Molecular level stochastic model for competence cycles in *Bacillus subtilis*

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The role of stochasticity and noise in controlling genetic circuits is investigated in the context of transitions into and from competence in *Bacillus subtilis*. Recent experiments have demonstrated that bistability is not necessary for this function, but that the existence of one stable fixed point (vegetation) and an excitatory unstable one (competence) is sufficient. Stochasticity therefore plays a crucial role in this excitation. Noise can be generated by discrete events such as RNA and protein synthesis and their degradation. We consider an alternative noise source connected with the protein binding/unbinding to the DNA. A theoretical model that includes this "nonadiabatic" mechanism appears to produce a better agreement with experiments than models where only the adiabatic limit is considered, suggesting that this unconventional stochasticity source may be important for biological functions.

stochasticity | nonadiabaticity | gene networks | competence

Bacteria encode in their genetic material many different strategies of responding to environmental stress to ensure their survival. Examples of such strategies are motility, chemotaxis, antibiotic production, and ultimately sporulation. Many of these responses occur only in a fraction of an isogenic population: phenotypic variability can be obtained even from identical DNA sequences (2, 3). Thus, a population, without rare genetic mutations, can rapidly regenerate a variety of phenotypes from a single phenotype able to survive a certain condition. This variability most likely arises from fluctuations in the cell network that occur even under favorable conditions. This stresses the biological relevance of stochasticity in cellular processes, allowing functional plasticity by switching between phenotypes (4).

Competence is a differentiated state observed in many bacterial species that is an alternative survival strategy to sporulation. Competence occurs in populations with high cell densities under limited nutrient conditions, when the cells are in stationary growth. When cells become competent, DNA replication stops and cell division ceases. In this state, the cell becomes able to capture DNA from its surroundings. There is no sequence specificity for the subsequent DNA uptake. It has been proposed that the absorbed genetic material can provide templates for DNA repair or be a source of nutrients like phosphates or may simply be incorporated in the cell's genome. Competence is frequently linked to the production of antibiotics that can lyse nearby cells, releasing their genetic material to the environment. These extrinsic sources of noise would lead to variations in the rates of the chemical equations that describe the system. Traditionally, an adiabatic approximation is used to temporally average over the neglected dynamics of chemical substeps of the system, such as the degradation complex dynamics and binding and unbinding of proteins to the DNA (17, 18). Such adiabatic approximations usually consider that binding equilibrium is reached rapidly when compared with the other timescales of the system. We argue however that microscopic DNA binding events could be important sources of fluctuations in this system as was discussed earlier in a more abstract context (19). We investigate all these sources of noise, which may be important for biological functions.

ComK are tightly regulated by the presence of MecA, which recruits ComK for degradation by the ClpC/ClpP protease complex. The concentration of ComK is kept low by MecA/ClpC/ClpP degradation, but when a certain threshold is crossed, self-activation takes place and ComK is brought to high levels. Virtually all cells lacking a functional MecA protein produce ComK and go into competence. Two different pheromones involved in quorum sensing, ComX and PhrC, induce the sfA promoter. This promoter is linked both to the synthesis of the antibiotic surfactin and also to the synthesis of a small peptide ComS that binds to the MecA/ClpC/ClpP complex. As the expression of mecA and eplC does not change much over stationary growth phase, the production of ComS protects ComK from degradation, allowing ComK levels to reach the threshold for self-activation (5, 9, 10). Experiments have shown that overexpression of ComK is preceded by high levels of ComS. There is also experimental evidence for inhibition of comS by ComK (11) (Fig. 1).

