Optimal path to epigenetic switching

David Marin Roma, Ruadhan A. O'Flanagan, Andrei E. Ruckenstein, and Anirvan M. Sengupta

Department of Physics and Astronomy and BioMaPS Institute, Rutgers University, Piscataway, New Jersey 08854, USA

Ranjan Mukhopadhyay

Department of Physics, Clark University, Worcester, Massachusetts 01610, USA

We use large deviation methods to calculate rates of noise-induced transitions between states in multistable genetic networks. We analyze a synthetic biochemical circuit, the toggle switch, and compare the results to those obtained from a numerical solution of the master equation.

Fluctuations in biomolecular networks have been the subject of much research activity recently [1]. Studies on noise in gene expression [2–7], in signal transduction [8], and in biochemical oscillators [9–11] demonstrated that having a small number of molecules affects, sometimes critically, the behavior of cellular circuits. Stochastic aspects of the choice between lytic and lysogenic developmental strategies of bacteriophage lambda virus infection in *E. coli* were studied in an influential paper by Arkin, Ross, and McAdams [12].

One of the interesting aspects of developmental processes is that one could get multiple heritable cell fates without irreversible changes to the genetic information. Different cells with the same DNA sequence, showing different phenotypes that are stably maintained through cell divisions, namely epigenetic phenomena, have been represented as multiple stable attractors in deterministic descriptions of the biochemical dynamics. In this paper, we are concerned with the robustness of such attractors against spontaneous fluctuations which might induce transitions from one stable state to another. Previous work in this area has modeled the effects of fluctuations by adding Gaussian-distributed Langevin forces to the deterministic equations [13–15]. Although this description is appropriate in describing typical fluctuations when the number of molecules is sufficiently large [2,4,6,8], *rare events* involving occasional large departures from average behavior are typically outside the scope of the Langevin treatment (Gaussian approximation). The transition rate in a simplified model of the phage lambda switch has been studied [14,16] in this approximation. We wish to compute the transition rate using a more appropriate large deviation theory with special focus on the attempt frequency. Of course, one could get the transition rate from direct computer simulations. However, direct simulations of rare events is, obviously, time-consuming. Recent research in the lambda switch suggests that the simplified model lacks one very important physical interaction between distant regions of the lambda virus genome, changing dramatically the behavior of the switch [17]. Applying our tools to that question, among others, is the long-time goal of our research. However, we wish to test our methods on a simpler system. We will consider the artificially constructed toggle switch [18]. In this example, we find that the contributions to the transition rate coming from corrections to the Gaussian approximation can change the overall rate by several orders of magnitude and,

therefore, are important for comparison with experimental results.

The theory of transition rates is a well developed subject (see [19] as well as references therein). For a bistable system like the genetic switch we are considering, the transition probability from one stable point to the other is estimated by computing the probability of reaching the saddle point between stable states, and, from there, by following the deterministic trajectory to the other stable state rather than falling back to the initial state. The transition rate is given by an expression of the form

$$\text{rate} = \frac{\lambda_+}{2\pi} \left[\frac{\det A_{fp}}{|\det A_{sp}|} \right]^{1/2} * P(x_f, x_o), \qquad (1)$$

where λ_+ is the positive eigenvalue of the matrix describing the linearized equations of motion around the saddle point, A_{fp} and A_{sp} are the inverses of covariance matrices appearing in the quasistationary Gaussian approximation of the probability distribution in the starting stable point and in the saddle point, respectively, and $P(x_f, x_o)$ is the probability of finding the system at the saddle point state in a quasistationary distribution centered around the stable fixed point x_o. Note that A_{sp} has one negative eigenvalue and the Gaussian distribution around the saddle point is only a formal solution. A more precise definition of A appears later in the paper. For derivations of a very similar formula, see [20] or Sec. VII.D in the review [19].

