How noise statistics impact models of enzyme cycles

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Theoretical tools for adequately treating stochastic effects are important for understanding their role in biological processes. Although master equations provide rigorous means for investigating effects associated with fluctuations of discrete molecular copy numbers, they can be very challenging to treat analytically and numerically. Approaches based on the Langevin approximation are often more tractable, but care must be used to ensure that it is justified in each situation. Here, we examine a model of an enzyme cycle for which noise qualitatively alters the behavior of the system: fluctuations in the population of an enzyme can result in amplification and multistability in the distribution of its product. We compare master equation and Langevin treatments of this system and show that results derived previously with a white noise Langevin equation [M. Samoilov *et al.*, Proc. Natl. Acad. Sci. U.S.A. **102**, 2310 (2005)] are inconsistent with the master equation. A colored noise Langevin equation captures some, but not all, of the essential physics of the system. The advantages and disadvantages of the Langevin approximation for modeling biological processes are discussed. © *2008 American Institute of Physics*. [DOI: 10.1063/1.2929841]

I. INTRODUCTION

Experiments that yield information about single cells make clear that intrinsic noise in reactions involving low copy numbers of molecules can have important biological consequences.[1–6] Among the phenomena thought to be influenced by stochastic effects are reversible transitions made by unicellular organisms between states that are competent and noncompetent for uptake of DNA (Ref. 6) and processing of nutrients[4] and the specification of and commitment to different cell fates during development in multicellular organisms.[7,8] Quantitative analyses of fluctuations in molecular populations are important for understanding the fundamental bases of such phenomena. While such analyses often assume the fluctuations of interest to be symmetrically distributed about a mean,[1,2,9–12] more complicated situations exist in which distributions of copy numbers become strongly skewed or multimodal.[13–15] Care is needed to avoid artifacts in theoretical descriptions of such situations.

Master equations are generally regarded as the most well-founded means of specifying the evolution of the joint probability of molecular copy numbers in biological systems because discrete states can be incorporated in a straightforward fashion. In addition, the master equation directly specifies the evolution of the probability distribution and no additional noise variables need to be introduced. However, few master equations can be solved exactly;[16] simulations can be slow to converge (see Ref. 17 and references therein) and often provide only limited physical insight. The relative ease of analytical and numerical analysis of Langevin equations makes them popular alternatives. Protein concentrations are treated as continuous variables, and stochastic effects enter through terms that depend on additional random variables defined by their statistical properties. Commonly, it is as-

sumed that the fluctuations are uncorrelated in time ("white" noise),[2,14] although models with finite correlation times ("colored" noise) have also been investigated.[18] Langevin models can also be categorized based on whether the noise magnitude is independent or dependent on the values of the dynamical variables ("additive" and "multiplicative" noise, respectively). Although methods exist for systematically deriving Langevin equations from master equations,[19–21] in most cases, phenomenological forms are employed without formal justification.[2,14] As such, it is often difficult to determine whether the approximations involved in Langevin approaches are valid.

Recently, it was observed in simulations of an enzyme cycle comprised of competing chemical modification reactions that small fluctuations in the copy number of the enzyme that catalyzed the forward reaction resulted in a positive shift in the mode of the distribution of the number of product molecules; large fluctuations caused this distribution to become bimodal.[14] Based on a Langevin equation with multiplicative white noise, it was argued that fluctuations in the forward enzyme population would always amplify the population of products. Furthermore, the fact that the white noise Langevin equation exhibited a bistability was taken as evidence that the unimodal-to-bimodal transition observed in the master equation simulations did not derive from the interplay of fluctuations in enzyme copy numbers and the well-known ultrasensitivity of such an enzyme cycle.[22,23] However, it is not clear that the phenomenological Langevin equation employed by Samoilov *et al.*[14] adequately represents the discrete system of interest. Determining whether this is the case is important given that the enzyme cycle is a ubiquitous motif in biological networks and serves as a standard model for understanding nonlinear response in molecular systems.
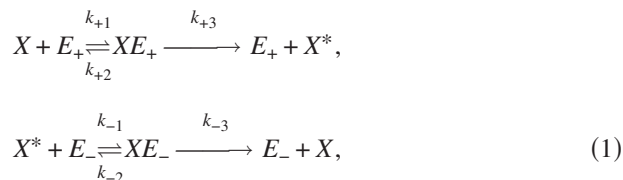
Here, we systematically examine the behavior of the en-

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zyme cycle as described by master and Langevin equations. In both cases, we vary the magnitude and correlation time of the variations in the forward enzyme population to derive "phase" diagrams. That for the master equation reveals that multistability does, in fact, result from the interplay of fluctuations in molecular copy numbers and the ultrasensitivity of the cycle; in the monostable region, fluctuations can either amplify or reduce the forward product depending on the specific choice of parameters. The Langevin phase diagram is qualitatively different and exhibits two independent bifurcations that can combine to give tristability in the product distribution. The differences between the behaviors of the master equation and white noise Langevin equation studied previously[14] derive from a breakdown of steady-state approximations employed in Michaelis–Menten kinetics in the limit that the correlation time of the fluctuations in the enzyme population goes to zero. Expansions of the master equation and general results for white-noise Langevin equations are discussed in Appendices. In addition to providing insight into the behavior of this paradigmatic system, our analysis illustrates the advantages and disadvantages of the Langevin approach.

II. ENZYME CYCLE

The enzyme cycle is comprised of the following reactions:

$$X + E_+ \underset{k_{+2}}{\overset{k_{+1}}{\rightleftharpoons}} XE_+ \xrightarrow{k_{+3}} E_+ + X^*,$$

$$X^* + E_- \underset{k_{-2}}{\overset{k_{-1}}{\rightleftharpoons}} XE_- \xrightarrow{k_{-3}} E_- + X, \tag{1}$$

where E_+ and E_- are enzymes that catalyze conversion between substrates X and X^* (below, we use these symbols to denote the concentrations of these species as well). When the concentrations of the enzymes are limiting, this system exhibits ultrasensitivity in the deterministic representation.[22] Namely, a small change in the ratio of the maximum forward to backward reaction velocities can cause the steady-state population to shift from $X^* \approx 0$ to $X^* \approx X_0 \equiv X + X^*$. Stochastic fluctuations in the cycle reactions reduce the sensitivity but do not shift the point at which the steady-state population of X^* becomes larger than that of X.[23] On the other hand, fluctuations in the total number of E_+ counterintuitively move the location of the peak of the probability distribution for X^* and can even create additional peaks in its distribution.[14]

III. LANGEVIN APPROACH

As mentioned in the Introduction, Langevin equations can be systematically derived from master equations or phenomenologically from physical considerations. Here, we consider a previously introduced phenomenological Langevin equation, which was used to make predictions about amplification and bistability for the enzyme cycle in the white-noise case.[14] Alternative Langevin equations derived systematically are discussed in Appendix A.

