Mathematical theory of stochastic phenotype switching and bet-hedging in bacteria: stochastic nonlinear dynamics and critical state identification

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Abstract

Fluctuating environments pose tremendous challenges to bacterial populations. It is widely observed in numerous bacterial species that individual bacterial cells will stochastically switch among multiple phenotypes to survive in rapidly changing environments. This phenotypic heterogeneity with stochastic phenotypic switching is generally assumed to be an adaptive bet-hedging strategy. To gain a deeper understanding how bet-hedging is achieved and the pattern and information behind experimental data, a mathematical model is needed. Traditional deterministic models cannot provide a correct description of stochastic phenotype switching, and besides, recent research has demonstrated that cellular processes during gene expression are inherently stochastic. In this article, we proposed a unified nonlinear stochastic model of multistable bacterial systems at the molecular level. We presented a mathematical explanation of phenotypic heterogeneity, stochastic phenotype switching, and bet-hedging within isogenic bacterial populations, and thus provided a theoretical framework for the analysis of experiment data at the cellular or molecular level. In addition, we also provided a quantitative characterization of the critical state during the transition among multiple phenotypes.

Introduction

Bacteria in the wild exist in ever-changing environments and have to surmount the challenges posed by environmental fluctuations. Numerous experiments have confirmed that multiple distinct phenotypes can coexist within an isogenic bacterial population \([1\text{-}10]\). This phenotypic heterogeneity in genetically identical cells has received increasing attention in recent years since it could help a bacterium to survive in rapidly changing environments. In the framework of traditional population genetics, a bacterial population enhances its fitness via genetic changes caused by mutation or recombination. However, the extracellular conditions can change so rapidly that adaptation only by mutation and recombination would be too slow. One solution to this problem is to allow individual cells to stochastically switch among multiple phenotypes without modification of the genotype, a phenomenon widely known as stochastic phenotype switching \([11\text{-}16]\).

Phenotypic heterogeneity is a widespread phenomenon in the bacterial realm. Examples of phenotypic heterogeneity include the lactose utilization in \textit{Escherichia coli} \([17]\), the lysis-lysogeny switch of bacteriophage \(\lambda\), competence development in \textit{Bacillus subtilis} \([18\text{-}20]\), sporulation in \textit{Bacillus subtilis} \([21\text{-}24]\), and persistence in \textit{Mycobacterium tuberculosis} \([25\text{-}27]\). The potential function of phenotypic heterogeneity with stochastic phenotype switching is generally understood to be a bet-hedging strategy \([7\text{-}28\text{-}29]\), a term originating from finance. In response to fluctuating environments, a heterogeneous bacterial population could optimize
its fitness by altering the proportion of cells in each subpopulation via stochastic phenotype switching to achieve an optimal ‘investment portfolio’.

Multiple distinct phenotypes within an isogenic bacterial population generally result from multiple steady-state expression levels of a group of stress-related genes. Such kind of gene expression pattern with two or more steady-state expression levels are widely known as bistability or multistability [3, 7]. Recent research has demonstrated that phenotypic heterogeneity within isogenic bacterial populations often results from the feedback circuitry of the gene regulatory network [30, 31]. However, feedback in itself does not guarantee multistability. In order to generate multistability, the gene network must include nonlinear interactions, which could originate from multimerization of transcription factors or cooperativity in DNA binding [2, 7].

To study the evolution of heterogeneous bacterial populations in detail, a number of Markov chain models have been proposed at the cellular level [1, 6, 12, 14, 15, 32–34]. These models assumed *a priori* that the bacterial population has multiple distinct phenotypes. In these Markov chain models, each phenotype is modeled as a state of the Markov chain and stochastic phenotypic switching is modeled as the state transition of the Markov chain. However, these models take phenotypic heterogeneity and stochastic phenotypic switching for granted and fail to account for their underlying molecular mechanisms.

To account for the molecular mechanism of phenotypic heterogeneity, a number of deterministic models have been proposed at the molecular level [17, 19, 25, 27, 35]. In these models, different steady states of gene expression are described as different stable fixed points (attractors) of a deterministic system composed of several ordinary differential equations which are written down based on the regulatory relationships behind the network. However, deterministic models cannot provide a correct description of many important experimental phenomena, such as stochastic phenotype switching and bet-hedging. In every deterministic model, if the expression level of an individual cell lies in the attraction basin of an attractor at a particular time, then it will never leave this attraction basin and thus phenotype switching will never occur.

