Stochastic simulations of genetic switch systems

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(Received 26 October 2006; published 9 February 2007)

Genetic switch systems with mutual repression of two transcription factors are studied using deterministic methods (rate equations) and stochastic methods (the master equation and Monte Carlo simulations). These systems exhibit bistability, namely two stable states such that spontaneous transitions between them are rare. Induced transitions may take place as a result of an external stimulus. We study several variants of the genetic switch and examine the effects of cooperative binding, exclusive binding, protein-protein interactions, and degradation of bound repressors. We identify the range of parameters in which bistability takes place, enabling the system to function as a switch. Numerous studies have concluded that cooperative binding is a necessary condition for the emergence of bistability in these systems. We show that a suitable combination of network structure and stochastic effects gives rise to bistability even without cooperative binding. The average time between spontaneous transitions is evaluated as a function of the biological parameters.

DOI: 10.1103/PhysRevE.75.021904 PACS number(s): 87.10.+e, 87.16.−b

I. INTRODUCTION

Recent advances in quantitative measurements of gene expression at the single-cell level [1,2] have brought new insight on the importance of stochastic fluctuations in genetic circuits [3]. The role of fluctuations is enhanced due to the discrete nature of the transcription factors and their binding sites, which may appear in low copy numbers [4,5]. As a result, populations of genetically identical cells may show significant variability. Stochastic behavior may invoke oscillations [6] and spatio-temporal patterns [7], which are unaccounted for by macroscopic chemical rate equations. Genetic circuits with feedback mechanisms may exhibit bistability, namely, two distinct stable states which can be switched by an external signal [8]. A low rate of spontaneous switching events may also take place. To qualify as a switch, this rate must be much lower than the rates of the relevant processes in the cell, namely transcription, translation, and degradation of transcription factors. Genetic switches, such as the phage λ switch, give rise to different cell fates [9]. In this switch, λ phages infect *E. coli* bacteria and can exist in two exclusive states, one called lysogeny and the other called lysis. When the phage enters its host, it integrates itself into the host's DNA and is duplicated by cell division. It codes for proteins that can identify stress in the host cell. In case of stress, the phage transforms into the lysis state. In this state, it kills the host cell, using its DNA to produce many copies of the phage, which are released and later infect other cells. Other switch circuits exist in the metabolic systems of cells. These switches determine which type of sugar the cell will digest [10]. The genetic switch may also serve as a memory unit of the cell, and help determine its fate during cell differentiation.

Recent advances enable the construction of genetic circuits with desired properties, that are determined by the network architecture. These networks are constructed from available components, namely genes and promoters. They do not require the manipulation of the structure of proteins and other regulatory elements at the molecular level. These genes and promoters are often inserted into plasmids rather than on the chromosome. A synthetic toggle switch, that consists of two repressible promoters with mutual negative regulation, was constructed in *E. coli* and the conditions for bistability were examined [11]. The switching between its two states was demonstrated using chemical and thermal induction. More recently, such circuit was found to exist in a natural system in which two mutual repressors regulate the differentiation of myeloid progenitors into either macrophages or neutrophils [12].

In this paper we analyze the genetic toggle switch using deterministic and stochastic methods. In this simple genetic circuit, two proteins, A and B, negatively regulate each other's synthesis. The regulation is performed at the transcription level, namely the production of protein A is negatively regulated by protein B, through binding of n copies of B to the A promoter (and vice versa). This process can be modeled by a Hill function, which reduces the production rate of A by a factor of $1+k[B]^n$, where $[B]$ is the concentration of B proteins in the cell, k is a parameter, and n is the Hill coefficient [13]. In the case that $n=1$, the binding of a single protein is sufficient in order to perform the negative regulation, while for $n>1$ the *cooperative binding* of two or more proteins is required.

One may expect this circuit to function as a switch, with two stable states, one dominated by A proteins and the other dominated by B proteins. When the population of A proteins is larger than the population of B proteins, the A proteins suppress the production of B proteins. Under these conditions, the production of A proteins will not be suppressed much by the small B population. Therefore, the system approaches a state rich in A proteins and poor in B proteins. Similarly, the system may approach a state rich in B proteins and poor in A proteins.

To qualify as a switch, the system should be bistable. In the deterministic description, bistability is defined as the existence of two stable steady state solutions of the rate equations. This description does not account for the possibility of spontaneous transitions between the two states. In the sto-

chastic description, spontaneous transitions do take place. Therefore, the condition for bistability is that the rate of spontaneous switching events (due to random fluctuations rather than an external signal) is much lower than the rates of all other relevant processes in the system.

Rate equations provide the average concentrations of A and B proteins in a population of cells. In these equations, bistability emerges at a bifurcation point, where two stable states emerge. Rate equations do not include fluctuations and do not account for the possibility of spontaneous transitions between the two states. The master equation provides the probability distribution of the populations of A and B proteins. The two bistable states appear as two distinct peaks in this distribution. Monte Carlo simulations enable to follow the fluctuations in a single cell and to evaluate the rate of spontaneous switching events.

We examine the conditions for the system to become a switch, and calculate the rate of spontaneous transitions between its two states. This is done for several variants of the toggle switch. In particular, we focus on switch systems in which the repression in done without cooperative binding (namely, $n=1$). Numerous studies have concluded, using rate equations, that cooperative binding is a necessary condition for the emergence of bistability [11,14–17]. Below we show, using a combination of deterministic and stochastic simulation methods, that this is not the case, namely a bistable switch can exist even in the absence of cooperative binding. In particular, we show that bound-repressor degradation (BRD) and protein-protein interactions (PPI) give rise to bistability, without cooperative binding, even at the level of rate equations. These results are confirmed by stochastic simulations using the master equation and Monte Carlo methods. We also consider the exclusive switch, in which the A and B repressors cannot be bound simultaneously due to overlap between their promoter sites. This system exhibits bistability only when stochastic fluctuations are taken into account. The rate of spontaneous transitions between the two states is calculated as a function of the biological parameters.

The paper is organized as follows. In Sec. II we consider the basic version called the general switch. Several variants of this circuit are considered in the sections that follow. The exclusive switch is studied in Sec. III, the BRD switch is considered in Sec. IV and the PPI switch is analyzed in Sec. V. The effects of cooperative binding are studied in Sec. VI. The response of toggle switch systems to external signals is examined in Sec. VII. The results are discussed in Sec. VIII and summarized in Sec. IX.

II. THE GENERAL SWITCH (WITHOUT COOPERATIVE BINDING)

The general switch consists of two transcription factors, A and B, that negatively regulate each other's synthesis [14,15]. A schematic description of this circuit is given in Fig. 1(a). The regulation is done by the binding of a protein to the promoter site of the other gene, blocking the access of the RNA polymerase and suppressing the transcription process. In this circuit there is no cooperative binding, namely the regulation process is performed by a single bound protein.

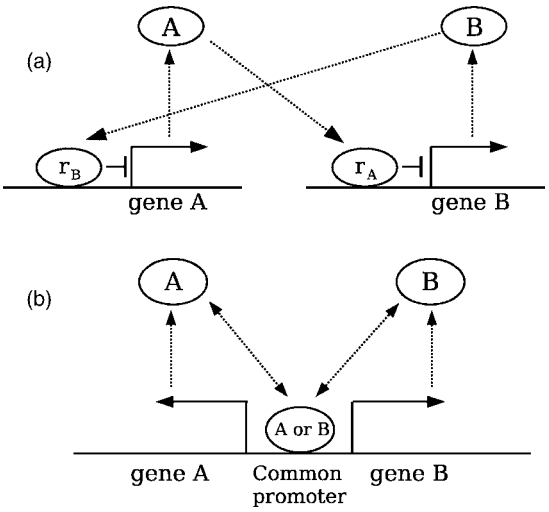

FIG. 1. Schematic illustrations of (a) the general switch circuit, that includes two transcription factors, A and B, which negatively regulate each other's synthesis; (b) the exclusive switch, in which there is an overlap between the promoter sites of A and B proteins, so they cannot be bound simultaneously.

The concentrations of free A and B proteins in the cell are denoted by $[A]$ and $[B]$, respectively (by concentration we mean the average copy number of proteins per cell). The copy numbers of the bound proteins, are denoted by $[r_A]$ and $[r_B]$, where r_A is a bound A protein that monitors the production of B, while r_B is a bound B protein that monitors the production of A. Note that there is at most one bound repressor of each type at any given time, and thus $0 \leq r_A, r_B \leq 1$. For simplicity, we ignore the mRNA level and combine the processes of transcription and translation as a single step of synthesis [18].