To derive their Langevin equation, Samoilov et al.[14] began with the deterministic rate equations for the system and relied on the assumption that the binding and unbinding reactions of enzyme and substrate are at local equilibrium. By exploiting conservation of mass, these rate equations can be combined into a single one,

$$\frac{dX^*}{dt} = \frac{k_+ E_+(t)(X_0 - X^*)}{K_+ + X_0 - X^*} - \frac{k_- E_- X^*}{K_- + X^*}, \tag{2}$$

where $k_\pm = k_{\pm 3}$ and $K_\pm = (k_{\pm 2} + k_{\pm 3})/k_{\pm 1}$. They then assumed that the dominant source of noise was in the concentration of the forward enzyme,

$$E_+(t) = \bar{E}_+ + \eta(t), \tag{3}$$

where $\eta(t)$ is a noise term discussed below in detail. Substitution of Eq. (3) into Eq. (2) gives a Langevin equation of the general form

$$\frac{dX^*}{dt} = A(X^*) + C(X^*)\eta(t), \tag{4}$$

where $A(X^*)$ is the right-hand-side of Eq. (2) with $E_+(t)$ replaced with its average value and $C(X^*)$ is the magnitude of the multiplicative noise,

$$C(X^*) = \frac{k_+(X_0 - X^*)}{K_+ + X_0 - X^*}. \tag{5}$$

This Langevin equation neglects other sources of noise such as those in the cycle reactions themselves. This is expected to be a valid assumption when the copy numbers of substrate molecules are sufficiently large.

As mentioned above, Samoilov et al.[14] considered the white-noise case in which, by definition, the statistics of $\eta(t)$ are

$$\langle \eta(t) \rangle = 0,$$

and

$$\langle \eta(t)\eta(t') \rangle = \Gamma \delta(t - t'), \tag{6}$$

where Γ is a parameter that controls the magnitude of the noise. In this case, the Langevin equation can be converted to a Fokker–Planck equation for the probability distribution, which can be solved exactly at steady state (Appendix B). This analysis shows that noise shifts the peak in the substrate-product distribution toward higher values of X^* relative to the deterministic fixed point.[14] We derive a general relation for multiplicative white noise Langevin equations in Appendix B that reveals that the physical basis for this amplification is that the shift in the distribution of X^* is in the direction of decreasing noise. In other words, since the noise magnitude $C(X^*)$ decreases as X^* increases [Eq. (5)], the system diffuses more slowly in X^* at higher values of that variable and thus spends more time at those points. To the best of our knowledge, this physical mechanism was not previously appreciated. As Γ becomes large, a new stable state is created such that the system is bistable in X^*.[14] Further increases in the magnitude of the noise destabilize the original deterministic stable state and the system is again monostable.
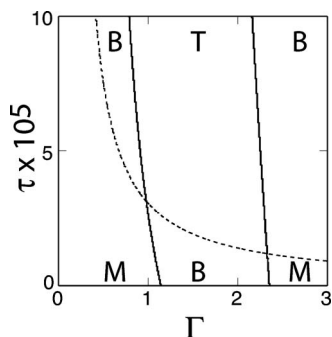
FIG. 1. Noise-induced phase diagram for the Langevin description of the enzyme cycle. Regions of monostability (M), bistability (B), and tristability (T) are denoted. At each combination of Γ and τ, the number of phases was determined from the number of maxima in the colored-noise probability distribution, the exponential form in Eq. (3.12) of Ref. 18. The dashed line marks the transition to multistability that results from the interplay of finite noise correlation times and ultrasensitivity; the solid lines mark the transitions present in the white-noise case. The parameters used were $k_+=k_-=10^4$, $K_+=K_-=50$, $X_0=2000$, and $\bar{E}_+=E_-=30$. All concentrations are in molecules per unit volume and time is in arbitrary dimensionless units.

The noise in the enzyme cycle physically represents the fluctuations in the concentration of the forward enzyme, which would have a finite lifetime in an actual system. To account for this fact, one can introduce a separate white noise Langevin equation for η,

$$\frac{d\eta(t)}{dt} = -\frac{\eta(t)}{\tau} + \xi(t), \qquad (7)$$

where ξ is a white noise with a mean of zero and a variance of Γ, and τ is a parameter that controls the correlation time of the noise. Equivalently, one can directly change the statistics of η such that it represents a colored noise of the Ornstein–Uhlenbeck form:

$$\langle \eta(t) \rangle = 0,$$

and

$$\langle \eta(t)\eta(t') \rangle = \frac{\Gamma}{2\tau}e^{-(t-t')/\tau}. \qquad (8)$$

Equation (6) is recovered in the limit $\tau \rightarrow 0$.

To understand the interplay of the magnitude and the correlation time of the noise on the enzyme cycle, we exploited an analytic solution (to first order in τ) for the steady-state probability distribution of a colored noise multiplicative Langevin equation[18] to construct a phase diagram (Fig. 1). This diagram shows that there are two ways bistability can arise within the Langevin framework. One is that mentioned previously in the white-noise case: the fluctuations are of intermediate magnitude (discussed further in Appendix B). The other is that the correlation time becomes long. In this case, the bistability results from the ultrasensitive response of the substrate distribution to fluctuations in the enzyme concentration; the enzyme cycle forms a switch which is flipped between two well separated substrate distributions. Because the two means of generating bistability are distinct, they can combine to yield a region of tristability (Fig. 1).