Although deterministic models can give rise to multiple attractors and attraction basins, they do not allow transitions among different attraction basins. One solution to this problem is to consider stochastic effects, which allow the system to switch among different attraction basins and drive stochastic phenotype switching. This fact is analogous to the simulated annealing techniques in optimization problems, in which noise is indispensable to make the search escape from the trap of local minimum points and reach the global minimum point. In order to better understand the role that stochastic effects played in bistable systems, H. Qian and his coworkers studied the relations between deterministic and stochastic nonlinear dynamics in great detail [36–41]. However, their models are sometimes so abstract and oversimplified that they cannot be directly applied to practical problems with experimental data and observations.

Stochastic effects are extremely important not only because they are indispensable for the model to generate phenotype switching, but also because recent developments of single-molecule experiments in living cells have demonstrated that many important cellular processes, such as transcription, translation, replication, and gene regulation, are inherently stochastic [42–51]. Due to the stochastic effects, the expression levels of the stress-related genes in a bistable or multistable system will have a bimodal or multimodal distribution.

In this article, we proposed a unified stochastic model of multistable bacterial systems at the molecular level based on a core double-positive feedback gene network. By studying its stochastic nonlinear dynamics, we showed that our model not only provides a clear description of phenotypic heterogeneity, stochastic phenotype switching, and bet-hedging within isogenic bacterial populations, but also provides some insights for the analysis of gene expression data.
We made it clear that each phenotype of a bacterial population corresponds to a monomodal distribution of gene expression within an attraction basin. The multiple phenotypes of a heterogeneous bacterial population then correspond to a multimodal distribution of gene expression. The gene expression data are generally distributed around the attractors and are rarely distributed around the boundaries of the attraction basins.

Next, we applied the mathematical tool of large deviation theory established by Freidlin and Wentzell to show that each multistable stochastic system can be approximated by a Markov chain with multiple states, each of which corresponds to a phenotype of the cell population. In this way, our stochastic model at the molecular level reduces to a Markov chain model at the cellular level. This justifies the wide applications of early Markov chain models at the cellular level, in particular, the Markov chain model proposed by E. Lander and his coworkers [27] about the dynamics of the phenotypic proportions in human breast cancer cell lines. The stochastic model and its Markov chain approximation further allow us to discuss the mean value and the distribution of the time for an individual cell to make a state transition.

In addition, we pointed out a misunderstanding on the analysis of gene expression data inspired by our recent (unpublished) work of antibiotic resistance in Escherichia coli. Early works often took for granted that phenotypic heterogeneity can be identified by the multistable expression of a single pivotal gene. However, phenotypic heterogeneity in most bacterial systems results from the interaction of a group of stress-related genes. We used simulation results to show that in many cases, the expression data of a group of genes give rise to an apparent multimodal distribution, however, we cannot observe the multistable expression if we only focus on the expression data of a single pivotal gene. This shows that the traditional method to identify phenotypic heterogeneity by the multistable expression of a single gene is sometimes ineffective.

Finally, we applied our stochastic model to provide an answer to the important question that of detecting the critical state by using noisy data of gene expression. In our stochastic model of multistable bacterial systems, there is a saddle lying on the boundary of two attraction basins. This saddle, which characterizes a critical state between two steady states of gene expression, is not targeted in early works since it is rarely observed in experiments and cannot be estimated by simple statistical analysis of gene expression data. However, the identification of the critical state has drawn increasing attention in recent years since it is closely related to the early diagnosis of complex diseases. Recently, L. Chen and his coworkers [52] developed a method of identifying the leading network in complex diseases by evaluating a kind of network entropy. Inspired by their creative idea, we defined a characteristic quantity, named as the variance function, which can be used to detect the critical state effectively. We proved from the theoretical point of view that the critical expression level of the pivotal gene can be identified through finding the maximum points of the variance function.

**Model**

In natural bacterial systems, the origin of phenotypic heterogeneity and stochastic phenotype switching is often the feedback circuitry of the regulatory network which governs a group of stress-related genes. In order to better understand the general principles behind phenotypic heterogeneity, we illustrate the gene regulatory networks that govern some best-understood multistable systems in bacteria (Fig. 1(a)-(d)). A crucial similarity shared by these examples is that the wiring of the gene regulatory network forms a double-positive feedback loop. In order to establish a unified model of these bacterial systems, we focus on the core double-positive
feedback network depicted in Fig. 1(e). Here, protein X is the product of a pivotal stress-related
gene, gene X, whose expression levels determine the phenotypes of individual cells within the
bacterial population. Protein Y is a transcription factor which activates the expression of gene
X. Protein A is an inducer whose levels reflect the fluctuations in extracellular environmental
conditions, such as fluctuations in temperature, pH, and concentrations of nutrients and toxins
[21, 25].