The maximal production rate of protein X is denoted by g_X (s^{-1}), $X=A,B$. The degradation rate of protein X is given by d_X (s^{-1}). While the structure of the circuits studied here is symmetric, the rate constants can be different for A and B. However, for simplicity we use symmetric parameters, i.e., $g=g_A=g_B$ and $d=d_A=d_B$. The binding rate of proteins to the promoter is denoted by α_0 (s^{-1}) and the dissociation rate by α_1 (s^{-1}).

A. Rate equations

The dynamics of the general switch circuit is described by the rate equations [19,20]

$$[\dot{A}] = g_A(1-[r_B]) - d_A[A] - \alpha_0[A](1-[r_A]) + \alpha_1[r_A],$$

$$[\dot{B}] = g_B(1-[r_A]) - d_B[B] - \alpha_0[B](1-[r_B]) + \alpha_1[r_B],$$

$$[\dot{r_A}] = \alpha_0[A](1-[r_A]) - \alpha_1[r_A],$$

$$[\dot{r_B}] = \alpha_0[B](1-[r_B]) - \alpha_1[r_B]. \quad (1)$$

It is commonly assumed that the binding-unbinding processes are much faster than other processes in the circuit,

namely $\alpha_0, \alpha_1 \gg d_X, g_X$. This means that the relaxation times of $[r_X]$ are much shorter than other relaxation times in the circuit. Under this assumption, one can take the time derivatives of $[r_X]$ to zero, even if the system is away from steady state. This brings the rate equations to the standard Michaelis-Menten form

$$[\dot{A}] = \frac{g}{1+k[B]} - d[A],$$

$$[\dot{B}] = \frac{g}{1+k[A]} - d[B], \tag{2}$$

where symmetric parameters are used, and $k=\alpha_0/\alpha_1$ is the repression strength. For a given population of free X repressors, the parameter k controls the value of $[r_X]$. The limit of weak repression, $[r_X] \ll 1$, is obtained when $k[X] \ll 1$, while the limit of strong repression, $[r_X] \simeq 1$, is obtained for $k[X] \gg 1$.

The meaning of bistability at the level of rate equations is that at steady state the equations exhibit two distinct positive solutions. In this particular class of circuits, one solution is dominated by A proteins and the other is dominated by B proteins. Starting from any initial state, the system will converge to one of these solutions. The solutions are stable, so the possibility of spontaneous transitions, induced by stochastic fluctuations, is not included in the rate equation description.

The steady state solutions of Eqs. (1) and (2) are identical. We will now show that these equations have only one positive steady-state solution. To this end, we first take $[\dot{A}]=[\dot{B}]=0$ in Eq. (2). We multiply each equation by the denominator of the Hill function that appears in it. We obtain

$$g - d[A] - kd[A][B] = 0,$$

$$g - d[B] - kd[A][B] = 0. \tag{3}$$

Subtracting one equation from the other we get $d([A]-[B])=0$ and therefore $[A]$ must be equal to $[B]$ at steady state. The steady state values of $[A]$ and $[B]$ can be easily found. Inserting $[A]=[B]$ into Eq. (3) we obtain a quadratic equation whose only positive solution is

$$[A] = [B] = \frac{-1+\sqrt{1+4kg/d}}{2k}. \tag{4}$$

Standard linear stability analysis shows that this solution is always stable.

As a result, we conclude that at the level of rate equations the general switch, without cooperative binding, does not exhibit bistability. In Sec. VI we consider the case of cooperative binding, where the rate equations do exhibit bistability.

B. Master equation

In order to account for stochastic effects and to obtain insight on the reason that this system is not bistable, the master equation approach is applied [3,21–24]. In this case, we consider the probability distribution function $P(N_A, N_B, r_A, r_B)$. It is the probability for a cell to include N_X copies of free protein X and r_X copies of the bound X repressor, where $N_X=0,1,2,\ldots$, and $r_X=0,1$. The master equation for the general switch takes the form

$$\begin{aligned}\dot{P}(N_A,N_B,r_A,r_B) &= g_A\delta_{r_B,0}[P(N_A-1,N_B,r_A,r_B) - P(N_A,N_B,r_A,r_B)] + g_B\delta_{r_A,0}[P(N_A,N_B-1,r_A,r_B) - P(N_A,N_B,r_A,r_B)] + d_A[(N_A \\ &+ 1)P(N_A+1,N_B,r_A,r_B) - N_A P(N_A,N_B,r_A,r_B)] + d_B[(N_B+1)P(N_A,N_B+1,r_A,r_B) - N_B P(N_A,N_B,r_A,r_B)] \\ &+ \alpha_0[(N_A+1)\delta_{r_A,1}P(N_A+1,N_B,r_A-1,r_B) - N_A\delta_{r_A,0}P(N_A,N_B,r_A,r_B)] + \alpha_0[(N_B+1)\delta_{r_B,1}P(N_A,N_B+1,r_A,r_B \\ &- 1) - N_B\delta_{r_B,0}P(N_A,N_B,r_A,r_B)] + \alpha_1[\delta_{r_A,0}P(N_A-1,N_B,r_A+1,r_B) - \delta_{r_A,1}P(N_A,N_B,r_A,r_B)] + \alpha_1[\delta_{r_B,0}P(N_A,N_B \\ &- 1,r_A,r_B+1) - \delta_{r_B,1}P(N_A,N_B,r_A,r_B)],\end{aligned} \tag{5}$$

where $\delta_{i,j}=1$ for $i=j$ and 0 otherwise. The g_X terms account for the production of proteins. The d_X terms account for the degradation of free proteins, while the α_0 (α_1) terms describe the binding (unbinding) of proteins to (from) the promoter site. In numerical integration, the master equation must be truncated in order to keep the number of equations finite. This is done by setting suitable upper cutoffs, $N_A^{\max}$ and $N_B^{\max}$, on the populations sizes of free proteins. In order to maintain the accuracy of the calculations, the probability of population sizes beyond the cutoffs must be sufficiently small.

The master equation has a single steady state solution, which is always stable [25]. The criterion for bistability is that the steady state solution $P(N_A,N_B,r_A,r_B)$ exhibits two distinct regions (peaks) of high probabilities, separated by a gap in which the probabilities are very small. These two regions correspond to the two states in which the system is likely to be. If the transition rate between the peaks is small enough, the system is indeed a bistable switch. Note, that in this case, averages of the form

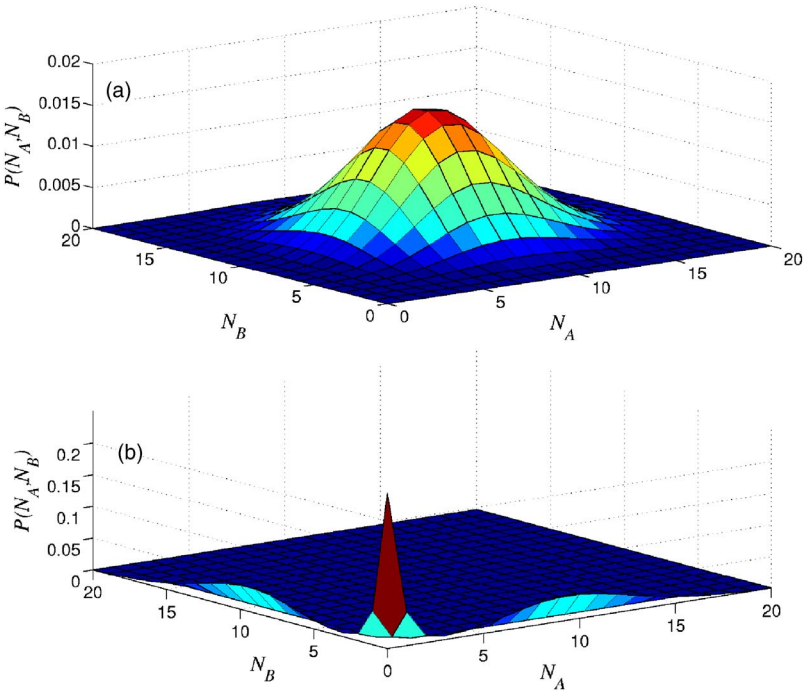

FIG. 2. (Color online) The probability distribution $P(N_A,N_B)$ for the general switch, under conditions of (a) weak repression ($k=0.005$) where there is one symmetric peak; and (b) strong repression ($k=50$) where three peaks appear, one dominated by A, the second dominated by B and the third in which both species are mutually suppressed. The weights of the three peaks are about the same.

$$\langle N_X \rangle = \sum_{N_A=0}^{N_A^{\max}} \sum_{N_B=0}^{N_B^{\max}} \sum_{r_A=0}^{1} \sum_{r_B=0}^{1} N_X P(N_A,N_B,r_A,r_B), \tag{6}$$

where $X=A$, B, do not reflect the complex structure of the probability distribution. These can be considered as averages over many cells, some dominated by A and others dominated by B proteins, such that the total populations of the two species are about the same.