Even in optimal conditions, only ~10% of the vegetative cells overexpress ComK and become competent, and this percentage is independent of neighboring cells or family history. Interestingly, the overexpression of ComK has a more or less defined duration. Cells that become competent come back to vegetative state and divide after ~20 h, suggesting that competence is not necessarily a stable state (5). Previous studies have proposed a model where the system shows a stable state and an excitable unstable state (12, 13). The system can be thrown out of the stable state (vegetative) and then make an excursion around the unstable fixed point (competence), only to come back to the stable state after some time. The lack of stability of the competent state would make its duration less variable. These facts strongly suggest that noise plays a crucial role in competence control, exciting the system into the competent state. The noise can have many origins: the synthesis of RNA proteins, the degradation of the proteins through the MecA/ClpC/ClpP complex, and the binding of proteins to the DNA promotor, which are known as intrinsic noise sources (14–16). Noise could also come from extrinsic sources, which are fluctuations in the environment. These extrinsic sources of noise would lead to variations in the rates of the chemical equations that describe the system. Traditionally, an adiabatic approximation is used to temporally average over the neglected dynamics of chemical substeps of the system, such as the degradation complex dynamics and binding and unbinding of proteins to the DNA (17, 18). Such adiabatic approximations usually consider that binding equilibrium is reached rapidly when compared with the other timescales of the system. We argue however that microscopic DNA binding events could be important sources of fluctuations in this system as was discussed earlier in a more abstract context (19). We investigate all these sources of noise, which may be important for biological functions.

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Determining their effect in the competence cycle. A stochastic model was developed with the goal of determining the importance of different sources of noise (20–27).

Initially, we examined the deterministic limit to orient our understanding of how different dynamical regimes are obtained for different parameters (12, 13). Once these regimes leading to the excitable mechanism are determined, we could then explore the full stochastic approach, analyzing many possible sources of noise. We express the results of our simulations through sample trajectories plotted over the effective potential surface, obtained from the average of many trajectories. These surfaces, although not denoting a rigorously proper potential field (27), help visualizing the basins of attractions and provide a systematic way of visualizing the probability distribution. To connect our theoretical work to studies in the laboratory, we address two variables that can be measured in vivo in the laboratory, we address two variables that can be measured in vivo in the laboratory, we address two variables that can be measured in vivo in the laboratory. For better visualization, instead of the probability distribution of the system. We will express our results through trajectories generated over effective potential surfaces. Trajectories are generated from the stochastic reactions using the Gillespie algorithm. In the simulations, the system starts at the vegetative stable state and is followed until a whole competence cycle is completed. The average of many trajectories results in average vegetative times and average competence times, as well as a probability distribution for the states of the system. For better visualization, instead of the probability distributions we plot “effective potential” surfaces, obtained from $V_{eff} = -k_B T$. These surfaces, although not denoting a “real” potential, help visualize the basins of attraction in the system.

Exploring the Ensemble of Possible Dynamical Behaviors

To characterize when the model shows excitable dynamics, we first simplify our model using the deterministic limit (12, 13). We discuss the kinetic rate equations for $k$ and $s$ under the assumption that the degradation complex binding to ComK and ComS and the binding/unbinding of ComK to DNA are fast compared with synthesis and degradation reactions. Assuming that the time scales for the reactions involving the binding of the degradation complex to ComK and ComS are overwhelmingly faster than the net degradation rate, they can be considered as being in equilibrium. Considering that the total number of degradation complexes is constant

$$h(k) = \frac{h(k) = \frac{g(k)}{1 + (k/A)f_s}}{g(k)}$$

$$h(k) = \frac{h(k)}{g(k)}$$

These in turn, can lead to the degradation of ComK or ComS and subsequent release of free units of the degradation complex. We denote the total number of degradation complexes as $A$. Free units of the degradation complex are denoted as $A_k$ and complexes formed with ComK and ComS are denoted as $A_{ik}$ and $A_{ik}$, respectively. This model can be expressed by the following chemical reactions