![Diagram of the feedback network]

Figure 1. Schematic models of bacterial systems with phenotypic heterogeneity. a-d. Examples of
naturally occurring bacterial systems with phenotypic heterogeneity. a. Lactose utilization in E. coli. b.
Competence development in B. subtilis. c. Sporulation in B. subtilis. d. Persistence in M. tuberculosis.
e. The core positive feedback gene regulatory network shared by a-d. X (red) is a key protein (or mRNA)
whose expression level determines the phenotype of the bacteria. Y (blue) is the key transcription factor
of gene X. A (green) is an inducer whose concentration can affect the distribution of gene expression and
the transition among cellular states.

We use lowercase letters $x$, $y$, and $a$ to denote the concentrations of X, Y, and A, respectively.
Since gene expression in living cells is inherently stochastic, the dynamics of $x$ and $y$
can be described by the following two-dimensional system of stochastic differential equations:

\[
\begin{align*}
\dot{x} &= -\alpha(x - F(a, y)) + \sqrt{2}\epsilon_x, \\
\dot{y} &= -\beta(y - G(x)) + \sqrt{2}\eta_y.
\end{align*}
\]  

(1)

Here, $F(a, y)$ describes the activation of protein X by inducer A and protein Y. $G(x)$ describes
the activation of protein Y by protein X. $\alpha$ and $\beta$ are two parameters characterizing the response
speeds of the proteins X and Y, respectively. In addition, $\epsilon_x$ and $\epsilon_y$ are two independent standard
white noises. Since the fluctuations in the levels of proteins X and Y can be different, we use
two noise levels, $\epsilon$ and $\eta$, to characterize the stochastic fluctuations in the levels of proteins X and Y, respectively.

If we ignore stochastic effects, then the stochastic system (13) reduces to the following deterministic system as the two noise levels, $\epsilon$ and $\eta$, tend to zero:

$$\dot{x} = -\alpha(x - F(a, y)), \quad \dot{y} = -\beta(y - G(x)).$$

(2)

We notice that the fixed points of the deterministic system are the solutions to the following equations: $x = F(a, G(x)) = 0$ and $y = G(x)$. In natural bacterial systems, the most common expression of $F(a, G(x))$ has the following form:

$$F(a, G(x)) = \frac{\gamma x^n}{K + x^n} + (\mu a + \delta),$$

(3)

where the Hill function $\gamma x^n/(K + x^n)$ with $n > 1$ describes the activation of gene X by protein Y, the term $\mu a$ describes the activation of protein X by inducer A, and the term $\delta$ describes a basal expression of gene X independent of the activation of protein Y. From Fig. 2(a), we see that the deterministic system (2) has one or three fixed points under different inducer concentrations. To be specific, the inducer concentration $a$ has two threshold levels, $a_0$ and $a_1$. If $a < a_0$ or $a > a_1$, the system has only one fixed point (Fig. 2(a)). If $a < a_0$, the only attractor $(x_L, y_L)$ describes a low-expressing phenotype in which gene X is inactivated. If $a > a_1$, the only attractor $(x_H, y_H)$ describes a high-expressing phenotype in which gene X is activated. If $a_0 < a < a_1$, however, the system has three fixed points, including two attractors, $(x_L, y_L)$ and $(x_H, y_H)$, and a saddle, $(x_M, y_M)$ (Fig. 2(a),(b)). The two attractors, $(x_L, y_L)$ and $(x_H, y_H)$, describe the low- and high-expressing phenotypes, respectively, whereas the saddle $(x_M, y_M)$ describes a critical state between the two steady states of gene expression. Mathematically, each attractor of a deterministic system has an attraction basin, and two adjacent attraction basins are separated by a boundary (Fig. 2(b)).

**Phenotypic heterogeneity and bet-hedging**

In recent decade, live-cell experiments with single-molecule sensitivity have made significant progresses and have confirmed that cellular processes during gene expression in single cells are inherently stochastic [50]. In addition, deterministic models have some significant flaws. Many important dynamic phenomena, such as stochastic phenotype switching and bet-hedging, fail to be correctly described by deterministic models.