Much of the rest of the paper is devoted to the computation of $P(x_f, x_o)$ by large deviation methods. There are two related ways. In one approach, one keeps track of the trajectories in the space of numbers of different molecules, distributed according to a state-dependent Poisson process, and computes time-dependent transition probabilities as a sum of the probabilities of all paths connecting the initial and final points, which leads naturally to a path integral formulation of the stochastic process. In this way, the transition probability is evaluated as the exponential of the "action." This action can be computed in a perturbation expansion (using the volume of the system as a parameter), in which the leading-order correction is the line integral along the path that minimizes the action (optimal path) of a Lagrangian function. This calculation naturally gives rise to a Hamiltonian that

corresponds to the evolution operator in the master equation [21], written in terms of numbers and raising and lowering operators expressed as exponentials of the phase variables conjugate to the numbers.

An alternative but exactly equivalent approach is to start directly from the master equation and solve it in the Eikonal approximation [22]. We will present our arguments in this article using this approach, which is easier to explain mathematically, and provides an easier way to compute the next-order correction in the volume expansion, a term which has not been computed before in relation to these genetic switches. As we will see, in the case of the toggle switch, the next-order term in $\ln P(x_f, x_o)$ makes an important contribution to the overall rate of transition.

The general ideas are developed in the context of the simple example of the toggle switch. This artificially realized switch consists of two genes that repress each others' expression, placed in a high copy plasmid in *E. coli*. Once expressed, each protein can bind particular DNA sites upstream of the gene which codes for the other protein, thereby repressing its transcription. If we denote the ith protein concentration by x_i, the deterministic system is described by the equations

$$\dot{x}_1 = \frac{a_1}{1 + (x_2/K_2)^n} - \frac{x_1}{\tau}, \tag{2}$$

$$\dot{x}_2 = \frac{a_2}{1 + (x_1/K_1)^m} - \frac{x_2}{\tau}. \tag{3}$$

The constants a_1 and a_2 incorporate all aspects of transcription and translation reactions. The Hill exponents, m and n, represent the degree of cooperative binding of proteins to DNA, and τ^{-1} is the protein degradation/dilution rate (assumed equal for the two proteins). K_1 is the effective dissociation constant for binding of protein 1 in the promoter of gene 2. K_2 is the corresponding parameter for protein 2. For some regions of parameter space, the system has three stationary points: two stable ones and a saddle point [18].

For the purposes of this discussion, we model the stochastic evolution of the protein concentrations in the system by a birth-death process in which protein i is made in short-lived bursts of size b_i and proteins are diluted or degraded at a rate τ^{-1}. A more detailed description involving proteins and RNA will be published elsewhere. It is worth noting that, while both the burst size b_i and the RNA production rate show up as parameters in the stochastic modeling, only their product, a_i, shows up in the effective deterministic equations (2) for the protein levels.

To compute the rate of transition from one fixed point to the other, we must solve the master equation [21], which describes the time evolution of the probability distribution of protein concentrations. The qualitative behavior of the stationary solution for the bistable system can be described in simple intuitive terms: the solution displays two peaks centered around the stable points. If we start with probability 1 around one of the stable points, rare transitions lead to a long tail which leaks into the domain of attraction of the other stable point, in very much the same way in which the prob-

ability amplitude extends beyond the classically allowed region in quantum-mechanical tunneling through a barrier. This analogy motivates the Eikonal approximation to the solution of the master equation [22]. The master equation is given by

$$\frac{\partial P}{\partial t} = \Omega \sum_e [W_{\hat{e}}(\boldsymbol{x} - \hat{e}/\Omega) P(\boldsymbol{x} - \hat{e}/\Omega, t) - W_{\hat{e}}(\boldsymbol{x}) P(\boldsymbol{x}, t)], \tag{4}$$

where Ω is the volume of the system, and $\hat{e}/\Omega = \Delta \vec{x}$ is the concentration change associated with individual reaction events, the rate of which is given by $\Omega W_{\hat{e}}(x)$. Assuming that the distribution is quasistationary in the region of interest, we consider solutions of the WKB form,