$$k_1 + k \xrightarrow{A_1} S \xrightarrow{S} A_1 + A_2$$

$$k_2 \xrightarrow{A_2} k_3 + k \xrightarrow{A_2} A_1 + A_2$$

$$k_4 \xrightarrow{A_4} k_5 + k \xrightarrow{A_4} A_1 + A_2$$

$$k_6 \xrightarrow{A_6} k_7 + k \xrightarrow{A_6} A_1 + A_2$$

$$k_8 \xrightarrow{A_8} k_9 + k \xrightarrow{A_8} A_1 + A_2$$

Little is known with certainty about the rates associated with these individual reactions. In this work we will highlight the changes in dynamical behavior that depend on the relative speed of three processes: binding/unbinding events, MecA/CipC/CipP dynamics and synthesis/degradation of proteins, and discuss the implication of the relative kinetics in the stochastic context. The first two processes (binding/unbinding and degradation dynamics) are usually assumed to be extremely fast, but in a system where noise plays a decisive role, the fluctuations induced even by fast processes can become important. We will use different stochastic models to explore different sources of stochasticity and observe their effect on the system. We will express our results through trajectories generated over effective potential surfaces. Trajectories are generated from the stochastic reactions using the Gillespie algorithm. In the simulations, the system starts at the vegetative stable state and is followed until a whole competence cycle is completed. The average of many trajectories results in average vegetative times and average competence times, as well as a probability distribution for the states of the system. For better visualization, instead of the probability distributions we plot “effective potential” surfaces, obtained from $V_{eff} = -k_B T$. These surfaces, although not denoting a “real” potential, help visualize the basins of attraction in the system.
(A = A_1 + A_k + A_s), we can set dA_1/dt, dA_k/dt, and dA_s/dt equal to zero, yielding A_1 = A(k, s), A_k = Aϕ(k, s)ψ/T_s, and A_s = Aϕ(k, s)ψ/T_s, where \( k_1 = (λ_1 + λ_2)λ_{12} \) and \( T_1 = (λ_1 + λ_2)λ_{12} \) are the concentrations of \( k \) and \( s \) for half-maximal degradation and degradation of \( \phi(k, s) = A/k = 1/(1 + k/T_s + s/T_s) \) is a measure of the availability of the degradation complex. Assuming that the binding/unbinding rates are also faster than the synthesis/degradation rates, the synthesis rate of \( A \), \( \tau_1 \), and \( \tau_2 \), is also faster than the synthesis/degradation rates, the effective surface for a near-equilibrium system is unstable fixed points and still others for situations where there are three fixed points: one stable point, one saddle point, and one unstable point. An unstable fixed point, corresponding to high concentration of ComK, implies that lengthy excursions into competence usually start with the system having one stable state, as pointed out in the pioneering work of Elowitz et al. (12, 13). Histograms of the values of the sets of parameters found to result in an excitable system show wide Gaussian-like distributions. The region in the parameter space corresponding to the excitable system appears to be compact and narrow. If we choose from here a standard set of parameters corresponding to the centers of the distributions on the histograms.

### Simulating the Full Stochastic Model

Using Eq. 1 as a basis for a stochastic treatment, simulations were performed using the Gillespie algorithm (29), starting in the vegetative state and simulated through many cycles. The Gillespie method accounts for noise by explicitly dealing with the fate of small number of particles actually involved in gene regulation in an individual cell. Simulations were performed for several sets of parameters: some corresponding to the system having one stable fixed point, others for the case having one stable and one unstable fixed points and still others for situations where there are two stable fixed points. Sample trajectories were generated and are displayed over the “effective potential surface.” The effective potential surface is calculated directly from the probability distribution that results from the averaging over many trajectories. The effective surface for a near-equilibrium system is \( V_{eff} = -\ln P \) and we use the same construct to orient our thinking in the far-from-equilibrium situation relevant here (Fig. 1).

Excursions into competence usually start with the system having a higher concentration of ComS then the stable vegetative fixed point, due to fluctuations. The ComK concentration then starts to increase and the trajectory begins to drift toward the vicinity of the unstable fixed point. The concentration of ComS then decreases at high levels of ComK because of the repression. Finally, ComK returns to its basal levels and the ComS levels are restored. The effective potential surface exhibits a well that corresponds to the vegetative state and a rather flat region that corresponds to the excursions into competence exploring the neighborhood of the unstable fixed point. To compare our results with the laboratory experiments, we will use the models to compute the probability that a cell under stress (presence of ComS) will go into competence \( (P_1) \) and also will compute the time spent in competence cycles (\( T_c \)). To compare the results of these calculations with observations, we note that cells in the vegetative state have a life cycle of \( \approx 4 \) h. However, most vegetative \( B. subtilis \) cells in a culture under stationary growth conditions eventually sporulate, providing a limiting time cutoff. Some cells go into competence instead. These cells have cell division arrested until they return to vegetative state, when the cells grow, septate, and divide. Considering that each cell division event in the culture leads to a vegetative cell, the total number of competence events observed divided by the total number of cell divisions will give the proportion \( P_1 \) of vegetative cells that are said to go into competence. Our simulations do not explicitly contain the mechanism of sporulation. The proportion of vegetative cells going into competence corresponds then to the probability of entrance into competence within the average time of a vegetative life cycle.

