Multiple binding sites for transcriptional repressors can produce regular bursting and enhance noise suppression

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Cells may control fluctuations in protein levels by means of negative autoregulation, where transcription factors bind DNA sites to repress their own production. Theoretical studies have assumed a single binding site for the repressor, while in most species it is found that multiple binding sites are arranged in clusters. We study a stochastic description of negative autoregulation with multiple binding sites for the repressor. We find that increasing the number of binding sites induces regular bursting of gene products. By tuning the threshold for repression, we show that multiple binding sites can also suppress fluctuations. Our results highlight possible roles for the presence of multiple binding sites of negative autoregulators.

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I. INTRODUCTION

The state of living cells is determined by the molecules they produce and their numbers [1]. Cells can control the production of molecules by means of gene regulatory networks [2]. In these networks, transcription factors are key proteins that bind DNA to activate or repress synthesis [1,2]. Negative autoregulation is a common component of these networks in which a transcription factor binds a specific site in the DNA and prevents its own synthesis [2]. In \textit{E. coli}, about 40\% of transcription factors are negatively autoregulated [2–4].

Since the number of some molecules in the cell can be small and the process of molecular synthesis is subject to fluctuations in the cellular environment, the resulting molecule numbers are in general noisy [5–9]. It is thought that negative autoregulation can speed up response times [2,4] and tame fluctuations [6,10,11]. Such circuits can suppress noise by regulating the number of molecules that a cell produces, permitting or repressing synthesis depending on the relative amount of the molecule [10,11]. However, negative autoregulation can also boost fluctuations if the transcription factor binding is too strong [12]. A balance of time scales is key for negative autoregulation to be able to act as a noise suppressor [13,14]. Furthermore, it has been argued that the cost of suppressing noise by negative autoregulation may be high due to fluctuations in intermediate signaling events [15]. Such intermediate events may also introduce effective delays and cause oscillations in molecule numbers [16–18].

Despite its ubiquity, the effects of noise on negative autoregulation are still poorly understood. Theoretical studies often assume a single binding site for a transcriptional repressor or an effective Hill function. However, multiple binding sites for the same transcription factor are known to form clusters within regulatory domains [19–22]. This feature has been widely observed in \textit{E. coli} [21], eukaryotes [23,24], invertebrates [25,26], and vertebrates [27]. In fact, more than half of human genes contain clusters of binding sites for the same transcription factor [27]. This suggests that multiple binding sites for transcription factors may play an important role in gene regulation [27,28].

The architecture of regulatory domains can affect bursting kinetics [29,30]. In the presence of multiple binding sites, their occupation can have multiple states. We hypothesize that stochastic switching will occur between these states and that this will set an additional time scale that affects fluctuations. Here we study how fluctuations are affected by multiple binding sites in the framework of a stochastic binding theory in which a single transcription factor represses its own production, Fig. 1.

II. MULTIPLE STOCHASTIC BINDING

We consider a single gene encoding a protein that can bind to any of \(N\) sites to repress its own synthesis. We introduce negative autoregulation as a dependence of the production rate with the number of DNA bound proteins, \(r = r(m)\), Fig. 1. We focus on noncooperative binding, that is, the affinity of proteins for binding sites \(k_b\) is not affected by how many molecules are bound already. Bound proteins fall off from the binding site with a rate \(k_d\) per molecule. Bound proteins have a decay rate \(d_f\) and free proteins have a decay rate \(d_b\) per molecule.

The statistics of the stochastic process can be described by the probability distribution \(P(n,m,t)\) to have \(n\) free proteins and \(m\) bound proteins at time \(t\) [31]. This probability distribution obeys the master equation

\[
\frac{dP(n,m,t)}{dt} = r(m)[P(n-1,m,t) - P(n,m,t)]
+ k_b[(N - (m-1))(n+1)P(n+1,m-1,t)]
- k_b[(N-m)nP(n,m,t)]
+ k_d[(m+1)P(n-1,m+1,t) - mP(n,m,t)]
+ d_f[(n+1)P(n+1,m,t) - nP(n,m,t)]
+ d_b[(m+1)P(n,m+1,t) - mP(n,m,t)].
\]  

(1)
The first line accounts for molecule production, the second and third for the binding process, the fourth for the unbinding process, and the two last lines for degradation of free and bound molecules.