To examine the existence of bistability we consider the marginal probability distribution

$$P(N_A,N_B) = \sum_{r_A=0}^{1} \sum_{r_B=0}^{1} P(N_A,N_B,r_A,r_B). \tag{7}$$

This probability distribution was calculated for a broad range of parameters. Two representative examples are shown in Fig. 2.

Under conditions of weak repression (small k), $P(N_A,N_B)$ exhibits a single peak for which $N_A \approx N_B \approx g/d$ [Fig. 2(a)], in agreement with the rate equations. This is due to the fact that the repression is weak, and the A and B populations are almost uncorrelated. In this case, the cell will contain roughly the same amount of A and B proteins.

For strong repression, the distribution $P(N_A,N_B)$ exhibits a peak dominated by A proteins and a peak dominated by B proteins, as expected for a bistable system. However, a third peak appears near the origin, in which both populations of free proteins are suppressed [Fig. 2(b)]. This peak represents a dead-lock situation, caused by the fact that both A and B repressors can be bound simultaneously, each bringing to a halt the production of the other specie. The third peak provides a corridor through which the probability can flow between the other two peaks. As a result, the system can quickly switch between the A-dominated and the B-dominated states.

In addition to the solution of the master equation, Monte Carlo simulations have been performed. In these simulations one can follow the time evolution of the populations of free and bound proteins in a single cell. In Fig. 3(a) we present the population sizes of free proteins vs time for the general switch. It is clear that the cell can indeed be in one of three states: a state rich in A, a state rich in B, and a state in which both proteins are in very low copy numbers. We conclude that a necessary condition for the system to become a switch is to prevent this dead-lock situation in which both protein populations are suppressed simultaneously. Below we present several variants of the circuit in which the third peak is suppressed, giving rise to a bistable switch.

III. THE EXCLUSIVE SWITCH

The first variant we consider is the exclusive switch, depicted in Fig. 1(b). In this circuit there is an overlap between the promoters of A and B. As a result, there is no room for both A and B proteins to be bound simultaneously. Exclusive binding is encountered in nature, for example, in the lysis-lysogeny switch of phage λ [9].

It was shown that in presence of cooperative binding, the exclusive switch is more stable than the general switch [14,15]. This is because in the exclusive switch the access of the minority proteins to the promoter site is blocked by the dominant proteins. Here we show that in the exclusive switch, stochastic effects give rise to bistability even without cooperativity between the transcription factors. The dead-lock situation in prevented in this case, since A and B repressors cannot be bound simultaneously.

A. Rate equations

To model the exclusive switch, recall that the variable $[r_A]$ ($[r_B]$) is actually the fraction of time in which the promoter is

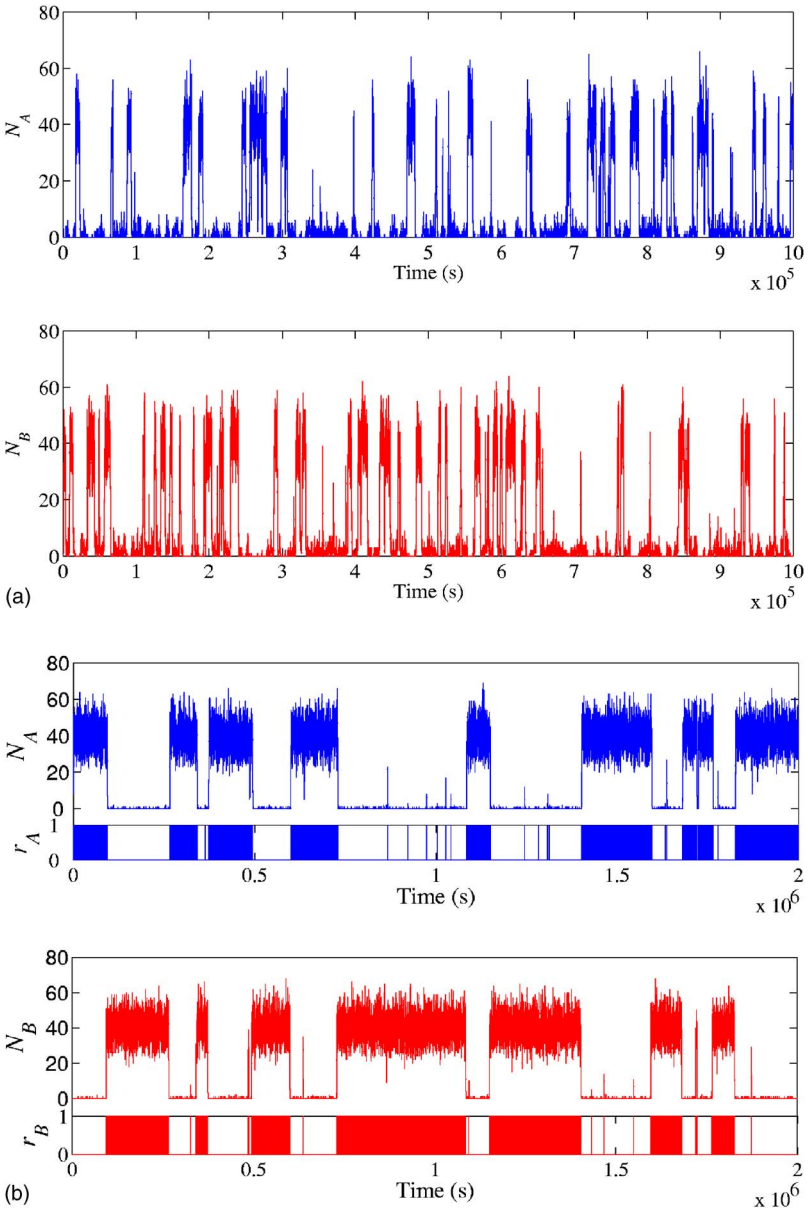

FIG. 3. (Color online) The population sizes of free A and B proteins vs time obtained from a Monte Carlo simulation (a) for the general switch, where the system exhibits fast transitions between its three states; (b) for the exclusive switch. The bistable behavior is clearly observed, where the population size of the dominant specie is between 20–60 and the other specie is nearly diminished. Failed switching attempts are clearly seen. The typical switching time is in the order of 10^5 (s) or roughly 1 day. Bound proteins are also shown. Their fast binding and unbinding events cannot be resolved on the time scale that is presented. In both cases, $g=0.2$, $d=0.005$, $\alpha_0=0.2$, and $\alpha_1=0.01$ (s^{-1}).

occupied by a bound A (B) protein [19]. The fraction of time in which the promoter is vacant is thus $1-[r_A]-[r_B]$. Incorporating this into Eq. (1) gives rise to the following modification: in the α_0 terms, each appearance of $[r_A]$ or $[r_B]$ should be replaced by $[r_A]+[r_B]$. With this modification, the rate equations take the form

$$[\dot{A}] = g(1-[r_B]) - d[A] - \alpha_0[A](1-[r_A]-[r_B]) + \alpha_1[r_A],$$

$$[\dot{B}] = g(1-[r_A]) - d[B] - \alpha_0[B](1-[r_A]-[r_B]) + \alpha_1[r_B],$$

$$[\dot{r_A}] = \alpha_0[A](1-[r_A]-[r_B]) - \alpha_1[r_A],$$

$$[\dot{r_B}] = \alpha_0[B](1-[r_A]-[r_B]) - \alpha_1[r_B]. \quad (8)$$

Under steady state conditions, the rate equations can be reduced to the Michaelis-Menten form

$$[\dot{A}] = \frac{g}{1+k[B]/(1+k[A])} - d[A],$$

$$[\dot{B}] = \frac{g}{1+k[A]/(1+k[B])} - d[B], \quad (9)$$

where, as before, $k=\alpha_0/\alpha_1$. We will now show that even for the case of the exclusive switch, the rate equations still exhibit a single solution, thus there is no bistability. This is done by taking $[\dot{A}]=[\dot{B}]=0$ and getting rid of the denominators, by repeated multiplications. The resulting equations are

$$g + (kg-d)[A] - kd[A]([A]+[B]) = 0,$$

$$g + (kg-d)[B] - kd[B]([A]+[B]) = 0. \quad (10)$$

By subtraction of one equation from the other, we find that

$$\{kg - d - kd([A] + [B])\}([A] - [B]) = 0. \tag{11}$$

The positive, symmetric solution, $[A]=[B]$, is given by

$$[A] = \frac{(kg - d) + \sqrt{(kg + d)^2 + 4kgd}}{4kd}. \tag{12}$$

The other, nonsymmetric solution, given by

$$kg - d - kd([A] + [B]) = 0 \tag{13}$$

is inconsistent with Eq. (10) unless $g=0$, namely there is no production of A and B proteins, which immediately leads to $[A]=[B]=0$. Under these conditions the solution of Eq. (13) is $[A]+[B]=-1/k$, which requires a negative population size and thus makes no physical sense. Therefore, the only solution for $g>0$ is the symmetric solution, $[A]=[B]$. Thus, the rate equations do not support a bistable solution for the exclusive switch for any choice of the parameters.