To determine if the tristability persisted at high τ, where the analytic solution[18] is no longer valid, we numerically
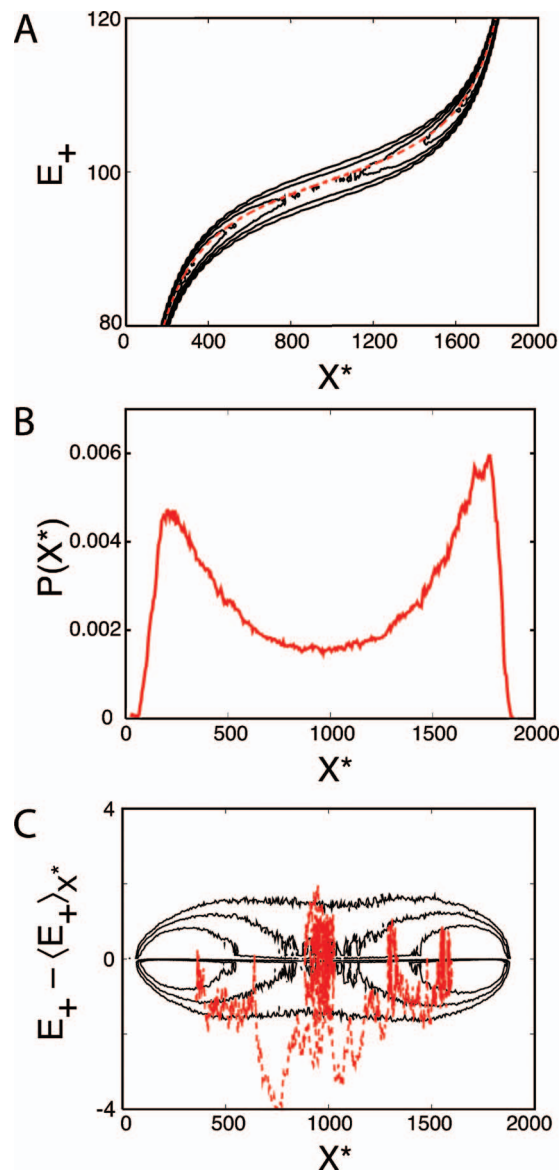

FIG. 2. (Color) Numerical integration of Langevin equations at $\tau=1$ and $\Gamma=300$. (A) Probability of observing X^* and E_+ at steady-state obtained by integration of Eqs. (2) and (7). Contours are spaced logarithmically by factors of 2. (B) Projected X^* distribution. (C) Values of E_+ are mapped to their deviation from the mean of E_+ for each X^* value [red dashed line in (A)]. A representative trajectory transitioning from high to low substrate concentrations is shown (red dashed line). The same parameters as in Fig. 1 were used except $E_-=99$ and $\bar{E}_+=100$ to avoid the enzyme concentration crossing the boundary at zero enzyme concentration.

integrated Eqs. (2) and (7) with an algorithm introduced by Sancho *et al.*[18] (Fig. 2). We scanned parameter combinations $50 \leq \Gamma \leq 1000$ in increments of 50 for $\tau=1$. Note that we employed much larger values of Γ than in the phase diagram because the magnitude of the noise is $\Gamma/2\tau$ so that increasing the value of τ necessitated increasing the value of Γ for the noise to have a discernible effect on the process. Although the system transitioned from monostability to bistability in going from $\Gamma=50$ to $\Gamma=100$, no tristability was observed. Since the distribution is bistable at large τ [Fig. 2(b)], it is of interest to examine the transition paths between the steady states. We found that the system took different paths in the forward and reverse directions, which is a common feature of
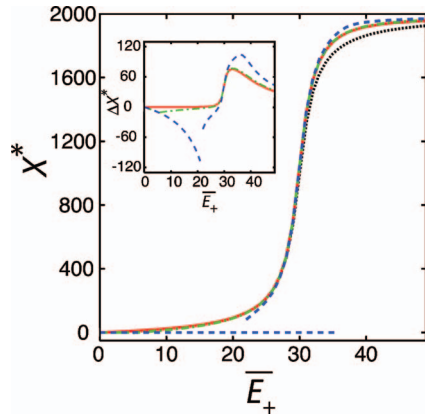
FIG. 3. (Color) Stochastic amplification in the Langevin equation. Shown are the deterministic steady-state solution for the concentration of substrate (black dotted line) and concentration of substrate at the peak of the probability distribution for white noise (red solid line) and colored noise with correlation times of $\tau=10^{-5}$ (green dotted-dashed line) and $\tau=10^{-4}$ (blue dashed line). Note that at $\tau=10^{-4}$ the distribution has two peaks for some values of E_+. (Inset) Difference between the deterministic result and the concentration of substrate at the peak(s) of the probability distributions. For clarity, where there are two branches for the dashed curve, only the upper branch in the main figure is shown. The same parameters as in Fig. 1 were used with $\Gamma=0.5$.

systems which do not obey detailed balance[24] [Fig. 2(c)]. In summary, at small values of τ, the Langevin equation has two qualitatively different bistability transitions. At large τ, only the bistability which results from the fact that fluctuations in the enzyme concentration are sufficiently slow that the substrate distribution can reach quasisteady states at high and low values of E_+ persists. That which is present in the white noise case is not seen in this regime.

Before turning to the master equation, we return to consider the amplification of X^* in the monostable portion of Fig. 1 with small Γ. As noted above, in the white-noise case, the peak in the distribution of substrate was always at a greater concentration than the deterministic result (Fig. 3, compared dotted and solid lines; inset, red solid line), which is in agreement with the analytical results in Ref. 14 and in Appendix B. As the correlation time increases, the transition from the peak of the distribution at low substrate concentration to high concentration becomes sharper, and the noise amplifies the signal only for high values of $\bar{E}_+$ (Fig. 3, green dotted-dashed lines). At low values of $\bar{E}_+$, the noise silences the signal. In other words, the colored and white noises have qualitatively different amplification properties. Consistent with the results above, the distribution becomes bistable for sufficiently large τ (Fig. 3, blue dashed lines).

IV. MASTER EQUATION

The analysis above reveals that there are two means of generating bistability in the Langevin framework: that accessible in the white noise case, which was observed previously for the enzyme cycle,[14] and that obtained due to a rapid response of the substrate-product distribution to slow fluctuations in the enzyme population. To determine which, if either, corresponds to the bistability observed in chemical

master equation simulations,[14] we sought to construct a phase diagram for the enzyme cycle in the master equation representation.