The synthesis rate $r(m)$ can be adjusted to describe different regulatory mechanisms. For simplicity, here we consider the case of a sharp threshold: synthesis occurs at a rate $r_0$ while there are $M$ or fewer bound proteins, and it is fully repressed when there are more than $M$ bound proteins,

$$r(m) = \begin{cases} r_0 & \text{if } 0 \leq m \leq M, \\ 0 & \text{if } M + 1 \leq m \leq N. \end{cases}$$

Although a function $r(m)$ has not been determined experimentally, elegant experiments have determined the regulatory relationship between transcription factor concentration and gene activity [32]. The form of the synthesis rate as a function of the number of bound transcription factors that we chose here gives rise to effective regulatory functions that are consistent with these experimental observations [33]. Additionally, below in Sec. IV we discuss the case of a graded synthesis rate to show that our conclusions do not depend strongly on this choice.

In general, changing the regulatory domain architecture will affect the mean value and fluctuations of the total number of proteins. Here we are interested in how changes to regulatory domain architecture affect fluctuations, under the assumption that autoregulation controls the level of proteins to keep it at some constant functional value in steady state [2]. With similar mean expression levels, noise can vary by more than one order of magnitude when the regulatory domain architecture is changed [34]. Because adding binding sites to the regulatory domain may change the mean number of molecules, $\langle n_T \rangle = (n + m)$, to keep it constant we adjust the synthesis rate $r_0$. In the following, we choose the value $\langle n_T \rangle = 20$ for illustration [12,14,35].

We generate trajectories satisfying Eq. (1) by means of a standard Gillespie algorithm [36,37]. We obtain the free $n(t)$, bound $m(t)$, and total $n_T(t) = n(t) + m(t)$ number of proteins. The total number of proteins $n_T$ is useful to compare theoretical results with experiments that do not distinguish free and bound molecules.

III. REGULAR BURSTING

We first study the case $M = 0$ in which a single bound protein fully represses production, and we analyze the effects of varying the number of binding sites $N$. We consider the case in which bound proteins are degraded at the same rate as free proteins, $d_f = d_b = 1$. Deviations of the total number of proteins $n_T$ from its mean value $\langle n_T \rangle$ increase with $N$, Figs. 2(a)–2(c). Adding binding sites to the system leads to bursty kinetics, with increasing burst size and consequently longer decay times. This is reflected in an increasing Fano factor, defined as the variance to mean ratio $F = \sigma_{n_T}^2 / \langle n_T \rangle$, Fig. 2(j).
These bursts occur with some temporal regularity. The time interval between two consecutive maxima naturally defines burst duration $T$. Varying $N$, we compute the burst duration distribution from stochastic simulations, Figs. 2(d)–2(f). Burst duration becomes longer while the relative dispersion of the distribution around the mean decreases with increasing $N$, as reflected in the coefficient of variation, $CV = \sigma_T / \langle T \rangle$, Fig. 2(k). Thus, the characteristic time governing burst timing becomes both longer and more precise.

Temporal precision can be further characterized by the quality factor

$$Q = \frac{f_0}{\pi \Delta f},$$

where $\Delta f$ is the bandwidth and $f_0$ is the main frequency. Higher values of $Q$ indicate a narrower frequency spectrum and a more defined characteristic time [16]. To estimate the quality factor, we fit a Lorentzian distribution to the power spectral density; see Appendix A for the details. From this fit, Figs. 2(g)–2(i), we determine the main frequency $f_0$ and half power bandwidth $\Delta f$. The quality factor increases with $N$ reflecting the observed increased regularity of burst duration, Fig. 2(l). This indicates an increase in bursting temporal precision, although the values of the quality factor remain low.