$$P(\boldsymbol{x}, t) = C \exp[-\Omega S(\boldsymbol{x})], \quad S(\boldsymbol{x}_o) = 0, \tag{5}$$

x_o being the initial stable point. In the same way the wave function in quantum mechanics is computed using an expansion in powers of $\hbar$, it customary to find the probability $P(\boldsymbol{x}, t)$ by expanding $S(\boldsymbol{x})$ in powers of inverse volume, which plays the same role as $\hbar$ in quantum mechanics, since the bigger the volume, the less likely are fluctuations to happen. Then, to first order in Ω^{-1}, we write

$$S(\boldsymbol{x}) = S_0(\boldsymbol{x}) + \Omega^{-1} S_1(\boldsymbol{x}) + O(\Omega^{-2}).$$

Assuming that the scaled transition rates $W_{\hat{e}}(\boldsymbol{x})$ are smooth functions of $\boldsymbol{x}$, and expanding S to first order, $S(\boldsymbol{x} - \hat{e}/\Omega) = S(\boldsymbol{x}) - (\hat{e}_i/\Omega) \cdot (\partial/\partial x_i) S(\boldsymbol{x})$, collecting the terms which do not contain powers of Ω, we have

$$\frac{\partial P(\boldsymbol{x}, t)}{\partial t} = H P(\boldsymbol{x}, t), \tag{6}$$

$$H(\boldsymbol{x}, \boldsymbol{p}) = \sum_{\hat{e}} [W_{\hat{e}}(\boldsymbol{x})(e^{(\hat{e} \cdot \boldsymbol{p})} - 1)], \tag{7}$$

where H is the Hamiltonian describing the time evolution of the probability distribution, and we define the momentum p_i as

$$p_i = \frac{\partial}{\partial x_i} S_0(\boldsymbol{x}). \tag{8}$$

If we expand the Hamiltonian (7) in $\boldsymbol{p}$ and keep terms up to second order in $\boldsymbol{p}$, we recover the Gaussian approach used in [13,14]. Since we are considering a situation where the transitions are so rare that the probability does not change much in time, the Hamiltonian will be very small.

The main contribution to the transition probability is obtained by evaluating P along a particular trajectory [22]. This trajectory, called the optimal path, is the solution to Hamilton's equations derived from Eq. (7),

$$\dot{x}_i = \frac{\partial H(\boldsymbol{x}, \boldsymbol{p})}{\partial p_i} = \sum_{\hat{e}} [\hat{e}_i W_{\hat{e}}(\boldsymbol{x}) e^{(\hat{e}_a p_a)}], \tag{9}$$

$$\dot{p}_i = -\frac{\partial H(\boldsymbol{x}, \boldsymbol{p})}{\partial x_i} = -\sum_{\hat{e}} \left[\frac{\partial W_{\hat{e}}(\boldsymbol{x})}{\partial x_i} (e^{(\hat{e}_a p_a)} - 1) \right]. \tag{10}$$

For the toggle switch example we have four $\hat{e}_i$'s describing jumps to the right, left, up, or down, given by $b_1\hat{x}_1$, $-\hat{x}_1, b_2\hat{x}_2$, and $-\hat{x}_2$, respectively. The relevant Hamiltonian defined on times long compared to the inverse binding/unbinding rates of proteins at the two promoters is given by

$$H = \frac{a_1/b_1}{[1+(x_2/K_1)^n]}(e^{b_1 p_1}-1) + \frac{x_1}{\tau}(e^{-p_1}-1)$$

$$+ \frac{a_2/b_2}{[1+(x_1/K_2)^m]}(e^{b_2 p_2}-1) + \frac{x_2}{\tau}(e^{-p_2}-1). \quad (11)$$

As already mentioned above, $K_{1,2}$ are the effective dissociation constants for binding of proteins 1,2 at the promoter of gene 2,1, respectively, b_i is the burst size of protein i, and the ratio a_i/b_i is a measure of the RNA production rate associated with the transcription of the gene i.

