and not too close to 1), and $(\bar{X}, \bar{S}) = [(k-1)A/\eta, (k-1)A]$. The time scale separation occurs at $\beta \gg 1$. At the level of the CME (2) we again assume a rapid adjustment of the S species to the m -dependent Poisson distribution. The effective stochastic rate g_m in Eq. (3) is

$$g_m = \sum_{n=0}^{\infty} P_{n|m}^{(P)} g_{m,n} = k m e^{-\eta m} {}_1F_1(A, A+1, \eta m), \quad (11)$$

where ${}_1F_1(a, b, z)$ is the Kummer confluent hypergeometric function [20]. Using this effective rate, Paulsson and Ehrenberg [4] calculated the QSD numerically. We have found it analytically from the recursion relation [10,15], by assuming $g_1 \gg \mu_1 = 1$. The result is $q_m/q_1 \simeq (1/m!) \times \prod_{j=1}^{m-1} g_j$. Again, $q_1^{-1} = \tau$ can be found by normalizing the QSD to unity. Therefore, the PDF of having m plasmids at time t , and the MET, are

$$\pi_{m>0}(t) \simeq \frac{e^{-t/\tau}}{\tau m!} \prod_{j=1}^{m-1} g_j, \quad \tau \simeq \sum_{m=1}^{\infty} \frac{1}{m!} \prod_{j=1}^{m-1} g_j. \quad (12)$$

Comparisons between these predictions for the fully sto-

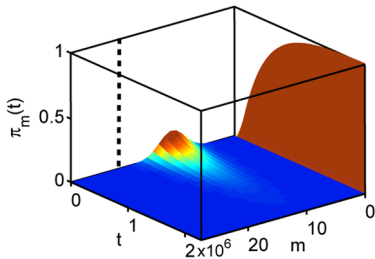


FIG. 2 (color online). The PDF $\pi_m(t)$ found by solving numerically a truncated CME (2) for the exponential inhibition with $k = 13$, $A = 4$, and $\alpha = \beta = 400$. The dashed line shows the initial distribution: a Kronecker delta at $m = n = 18$.

chastic and SD cases [with truncated sums in Eq. (12)] and numerical solutions of the truncated CMEs (2) and (3), respectively, are shown in Fig. 4(a) and 4(b), and very good agreement is observed [18].

The extreme case of very strong inhibition, $A \ll \ln k/k$, can be further simplified. It can be checked *a posteriori* that here, for all m that contribute to the normalization of π_m , and hence to the MET, the effective rate (11) is well approximated by the first term: $g_m \simeq k m e^{-\eta m}$. This rate formally coincides with that given by Eq. (4) for the exponential inhibition, where one must put $r = 1$. Therefore, the most interesting case of strong inhibition $A \ll \ln k/k$ (when the stabilizing effect of noise in the S molecules on the plasmid fluctuations and persistence is the largest) is also the simplest. Furthermore, the *exponential* inhibition model formally describes the strong *hyperbolic* inhibition limit. By additionally assuming that $A \ll \eta/k \ll \ln k/k$, one can show after some algebra that the QSD and $\pi_m(t)$ from Eq. (12) reduce to Eqs. (6) and (9), respectively (with $r = 1$). The slowly decaying average of this PDF [Eq. (10) with $r = 1$] again strongly deviates, already at $t \ll \tau$, from the RRE prediction $\bar{X} = (k-1)A/\eta$. The corresponding MET [Eq. (6) with $r = 1$] can again be compared with the SD MET, obtained by using the SD rate $g_m^{\text{SD}} = k m (1 + \eta m/A)^{-1}$ which assumes $n = \eta m$. For $A \ll \eta/k$ one has $g_1^{\text{SD}} \ll 1$, so $\tau_{\text{SD}} = \mathcal{O}(1)$, as the decay dominates over the replication. In contrast, the asymptotic result for the stochastic MET, for $\eta \ll 1$,

$$\tau \simeq \frac{\sqrt{2\pi\eta}}{\sqrt{k} \ln k} e^{(\ln k)^2/(2\eta)}, \quad (13)$$

is exponentially large. Therefore, the noise in the number of the S molecules again causes, at $A \ll \eta/k$, exponential enhancement of the persistence of the plasmids. Equation (13) compares well with numerics, see Fig. 4(c).