B. Master equation

To account for the effects of fluctuations, we now describe the exclusive switch using the master equation. It is similar to master equation for the general switch given by Eq. (5), except for the following modifications: (a) In the α_0 and α_1 terms, each time $\delta_{r_A,j}$ $(\delta_{r_B,j})$, $j=0,1$, appears it should be multiplied by $\delta_{r_B,0}$, $(\delta_{r_A,0})$; (b) the constraint $P(N_A,N_B,1,1)=0$ should be imposed. Implementing these changes we obtain the following equation:

$$\begin{aligned}\dot{P}(N_A,N_B,r_A,r_B) = {} & g_A\delta_{r_B,0}[P(N_A-1,N_B,r_A,r_B) - P(N_A,N_B,r_A,r_B)] + g_B\delta_{r_A,0}[P(N_A,N_B-1,r_A,r_B) - P(N_A,N_B,r_A,r_B)] + d_A[(N_A \\ & + 1)P(N_A+1,N_B,r_A,r_B) - N_A P(N_A,N_B,r_A,r_B)] + d_B[(N_B+1)P(N_A,N_B+1,r_A,r_B) - N_B P(N_A,N_B,r_A,r_B)] \\ & + \alpha_0\delta_{r_B,0}[(N_A+1)\delta_{r_A,1}P(N_A+1,N_B,r_A-1,r_B) - N_A\delta_{r_A,0}P(N_A,N_B,r_A,r_B)] + \alpha_0\delta_{r_A,0}[(N_B+1)\delta_{r_B,1}P(N_A,N_B \\ & + 1,r_A,r_B-1) - N_B\delta_{r_B,0}P(N_A,N_B,r_A,r_B)] + \alpha_1[\delta_{r_A,0}P(N_A-1,N_B,r_A+1,r_B) - \delta_{r_A,1}P(N_A,N_B,r_A,r_B)] \\ & + \alpha_1[\delta_{r_B,0}P(N_A,N_B-1,r_A,r_B+1) - \delta_{r_B,1}P(N_A,N_B,r_A,r_B)].\end{aligned} \tag{14}$$

For the exclusive switch, as for the general switch, under conditions of weak repression, $P(N_A,N_B)$ exhibits a single peak [Fig. 4(a)] that satisfies $N_A \approx N_B \approx g/d$. However, as the repression strength increases two distinct peaks begin to form. For intermediate values of k these peaks are still connected, by a corridor of nonvanishing probabilities [Fig. 4(b)]. Monte Carlo simulations show that for intermediate values of k, the system indeed exhibits two states, one rich in A and the other rich in B, but rapid transitions occur between them.

For strong repression, the distribution $P(N_A,N_B)$ exhibits two peaks which are separated by a region with vanishing probabilities [Fig. 4(c)]. In one peak the A population is suppressed, while in the other peak the B population is suppressed, as expected for a bistable system. The average population of the dominant protein specie in each peak is $\langle N_X\rangle \approx g/d$, while the population of the suppressed specie is $\langle N_X\rangle \approx 0$. Monte Carlo simulations show that in this case the average time between spontaneous transitions is much longer. The typical switching time for the case shown in Fig. 3(b) is around 10^5 seconds. It is much longer than the time scales of the transcription, translation, and degradation processes. It is also longer than the time between cell divisions which is of the order of 10^3–10^4 seconds. The Monte Carlo results clearly show a large number of failed attempts in which a protein of the minority specie binds to the promoter and then unbinds again, without causing the system to flip.

C. Analysis of switching times

To evaluate the switching times we performed the following procedure. We initialized the master equation in a state which is completely dominated by A proteins, namely, $P(N_A=\lfloor g/d\rfloor, N_B=0, r_A=0, r_B=0)=1$ (where $\lfloor\ \rfloor$ represents the integer part), and all other probabilities vanish. The master equation was then integrated numerically and $P(N_A,N_B)$ was calculated as a function of time. The function $f(t) = P(N_A>N_B) - P(N_A<N_B)$ was found to decay exponentially from its initial value, $f(0)=1$, to zero, according to $f(t) = \exp(-t/\tau)$. The time constant τ is defined as the switching time [26]. Its inverse, τ^{-1}, is referred to as the switching rate.

Using this procedure, we examined the dependence of the switching time, τ on the protein synthesis rate, g [Fig. 5(a)], the degradation rate, d [Fig. 5(b)], and the repression strength, k [Fig. 5(c)]. In the parameter range in which bistability takes place, we obtain that (a) $\tau \sim g$, (b) $\tau \sim 1/d^2$, and (c) $\tau \sim k$. Concerning Fig. 5(c), note that system exhibits bistability only in the regime in which k is large [27]. For $k<1$, $\tau \sim 100$–1000 (s), which is the typical time scale of other processes in the cell. Only for $k \gtrsim 10$, τ becomes significantly larger than the time scales of other processes, and the system can function as a stable switch. The scaling properties of the switching time can be summarized by

$$\tau \sim \frac{\alpha_0}{\alpha_1}\frac{g}{d^2}. \tag{15}$$

This result can be reproduced by a simple argument. Consider an initial state in which the system is dominated by A

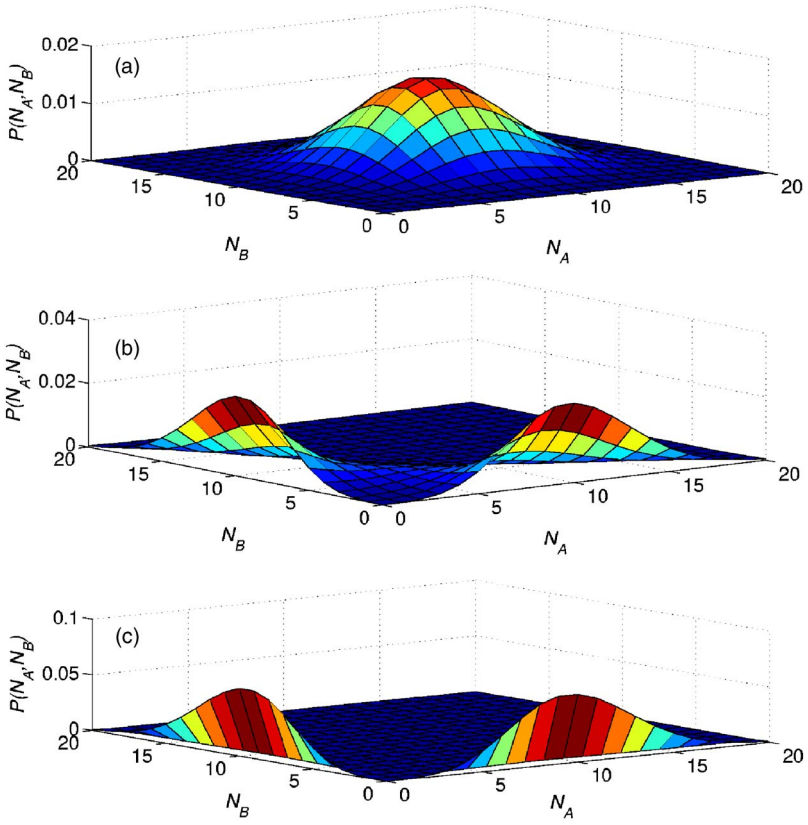

FIG. 4. (Color online) The probability distribution $P(N_A,N_B)$ for the exclusive switch, under conditions of (a) weak repression ($k=0.005$) where there is one symmetric peak (b) intermediate repression ($k=1$) where two distinct peaks begin to emerge but are still connected, and (c) strong repression ($k=50$), where bistability is observed.