We now consider the stochastic system (13) in which the two noise levels, $\epsilon$ and $\eta$, are strictly positive. We first study the steady-state behavior of the stochastic system. From the derivation in SI, we can obtain an approximate steady-state probability distribution (density) $p_s(a, x, y)$ of the stochastic system (13), which follows a Boltzmann distribution:

$$p_s(a, x, y) = \frac{1}{Z} \exp \left\{ -\frac{1}{\epsilon} U(a, x, y) \right\},$$

(4)

where $Z$ is a normalization constant and $U(a, x, y)$ is a potential (landscape) defined as

$$U(a, x, y) = \frac{\epsilon \beta}{2\eta} (y - G(x))^2 + \alpha \int_0^x (u - F(a, G(u))) du.$$  

(5)

We make a crucial observation that the fixed points of the deterministic system (2) are exactly the solutions to the equations, $\partial_x U(a, x, y) = \partial_y U(a, x, y) = 0$. This shows that the attractors of the deterministic system are the local minimum points of the potential $U(a, x, y)$ and...
are the local maximum points of the steady-state distribution \( p_s(a, x, y) \), according to Equation \( 23 \). From Fig. 2(c-e), we see that the steady-state distribution of levels of proteins X and Y is controlled by the inducer concentration \( a \). In the case of \( a < a_0 \), the steady-state distribution has only the low-expressing peak, suggesting that all bacterial cells will be in the low-expressing phenotype under favorable conditions (Fig. 2(c)). In the case of \( a_0 < a < a_1 \), the steady-state distribution has two peaks, each of which corresponds to a phenotype. With the increase of the inducer concentration, a larger fraction of bacterial cells will switch from the low-expressing to the high-expressing phenotype to maximize survival (Fig. 2(d)). In the case of \( a > a_1 \), the low-expressing peak of the steady-state distribution disappears, suggesting that all cells will switch to the high-expressing phenotype under unfavorable conditions (Fig. 2(e)). The above discussion clearly shows that how the bet-hedging strategy could help the bacterial population to better adapt to rapidly changing environmental conditions.

Generally speaking, the steady-state expression levels of a multistable system have a multimodal distribution. Intuitively, the multimodal distribution of gene expression can be viewed as the superposition of multiple monomodal distributions, each of which is concentrated within an attraction basin. The attractors are locally the most-probable states and thus are most likely to be observed in experiments. Based on the stochastic system \( 13 \), we simulate the time course of expression levels of proteins X and Y in a single cell. The simulation result (Fig. 2(b)) shows that the gene expression data are generally distributed around the attractors and are rarely distributed around the boundaries of the attraction basins. These results show that each phenotypic state of a bacterial population cannot be simply described as an attractor of the deterministic model, but should be understood as a monomodal distribution concentrated within an attraction basin. This understanding is similar to that in quantum mechanics, where a state of a particle cannot be viewed as its position and momentum, as in the case of classical mechanics, but should be viewed as a wave function in the phase space, whose modulus square represents the probability density of that particle.

**A misunderstanding on the analysis of gene expression data**

Phenotypic heterogeneity in most bacterial systems results from the interaction of a group of stress-related genes and biochemical species. Early works always took for granted that phenotypic heterogeneity can be identified by the multistable expression of a single pivotal gene. In experiments, however, it often happens that the histogram of steady-state expression data of a pivotal gene does not display multiple peaks, and only when the population is sorted to start from some extreme initial conditions, the multimodal distribution of gene expression could be seen at different times before reaching the steady state. Therefore it is rather difficult to decide whether the bacterial population has multiple phenotypes or not. We make it clear that phenotypic heterogeneity due to the interaction of a group of stress-related genes may be missed if we only focus on the expression data of a single pivotal gene. In our recent study on antibiotic resistance in *Escherichia coli* (unpublished work), we find that the expression data of the hydrolase gene does not lead to a multimodal distribution, whereas the expression data of three stress-related genes lead to an apparent multimodal distribution.

We now use our stochastic model to illustrate this interesting phenomenon. To this end, we simulate the steady-state levels of proteins X and Y in 500,000 cells according to the stochastic system \( 13 \) under a set of model parameters. From the simulation result (Fig. 2(f)), we see that the two-dimensional gene expression data are distributed around two attractors in the phase plane and lead to a bimodal distribution, which is the superposition of two monomodal dis-
Figure 2. **Stochastic description of multistability.**