To vary the rate and size of the fluctuations of the enzyme copy number in a controlled fashion, we exploited a set of auxiliary reactions that is conceptually simpler than that used by Samoilov *et al.*[14] The more complex auxiliary reaction scheme used in that study is capable of generating bistability even when coupled to a simple isomerization reaction due to a phenomenon in which the transient depletion of a species leads to an effective reduction in network topology.[25] Since we were interested in the properties of the enzyme cycle, we created the fluctuations in enzyme copy number using a simple genetic process which can be treated analytically in isolation.[9,26] Namely, we modeled production of the forward enzyme through the reactions:

$$\varnothing \xrightarrow{\;g_M\;} M \xrightarrow{\;k_M\;} \varnothing$$

$$M \xrightarrow{\;g_E\;} M + E_+$$

$$E_+ \xrightarrow{\;k_E\;} \varnothing. \tag{9}$$
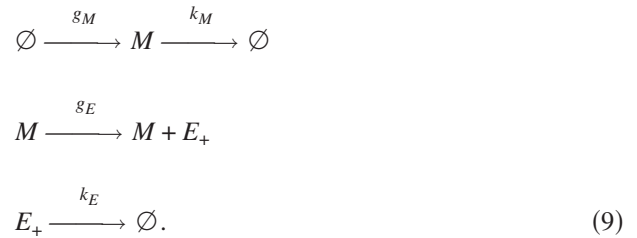
Here, $\varnothing$ denotes loss of one copy of a molecule and M denotes the mRNA used to produce the protein E_+ such that the first reaction physically represents transcription and mRNA degradation, the second reaction represents translation, and the third reaction represents protein degradation. For this set of reactions, the relative variance in the concentration of E_+ was shown to be[9,26,27]

$$\langle \delta E_+^2 \rangle / \langle E_+ \rangle = 1 + g_E/(k_E + k_M) = 1 + b/(1 + k_E/k_M), \tag{10}$$

where $b = g_E/k_M$ is the average number of proteins produced per mRNA molecule (the "burst size"). Equation (10) indicates that the burst size can be changed by scaling g_E. It can further be shown that the relaxation time of E_+ depends only on the degradation rates k_E and k_M (Ref. 26) such that the correlation time of the noise of interest in the cycle can be varied independently by scaling the rates of all the auxiliary reactions uniformly (in which case, the noise magnitude [Eq. (10)] remains constant). Unless otherwise stated, we choose the rate constants to be

$$g_M = s/b, \quad k_M = s/10, \quad g_E = bs/10, \quad k_E = s/2. \tag{11}$$

The scale factor s, which is defined by the above equations, is inversely proportional to the correlation time so that the correlation time and the burst size can be tuned independently.

The resulting phase diagram for the enzyme cycle response as a function of the magnitude (burst size in E_+) and correlation time (overall scale of the reaction rates) of the noise is shown in Fig. 4. While the coexistence curve that results from slow fluctuations of the enzyme population is present (dashed in Fig. 1), the white noise limit (bottom of Fig. 4) was found to be monostable over the entire range of burst sizes studied (eight orders of magnitude; note that the axes of the phase diagram are logarithmic), which suggests
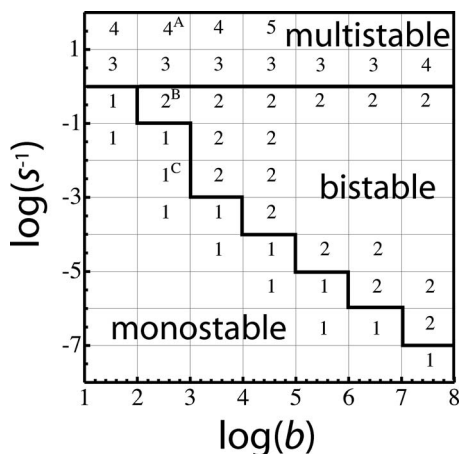
FIG. 4. Master equation phase diagram. A number in a square indicates the number of peaks in the probability distribution determined by simulation with the parameters taken at the lower left corner of the square. b is the burst size for protein creation, which gives a measure of the noise magnitude (see text) and s is a scale factor for all reaction rates in the enzyme creation module so that s^{-1} is proportional to the correlation time. Superscript letters refer to the panels in Fig. 5 where probability distributions for the substrate are shown for the parameters at these points in the phase diagram. The cycle parameters were $k_1 = k_{-1} = 200$, $k_2 = k_{-2} = 100$, and $k_3 = k_{-3} = 5000$. The parameters for the driving reactions are given in terms of s and b by Eq. (11). Note that in the multistable regime the exact number of distinct peaks is often difficult to determine by simulation. The number in each box reflects the number of peaks which were unambiguously distinct upon visually examining plots like those in Fig. 5.

that the Langevin model considered by Samoilov *et al.*[14] is qualitatively different from a discrete representation of the enzyme cycle.

    While both the master equation and the colored-noise Langevin equation display a bistability, which results from the response of the ultrasensitive switch to fluctuations in the enzyme, the master equation also displays additional multistabilities which result from discrete copy number effects. If the fluctuations in the enzyme concentration are sufficiently slow, each integral copy number of E_+ can give rise to a separate peak in the steady-state distribution of X^*, as can be seen by sorting the data according to the total number of enzyme molecules [Fig. 5(a)]. The physical reason for this behavior is that, as the fluctuations in the copy number of the enzyme become slow, the substrate-product distribution has sufficient time to reach a new quasisteady state at each enzyme copy number. Ultrasensitivity can enhance such effects, but it is not a prerequisite for this type of stochastic bistability. Indeed, such discrete copy number bistabilities can be observed in simple models without any ultrasensitivity.[25] Thus, when fluctuations are slow, there are discrete copy number peaks which result both from ultrasensitivity and those which are independent of it. When the fluctuations occur on intermediate time scales, only the bistability which results from ultrasensitivity remains [Fig. 5(b)]. Finally, when the noise occurs on a much faster time scale than the cycle reactions, the fluctuations are time averaged by the cycle and the system is monostable [Fig. 5(c)], as in the deterministic description. The idea that changes in enzyme copy number can enable an ultrasensitive cycle to fluctuate between well-separated steady states was first suggested by Berg *et al.*[23] There are parallels to a protein that
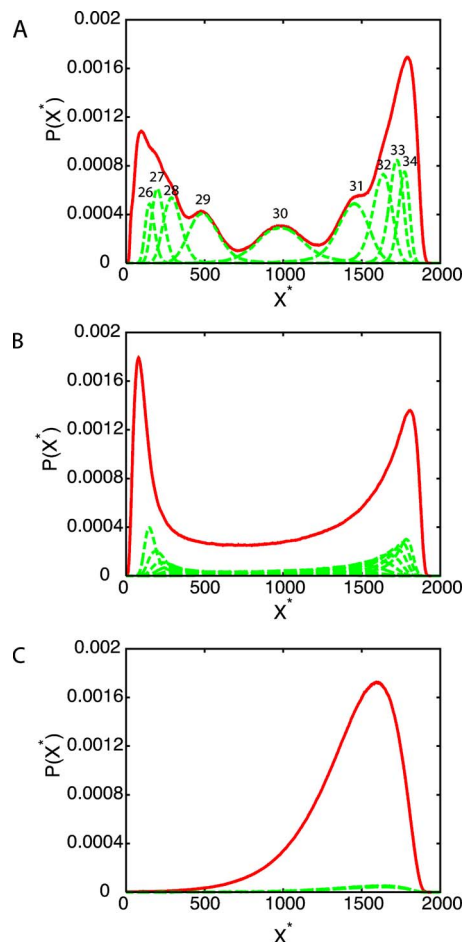