As indicated in Sec. II, we keep the mean value of the total number of proteins $\langle n_T \rangle$ fixed by adjusting the value of the synthesis rate, $r_0$. The large values of $r_0$ employed in Fig. 2 are required to balance the large values of binding affinity that occur as $N$ is increased; see Appendix B.

The multiple binding sites act as a buffer for the bound proteins: as long as the occupation number $m$ is larger than 1, there can be fluctuations in $m$ and the system is still repressed. In this way, multiple binding sites give rise to an effective time scale in the system, which manifests as the time taken to exponentially decay from the maximum to the minimum value of the total number of proteins. In Sec. VI, we further discuss the mechanism underlying this observation.

Degradation of bound proteins is not required for regular bursting

In eukaryotic cells, some proteins are actively degraded by the complex machinery of the proteasome [1]. The molecular machines of the proteasome recognize specific domains in the protein surface and target these proteins for destruction. When a transcription factor binds DNA, these protein domains may or may not become obstructed. Therefore, bound and free proteins can have different degradation rates. So far we have addressed the case in which both bound and free proteins are degraded at the same rate, $d_f = d_b = 1$. Here we study the case in which bound proteins cannot be degraded, setting $d_b = 0$.

Adding $N$ binding sites with fixed $M = 0$ also leads to bursty kinetics, Figs. 3(a)–3(c). Since there is no degradation of bound proteins, the number of bound proteins decreases only when there is an unbinding event. Thus, the probability of finding $m = 0$ is lower, leading to longer decaying times under the mean and higher burst maximums. The fluctuations around the mean quantified by the Fano factor increase with the number of binding sites $N$, Fig. 3(d). The bursty fluctuations have a characteristic time that becomes longer as $N$ increases. The dispersion around the mean of the maximum distribution decreases, indicating that the characteristic time becomes more precise although the decay times are longer, Fig. 3(e). This increased regularity is also reflected in the quality factor $Q$, Fig. 3(f).

IV. NOISE SUPPRESSION

We now turn to the effects of changing the threshold $M$ for a fixed value of the total number of binding sites, $N$. We first consider the case in which bound proteins are degraded at the same rate as free proteins, $d_f = d_b = 1$. As $M$ changes, we adjust the synthesis rate $r_0$ accordingly to keep the mean value $\langle n_T \rangle$ fixed.

Increasing the threshold $M$, fluctuations decrease to a minimum and then rise again, Figs. 4(a)–4(d). This minimum occurs when synthesis events are driven by stochastic binding and unbinding of proteins; see Sec. VI for a further discussion about the mechanism. For lower values of $M$, fluctuations around the mean value of bound proteins ($m$) are not sufficient to give rise to synthesis events because a small number of molecules is enough to overcome the low threshold. For higher values of $M$, fluctuations that bring $m$ below threshold are more frequent and the synthesis rate is slower, resulting in a lax control of the mean value of proteins $\langle n_T \rangle$. 
Multiple binding sites can reduce fluctuations without increasing the turnover. (a)–(e) Time series of the total number of proteins (left panels) and corresponding histograms (right panels). (a)–(d) \( N = 14 \) and \( M = 0, 4, 9, \) and 13, and (e) single binding site autoregulation \( N = 1, M = 0 \). The orange line indicates the mean value, and the shaded region indicates the variance bounds. (f) Fano factor and (g) number of molecules degraded per unit of time, obtained from time series. The single binding site result is displayed for reference (dashed magenta line). Parameters: \( k_b = k_u = 335.5, df = db = 1 \). In (f) and (g) we first computed the Fano factor/number of molecules from a time series of 200 arb. units long, and then averaged over 100 realizations. Dots correspond to the mean value, and error bars to the standard deviation. Dots are joined by a dashed line to guide the eye.

Noise suppression with multiple binding sites outperforms the single binding site control system; see its smaller variance and Fano factor, Figs. 4(c) and 4(e). This stronger noise suppression occurs for a range of \( M \) values, Fig. 4(f). Even in cases in which single binding site regulation \( N = 1 \) increases fluctuations [12,14,38], the presence of multiple binding sites may bring noise below the level for unregulated synthesis. Moreover, the turnover of molecules does not change with the threshold value, that is, the number of molecules degraded per unit time is constant for different values of \( M \), Fig. 4(g). This indicates that this noise suppression mechanism comes at no extra cost for the system.