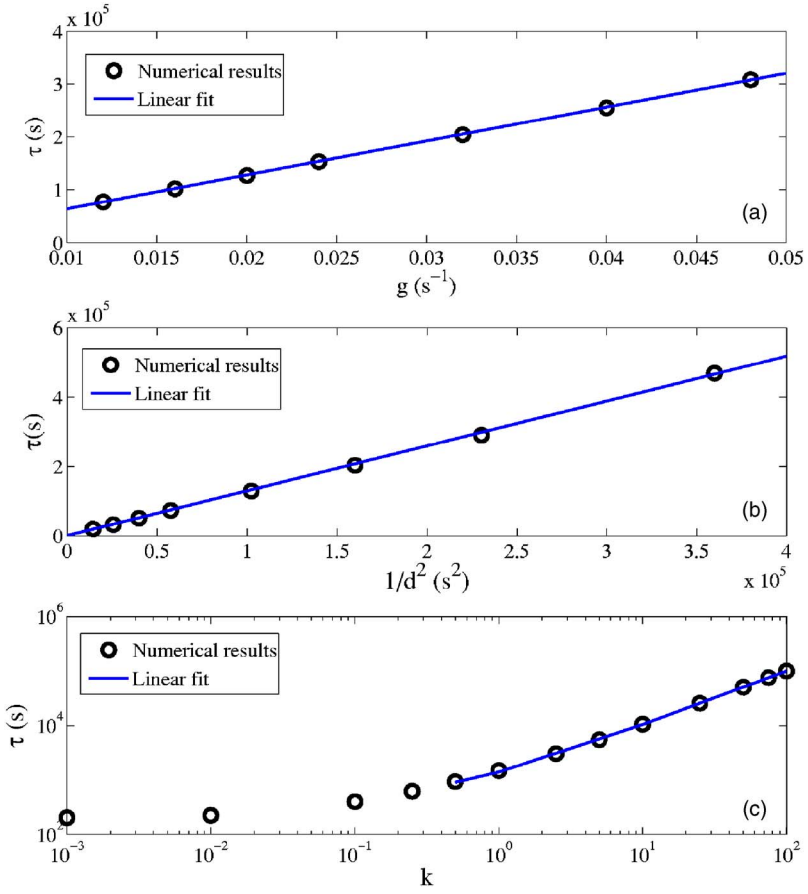

FIG. 5. (Color online) Scaling properties of the switching time τ for the exclusive switch vs the protein synthesis rate g, the degradation rate d and the repression strength k.

proteins, while the population of B proteins is suppressed, namely $[A] \gg [B]$. In this situation the promoter site is occupied by an A protein during most of the time. In order that the switch will flip, the bound A protein must unbind (at rate α_1). Then, a B protein (rather than an A protein) should bind to the promoter. The probability for this to happen is $\sim [B]/[A]$. This B protein should remain bound long enough in order to build up a sufficiently large population of B proteins. On average, the B protein stays bound $1/\alpha_1$ (s), during which g/α_1 proteins of type B are produced. After the B repressor will unbind, the probability that the next protein that binds will be of type B rather than A, is thus $\sim (g/\alpha_1)/[A]$ (neglecting the degradation of A proteins, because $\alpha_1 \gg d$). Following this argument, the switching rate is given by

$$\tau^{-1} \sim \alpha_1 \times \frac{[B]}{[A]} \times \frac{g}{\alpha_1 [A]} = g \frac{[B]}{[A]^2}. \tag{16}$$

From the Michaelis-Menten equations we obtain that

$$\frac{[B]}{[A]} = \frac{1}{1 + k[A]} \approx \frac{1}{k[A]}, \tag{17}$$

since for strong repression $k[A] \gg 1$. Inserting this result into Eq. (16) and using and $[A] \approx g/d$ we find that

$$\tau = \frac{kg}{d^2}. \tag{18}$$

This result can be considered as the leading term in the expansion of τ in powers of g, d, and k. This leading term turns out to provide a very good approximation to simulation results. For example, for $g=0.2$, $d=0.005$, $\alpha_0=0.2$, and $\alpha_1 = 0.01$ (s^{-1}) we get $\tau = 1.6 \times 10^5$ (s), which agrees perfectly with the results of Monte Carlo simulations.

From Eq. (16), and from the fact that the average copy number of the dominant specie is $[A] \approx g/d$, we find that when the production rate, g, is varied while keeping all other parameters fixed, $\tau \sim [A]$. Otherwise, when the degradation rate, d, is varied while all other parameters are fixed, $\tau \sim [A]^2$. In general, the switching time is $\tau = \tau(k, g, d)$, while the population size, $[A]$, of the dominant specie depends on both g and d. Thus, by a suitable variation of the rate constants, any desired dependence of τ on $[A]$ can be obtained. In particular, by increasing k, τ can be increased with no effect on $[A]$. A similar result is obtained when g and d are decreased by the same factor. We thus conclude that the population size is only one of several factors that affect the switching time. A complete description of the switching time should include all the relevant rate constants.

In Monte Carlo simulations of a switch system with cooperative binding, the switching time was found to depend exponentialy on the copy number [14,15]. This is consistent with the discussion above, but requires a well-defined protocol according to which the rate constants are varied.

IV. THE SWITCH WITH BOUND REPRESSOR DEGRADATION

Consider a different variant of the general switch, in which not only free repressors, but also bound repressors are affected by degradation. The bound-repressor degradation (BRD) tends to prevent the dead-lock situation in which both A and B repressors are bound simultaneously. This is due to the fact that degradation removes the bound repressor from the system, unlike unbinding, where the resulting free repressor may quickly bind again. It turns out that degradation of bound repressors induces bistability not only at the level of the master equation but even at the level of rate equations.

A. Rate equations

The rate equations that describe the BRD switch take the form

$$\begin{aligned}
[\dot{A}] &= g(1 - [r_B]) - d[A] - \alpha_0 [A](1 - [r_A]) + \alpha_1 [r_A], \\
[\dot{B}] &= g(1 - [r_A]) - d[B] - \alpha_0 [B](1 - [r_B]) + \alpha_1 [r_B], \\
[\dot{r_A}] &= \alpha_0 [A](1 - [r_A]) - \alpha_1 [r_A] - d_r [r_A], \\
[\dot{r_B}] &= \alpha_0 [B](1 - [r_B]) - \alpha_1 [r_B] - d_r [r_B],
\end{aligned} \tag{19}$$

where d_r is the degradation rate of the bound repressors. Assuming quasisteady state for the binding-unbinding processes we obtain the Michaelis-Menten equations

$$\begin{aligned}
[\dot{A}] &= \frac{g}{1 + k[B]} - \left(d + \frac{d_r k}{1 + k[A]} \right) [A], \\
[\dot{B}] &= \frac{g}{1 + k[A]} - \left(d + \frac{d_r k}{1 + k[B]} \right) [B],
\end{aligned} \tag{20}$$

where now $k = \alpha_0/(\alpha_1 + d_r)$. Note that the coefficients of $[A]$ and $[B]$ in the second terms in Eq. (20) can be considered as effective degradation rate constants.

For steady state conditions, Eq. (20) exhibits the symmetric solution

$$[A] = [B] = \frac{[(d + d_r k)^2 + 4dkg]^{1/2} - d - d_r k}{2dk}. \tag{21}$$

This solution exists for any choice of the parameters. In addition, in some parameter range, two nonsymmetric solutions exist. These solutions can be expressed as the solutions of the quadratic equation

$$dd_r k^2 [A]^2 + (gdk + dd_r k + d_r^2 k^2 - gd_r k^2)[A] + gd = 0. \tag{22}$$

The condition for the existence of two different solutions of this equation is

$$(g - d_r)[g(kd_r - d)^2 - d_r(kd_r + d)^2] > 0. \tag{23}$$

In order for them to be positive the condition $g > d_r$ must be satisfied. Thus, the bifurcation takes place at

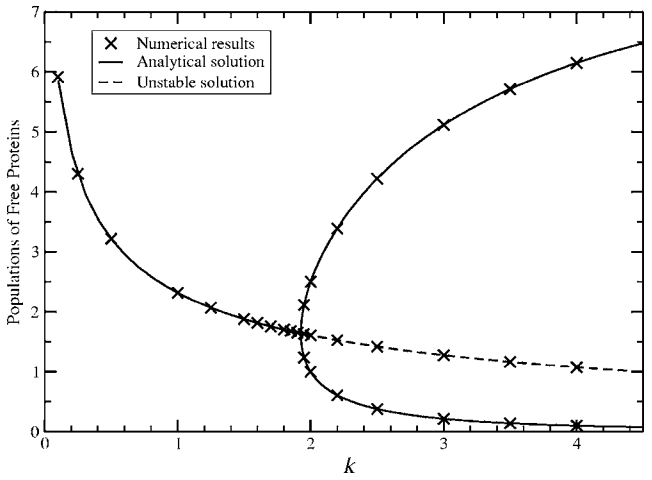

FIG. 6. Population sizes of the free A and B proteins vs k for the BRD switch obtained from the rate equations. The parameters are $g=0.05$, $d=d_r=0.005$, $\alpha_1=0.01$, and α_0 is varied. Here $k_c \approx 1.92$. Stable solutions are shown by solid lines and unstable solutions by dashed lines.

$$k_c = \frac{d(\sqrt{g}+\sqrt{d_r})}{d_r(\sqrt{g}-\sqrt{d_r})}, \tag{24}$$

and the nonsymmetric solutions exist for $k>k_c$. Linear stability analysis shows that whenever the nonsymmetric solutions exist they are stable, while the symmetric solution is stable only for $k \leq k_c$.