FIG. 5. (Color online) Master equation simulations. (A) Full probability distributions (solid curve) and sorted distributions (dashed curves) corresponding to enzyme copy numbers as indicated. Parameters are the same as in Fig. 4 with $s = 1$ and $b = 10$. (B) Same parameters as in (A) except $s = 100$. (C) Same parameters as in (A) except $s = 10^4$. The green curves in (B) and (C) correspond to the same range of enzyme copy numbers as in (A). In (C), the distributions of substrate concentrations for different enzyme copy numbers are nearly identical.

represses its own expression, a discrete model of which can be solved exactly,[16] and to a stochastic model of a genetic toggle switch without cooperative binding.[28]

    The discussion above shows that certain features of the behavior of the enzyme cycle can be obtained readily only from a discrete model. Indeed, this is particularly true of the dynamics when the burst size is large. In that case, the path the system takes in transitioning from low to high substrate concentration is drastically different from the path it traverses in the opposite direction (Fig. 6). This can be understood as follows. When the burst size is large and the correlation time is long, the production of a mRNA for E_+ leads to a large burst of E_+, which quickly exceeds the threshold to flip the ultrasensitive switch. Once the switch is flipped, the concentration of X^* quickly rises in response (Fig. 6, + symbols). The concentration of E_+ then decays slowly and the concentration of X^* remains nearly constant as long as E_+ is above the switch threshold. Once the threshold is crossed due to steady degradation, the levels of substrate begin to drop and the cycle begins again (Fig. 6, × symbols). Since the fluctuations which increase E_+ above the
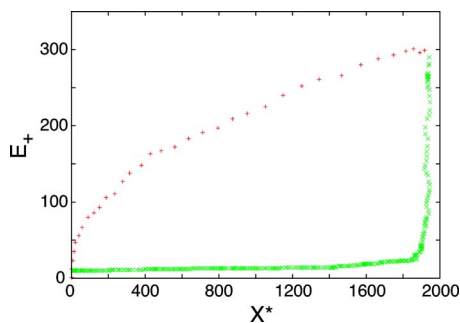
FIG. 6. (Color online) Transition paths in the master equation. One cycle between the steady states is shown with the trajectory from low to high $X*$ denoted by $+$ symbols and that from high to low denoted by $\times$ symbols. The symbols are evenly spaced in time. The same parameters as in Fig. 4 were employed with $s=b=1000$.

threshold are fast while those which decrease E_+ below it are slow compared to the reactions which form the enzyme cycle, the system takes different paths through the phase space in response to increases and decreases in the enzyme concentration. Although the colored-noise Langevin equation does exhibit a similar phenomenon at large τ, as evidenced by the fact that it tends to transition from low to high substrate concentrations above and below the average enzyme concentration [see representative trajectory in Fig. 2(c)], it is not nearly as dramatic as the master equation due to the fact that it is hard to make a continuous variable model with bursts without introducing additional nonlinearities by hand.

As a final comparison of the master and Langevin equations, we return to the shift in the peak of the probability distribution in the monostable region. We saw that the white noise always amplifies,[14] while at longer correlation times, the colored noise amplifies for high values of $\bar{E}_+$ and silences for low values (Fig. 3). For the master equation simulations, we compared the steady-state probability distribution obtained with the system size above with that in a system with the volume scaled by a factor of 10^3. In the latter, stochastic effects play little role. In agreement with the colored-noise Langevin equation, the driving reaction for E_+ results in a shift toward $X*$ at sufficiently high values of $\bar{E}_+$ [Fig. 7(a)] and a shift toward X at low $\bar{E}_+$ [Fig. 7(b)]. Putting all the results together, the colored-noise Langevin equation captures master equation features that result from finite-time fluctuations of the enzyme population but not those that depend on discrete copy numbers.

V. CONCLUSIONS

We have constructed phase diagrams for noise induced effects in an enzyme cycle represented by the Langevin and master equations and investigated the behavior of these representations in the different phases. A genetic module with well-defined properties[9,26] was instrumental for separately varying the magnitude and correlation time of the noise in the master equation case; a similar approach should be useful in quantifying the response of other biochemical systems to external fluctuations. We found that, while a colored-noise Langevin equation did not capture effects associated with discrete copy number fluctuations, it produced qualitatively
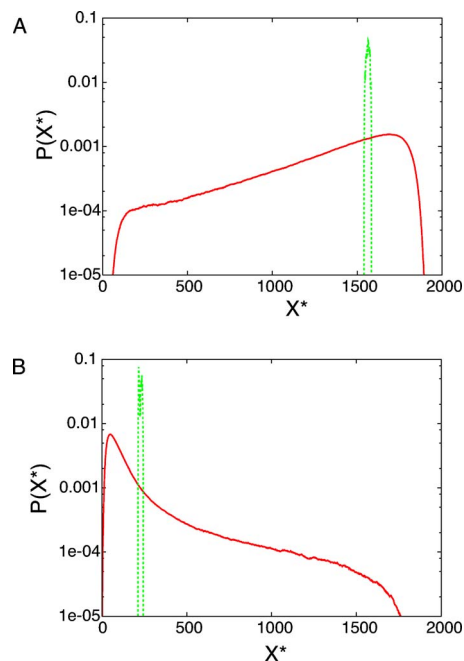

FIG. 7. (Color online) Amplification in the master equation. Probability distributions for the product of the cycle ($X*$) (solid curves) and the same system scaled by a factor of 10^3 (dashed curves). The same cycle parameters as in Fig. 4 were employed with (A) $s=100$ and $b=1$. (B) Same parameters as (A) except $g_M=20$.

correct results regarding multistability and stochastic amplification. In contrast, the white-noise Langevin equation introduced previously[14] exhibited significant artifacts, and its predictions were in qualitative disagreement with the more accurate master equation treatment.