To extract the values of the burst size parameters, the spontaneous transition rate has to be measured experimentally for more than one condition. Since this has not yet been done, we will compare the results of the Eikonal approximation to the solution obtained by direct diagonalization of the Hamiltonian (11). For simplicity, we will set the parameters $K_i=1, b_i=1$.

The optimal path for the transition from one stable point to the other starts near one stable point, proceeds to the saddle point, and from there it follows the deterministic trajectory to the other stable point. Thus we must first find solutions of Eqs. (9) and (10) which start at (near) the initial stable point and end at the saddle point. At the end points we have $p_1=p_2=0$, and $H=0$. This also implies that if the system is at the stable point, it will remain there. So, the optimal path must instead start at a point very close to but not exactly at the fixed point. In this case, the Hamiltonian will be a very small number (and constant). In what follows, we will make the approximation $H=0$. The initial conditions for the momentum equations can be obtained by approximating the probability around the stable point by a Gaussian distribution $P=e^{-\Omega S_g}$ with $S_g=\frac{1}{2}A_{ij}\delta x_i \delta x_j$ (note that we use summation convention, i.e., repeated indices are summed over). Then $p_i=\partial S_o/\partial x_i = A_{ij}\delta x_j$, and we expand the equation $H=0$ around the stable point to find A_{ij}. Then we have a two-point boundary value problem which can be solved by various methods [23]. The solution of the equations of motion (9) and (10) for a set of parameters, projected to concentration space, is shown in Fig. 1. We integrate Eqs. (8) along the optimal path C to obtain $S_0=\int_C p_i dx_i$.

The S_1 factor can be viewed as a correction due to fluctuations around the optimal path and could be calculated following Refs. [24,25]. Collecting coefficients of powers of Ω in the Ω^{-1} expansion, we derive an equation for S_1,

$$\sum_{\hat{e}}\left[W_{\hat{e}}\hat{e}_i\frac{\partial S_1}{\partial x_i} - \frac{W_{\hat{e}}}{2}\hat{e}_i\hat{e}_j\partial_i p_j - \hat{e}_i\partial_i W_{\hat{e}}\right]e^{(\hat{e}_a p_a)} = 0. \quad (12)$$

In turn, after using the equations of motion to rewrite the first term as derivative along the optimal path $x_{op}(t')$, Eq. (12) can be transformed into
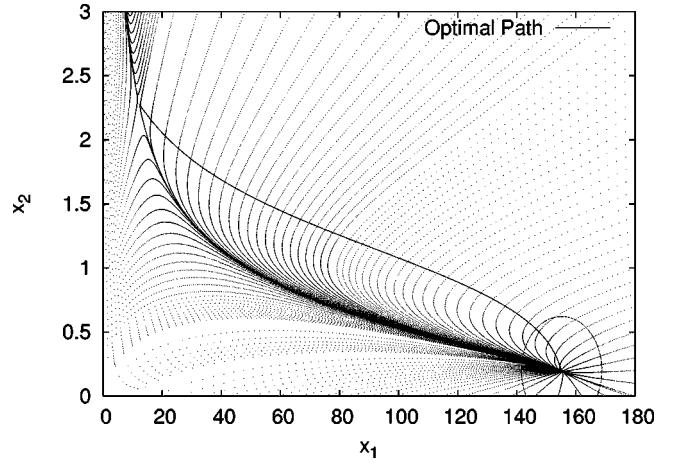

FIG. 1. Optimal path for the parameters, $a_1=156$, $a_2=30$, $n=3$, $m=1$, $K_1=K_2=1$, $b_1=b_2=1$, and $\tau=1$. xi are dimensionless. The ellipsoid indicates the orientation of the Gaussian spread around the stable point. The size of the spread scales like $\Omega^{-1/2}$.

$$\frac{d}{dt'}S_1 = \sum_{\hat{e}}\frac{1}{2}W_{\hat{e}}(\boldsymbol{x})\hat{e}_i\hat{e}_j\frac{\partial p_j}{\partial x_i}e^{\hat{e}_i p_i} + \sum_{\hat{e}}\hat{e}_i\frac{\partial W_{\hat{e}}(\boldsymbol{x})}{\partial x_i}e^{\hat{e}_i p_i}. \quad (13)$$