### FIG. 5. Multiple binding sites can reduce fluctuations without increasing the turnover when bound proteins are not degraded.

(a)–(e) Time series of the total number of proteins (left panels) and corresponding histograms (right panels). (a)–(d) \( N = 14 \) and \( M = 0, 4, 9, \) and 13, and (e) single binding site autoregulation \( N = 1, M = 0 \). The orange line indicates the mean value, and the shaded region indicates the variance bounds. (f) Fano factor and (g) number of molecules degraded per unit of time, obtained from time series (blue line) for \( N = 14 \). The single binding site result is displayed for reference (dashed magenta line). Parameters: \( k_b = k_u = 335.5, df = 1, db = 0 \). In (f) and (g) we first computed the Fano factor/number of molecules from a time series of 200 arb. units long, and then averaged over 100 realizations. Dots correspond to the mean value, and error bars to the standard deviation.

#### A. Bound protein degradation is not required for noise suppression

So far we have analyzed noise suppression in a situation in which bound and free proteins are degraded at the same rate. Next we analyze the case in which bound proteins are not degraded, that is, \( db = 0 \). For fixed \( N \), noise suppression can be achieved by changing the value of the regulatory threshold \( M \), Figs. 5(a)–5(d). There is a value that optimizes the noise for fixed \( N \), and there is a range of values for which the noise suppression is larger than that for the single site case, Figs. 5(e) and 5(f). Turnover decreases as the regulatory threshold \( M \) increases due to a sequestration effect, since bound proteins are not degraded, Fig. 5(g).
B. A sharp threshold synthesis rate is not required for noise suppression

In the previous sections, we analyzed the case of a synthesis rate \( r(m) \) with a sharp regulatory threshold, Eq. (2). Here we analyze the case of a graded synthesis rate that decreases linearly with the number of bound proteins,

\[
r(m) = \begin{cases} 
  r_0[1 - m/(M+1)] & \text{if } 0 \leq m \leq M, \\
  0 & \text{if } M+1 \leq m \leq N. 
\end{cases}
\]  

(4)

The maximum value of bound proteins for which the system can synthesize new molecules is \( m = M \); if \( m > M \), the system is fully repressed [39].

Following the same approach as in the sharp regulatory threshold case, we analyze noise levels when changing \( M \) for a fixed value of \( N \). We analyze the case in which bound and free proteins have the same degradation rate \( d_f = d_b = 1 \). As elsewhere, we adjust the value of \( r_0 \) to keep the mean value \((\langle n_T \rangle)\) fixed for different values of \( M \). As \( M \) increases, the noise decreases and then increases again, Figs. 6(a)–6(d).

The noise suppression mechanism with a graded synthesis rate can enhance noise suppression and outperforms the single site case, \( N = 1 \) and \( M = 0 \), Fig. 6(e), for a range of values of \( M \), Fig. 6(f). The turnover does not change while \( M \) changes, Fig. 6(g). Thus, a sharp threshold is not required for noise suppression by multiple binding sites.

V. REGULAR BURSTING AND NOISE SUPPRESSION ARE ROBUST

Up to now we have focused on a set of parameters in which the binding and unbinding rates are equal, \( k_b = k_u \). We now lift this constraint and analyze the effects of multiple binding sites and changing the threshold \( M \) for different \( k_b \) and \( k_u \) ratios, Fig. 7. In the three different cases spanning three orders of magnitude, adding multiple binding sites generates regular bursting for low \( M \), Figs. 7(a)–7(c). The smaller the ratio \( k_u/k_b \), the larger the quality factor and the region in \((N,M)\) space where regular bursting is enhanced. Increasing the binding affinity generates longer decay times of bursts together with a larger memory effect.