The steady state populations of free A and B repressors vs k, for the BRD switch, are shown in Fig. 6. The results of numerical integration of the rate equations ($\times$) are in perfect agreement with the analytical results derived above (solid line). We conclude that the degradation of bound repressors induces bistability, even at the level of rate equations. The emergence of bistability can be attributed to the fact that the effective degradation rate for the minority specie in Eq. (20) is larger than the effective degradation rate for the dominant specie. This tends to enhance the difference between the population sizes and to destabilize the symmetric solution for $k>k_c$.

B. Master equation

The master equation for the BRD switch can be obtained from Eq. (5) by adding the term

$$d_r[\delta_{r_A,0}P(N_A,N_B,r_A+1,r_B) - \delta_{r_A,1}P(N_A,N_B,r_A,r_B)]$$
$$+ d_r[\delta_{r_B,0}P(N_A,N_B,r_A,r_B+1) - \delta_{r_B,1}P(N_A,N_B,r_A,r_B)]. \tag{25}$$

For steady state conditions we find that BRD tends to suppress the peak near the origin of $P(N_A,N_B)$. For a suitable range of parameters, two separate peaks appear, which qualitatively resemble those obtained for the exclusive switch. However, unlike the exclusive switch, there is a narrow corridor with small but nonvanishing probabilities that connects the two peaks via the origin. As a result, the switching time

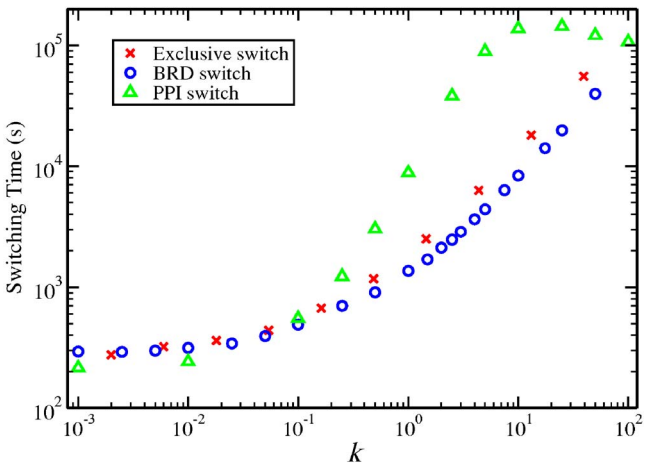

FIG. 7. (Color online) The switching time τ vs k for the exclusive ($\times$), BRD ($\circ$) and PPI ($\triangle$) switch systems. The parameters used are $g=0.05$, $d=d_r=0.005$, and $\gamma=0.1$ (s^{-1}).

for the BRD switch tend to be somewhat shorter than for the exclusive switch with the same parameters. The switching times, τ, vs the repression strength, k, are shown in Fig. 7.

We now examine in what range of parameters this circuit is indeed a switch according to the master equation. Unlike the rate equation where the condition for bistability is clear [Eq. (24)], in the case of the master equation the notion of bistability is more subtle. Thus, in the analysis below we use the following operational criterion. First we define the two states of the switch. The A-dominated state is defined as the set of all states in which $N_A>2$ and $N_B=0,1$. Similarly, the B-dominated state is defined by $N_B>2$ and $N_A=0,1$. The system is considered as a switch if, under steady state conditions, the total probability to be in either of these states is larger than 0.99. This leaves a probability of only 0.01 for all the intermediate states, which the system must visit in order to switch between the A-dominated and the B-dominated states. As a result, the switching rate is low.

We used this criterion in order to find the region in the (k,d_r) plane of the parameter space in which the BRD circuit exhibits bistability. It was found that the BRD switch exhibits bistability for large enough values of k, as long as the value of d_r is not too different from d. If $d_r/d \ll 1$, the process of bound-repressor degradation is negligible and cannot eliminate the dead-lock situation. If $d_r/d \gg 1$, proteins bind and quickly degrade. As a result, the population of the dominant specie is reduced and bistability is suppressed.

Within the parameter range in which the system exhibits bistability, we examined the dependence of the switching time τ of the BRD switch on the parameters g, d, α_0, and d_r. It was found that τ exhibits linear dependence on the production rate g and on the repression strength k (here, k was varied by changing α_0, keeping α_1 and d_r fixed). The dependence of τ on the degradation rate d was found to be approximately $1/d^2$. Note that as d was varied, we kept $d_r=d$ in order that the system remains bistable. Since k depends on d_r, it slightly varied as well.

Unlike the exclusive switch, where we managed to obtain the scaling properties of τ by a simple argument, the BRD switch turns out to be more complicated. This is due to the

fact that there are several processes that may lead to the flipping of the switch, such as the unbinding or the degradation of the bound repressor. A further complication is that the two repressors can be bound simultaneously. As a result, we have not managed to obtain an expression for τ in the BRD switch.

V. THE SWITCH WITH PROTEIN-PROTEIN INTERACTION

Consider a switch circuit which in addition to the mutual repression, exhibits protein-protein interactions, namely an A protein and a B protein may form a complex, AB. The AB complex is not active as a transcription factor.

A. Rate equations

The PPI switch can be described by the following rate equations:

$$[\dot{A}] = g(1-[r_B]) - d[A] - \alpha_0[A](1-[r_A]) + \alpha_1[r_A] - \gamma AB,$$

$$[\dot{B}] = g(1-[r_A]) - d[B] - \alpha_0[B](1-[r_B]) + \alpha_1[r_B] - \gamma AB,$$

$$[\dot{r_A}] = \alpha_0[A](1-[r_A]) - \alpha_1[r_A],$$

$$[\dot{r_B}] = \alpha_0[B](1-[r_B]) - \alpha_1[r_B]. \tag{26}$$

The parameter γ is the rate constant for the binding of a pair of A and B proteins. The Michaelis-Menten equations take the form

$$[\dot{A}] = \frac{g}{1+k[B]} - d[A] - \gamma[A][B],$$

$$[\dot{B}] = \frac{g}{1+k[A]} - d[B] - \gamma[A][B]. \tag{27}$$

For steady state conditions, these equations exhibit a symmetric solution, $[A]=[B]$, for any choice of the parameters. It is the solution of

$$\gamma k[A]^3 + (\gamma + dk)[A]^2 + d[A] - g = 0. \tag{28}$$

Since all the coefficients of powers of $[A]$ are positive, this equation has only one positive solution. Also, within some range of parameters there exist nonsymmetric solutions, given by the solutions of

$$d\gamma k[A]^2 + (d\gamma + d^2k - g\gamma k)[A] + d^2 = 0. \tag{29}$$

The nonsymmetric solutions exist only for the range of parameters in which Eq. (29) has two positive solutions. The condition for this can be easily expressed in terms of the coefficients in Eq. (29).

As in the case of the BRD switch, bistability is observed even at the level of rate equations. Again, the emergence of bistability can be attributed to the fact that the effective degradation rate constant for the minority specie is larger than for the dominant specie, thus enhancing the difference between the population sizes [note that the effective degradation rate constant for A is $(d+\gamma[B])$, while for B it is $(d+\gamma[A])$].

B. Master equation

The master equation for the PPI switch can be obtained from Eq. (5) by adding the term

$$\gamma[(N_A+1)(N_B+1)P(N_A+1,N_B+1,r_A,r_B) - N_AN_BP(N_A,N_B,r_A,r_B)]. \tag{30}$$

For a suitable range of parameters the steady state solution of the master equation exhibits two separate peaks. To draw the range of parameters in which bistability takes place we apply the operational criterion used above for the BRD switch. We fix g, d, and α_1 and examined the system in the (k,γ) plane. The results are plotted in Fig. 8 (solid line).