### Adiabaticity in Competence Control

To study the effects of different sources of noise on the system, we use the approach of focusing in a specific source while silencing other potential sources of noise. To investigate the importance of considering explicitly the equilibrium of the complex formation between MecA/ClpC/ClpP and ComK/ComS, we silence the noise coming from ComK binding/unbinding to the DNA by using the adiabatic limit of \( g(k) = g_1 P(K_1) + g_0 P(K_0) \). The system then shows stochastic noise coming only from the synthesis and degradation of \( \text{ComK/ComS} \), and from the degradation complex equilibrium. Simulations performed for different values of a degradation adiabaticity parameter showed that neither the competence times nor the probabilities of initiation were significantly affected by different levels of noise (see SI Appendix). The effective potential surfaces also showed similar qualitative properties. These results suggest that an adiabatic approximation is valid for the degradation complex equilibrium. Making this approximation, simple stochastic reactions referring to the degradation of ComK and ComS would have \( k_s \) and \( s \)-dependent rates of \( \phi(k, s)_\psi \) and \( \phi(k, s)_\phi \).

To study the importance of explicitly considering the binding and unbinding of ComK to the DNA, we silence the noise coming from the degradation complex equilibrium by using the adiabatic approximation discussed above. The sources of noise are now the overall synthesis and degradation of ComK and ComS as well as the binding and unbinding of ComK to the DNA. To indicate the relative speed of these molecularly distinct processes, we introduce the binding/unbinding adiabaticity parameter \( \omega = k_{AB}/k_{S} \). Simulations were performed for many values of \( \omega \) and compared with the commonly used adiabatic limit approximation. In the adiabatic limit we use a \( k \)-dependent synthesis rate \( g(k) = (g_1 + (k^{\omega_{AB}/k_{S}}))(1 - k_{AB}/k_{S}) \) in substitution to the explicit binding and unbinding rates.

This approximation corresponds to the case where \( \omega = \infty \), meaning the binding/unbinding reactions are much faster than other time scales of the problem and can be averaged. In Fig. 2, we see samples of trajectories plotted over the effective potential surface for some values of \( \omega \). The surface is less flat around the
unstable fixed point for smaller values of \( \omega \). Trajectories do not approach the unstable fixed point and a clear path for entrance and exit from competence emerges. For values of \( \omega \) smaller than 1, expression of ComK equilibrates to the binding state of the \( comK \) promoter, and the system shows a “stable” competent state, which will persist for the lifetime of the ComK–DNA complex (19, 30, 31). In Fig. 3, we see the time evolution of ComK and ComS levels for different values of \( \omega \). Smaller values of \( \omega \) allow a faster transition to competence, as well as a higher expression of ComK and a longer time spent at maximum levels of ComK. When the effects of nonadiabaticity are considered, the probability of initiation of competence is much higher, even for values of \( \omega \) well above 1 (SI Appendix). Sets of parameters that do not exhibit competence when the adiabatic approximation is made, can show competence when there is nonadiabatic binding/unbinding of the promoter. We see, therefore, that nonadiabaticity can expand the region of the parameter space showing competence, allowing increased robustness of the system to parameter variation. These results manifest the idea of functional stochasticity, in which fluctuations increase the cell’s robustness.