The noise suppression mechanism discussed above is present for a wide range of \( k_u/k_b \) ratios, Figs. 7(d)–7(f). We introduce the relative Fano factor \( F_R \), defined as the Fano factor normalized to its single binding site value \((N = 1, M = 0)\). Values of \( F_R \) smaller than 1 reveal a region in \((N,M)\) space in which noise suppression outperforms the single binding site case, Figs. 7(d)–7(f). For any number of binding sites \( N \) there is a threshold \( M \) that reduces fluctuations. As the ratio \( k_u/k_b \) increases, the region of noise suppression becomes larger and the relative suppression effect becomes stronger. This is in line with the finding that weak binding is critical for the fidelity of autoregulation [14].

The particular form of the synthesis rate \( r(m) \), Eq. (2), is not crucial for regular bursting and noise suppression. Both phenomena are observed also with a graded synthesis rate that decreases linearly with \( m \) (Secs. III and IV and Fig. 6). While here we have chosen the same degradation rate for free and bound proteins, regular bursting and noise suppression also occur in a system in which bound proteins cannot be degraded.

FIG. 6. Multiple binding sites can reduce fluctuations without increasing the turnover in the case of a graded synthesis rate. (a)–(c) Time series of the total number of proteins (left panels) and corresponding histograms (right panels). (a)–(d) \( N = 14 \) and \( M = 0, 4, 9, \) and 13, and (e) single binding site autoregulation \( N = 1, M = 0 \). The orange line indicates the mean value, and the shaded region indicates the variance bounds. (f) Fano factor and (g) number of molecules degraded per unit of time, obtained from time series (blue line) for \( N = 14 \). The single binding site result is displayed for reference (dashed magenta line). Parameters: \( k_b = k_u = 335.5, d_f = d_b = 1 \). In (f) and (g) we first computed the Fano factor/number of molecules from a time series 200 arb. units long, and then we averaged over 100 realizations. Dots correspond to the mean value, and error bars to the standard deviation.

(Secs. III and IV and Figs. 3 and 5). These observations suggest that the reported phenomena are robust and do not depend on our simplifying assumptions.

VI. BOUND PROTEIN DYNAMICS DRIVES REGULAR BURSTING AND NOISE SUPPRESSION

This section discusses in more detail the mechanism behind regular bursting and noise suppression reported in the previous sections. We showed that adding multiple binding sites gives rise to regular bursting, Figs. 2 and 3. We argued that this occurs because multiple binding sites introduce an effective time scale in the system and act as a buffer of bound proteins.
We further showed that fluctuations are minimized for some value of the threshold $M$, Figs. 4, 5, and 6. We argued that the minimum occurs when synthesis events are short and frequent because the dynamics of the bound proteins $m$ is dominated by the processes of stochastic binding and falling off. Below we expand on these ideas and discuss how regular bursting and noise suppression emerge as a consequence of the dynamics of bound proteins in the presence of multiple binding sites.

With multiple binding sites and low values of $M$, bound protein $m$ fluctuations are not enough to cross the threshold, Figs. 8(a) and 8(d). No production events are possible while $m > M$, so the system is inhibited most of the time, Fig. 8(b). Only after degradation of most proteins does the number of proteins become very small and $m \leq M$, making synthesis events possible for a brief period of time, Figs. 8(d) and 8(e). During this short interval of time, a burst of synthesis occurs overshooting the total number of proteins $n_T$ over the mean, Figs. 4(a) and 4(b) and Fig. 8(e). This overshoot over the mean together with long degradation times leads to large fluctuations in the number of proteins and high noise. These excursions over the mean have a characteristic time scale set by the time needed for the bound proteins to go below $M$ after a synthesis burst, Fig. 8(d). Stochastic fluctuations of $m$ that do not cross the threshold $M$ do not lead to synthesis events. In this way, multiple binding sites confer a buffer of bound proteins that avoids synthesis events due to stochastic fluctuations.