To proceed, we need $\partial p_j/\partial x_i$ along the path. From Hamilton's equations (9) it follows that $\delta p(t)_a = M(t)_{ab}\delta x(t)_b$, and thus we can use the components of the matrix M in place of the derivative $\partial p_j/\partial x_i$ in Eq. (12). Moreover, Eq. (9) also implies that

$$\delta\dot{x}^a = \frac{\partial^2 H}{\partial p_a \partial x^i}\delta x^i + \frac{\partial^2 H}{\partial p_a \partial p_i}\delta p_i, \quad (14)$$

$$\delta\dot{p}_a = -\frac{\partial^2 H}{\partial x^a \partial x^i}\delta x^i - \frac{\partial^2 H}{\partial x^a \partial p_i}\delta p_i. \quad (15)$$

Combining this together with the time derivative of $\delta p(t)$,

$$\delta\dot{p} = \dot{M}\delta\boldsymbol{x} + M\delta\dot{\boldsymbol{x}}, \quad (16)$$

leads to the following set of coupled differential equations for M:

$$\dot{M}_{ab} + M_{ac}\frac{\partial^2 H}{\partial x^b \partial p_c} + M_{ac}\frac{\partial^2 H}{\partial p_c \partial p_d}M_{db} + \frac{\partial^2 H}{\partial x^a \partial p_c}M_{cb}$$

$$+ \frac{\partial^2 H}{\partial x^a \partial x^b} = 0 \quad (17)$$

with initial conditions $M_{ij}(t=0)=A_{ij}$ [defined below Eq. (11)]. Finally, solving these equations together with Eqs. (9) and (10), we integrate Eq. (13) to obtain S_1. Given the above values of S_0 and S_1, we compute the transition probability, $P(x_f,x_o)$, from the starting stable point, x_o, to the saddle point, x_f. Using Eq. (1), we can, therefore, find the transition rate for any large value of Ω. We now compare this calculation to the direct estimation of transition rates as described below.

From the master equation (4), it follows that the eigenvalues of H measure the decay rates of nonstationary states corresponding to eigenvectors of H with nonzero eigenval-

ues. The equilibrium state is represented by the "zero mode," i.e., the eigenvector of H with zero eigenvalue, the existence of which is guaranteed by the transition matrix character of the Hamiltonian and conservation of probability. To compute the eigenvalues of the Hamiltonian, we write the master equation in discrete form, replacing the continuous concentration variables (x_1, x_2) with a lattice with lattice parameter $1/\Omega$. Although the system displays infinitely many states, typically, the gap between the real parts of the eigenvalues for the first and second excited states is much larger than the absolute value of the real part of the first eigenvalue. This is because the gap between the first excited state and the second or the third excited states is governed by the local relaxation rate around the two fixed points, but the gap between the ground state and the first excited state is governed by the transition rate between the two stable fixed points. The local relaxation rates are order 1 in Ω, whereas the transition rate is exponentially small for large Ω (in practice, we find the ratios of the real parts to be about 10^3). Thus an arbitrary probability distribution rapidly decays into a linear combination of the stationary state and the first excited state. Equivalently, the state could be described as a linear combination of two states, each representing a quasistationary distribution around a stable fixed point. From then on, we can project the evolution to this two-state system. If we start with probability p_o of being in the state $(1,0)^T$, then the Master equation gives

$$\frac{d}{dt}\begin{pmatrix} p_o \\ p_f \end{pmatrix} = \begin{pmatrix} -r_{12} & r_{21} \\ r_{12} & -r_{21} \end{pmatrix}\begin{pmatrix} p_o \\ p_f \end{pmatrix}.$$

The two-by-two effective transition matrix has columns which sum to zero ensuring probability conservation. Also, the trace $0 + \epsilon_1 = r_{12} + r_{21}$, where ϵ_1 is the eigenvalue of the first excited state. Therefore, the first excited eigenvalue will be the sum of the forward and backward rates. In the case of the asymmetric systems, one rate is usually far greater than the other. Consequently, the larger rate among r_{12} and r_{21} will be approximately given by ϵ_1, which we computed numerically using the MATLAB routine "eigs" for sparse matrices as well as by the Lanczos algorithm [26]. For a symmetric choice of parameters for the two proteins, each rate is just $\epsilon_1/2$.