For small values of γ (weak PP interaction), the circuit does not exhibit bistability. As the interaction strength increases the circuit behaves as a switch for a certain range of repression strength k. This range broadens as γ is increased. Unlike the switch systems discussed above, in which the bistability gets stronger as k is increased, the PPI switch is bistable for intermediate values of k. This can be understood as follows. Recall that the key to the formation of a switch is the elimination of the dead-lock situation. The exclusive and the BRD switches deal with this situation directly at the bound repressor level. However the PP interaction does not directly affect the bound repressor. To prevent the possibility of two proteins bound simultaneously, one of them should unbind and form a complex with a protein of the other specie. In order for this to happen, the repressors must not be bound too strongly. Therefore, the PPI switch works at intermediate repression strength. As the PPI becomes more effective (larger γ) this mechanism applies at larger values of k.

Enhanced switching properties can be obtained by considering a hybrid system that combines PPI and exclusive binding. The resulting switch exhibits bistability in a broader range of parameters than the exclusive or PPI switches alone. The master equation for the exclusive-PPI switch is obtained from Eq. (14) by adding the term

$$\gamma[(N_A+1)(N_B+1)P(N_A+1,N_B+1,r_A,r_B) - N_AN_BP(N_A,N_B,r_A,r_B)], \tag{31}$$

which accounts for the PP interaction. Numerical results, shown in Fig. 8, indicate that indeed as expected the exclusive-PPI switch is a better switch than either the PPI or the exclusive switch. The parameter range in which it exhibits bistability is broader. Thus, it is more robust to variations in the parameters than the exclusive or PPI switches.

VI. COOPERATIVE BINDING

Cooperative binding is found in genetic switch systems such as the phage λ switch [9]. In this case, transcription regulation is obtained only when several copies of the repressor are bound simultaneously. This situation can be achieved in two ways. One possibility is that repressors bind to each

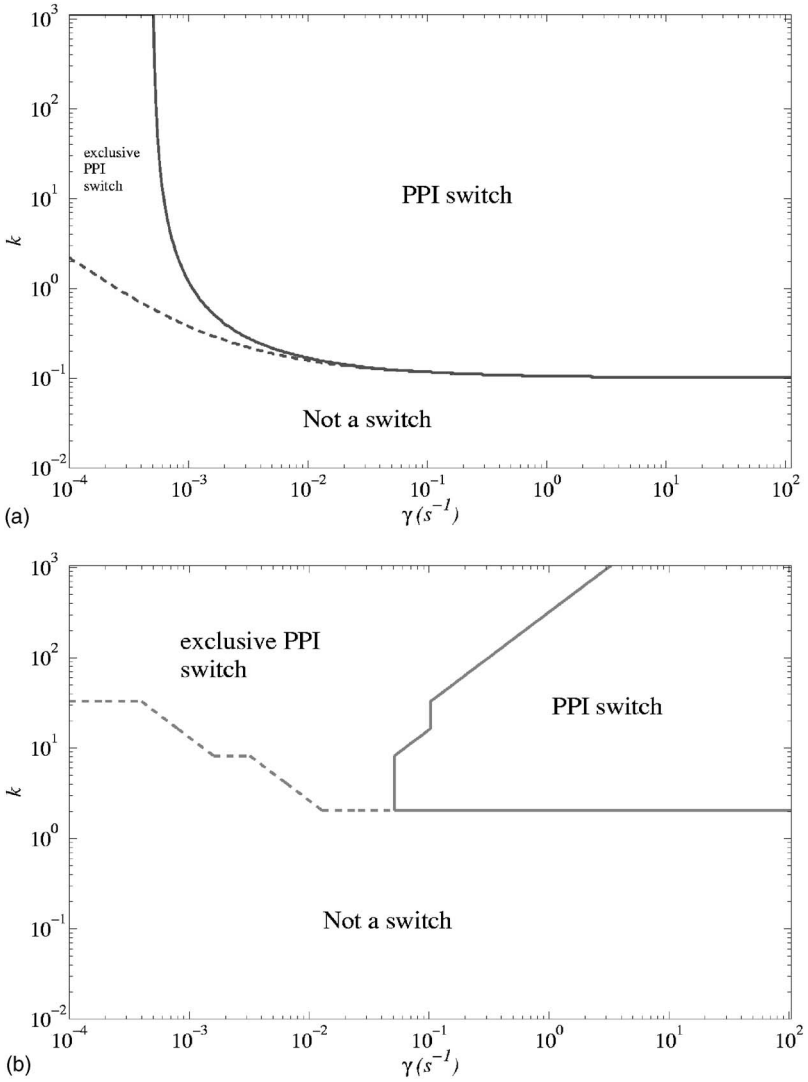

FIG. 8. The range of parameters in the (γ,k) plane in which bistability takes place in the PPI switch (solid line) and in the exclusive-PPI switch (dashed line), using rate equations (a) and using the master equation (b). The other parameters are $g=0.05$ and $d=0.005$ (s^{-1}).

other and form a complex, which then binds to the promoter. The other possibility is that the repressors bind separately, but those already bound assist the other ones to bind more effectively. In the case of cooperative binding, bistability turns out to appear even at the level of rate equations [17].

A. Rate equations

Switch systems with cooperative binding are commonly described by

$$[\dot{A}] = \frac{g}{1+k[B]^n} - d[A],$$

$$[\dot{B}] = \frac{g}{1+k[A]^n} - d[B], \tag{32}$$

where n is the Hill coefficient. It corresponds to the number of copies of the transcription factor which are required in order to perform the repression process. Here we focus on the case $n=2$, and show that these equations exhibit two stable steady state solutions for some range of parameters. Imposing $[\dot{A}]=[\dot{B}]=0$ in Eq. (32), we obtain

$$g - d[A] - kd[A][B]^2 = 0,$$

$$g - d[B] - kd[B][A]^2 = 0. \tag{33}$$

Subtracting one of these equations from the other we find that

$$-d([A]-[B]) - kd[A][B]([B]-[A]) = 0. \tag{34}$$

Looking for a nonsymmetric solution for which $[A]\neq[B]$, we divide Eq. (34) by $[A]-[B]$. We find that $k[A][B]=1$, or $[B]=1/k[A]$. Inserting this into Eq. (33) we get an equation for $[A]$:

$$dk[A]^2 - gk[A] + d = 0. \tag{35}$$

This equation exhibits two distinct stable solutions

$$[A] = \frac{gk \pm \sqrt{g^2k^2 - 4d^2k}}{2dk}, \tag{36}$$

for $k > 4d^2/g^2$. This means that the system becomes bistable at the bifurcation point, $k=4d^2/g^2$. In addition to these solutions, the symmetric solution $[A]=[B]$ exists for any choice

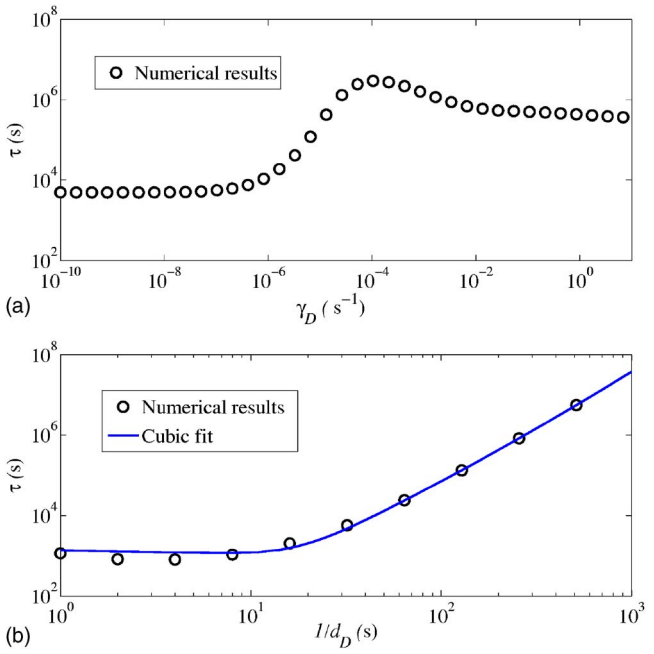

FIG. 9. (Color online) The dependence of the switching time τ for the dimers exclusive switch on the dimers degradation rate d_D (a) and the dimerization rate γ_D (b).

of the parameters. This symmetric solution is stable for $k < 4d^2/g^2$ and becomes unstable at the bifurcation point.