For high values of $M$, bound protein $m$ fluctuations are enough to cross the threshold, and the probability of finding the system active is high, Figs. 8(a) and 8(b). Furthermore, for the highest values of $M$, the mean value is below threshold, $(m) \leq M$, Figs. 8(a) and 8(h), indicating that most of the time the system can produce new molecules. In this situation, the probability of finding the gene active is high, and the synthesis rate $r$ is small in order to keep $(n_T) = 20$, Fig. 8(i). On the one hand, this small synthesis rate leads to a slow response that keeps the total number of proteins below the mean. On the other hand, since the number of free binding sites is typically low in this situation, binding events that overcome the threshold and inhibit the synthesis are not frequent, Fig. 8(h). This small number of free sites and the lack of inhibition keep synthesis going even when the number of proteins is above the mean value, $n_1 \geq (n_1)$. The slow response in both situations results in a lax control, which increases deviations from the mean. This causes poor noise regulation for high values of regulatory threshold $M$. 

FIG. 7. Regular bursting and noise suppression are robust to different binding or unbinding ratios. (a)–(c) Quality factor maps for different numbers of binding sites $N$ and threshold $M$. (d)–(f) Relative Fano factor $F_R$. Orange lines correspond to $F_R = 1$. Parameters: (a) $k_b = 1$, $k_d = 10$; (b) $k_b = k_d = 335$; and (c) $k_b = 10$, $k_d = 1$. In all panels, $d_f = d_b = 1$. Mean values of quality and Fano factor are averaged over $N_i = 100$ stochastic simulations.

FIG. 8. Bound protein dynamics. (a) Mean value and confidence interval of bound protein $m$. The solid blue line indicates the mean value of $m$ for the different regulatory thresholds $M$. The dashed orange line indicates the reference value of the regulatory threshold $M$. Shaded boxes indicate 90% (dark) and 99% (light) confidence intervals. The color scale goes from highest (black) to lowest (white) Fano factor. (b) Probability $\text{Prob}(m \leq M)$ of finding synthesis un-repressed for the case $N = 14$, solid blue line. The dashed magenta line indicates a reference value of the probability for $N = 1$ and $M = 0$. (c) $p$ value of two sample Kolmogorov-Smirnov test comparing the distribution of bound proteins in two situations, with $(r, d_f, d_b) = (1, 1, 1)$ and without synthesis and degradation $(r = d_f = d_b = 0)$. (d,f,h,j) Time series of the bound number of proteins $m(t)$ (e,g,i,k) Time series of total number of proteins $n_T(t)$. In panels (d)–(k) the shaded region (gray or magenta) indicates when $m(t) \leq M$. Parameters: $k_b = k_d = 1$, $d_f = d_b = 1$. (a)–(c) Statistics computed over 100 realizations of 200 arb. units for every $M$. 

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When the mean value of bound proteins is close to the threshold, noise suppression is optimal, Figs. 8(a) and 8(f). Fluctuations around the mean value are enough to cross the threshold frequently, and the system is able to synthesize new molecules. These excursions of the bound proteins are mainly due to stochastic binding and unbinding events. To show this, it is instructive to consider the particular case in which there is no degradation and no production, with fixed \( n_T = \langle n_T \rangle \). This particular case provides a reference distribution, where the dynamics of \( m(t) \) is only due to stochastic binding and unbinding events. We can compare this reference distribution with the distribution of \( m(t) \) for different values of thresholds \( M \) and \( N = 14 \) in the general case. We compare the distributions for different \( M \) with the particular case with a two-sample Kolmogorov-Smirnov test. We test whether the values of \( m \) of the different cases and the reference case come from the same distribution. High \( p \) values indicate that we cannot reject the hypothesis that the distributions of the two samples are the same. Where the noise suppression is minimum, we see that the \( p \) values are higher, Fig. 8(c). This indicates that excursions of the bound proteins above and below threshold are mainly due to stochastic binding and unbinding, and not because of changes in the total number of proteins due to synthesis and degradation of molecules. Thus, since the mean value and the threshold are close, the excursions and the short and frequent synthesis events are due to fluctuations in the bound proteins.

We include the one-binding-site case \( N = 1 \), \( M = 0 \), Figs. 8(b), 8(j), and 8(k), for reference to highlight the effects of adding multiple binding sites. Synthesis events occur in bursts, and fluctuations from the mean value are large since synthesis events are rare. However, there is no characteristic time scale in the system since fluctuations in the bound proteins drive the system in and out from synthesis and there is no buffer, Fig. 8(j).