What is the source of these artifacts? Reducing the system to a single Langevin equation requires assuming that the enzyme-substrate binding is at equilibrium which enables the equation to be brought to the Michaelis–Menten form. For this assumption to be valid, the binding and unbinding of enzyme to substrate should be the fastest process under consideration. However, if the concentration of enzyme is assumed to fluctuate with zero correlation time, these fluctuations occur on a faster time scale for any finite rates for binding and unbinding. Due to these fluctuations, no equilibrium in the binding reactions can be achieved. Thus, the assumptions which lead to a single white-noise Langevin equation are mutually inconsistent. This is in agreement with standard deterministic treatments of the Michaelis–Menten enzyme reaction[29] which show that the Michaelis–Menten form is only valid if the concentration of substrate can be considered to be constant over the length of time necessary for the concentration of the enzyme-substrate complex to reach equilibrium. Although such treatments do not explicitly consider fluctuations in the concentration of enzyme, the same considerations should apply to the concentration of enzyme as to that of the substrate. When the concentration of enzyme is assumed to fluctuate infinitely fast, this condition is violated. The conclusion that making both the white-noise and Michaelis–Menten assumptions together is the source of the discrepancy is supported by the agreement between the colored noise results and the master equation when τ is sufficiently large.

Fluctuations occurring on a fast time scale are time averaged by slower downstream processes and, therefore, it is generally valid to ignore such fluctuations and replace the fluctuating quantity by its average value. Indeed, the simulations in Fig. 4 show that, in the master equation, fast fluctuations have no effect on the number of stable states; the system is always monostable in the white-noise limit. Why then do infinitely fast fluctuations in the concentration of enzyme impact the validity of the Michaelis–Menten assumption in the present study? The artifacts in the case of the Langevin equation considered above arise because the noise is added in an *ad hoc* fashion *after* the Michaelis–Menten assumption has already been made. In the case that fluctuations in the enzyme population are infinitely fast, they can be meaningfully neglected by treating the system deterministically, not with a white-noise Langevin equation. The quasi-steady-state approximation can then hold for an average enzyme concentration which is small in comparison to the initial substrate concentration.

One of the central open questions in the stochastic treatment of biological systems is under what circumstances can the accurate but cumbersome master equation treatment be replaced with simpler treatments which are more amenable to analytical analysis and less costly to simulate computationally. Our results show that care must be taken to avoid artifacts in employing a Langevin treatment. For numerical investigations, there are approximate methods for accelerating master equation simulations (reviewed in Ref. 30) or numerically reducing the system to a Fokker–Planck equation.[31] One strategy to proceed analytically has been suggested by Gillespie,[20] and it enables one to write a Langevin equation which is valid under well defined circumstances directly from the master equation. In particular, there must exist a time interval small enough that the propensities for the reactions to occur can be considered constant, but large enough that each reaction fires many times. This method is not generally applicable to the enzyme cycle because, in the ultrasensitive regime, executing even a few reactions is enough to drastically change the propensities. A second method is van Kampen's systematic expansion of the master equation in a small parameter, usually taken to be the inverse volume of the system (Ω^{-1}).[19] If the system size is sufficiently large that only the lowest order terms need be examined, the master equation can be replaced with a Fokker–Planck equation which can be used to construct an equivalent Langevin equation. The Kramers–Moyal expansion is a related expansion approach.[19,32,33] Because it does not involve a small parameter, it can be difficult to determine whether truncation of the expansion at a particular order is valid.

In Appendix A, we examine the results of applying these expansion methods to the enzyme cycle. In both of these methods, the noise in the enzyme concentration is never transmitted to the substrate concentration as white noise and, therefore, the artifacts discussed above are avoided. However, because the Ω expansion separates the solution into deterministic and fluctuating portions, it is not suitable for cases where the stochastic behavior can qualitatively alter the system behavior, and the Langevin equation derived from this expansion fails to reproduce the bistability seen in the master equation treatment. Furthermore, the lowest order of the Ω expansion gives rise to linear Langevin equations which do not show any amplification. However, we expect that including higher order terms would enable one to calculate the magnitude of the shift using this method. In contrast, the lowest order of the Kramers–Moyal expansion gives very similar results to the colored-noise Langevin equation studied above regarding both amplification and bistability.

More generally, under what circumstances can we expect the stochastic description of a system to differ qualitatively from the deterministic one? In this paper, we have shown that, in the master equation description, the enzyme cycle displays a noise-induced bifurcation which results from slow fluctuations in enzyme concentration. However, this bifurcation is not truly "deviant"[34] from the deterministic behavior because it arises from the ultrasensitive response of the substrate to changes in enzyme concentration which is present in the deterministic description as well. In fact, this bistability was predicted in an earlier study.[23] In contrast, the Langevin treatment shows an additional stochastic bifurcation which would not have been predicted from examining the deterministic portion of the equation alone. In Appendix B, we discuss the necessary conditions for a Langevin equation to give rise to such bifurcations. Novel behavior resulting from stochastic fluctuations is not limited to phenomenological Langevin descriptions but has also been demonstrated in master equation treatments of simple models of an autorepressing gene and a signaling cascade which can be solved exactly.[15,16] In the signaling case, the authors identify irreversibility, branching, and feedback as necessary conditions for this stochastic transition. It will be interesting to see whether there are other general mechanisms by which fluctuations can give rise to behavior deviant from the deterministic description.
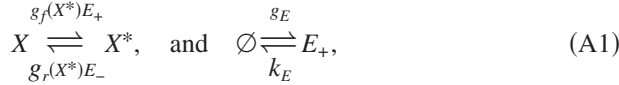
ACKNOWLEDGMENTS

APPENDIX A: SYSTEMATIC DERIVATION OF LANGEVIN EQUATIONS FROM THE MASTER EQUATION

To avoid artifacts which result from phenomenological approaches, one can derive Langevin equations systematically from the master equation. Here we examine the results of two common expansion methods: the Ω (Ref. 19) and the Kramers–Moyal.[19,32,33] These expansion methods yield Fokker–Planck equations from which equivalent Langevin equations are easily obtained.