B. Monte Carlo simulations

Consider a switch system with cooperative binding with $n=2$, in which two proteins of the same specie bind together to form a complex or dimer. The repression of A synthesis is done by dimers composed of two B proteins and vice versa. For example, consider an exclusive switch, in which the dimers of A and B cannot be bound simultaneously. To account for stochastic effects, we have studied this system using Monte Carlo simulations.

The rate constant for the formation of dimers is denoted by γ_D. It is assumed that dimers cannot dissociate into single proteins, but they can degrade. The degradation rate of dimers is denoted by d_D. We examined the dependence of the switching time τ on all the parameters. We found the following properties. The dependence of τ on g was found to be linear as for the exclusive and BRD switch. The dependence on d is very weak, except for the limit in which d is very large. This is because the proteins tend to form dimers before they have a chance to degrade. The dependence of τ on $k = \alpha_0/\alpha_1$ is found to be well fitted by a quadratic polynomial. This means that for sufficiently strong repression, $\tau \sim k^2$.

The dependence of τ on the dimerization rate γ_D [Fig. 9(a)] exhibits interesting behavior. For small values of γ_D the system is not really a switch, because almost no dimers are formed. Therefore the switching time is short. For larger values of γ_D the dimer population increases and the system starts to function as a switch. The switching time τ increases as the switch becomes more stable. But from some point, increasing γ_D causes τ to decrease. This is because, very fast dimerization helps the minority specie to form dimers, making it more likely to flip the switch.

The dependence of τ on d_D [Fig. 9(b)] was found to be well fitted by a cubic polynomial in $1/d_D$. This means that in the limit of slowly degrading dimers, $\tau \sim 1/d_D^3$. In the limit of fast dimer-degradation the system is not bistable, because the population of dimers is too small to make the repression effective.

The switching time for this system was also studied in Ref. [14], where τ was presented as a function of the average copy number of the dominant specie. However, the copy number depends in a nontrivial way on the parameters and cannot be directly controlled. Therefore, we believe that in a systematic study of the switching times, it is more practical to examine the dependence of τ on the parameters themselves.

Note that there is another important realization of cooperative binding in which the promoter consists of two binding sites. When a protein binds to one of them it facilitates the binding of another protein to the second site. The effect of this mechanism is qualitatively similar to the one shown above for dimers. In general cooperative binding induces bistability because it forces the minority specie to recruit at least two proteins in order to flip the switch. As a result, cooperative binding helps to remove the dead-lock situation in which both species are suppressed simultaneously.

VII. RESPONSE TO EXTERNAL SIGNALS

Until now our discussion considered only spontaneous transitions between the two states of the switch. Here we demonstrate how an external signal may lead to the flipping of the switch. In case of the λ switch, such an external signal may be, for example, the exposure of *E. coli* infected by phage λ to UV light. In the *lac* circuit, the external signal indicates the presence of lactose. We assume that the effect of the external signal is that one of the proteins undergoes a conformal change that prevents its binding to the promoter. When the signal affects the dominant specie, this may lead to the flipping of the switch. We assume that the conformal change is fast and that it lasts for a period of time determined by the duration of the external signal.

We have performed Monte Carlo simulations, where the binding rate α_0 of the dominant specie was set to zero for some period of time (the length of external signal). We calculated the probability for a flipping of the switch during 1800 (s), which is roughly the time between divisions of *E. coli*, as a function of the signal length. The results are shown in Fig. 10.

For short duration of the signal, the switch has a small chance to flip. As the duration increases the probability to flip increases too, and so for a long enough signal, the switch will eventually flip as expected (the actual switching time depends on the parameters of the switch, like the production rate g or the unbinding rate α_1). Here we just demonstrated that in principle the switch will flip states in response to an external signal.

VIII. DISCUSSION

In the rate equations, the meaning of bistability is clear. It typically appears as a result of a bifurcation. Below the bi-

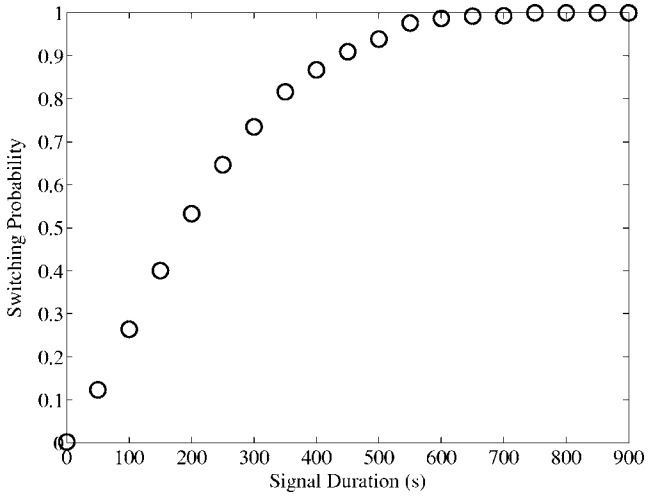

FIG. 10. Probability for the exclusive switch to flip during 1800 (s) after the initiation of the signal, as a function of the external signal duration. The parameters used were $g=0.2$, $d=0.005$, $\alpha_1=0.01$, and $\alpha_0=0.2$ or zero during the signal.

furcation there is a single, stable solution, which becomes unstable at the bifurcation point, where two stable solutions emerge. In case of the toggle switch, one of these solutions is dominated by A proteins and the other is dominated by B proteins. Since both solutions are stable, the possibility of spontaneous transitions between them due to stochastic fluctuations is not included in the rate equation model.

The objects that participate in regulatory processes in cells, namely genes, mRNAs, proteins and promoter sites are discrete objects, and some of them often appear in low copy numbers. This, together with the fact that many of the relevant processes such as diffusion, degradation as well as binding and unbinding of transcription factors are of stochastic nature, requires to consider the role of stochastic fluctuations in these regulatory processes. This can be done by using the master equation or Monte Carlo simulations.

In the master equation, bistability is characterized by two separate peaks in the probability distribution. These peaks should be sufficiently far from each other, with low probabilities in the domain between them. As a result, the flow of probability between the two peaks is low and the time between spontaneous switching events is long. In order to qualify as a switch, the average time between spontaneous switching events must be much longer than the time constants of the transcription, translation, and degradation processes in the cell.

For the systems studied here it was found that the general switch without cooperative binding does not exhibit bistability either with the rate equations or with the master equation. Two other variants, the BRD and the PPI switch systems, were found to exhibit bistability both with the rate equations and with the master equation. However, the exclusive switch, which is not bistable at the rate equation level, was found to exhibit bistability with the master equation. Thus, in case of the exclusive switch it is clear that stochastic fluctuations play a crucial role in making the system bistable. For this system we also found an exact phenomenological expression for the switching time in terms of the rate constants of the relevant processes.

Stochastic analysis of genetic networks can be done either by direct integration of the master equation or by Monte Carlo simulations. The master equation provides the probability distribution of the population sizes of all the mRNA's and proteins in the simulated circuit. It can be considered as a distribution over a large number of genetically identical cells. The average population sizes and the rates of processes are expressed in terms of moments of this distribution. To obtain such distributions from Monte Carlo simulations, one needs to repeat the simulations a large number of times and average over them. This may be inefficient in terms of computer time, and the statistical errors may be significant. On the other hand, unlike the master equation, Monte Carlo simulations enable to follow the time evolution of a single cell and directly evaluate quantities such as switching times and oscillation periods.

The number of equations in the master equation set increases exponentially with the number of proteins and mRNAs included in the simulated circuit. As a result, the master equation becomes infeasible for complex networks. Recently, we have shown that for reaction networks described by sparse graphs, one can use suitable approximations and dramatically reduce the number of equations [28].

A related circuit, the mixed feedback loop, in which A is a repressor to B and the A and B proteins bind to form a complex was recently studied using rate equations [29,30]. It was found to exhibit bistability within a range of parameters.

IX. SUMMARY

Genetic switch systems with mutual repression of two transcription factors, have been studied using a combination of deterministic and stochastic methods. These systems exhibit bistability, namely two stable states such that spontaneous transitions between them are rare. Induced transitions take place as a result of an external stimulus. We have studied several variants of the genetic switch, which exhibit cooperative binding, exclusive binding, protein-protein interactions and degradation of bound repressors. For each variant we examined the range of parameters in which bistability takes place. Numerous studies have concluded that cooperative binding is a necessary condition for the emergence of bistability in these systems. We have shown that a suitable combination of network structure and stochastic effects gives rise to bistability even without cooperative binding. The average time τ between spontaneous transitions was evaluated as a function of the biological parameters.

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