To explicitly extract the S_0 and S_1 contributions to the rate from the Lanczos results, we rescale the volume of the system $\Omega \to \nu\Omega$, which, in turn, leads to a rescaling of rates of individual reaction events as $f(x) \to \nu f(x)$. As a function of volume scale factor, ν, the logarithm of the rate has the form $\ln(r) = S_0\nu + b$, where b includes both S_1 and the logarithm of the prefactor of $P(x_f, x_o)$ in Eq. (1). The results and comparison with the Eikonal approximation are shown in Fig. 2. The dotted line is a fit to the data points obtained from calculation of the eigenvalues, and we see that the slope and intercept
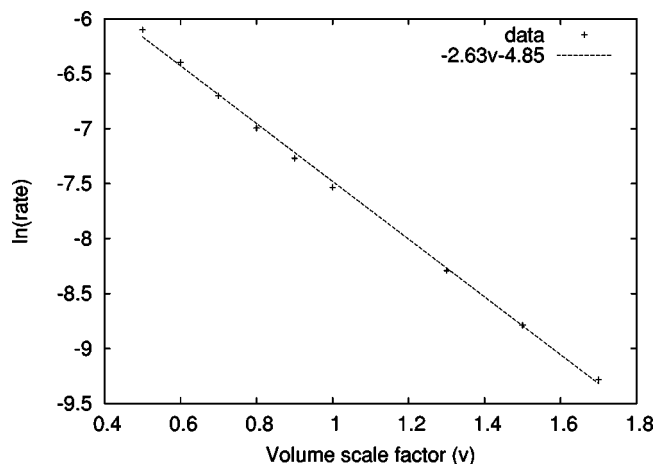

FIG. 2. Scaling with volume: estimates from direct computation of eigenvalues are $S_0 = 2.63$, $S_1 + \ln(\text{pref}) = 4.85$, whereas optimal path calculation gives $S_0 = 2.47$, $S_1 = 3.5$, $\ln(\text{pref}) = 1.5$. In this example, the backward rate is 1000 times smaller than the forward rate, so the lowest nonzero eigenvalue is very close to the rate of switching.

computed from Eqs. (1) and (5) are in good agreement with these values. Note that, in this example, S_1 and the prefactor are significant contributions to the transition rate.

When we perform these calculations for the "standard" model of the lambda switch [14,27], we find a rate three orders of magnitude higher than the observed rate of 10^{-7} per generation [28]. In retrospect, it is clear that accounting for the stability of the lysogenic state requires a more complex model which should include the effect of DNA looping [17]. Whether the stability is due to suppression of fluctuation or due to disappearance of the lytic "fixed" point [29] remains an open question.

Optimal path methods are routinely used for studying rare events related to failure of communication networks modeled as birth and death processes [30]. Such large deviation methods are likely to be important in the context of robustness and adaptability of biological networks. This paper illustrates the power of an approach to fluctuations based on the Eikonal approximation to solutions of the master equation. The scheme incorporates large deviations in a natural way and provides a quantitative method scalable to large networks. We also hope that beyond being an efficient computational tool, this method will provide further insight into the stability of epigenetic states of complex genetic networks.

[1] C. V. Rao, D. M. Wolf, and A. P. Arkin, Nature (London) **420**, 237 (2002); **421**, 190E (2003).

[2] T. B. Kepler and T. C. Elston, Biophys. J. **81**, 3116 (2001).