For simplicity, we make the Michaelis–Menten approximation and consider a master equation for only two species, the substrate and the enzyme which converts the substrate into the modified form. Although the Michaelis–Menten approximation introduced artifacts to the Langevin description above, these are only present when an additional white-noise

assumption is made. We expect the Michaelis–Menten approximation to be valid whenever the rates for binding and unbinding of enzyme to substrate are significantly faster than all other processes. Here, we assume that this condition is satisfied and make no assumptions regarding the correlation time of the noise. Instead, we allow the fluctuations in the copy number of the forward enzyme to arise naturally from the master equation. We assume that the amount of enzyme for the reverse reaction is constant. We also restrict ourselves to a simpler system for generating the fluctuations, that of a simple birth-death process. Then, the reaction system consists of four reactions

$$X \underset{g_r(X^*)E_-}{\overset{g_f(X^*)E_+}{\rightleftharpoons}} X^*, \quad \text{and} \quad \varnothing \underset{k_E}{\overset{g_E}{\rightleftharpoons}} E_+, \tag{A1}$$

where $g_f(X^*)$ and $g_r(X^*)$ are the nonlinear reaction rates which result from making the following Michaelis–Menten approximations:

$$g_f(X^*) = \frac{k_+(X_0 - X^*)}{K_+ + (X_0 - X^*)}, \tag{A2a}$$

$$g_r(X^*) = \frac{k_- X^*}{K_- + X^*}. \tag{A2b}$$

Then, the master equation describing the time evolution of the system is given by

$$\frac{dP(X^*, E_+)}{dt} = (\hat{Q}_{X*}^{-1} - 1)g_f(X^*)E_+ P(X^*, E_+)$$

$$+ (\hat{Q}_{X*} - 1)g_r(X^*)E_- P(X^*, E_+)$$

$$+ g_E(\hat{Q}_{E_+}^{-1} - 1)P(X^*, E_+)$$

$$+ k_E(\hat{Q}_{E_+} - 1)E_+ P(X^*, E_+), \tag{A3}$$

where $\hat{Q}_A$ is a raising operator introduced for notational convenience. It is defined by $\hat{Q}_A f(A) = f(A+1)$ where $f(A)$ is an arbitrary function of A.

In the Ω expansion, each variable is written as the sum of deterministic and fluctuating terms. It is assumed the fluctuating term is smaller than the deterministic term by a factor of $\Omega^{1/2}$, where Ω is the system volume. Thus, we write

$$X^* = \Omega \phi_{X*} + \Omega^{1/2} \xi_{X*} \tag{A4}$$

where ϕ_{X*} and ξ_{X*} are the deterministic and fluctuating terms in X^*, respectively. A systematic expansion is then performed in the variable Ω^{-1}. To the lowest order, one need only retain terms with $\Omega^{1/2}$ or Ω^0. Setting the terms of order $\Omega^{1/2}$ to zero yields an equation containing only the deterministic portion of the variable concentrations. The terms of order Ω^0 give a linear Fokker–Planck equation which can then be used to write equivalent Langevin equations for the fluctuating terms. The results of performing this analysis are that ϕ_{X*} and ϕ_{E_+} satisfy the deterministic equations

$$\frac{d\phi_{X*}}{dt} = g_f(\phi_{X*})\phi_{E_+} - g_r(\phi_{X*})E_-, \tag{A5a}$$

$$\frac{d\phi_{E_+}}{dt} = g_E - k_E \phi_{E_+}, \tag{A5b}$$

and ξ_{X*} and ξ_{E_+} satisfy the Langevin equations

$$\frac{d\xi_{X*}}{dt} = g_f(\phi_{X*})\xi_{E_+} + \eta_{X*}(t), \tag{A6a}$$

$$\frac{d\xi_{E_+}}{dt} = -k_E \xi_{E_+} + \eta_{E_+}(t), \tag{A6b}$$

where η_{X*} and η_{E_+} are noise terms with zero average and

$$\langle \eta_{X*}(t)\eta_{X*}(t')\rangle = (g_f(\phi_{X*})\phi_{E_+} + g_r(\phi_{X*})E_-)\delta(t - t'), \tag{A7a}$$

$$\langle \eta_{E_+}(t)\eta_{E_+}(t')\rangle = (g_E + k_E \phi_{E_+})\delta(t - t'). \tag{A7b}$$

Combining the deterministic and stochastic portions of these equations yields Langevin equations for the variables X^* and E_+ similar to the Langevin equations studied above. Importantly, in these equations, the magnitude of the fluctuations depends only on the value of the deterministic portion, not on the current value of the fluctuating quantity. At steady state, the magnitude of the fluctuations is constant. In addition, the coefficient of the term, which transmits the fluctuations in the enzyme population to the substrate distribution [the first term on the right hand side of Eq. (A6a)], is constant in magnitude. These facts mean that no bistabilities will be present in these Langevin equations. Although the artificial white-noise bistability is avoided, the separation between the deterministic portion of the variable, which displays ultrasensitivity and the fluctuating portion, prevents one from examining the interaction between the fluctuations and ultrasensitivity. In addition, as a result of the constant noise magnitude, the peak of the probability distribution is always at the deterministic solution. However, while bistability cannot emerge at any order in the Ω expansion, amplification would at higher orders. We thus see that the Ω expansion is useful for examining the quantitative properties of fluctuations around an average value,[9,19,35] but it cannot yield information about cases in which the fluctuations alter the qualitative behavior of the system.

Applying the Kramers–Moyal expansion is procedurally very similar to applying the Ω expansion. The main difference is that no small parameter is introduced so the number of terms to include is arbitrary. However, because there is no separation made between the stochastic and deterministic portions of the equation, it is possible for this description to capture effects not accessible to the Ω expansion. To obtain a Fokker–Planck equation, we only retain terms with first and second derivatives of the probability distribution. Transforming this Fokker–Planck equation to a Langevin equation yields

$$\frac{dX^*}{dt} = g_f(X^*)E_+ - g_r(X^*)E_- + \eta_{X*}(t), \tag{A8a}$$

$$\frac{dE_+}{dt} = g_E - k_E E_+ + \eta_{E_+}, \tag{A8b}$$

where the noise terms are given by

$$\langle \eta_{X*}(t) \eta_{X*}(t') \rangle = (g_f(X^*)E_+ + g_r(X^*)E_-)\delta(t - t'), \tag{A9a}$$

$$\langle \eta_{E_+}(t) \eta_{E_+}(t') \rangle = (g_E + k_E E_+)\delta(t - t'). \tag{A9b}$$

Note that these equations are similar to what is obtained from the Ω expansion except that the entire fluctuating variable is present in the noise magnitudes rather than solely its deterministic portion. If we assume the number of substrate molecules is sufficiently large that η_{X*} can be neglected, this equation is nearly identical to the colored-noise equation studied above. The only difference is that the magnitude of the fluctuations in the enzyme concentration depends on the current value of the enzyme concentration. Nonetheless, since this dependence is relatively simple, we do not expect that it will alter the qualitative behavior of the system, and thus these equations should capture many of the qualitative features of the master equation description. In summary, while both systematic expansion methods provide a means for deriving a Langevin equation which does not contain artificial bistabilities, the Ω expansion is not suitable for cases in which the fluctuations qualitatively alter the behavior of the system. In contrast, the Kramers–Moyal expansion produces a Langevin equation similar to the colored-noise Langevin equation studied above.