### Binding and unbinding kinetics

We have discussed how the regulatory domain architecture specified by the number of binding sites \( N \) and the regulatory threshold \( M \) controls the mechanism giving rise to regular bursting and noise suppression. Here we show how binding kinetics affects this mechanism by varying the rates \( k_b \) and \( k_u \) for a fixed regulatory architecture.

Small \( k_u/k_b \) ratios increase the Fano and the quality factor, Figs. 9(a) and 9(b). The increase in the Fano factor indicates that there is more dispersion in the total number of proteins around the mean value. The increase in the quality factor indicates that this dispersion comes with increased temporal precision and a characteristic time scale. As the ratio \( k_u/k_b \) decreases, binding becomes more stable. A stronger binding than unbinding rate generates more stably bound proteins and larger decay times, setting a characteristic time scale or increasing its length.

At large \( k_u/k_b \) ratios, binding is unstable, Figs. 9(a) and 9(b). In this scenario, there are large fluctuations in the number of bound proteins under the threshold \( M \). Production events are frequent, but the number of molecules produced in each event is small. This leads to small fluctuations around the mean value of the total number of proteins. Since production events are driven mainly by stochastic fluctuations of \( m \) below \( M \), there is no typical characteristic time in the system. Thus, a stronger unbinding than binding rate generates small fluctuations without a characteristic time scale and with a smaller Fano factor.

For \( k_b = k_u = k \), the Fano and quality factor exhibit a nonmonotonic behavior as a function of \( k \), Figs. 9(c) and 9(d). For low values of \( k \), the binding probability is low. Binding events are not so frequent, and \( \langle m \rangle \) is small. Since \( \langle m \rangle \) is small and \( M = 1 \), fluctuations of bound proteins that cross the threshold are frequent and there is no characteristic time scale in the system. When we increase the value of \( k \), the probability of binding increases. The mean value of bound proteins \( \langle m \rangle \) is not close to the threshold \( M \) as before. This gives rise to a memory effect in the bound proteins and a characteristic time scale in the system. For even larger values of \( k \), both fluctuations and quality factor decrease. The probability of binding and unbinding is large, so binding and unbinding events are frequent. This generates high-frequency fluctuations in the number of bound proteins. As the burst decays and the number of bound proteins becomes low, the probability of crossing the threshold increases because of large high-frequency fluctuations in \( m \). Therefore, the memory is shorter, and the precision of the characteristic time scale is lower.

In summary, the regulatory domain architecture set by \( N \) and \( M \) together with binding kinetics determine the bound protein dynamics, the negative feedback mechanism, and the dynamics of the total number of proteins, \( n_T(t) \).
VII. DISCUSSION

We studied the stochastic dynamics of a negatively autoregulated gene with multiple binding sites for the repressor, Fig. 1. We showed that increasing the number of binding sites induces regular bursting of gene products, Fig. 2. In deterministic systems, explicit time delays or multiple intermediate steps are required together with strong nonlinearity in order to generate biochemical oscillations [40,41]. The system we study here does not include such intermediate steps or delays, yet it produces regular bursting [42]. The stochastic dynamics of multiple binding site occupation induces an additional effective time scale that triggers regular bursting. The vertebrate segmentation clock [43] may be a good model system to test our results. This genetic oscillator acting during embryonic development is thought to be driven by negative autoregulation of her/Hes genes, which have several regulatory binding sites for the repressors [19,44]. In zebrafish these genetic oscillations appear to be cell autonomous [45]. Although it is thought that autoregulation of her genes involves effective delays [44,46], it is possible that the presence of multiple binding sites enhances oscillations [33]. In the mouse it has been proposed that collective rhythms are not cell-autonomous, rather they arise as a consequence of intercellular communication from the stabilization and synchronization of bursts [47]. The regular bursting induced by multiple binding sites we observe here may underlie these noisy biochemical oscillations and collective rhythms in the mouse segmentation clock. In summary, our results may establish paradigms for biochemical oscillations that either generate oscillations [49,50] or tightly control fluctuations around the mean number of molecules [10,11].