### Changing Basal Rates of Expression of ComK and ComS

Laboratory experiments have been performed where the basal rates of expression of ComK and ComS (\( g_1 \) and \( g_s \), respectively) were controlled (13). These studies reveal interesting effects on both competence times (\( T_c \)) and probabilities of initiation of competence cycles. The basal rate of expression of ComK has been shown to control the probability of initiation of competence events, whereas the basal rate of expression of ComS controls mostly the duration of such events. To see the effects of adiabaticity on this control, simulations were performed for different values of the adiabaticity parameter \( \omega \), using the standard set of parameters and changing only the basal rates of expression. These effects are shown in Fig. 4. As observed in the laboratory, increasing the basal ComK expression \( g_1 \) does not change the competence time, but does increase the probability of initiation (13). Increasing the basal ComS expression \( g_s \) indeed increases the competence times. However, the effect of \( g_s \) on the probability of initiation depends on the adiabaticity. In the adiabatic limit or for high values of \( \omega \) there is no change in \( P_e \). For lower values of \( \omega \), there is a slight increase in \( P_e \), whereas for \( \omega = 1 \), there is a large increase. Laboratory experiments indeed do observe such an increase in \( P_e \) as the basal expression of ComS is made larger. This finding suggests the presence of significant nonadiabatic effects in competence control.

To better illustrate these effects, we plot in Fig. 5 the time evolution of ComK and ComS levels obtained from the simulations. Increased \( g_s \) does not change the width of the peaks of ComK expression, but does increase their frequency, eventually leading to oscillatory behavior. This oscillatory behavior corresponds to another region of the parameter space, where there is only one unstable competent fixed point. Increasing \( g_1 \) causes the width of the peaks to increase. The frequency of the peaks initially stays the same, but increases for higher values of \( g_s \) in the case of \( \omega = 10 \) considered here. For lower values of adiabaticity, there is an increased probability of higher levels of ComS during vegetation (SI Appendix). Higher levels of ComS start competence events, so this change accounts for the increase in the probability of competence initiation observed for nonadiabatic scenarios.

### Entrance into and Exit from Competence

Here, we look more carefully into the events of entering into competence and the exit from competence. We look only at the variations of the ComK level \( k \), considering the level of ComS to be fixed (\( s \) is a parameter). We focus the analysis on the behavior of the feedback loop of ComK having a fixed concentration of ComS. This situation arises where ComS levels vary slowly or are deter-
minded by other cell processes. The dynamics of the degradation complex are taken to be in the deterministic limit, leaving a stochastic model with noise only from synthesis and degradation of ComK, and binding and unbinding of ComK to the DNA. We then analyze two simplifications of this model, corresponding to the deterministic limits of the synthesis/degradation and the binding/unbinding processes. First, we will consider a model where binding/unbinding processes occur at a deterministic rate and synthesis/degradation are stochastic events. This results in a birth–death process where the rates depend on $k$. This model corresponds to the case where binding/unbinding events are fast. The more comprehensive model treats the binding/unbinding events explicitly in a stochastic manner and the protein number respond deterministically to the DNA binding state. In this case, the probability of having a binding or unbinding event depends on the number of proteins at that time. This model would correspond to the case where binding/unbinding events are especially slow.

**Fast Binding/Unbinding**

Considering synthesis/degradation and binding/unbinding deterministically, we write an equation for the probability of having precisely $k$ molecules of ComK:

$$
\frac{d}{dt} P(k) = g(k - 1)P(k - 1) - g(k)P(k) + \lambda (k + 1)P(k + 1) - \lambda (k)P(k),
$$

where $g(k)$ and $\lambda (k) = \phi(k) \Gamma_k$ are the $k$-dependent synthesis and degradation rates discussed in the adiabatic limit. $\phi(k)$ is $\phi(k,s)$ with $s$ fixed.

The steady-state solution can be obtained iteratively by $P(k + 1) = (g(k)/\lambda (k + 1))P(k)$. A time-dependent solution can be obtained by expressing the equation through a transition matrix $M$ such that $dP/dt = MP$, giving the matrix exponential solution $P(t) = \exp(Mt)P_0$ (19).

We analyze this approximation by comparing it to simulations performed for different values of the adiabaticity parameter $\omega$ (Fig. 6). We consider two scenarios: entrance into competence ($s = 100$) and exit from competence ($s = 20$). In both cases, it is clear that nonadiabaticity has a large influence on the stabilization of unfavorable states. Nonadiabaticity allows bistability or excitability in many cases where the adiabatic limit does not allow such behavior, as can be observed from the preservation of the vegetative well in the entrance case and the flat competent region in the exit case. The adiabatic approximation can, however, successfully describe the most stable well in both cases.