APPENDIX B: GENERAL RESULTS FOR WHITE-NOISE LANGEVIN SYSTEMS

In this section, we derive general results for amplification and bistability in white-noise Langevin systems. In particular, we show that the peak of the probability distribution is always shifted in the direction of decreasing noise magnitude and provide a general description of the white-noise induced bifurcations of the type seen in the enzyme cycle. The analysis here is more general than that in Ref. 14, in which only the specific case of the enzyme cycle with white noise was examined. As a result, we are able to obtain a simple heuristic rule as to whether noise is amplifying or silencing in a given system.

A Langevin equation of the form Eq. (4) arises whenever the time derivative of one component of a system depends on another variable that varies stochastically. We thus take Eq. (4) with arbitrary smoothly varying functions $A(q)$ and $C(q)$ as the starting point for our analysis here. In the Stratonovich prescription, Eq. (4) with white noise is equivalent to the Fokker–Planck equation for the probability distribution[19]

$$\frac{\partial P}{\partial t} = -\frac{\partial}{\partial q}G(q)P + \frac{1}{2}\frac{\partial^2}{\partial q^2}B(q)P, \tag{B1}$$

where $P(q,t)$ is the probability distribution of q, $G(q) = A(q) + \Gamma C'(q)C(q)/2$, $B(q) = \Gamma[C(q)]^2$, and primes denote differentiation. The steady-state solution to Eq. (B1) is
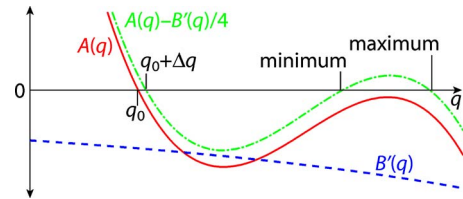

FIG. 8. (Color online) Schematic of stochastic amplification and noise induced bistability. Nonmonotonic A (shown) or B' (not shown; the enzyme cycle is an example) allows for the creation of additional extrema in the probability distribution (labeled "minimum" and "maximum") through a noise-induced bifurcation.

$$P_0(q) = \frac{C_0}{B(q)}\exp\left[2\int_0^q \frac{G(\tilde{q})}{B(\tilde{q})}d\tilde{q}\right], \tag{B2}$$

where C_0 is a normalization constant. From $P_0'(q) = 0$, the extrema of $P_0(q)$ satisfy the condition,[35]

$$A(q) - \frac{B'(q)}{4} = 0. \tag{B3}$$

This equation shows how new noise-induced stable states can be generated by white noise (Fig. 8). In cases where $A(q) = 0$ only has a single solution, nonmonotonic behavior in either $A(q)$ (depicted schematically in Fig. 8) or $B(q)$ (the case for the enzyme cycle), can give rise to new stable states. That is, if either the deterministic derivative or the magnitude of the noise are nonmonotonic functions of the coordinate q then new states can be stabilized by the noise.

To understand the amplification, we assume the amplitude of the noise is small and expand $A(q)$ and $B'(q)$ in Eq. (B3) to first order and solve for $\Delta q = q - q_0$, where q_0 is the fixed point of the deterministic equation of motion [$A(q_0) = 0$]. The peak in the distribution of q shifts by

$$\Delta q = \frac{B'(q_0)}{4A'(q_0) - B''(q_0)} \approx \frac{B'(q_0)}{4A'(q_0)}, \tag{B4}$$

where the approximate equality comes from retaining only terms to leading order in Γ. Use of the Itô prescription[19] results in an identical expression, except with a 2 in place of the 4. Equation (B4) provides us with a simple means for determining when (white) noise amplifies a signal.

To understand Eq. (B4), we examine the factors $A'(q_0)$ and $B'(q_0)$. For the fixed point q_0 to be stable, $A(q) > 0$ for all $q < q_0$, and $A(q) < 0$ for all $q > q_0$. Consequently, $A'(q_0) < 0$, and the sign of Δq, in general, is opposite that of $B'(q_0)$. The magnitude of the change in Δq depends on the deterministic restoring tendency: larger values of $|A'(q_0)|$ correspond to smaller shifts in the peak of the probability distribution. Now, consider the function $B'(q_0) = \Gamma\partial_q[C(q)]^2|_{q=q_0}$. $[C(q)]^2$ monotonically increases with the amplitude of the noise by definition, so the dependence of the amplitude of the noise on q determines the sign of B'. Specifically, $B'(q) > 0$ when the amplitude of the noise increases with q, and $B'(q) < 0$ when it decreases. Because, as discussed above, $A'(q_0) < 0$, the latter case corresponds to stochastic amplification. More generally, this result shows that the shift is always opposite in sign to $B'(q_0)$ and is therefore in the direction of decreasing noise.

Although Eq. (B4) is premised on small fluctuations, the reasoning above can be extended to show that Δq is opposite in sign to $B'(q)$ so long as $B(q)$ is a monotonic function of q and there is only a single deterministic fixed point. As schematically shown in Fig. 8, for $B(q)$ monotonically decreasing such that $B'(q) < 0$ everywhere, $A(q) - B'(q)/4 > 0$ for all $q < q_0$ [remember, $A(q) > 0$ for $q < q_0$]. Thus, there can be no extremum of the probability distribution in this range of q. On the other hand, for $q > q_0$, $A(q) < 0$, and addition of a positive number $(-B'/4)$ can result in solutions to Eq. (B3) that represent stochastic amplification. In other words, the peak in q must be to the right of q_0. By the same token, additional peaks that arise in the steady-state distribution must be at $q > q_0$ and thus correspond to $\Delta q > 0$ as well (Fig. 8). Because the elementary steps of reaction networks are often well-described by linear, Michaelis–Menten, and Hill functions, which are monotonic in the concentrations of species, we expect this reasoning to be generally applicable.

Finally, it is worth noting that in the case that the noise is independent of the concentrations of participating species, $B' = 0$, and the peak of the steady-state distribution is unchanged. Fluctuations obeying standard signal-to-noise scaling ($C(q) \sim \sqrt{q}$) give $B' \sim 1$ for all values of q, so the noise lowers the steady-state value of q.

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