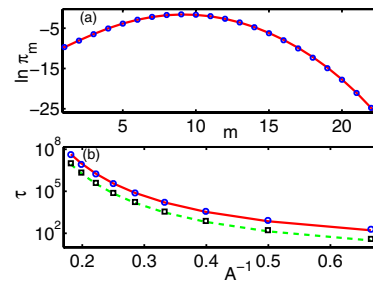


FIG. 3 (color online). (a) The PDF (9) of the metastable state for the exponential inhibition (solid line) and numerical solution of the CME (2) (\circ) for $t \ll \tau$. The parameters are $k = 13$, $A = 3$, and $\alpha = \beta = 500$. (b) The MET vs A^{-1} , the rest of parameters the same as in (a). Solid line: Eq. (6), \circ : numerical solutions for the fully stochastic case, dashed line: Eq. (7), \square : numerical solution for the SD case, see text for details. The ratio of the fully stochastic and SD METs increases with the inhibition strength $1/A$.

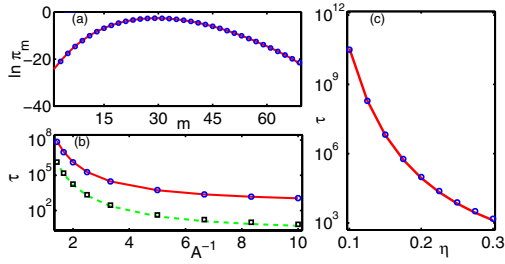


FIG. 4 (color online). (a) The PDF (12) of the metastable state for the hyperbolic inhibition (solid line) and numerical solution of the CME (2) (\circ) for $t \ll \tau$. The parameters are $k = 15$, $A = 1$, $\alpha = 350$, and $\beta = 700$. (b) The MET vs A^{-1} , the rest of parameters the same as in (a). Solid line: Eq. (12), \circ : numerical solutions for the fully stochastic case, dashed line: Eq. (12) with the SD g_m , \square : numerical solution for the SD case, see text for details. The ratio of the fully stochastic and SD METs grows with the inhibition strength $1/A$. (c) Eq. (13) for the MET (solid line) and numerical solutions of the CME (2) (\circ) at different η for $k = 10$, $A = 10^{-3}$, and $\beta = 2 \times 10^3$.

Discussion.—We have shown, in a simple CNC model, that intrinsic discrete noise of the signal molecules can greatly increase the average number of regulated molecules and therefore enhance the persistence of the regulated component. Although we assumed that $P_{n|m}$ is Poisson distributed, we expect these findings to hold, for sufficiently strong inhibition, for other signal molecule kinetics as well. What is the mechanism behind the noise-enhanced persistence? The autocatalytic production rate of the plasmids is largest at $S = 0$; therefore, the rare events of having a very small number of S molecules strongly dominate the effective stochastic growth rate g_m ; see, e.g., Eq. (4). As a result, the average number of plasmids in the metastable state greatly increases, and this enhances the plasmid persistence. As the mode and the average for the plasmid PDF π_m coincide, this mechanism of failure of the RRE is different from that discussed previously [7].

The noise-enhanced persistence that we predict here should be observable in experiment, *in vitro* and *in vivo*, due to recent advances in single-molecule signal measurements. Finally, the effect is not system specific, and should appear in a host of other birth-death-type systems where negative feedback is at work.

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 [17] For the single-step birth-death process (3), the MET, when starting from a state m_0 , is given by [10]:

$$\tau(m_0) = \sum_{i=0}^{m_0} \prod_{j=1}^i \frac{\mu_j}{g_j} \sum_{l=i}^{\infty} \left(g_l \prod_{s=1}^l \frac{\mu_s}{g_s} \right)^{-1}. \quad (14)$$

For the exponential inhibition the products can be easily calculated: $\prod_{i=1}^m (\mu_i/g_i) = k^{-m} e^{r\eta m(m-1)/2}$. Then, for $r\eta \ll 1$, both sums in (14) can be replaced by integrals. For $m_0 \gg 1$ the inner and outer integrals receive their main contributions from the vicinity of $l = \ln k / (r\eta)$ and $i = 0$, respectively. Therefore, we can use the saddle point method for the inner integral and a Taylor expansion for the outer one. The final result coincides with Eq. (6).

- [18] Note that the CME may require a different criterion of time scale separation between the S and X dynamics, compared to the RRE. To get an accurate *a posteriori* criterion, one can use the RRE with the effective stochastic growth rate g_m . For the exponential inhibition this yields $\beta \gg \ln k$, as in the deterministic case. For the hyperbolic inhibition one obtains $\beta \gg 1$ (as in the deterministic case) for $A \gtrsim \ln k/k$, and $\beta \gg \ln k$ (as for the exponential inhibition) for $A \ll \ln k/k$.
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