**Slow Binding/Unbinding**

We consider now the scenario where switching events between binding states are slow and happen stochastically, whereas synthesis and degradation are modeled in a deterministic fashion. If the system is in a given binding state with synthesis rate $g$, the ComK level $k$ varies deterministically in time as

$$
\frac{dk}{dt} = g - \phi(k) \Lambda_k k.
$$

This equation is separable, and a solution $k(t)$ can be found. With this solution in hand, given an initial binding state and an initial level of ComK $k$, we can calculate the probability of switching binding states at any given time based on the time evolution of $k$. If the system starts with the gene unbound and typical low values of $k$, we will call $t_{up}$ the time that it takes for the system to reach the threshold to transition to competence, following a binding event. $t_{down}$ is then the duration of switches in the binding state necessary for transitions from vegetation to competence to occur. The probability of reaching $t_{down}$ following a binding event will be called $P_{up}$, the probability that the gene will stay bound long enough for the transition to happen. The unbinding rate $f$ is independent of $k$, so $P_{up} = e^{-t_{down}}$. If, instead, the system starts with the gene bound and typical competence values of $k$, we will call $t_{down}$ the time that it takes to reach the vegetative state after an unbinding event. The probability of reaching $t_{down}$ following an unbinding event has to take into consideration the decreasing levels of $k$ when the gene is unbound, since the binding rate $h(k)$ is $k$-dependent. We have therefore $P_{down} = e^{-m(t_{down})}$, where $m(t) = \int \mu(t') dt'$.

We know the probabilities that a switch in the binding state will result in a transition between competence/vegetation ($P_{up}$ and $P_{down}$). We can now calculate the average times in the bound and unbound states by $t_{bound} = 1/f$ and $t_{unbound} = \int \mu(t') e^{-mt'} dt'$. Finally, we can calculate the time that the system will spend between

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**Fig. 5.** Trajectories showing the effects of changes in the basal rates of expression of ComK and ComS. Higher values of the basal rate of expression of ComK $G_1$ do not alter the width of ComK peaks, but peaks happen more frequently. Higher values of the ComS rate of expression $G_3$ result in wider peaks of expression of ComK. For the adiabaticity parameter $\omega = 10$ considered here, peaks also occur slightly more often.

**Fig. 6.** Fast and slow approximations for binding/unbinding of ComK to DNA. (Upper) Effective potentials for the case of fixed expression of ComS and fast binding/unbinding, considering $s = 100$ for entrance into competence and $s = 20$ for exit from competence. The thick red line denotes the adiabatic limit. Nonadiabaticity is a cause of bistability and bimodality, as seen for the effective potentials in the cases of lower $\omega$. (Lower) Comparison of simulated and calculated competence times and probabilities of initiation for the model with slow binding/unbinding. Using the slow-binding method allows good agreement for low values of the adiabaticity parameter $\omega$. 

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competence cycles as $T_b = (t_{bound} + t_{unbound})/P_{up}$ and the time that
the system spends in competence cycles as $T_c = (t_{bound} + t_{unbound})/P_{down}$.

We compare the results of these intuitive approximations with
the values of competence times and probabilities of initiation
obtained from simulations (Fig. 6). The calculated values start
agreed with the simulations for values of $\omega$ as low as 1. Even
though such strong nonadiabaticity might not apply to the case
of the competence module, the present approximation scheme
could be useful for other cellular processes that are extremely
nonadiabatic.

Discussion

A stochastic model of the self-activating loop of ComK with
competitive degradation of ComS is able to reproduce most of the
features observed of this system observed in nature. Our analysis of
this model indicates the likelihood that nonadiabaticity accompa-
nying binding/unbinding events is important for this noise-
dominated system. Specifically, nonadiabatic noise from binding
and unbinding of ComK to the DNA probably plays an important
role on determining the region of the parameter space able to
exhibit excitable behavior, as well as in robustness to parameter
variation. Comparison of our model with laboratory experiments
where the basal rates of ComS were varied suggests the presence of
nonadiabatic noise in ComK binding/unbinding. Competence in
bacteria is a beautiful example of a cell process where noise plays
an important constructive role. Understanding the microscopic
origins of biochemical noise will be as essential for cell biology in
the future as understanding the average behavior of biomolecular
subprocesses has been in the past.