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APPENDIX A: POWER SPECTRAL DENSITY AND QUALITY FACTOR ESTIMATION

Here we outline with more detail the calculation of the power spectral density and the quality factor. We run \(N_s = 100\) stochastic simulations for every set of parameters \(\{N, M, r, d_f, k_b, k_u, d_b\}\). We use the Hilbert transform to obtain a signal without amplitude fluctuations. We first compute the Hilbert transform of the time series \(n_T(t)\) minus the median, \(\hat{H}(n_T(t)) - \text{Med}(n_T)\) [57], using the signal processing package SCIPY of PYTHON. From the Hilbert transform we extract the analytical phase value and take the cosine of this phase to obtain \(h_T(t)\). We then compute the Fourier transform \(\hat{h}_T(f)\) of the time series \(h_T(t)\) using the fast Fourier transform algorithm implemented in the package SCIPY. We estimate its power spectral density \(S_{\hat{n}} = |\hat{h}_T(f)|^2\) [58]. We average the power spectral density over the \(N_s\) realizations, and fit by least squares the resulting average with a Lorentzian with transcription factor binding sites [55,56]. Our results could be useful in the design of synthetic control systems that either generate oscillations [49,50] or tightly control fluctuations around the mean number of molecules [10,11].

FIG. 10. Large binding rates require large synthesis rates in order to fix the average number of proteins. (a) Rate of synthesis \(r_0\) for \(m \leq M\) for different values of binding \(k_b\) and unbinding \(k_u\) rates. (b) Average binding propensity evaluated at burst peaks for different values of binding \(k_b\) and unbinding \(k_u\) rates. We introduce \(\bar{n}\), the average of \(n\) values at burst peaks. (c) Propensity ratio for different values of binding \(k_b\) and unbinding \(k_u\) rates. Parameters: \(N = 11\), \(M = 1\), \(d_f = d_b = 1\).
distribution $L(x) = A y^2/((\pi y)((x - f_c)^2 + y^2))$, where $A$ is a scale coefficient, $f_c$ specifies the peak location, and $y$ is a dispersion parameter that specifies the half-width at half-maximum. From the fit we compute the quality factor $Q$ with main frequency $f_0 = f_c$ and half-power bandwidth $\Delta f = 2y$.

**APPENDIX B: BALANCING SYNTHESIS AND BINDING PROPENSITIES TO FIX THE AVERAGE NUMBER OF MOLECULES**

To keep the mean number of proteins constant, as we increase the number of binding sites $N$ we also increase the value of the synthesis rate $r_0$. Since the binding propensity $a_{\text{bin}}(n,m) = k_b(N - m)n$ grows with the number of binding sites $N$, increasing $N$ increases the mean value of bound proteins ($m$). Therefore, the probability of finding the system open for synthesis $P(m \leq M)$ decreases. As a consequence, there are fewer synthesis events, so keeping $\langle n_T \rangle$ constant requires a larger synthesis propensity $a_{\text{syn}}(n,m) = r(m)$. This balance of increased synthesis rate together with a larger ($m$) maintains the average number of proteins fixed, as required.

For fixed $N$, changing the values of the binding and unbinding rates also requires a change in the value of $r_0$ since they modify ($m$) and $P(m \leq M)$. As $k_b$ or $k_u$ vary, the value of $r_0$ spans several orders of magnitude, but the binding propensity also does, Figs. 10(a) and 10(b). The order of magnitude of the ratio $r_0/a_{\text{bin}}$ remains constant in the region where the noise in the system is higher and the value of $r_0$ is larger, Fig. 10(c). High synthesis rates are required due to the low probability of synthesizing arising from the high binding rate.

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[39] For $M = 0$ this graded synthesis rate is equivalent to a sharp threshold, Eq. (4). For this reason, we do not discuss regular bursting with a graded synthesis rate in Sec. III. However, with multiple binding sites $N > 1$ and $M > 0$, Eq. (4) is different from Eq. (2).