[3] M. Thattai and A. van Oudenaarden, Proc. Natl. Acad. Sci. U.S.A. **98**, 8614 (2001).

[4] E. M. Ozbudak, M. Thattai, I. Kurtser, A. D. Grossman, and A. van Oudenaarden, Nat. Genet. **31**, 69 (2002).

[5] M. B. Elowitz, A. J. Levine, E. D. Siggia, and P. S. Swain, Science **297**, 1183 (2002).

[6] P. S. Swain, M. B. Elowitz, and E. D. Siggia, Proc. Natl. Acad. Sci. U.S.A. **99**, 12 795 (2002).

[7] W. J. Blake, M. Kærn, C. R. Cantor, and J. J. Collins, Nature (London) **422**, 633 (2003).

[8] P. B. Detwiler, S. Ramanathan, A. Sengupta, and B. I. Shraiman, Biophys. J. **79**, 2801 (2000).

[9] N. Barkai and S. Leibler, Nature (London) **403**, 267 (2000).

[10] J. M. Vilar, H. Y. Kueh, N. Barkai, and S. Leibler, Proc. Natl. Acad. Sci. U.S.A. **99**, 5988 (2002).

[11] D. Gonze, J. Halloy, and P. Gaspard, J. Chem. Phys. **116**, 10 997 (2002).

[12] A. P. Arkin, J. Ross, and H. H. McAdams, Genetics **149**, 1633 (1998).

[13] W. Bialek, in *Advances in Neural Information Processing 13*, edited by T. K. Leen, T. G. Dietterich, and V. Tresp (MIT Press, Cambridge, 2001), p. 103.

[14] E. Aurell and K. Sneppen, Phys. Rev. Lett. **88**, 048101 (2002).

[15] F. J. Isaacs, J. Hasty, C. R. Cantor, and J. J. Collins, Proc. Natl. Acad. Sci. U.S.A. **100**, 7714 (2003).

[16] X. M. Zhu, L. Yin, L. Hood, and P. Ao, Funct. Integr. Genomics, e-publication Feb. 5 (2004).

[17] I. B. Dodd, K. E. Shearwin, A. J. Perkins, T. Burr, A. Hochschild, and J. B. Egan, Genes Dev. **18**, 344 (2004).

[18] T. S. Gardner, C. R. Cantor, and J. J. Collins, Nature (London) **403**, 339 (2000).

[19] P. Hänggi, P. Talkner, and M. Borkovec, Rev. Mod. Phys. **62**, 251 (1990).

[20] J. S. Langer, Phys. Rev. Lett. **21**, 973 (1968).

[21] N. G. van Kampen, *Stochastic Processes in Physics and Chemistry*, 2nd ed. (North Holland, Amsterdam, 1992).

[22] M. I. Dykman, E. Mori, J. Ross, and P. M. Hunt, J. Chem. Phys. **100**, 5735 (1994).

[23] W. H. Press, B. P. Flannery, S. A. Teukolsky, and W. T. Vetterling, *Numerical Recipes in C: The Art of Scientific Computing*, 2nd ed. (Cambridge University Press, New York, 1992).

[24] B. J. Matkowsky, Z. Schuss, C. Knessl, C. Tier, and M. Mangel, Phys. Rev. A **29**, 3359 (1984).

[25] R. S. Maier and D. L. Stein, Phys. Rev. E **48**, 931 (1993).

[26] J. K. Cullum and R. A. Willoughby, *Lanczos Algorithms for Large Symmetric Eigenvalue Computations* (Birkhäuser, Boston, 1985).

[27] M. A. Shea and G. K. Ackers, J. Mol. Biol. **18**, 211 (1985).

[28] J. W. Little, D. P. Shepley, and D. W. Wert, EMBO J. **18**, 4299 (1999).

[29] M. Santillan and M. C. Mackey, Biophys. J. **86**, 75 (2004).

[30] A. Shwartz and A. Weiss, *Large Deviations for Performance Analysis: QUEUES, Communication, and Computing* (Chapman and Hall, London, 1995).