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Supporting Information (SI) Appendix

Exploring the Ensemble of Possible Dynamical Behaviors

In order to study the different kinds of behavior of these equations for different parameters, we will plot nullclines to determine the system’s fixed points. The variables in the differential equations can be made dimensionless by expressing the synthesis rates in units of the maximal degradation rates and concentrations in units of $\Gamma_k$ and $\Gamma_s$: $K = k/\Gamma_k$, $X_k = x_k/\Gamma_k$, $X_s = x_s/\Gamma_k$, $G_1 = g_1/\Lambda_k\Gamma_k$, $G_0 = g_0/\Lambda_k\Gamma_k$ and $S = s/\Gamma_s$, $G_s = g_s/\Lambda_s\Gamma_s$. The $k$ and $s$ axis are now rescaled by $\Gamma_k$ and $\Gamma_s$, respectively.

\[
\frac{dS}{dt} = \frac{G_s}{1 + (K/X_s)^n_s} - \frac{S}{1 + K + S} \tag{6}
\]

\[
\frac{dK}{dt} = \frac{G_1 + (K/X_k)^n_kG_0}{1 + (K/X_k)^n_k} - \frac{K}{1 + K + S} \tag{7}
\]

Constraints can be added to the dimensionless variables to limit our parameter search. In order to have the nullclines $dK/dt = 0$ and $dS/dt = 0$ in positive values of $K$ and $S$, $G_s, G_0$ have to be less than 1. $X_k$ and $X_s$ should also be in the same range as $G_s, G_0$ to have an important effect in the system. We generated 500000 different sets of

Figure 7: Plot of the nullclines of the deterministic kinetic rate equations governing the productions of ComK (red) and ComS (green) for the model including the repression of ComS by ComK. Depending on the parameters, the system can exhibit a variety of behaviors depending on the quantity and nature of its fixed points. The different plots used the average parameters obtained for each indicated behavior. The frequency $f$ denotes the percentage of randomly generated parameters to show that specific behavior.
random parameters and analyzed the fixed points of the system for each set. Exponents $n_k$ and $n_s$ will be chosen from $\{2,3,4,5\}$. $G_1$ will be chosen from $[0,0.2]$ and other parameters from $[0,1]$. The sets of parameters were then categorized according to their fixed points. For each of these categories the nullclines were plotted with the average set of parameters within the category (figure 7).

The excitable system discussed earlier corresponds to the category where there are three fixed points: one stable point, one saddle point and one unstable point. An unstable fixed point, corresponding to high concentration of ComK, implies that lengthy excursions of the trajectories to that unstable, but slow, region will be observed, but that no true basin of attraction exists in that region. An excitable system can show long competence cycles around the unstable fixed point without having a stable competent state, as pointed out in the pioneering work of Elowitz et al. For the sets of parameters found to result in an excitable system we plot histograms of the values found for the parameters to find out the range of parameters where excitable behavior emerges (figure 8). Except for the basal expression of ComK $G_0$, the histograms have wide gaussian-like distributions. This indicates that the region in the parameter space corresponding to the excitable system is probably compact and large enough to be robust to fluctuations on the parameters. This also indicates robustness to extrinsic noise, which can ultimately be translated into variations in the rates of this model of the module. We choose from here on a standard set of parameters corresponding to the centers of the distributions on the histograms.
The Deterministic Limit of MecA/ClpC/ClpP Dynamics

To study the effects of different sources of noise on the system, we use the approach of focusing in a specific source while silencing other potential sources of noise. To study the importance of considering explicitly the equilibrium of the complex formation between MecA/ClpC/ClpP and ComK/ComS, we silence the noise coming from ComK binding/unbinding to the DNA by using the adiabatic limit of \( g(k) = g_1 P(K_1) + g_0 P(K_0) \), where \( P(K_1) = f/(h(k) + f) \) and \( P(K_0) = h(k)/(h(k) + f) \). The system then shows stochastic noise coming only from the synthesis and degradation of ComK/ComS and from the degradation complex equilibrium. The relative speed of the degradation complex equilibrium to the other timescales of the system depends on the rates \( \lambda_{1k}, \lambda_{0k} \) and \( \lambda_{1s}, \lambda_{0s} \). We can analyze the importance of noise originated in this equilibrium by reducing these rates while maintaining \( \lambda_{1k}/\lambda_{0k} \) and \( \lambda_{1s}/\lambda_{0s} \) constant. To measure this effect we introduce the degradation adiabaticity parameter \( \omega_d = \lambda_{0k}/\lambda_k = \lambda_{0s}/\lambda_s \). Simulations performed for different values of \( \omega_d \) showed that neither the competence times nor the probabilities of initiation were significantly affected by different levels of noise (figure 9). The effective potential surfaces also showed similar qualitative properties. These results suggest that an adiabatic approximation is valid for the degradation complex equilibrium. Making this approximation, simple stochastic reactions referring to the degradation of ComK and ComS would have \( k \) - and \( s \)-dependent rates:

\[
\begin{align*}
    k & \quad \xrightarrow{\Lambda_{kk} + k} \quad \emptyset \\
    s & \quad \xrightarrow{\Lambda_{ss} + s} \quad \emptyset
\end{align*}
\]

Figure 9: Simulations for different values of the degradation adiabaticity parameter \( \omega_d \). There are no significant changes on neither the competence time nor the probability of initiation.
Nonadiabaticity in Binding/Unbinding

In figure 10 we see the effects of non-adiabaticity in binding/unbinding of the protein to the DNA in competence times and probability of initiation. When non-adiabaticity is considered, the probability of initiation of competence is much higher, even for values of $\omega$ well above 1. The probability of initiation is highest for an adiabaticity of $\omega \approx 1$, and then decreases for lower values. Sets of parameters that do not exhibit competence when the adiabatic approximation is made, can show competence when there is non-adiabatic binding/unbinding of the promoter. We see therefore that non-adiabaticity can expand the region of the parameter space showing competence, allowing increased the robustness of the system to parameter variation. These results manifest the idea of functional stochasticity, in which fluctuations increase the cell’s robustness. Competence times are longer for smaller values of $\omega$.

In figure 11 we show the effective potential surfaces for different values of $\omega$ and basal rates of expression. In the case of increasing the basal ComK expression we see increased population of the high-ComK region, corresponding to competent behavior, while the stable vegetative well remains unchanged. This corresponds to the observation of more competence cycles. In the case of increased basal ComS expression we observe larger cycles, corresponding to increased competence times. For lower values of adiabaticity the vegetative well starts to populate high-ComS regions. Higher levels of ComS start competence events, so this change accounts for the increase in the probability of competence initiation observed for non-adiabatic scenarios.

![Figure 10: Effects of non-adiabaticity in competence time $T_c$ and probability of initiation $P_i$, calculated in relation to the adiabatic approximation. Non-adiabaticity results in higher probabilities of initiation and higher competence times.](image-url)
Figure 11: Effective potential surfaces plotted for different values of the basal rates of expression of ComK and ComS. For the case of increased basal rate of expression of ComK, the high-ComK region corresponding to competent behavior is more populated while the stable well corresponding to the vegetative state remains unchanged, indicating more competence cycles (increased probability of initiation). For the case of increased basal rate of expression of ComS there are larger competence cycles, resulting on increased competence times. For lower values of the adiabaticity parameter $\omega$ we see the vegetative well populate high-ComS regions, resulting in an increased probability of initiation, as well.

**Fast Binding/Unbinding Approximation for Fixed ComS Levels**

Considering the fast binding/unbinding approximation considered in the main text, time evolutions of the calculated effective potentials can be calculated for both entrance to and exit from competence (figure 12).

Figure 12: Effective potentials for the case of fixed expression of ComS and fast binding/unbinding, considering $s = 100$ for entrance into competence and $s = 20$ for exit from competence. Graphs show the time evolution of the effective potentials for entrance into and exit from competence.