**a.** Numbers of fixed points under different inducer concentrations. The curve represents the Hill function $y = \gamma x^n/(K + x^n)$ and the lines represent the function $y = x - (\mu a + \delta)$. The intersections of the curve and the line give the positions of the fixed points. **b.** Simulation of the time course of levels of proteins X and Y in a single cell. When $a_0 < a < a_1$, the deterministic model has three fixed points, including two attractors and a saddle. The saddle lies on the boundary of two attraction basins. The expression levels of the cell stay around the two attractors at most times and cross the boundary around a unique point, which is exactly the saddle of the deterministic model. **c-e.** The steady-state probability distribution of the levels of proteins X and Y. **c.** When $a < a_0$, the steady-state gene expression has a monomodal distribution, which attains its unique maximum at the low-expressing attractor. **d.** When $a_0 < a < a_1$, the steady-state gene expression has a bimodal distribution, which attains two maxima at both the low- and high-expressing attractors. **e.** When $a > a_1$, the steady-state gene expression has a monomodal distribution, which attains its unique maximum at the high-expressing attractor. **f.** The simulation data of steady-state levels of proteins X and Y in 500,000 cells under a set of appropriately chosen model parameters. The two-dimensional gene expression data are distributed around two attractors in the phase plane, and thus lead to an apparent bimodal distribution. **g.** The marginal distribution of the two-dimensional gene expression data. The blue and red curves represent the marginal distributions of cells in the low- and high-expressing states, respectively. The black curve represents the marginal distribution of all cells. There is much overlap between the blue and red curves, resulting in a monomodal marginal distribution.
tributions. Although these two monomodal distributions are concentrated within two different attraction basins in the phase plane, there is much overlap between their marginal distributions, whose superposition, which represents the steady-state distribution of the level of protein Y, has only one peak (Fig. 2(g)). This clearly shows that the traditional idea to identify phenotypic heterogeneity by the multistable expression of a single pivotal gene is sometimes ineffective.

From the molecular level to the cellular level

We have seen that the stochastic model proposed in this article can account for the origin of phenotypic heterogeneity and bet-hedging with isogenic bacterial populations at the molecular level. However, more widely-used models in early works are Markov chain models at the cellular level. These models assume a priori that the bacterial population has multiple distinct phenotypes, each of which corresponds to a state of the Markov chain and can make a state transition to other phenotypes with certain transition rates. This raises the question that whether the models at these two different levels, our stochastic model at the molecular level and the Markov chain models at the cellular level, are linked in some way or not? We now use the mathematical tool of large deviation theory established by Freidlin and Wentzell [53] to answer this question.

The Freidlin-Wentzell theory is mainly concerned about the dynamic behavior of a system of stochastic differential equations when the noise level is not too large. To make the readers understand this useful mathematical tool, we now list some of the main results of the Freidlin-Wentzell theory:

• If a stochastic system has multiple attractors, no matter how small the noise level is, the accumulation of the stochastic effects will make the system switch among multiple attraction basins. Before the system escapes from the trap of an attraction basin, it will spend most of the time staying around the attractors and will spend little time staying around the boundaries of the attraction basins.

• Each point in an attraction basin has a potential, which is referred to as the quasipotential. At the moment that the system escapes from the trap of an attraction basin, it will cross the boundary around those local minimum points of the quasipotential (with almost probability 1 when the noise level is small). In most cases, the local minimum points of the quasipotential are saddles of the deterministic counterpart of the stochastic system.

• The time needed for the stochastic system to leave an attraction basin is referred to as the escape time. The escape time of a stochastic system with noise level $\epsilon$ approximately follows an exponential distribution with a approximate time constant $\exp(U/\epsilon)$, where $U$ is the minimum of the quasipotential on the boundary.

• Since the escape time from each attraction basin approximately follows an exponential distribution, each attraction basin of a stochastic system can be considered as a state and a stochastic system with multiple attractors can be approximated by a Markov chain with multiple states at the time scale of $\exp(1/\epsilon)$. When the noise level $\epsilon$ is small, $\exp(1/\epsilon)$ becomes very large. This shows that the approximate Markov chain reflects the long-term dynamic behavior of the stochastic system.

In our stochastic model (13), the system has two attraction basins if the inducer concentration $a$ is between $a_0$ and $a_1$, and thus can be approximated by a Markov chain with two states, according to the Freidlin-Wentzell theory. If the feedback architecture of the underlying gene network is more complicated, then the system can give rise to more than two attraction basins and thus can be approximated by a Markov chain model with more than two states. For exam-
ples of Markov chain models with two, three, or four states, the reader may refer to [14, 15, 33]. In this way, the stochastic model at the molecular level reduces to a Markov chain model at the cellular level. We see clearly from the above discussions that the models at two different levels are essentially the same and reflect the dynamic behavior of the stochastic system at two different time scales.

**Stochastic phenotype switching**

Fluctuating environments pose tremendous challenges to bacterial populations. A heterogeneous bacterial population may survive in rapidly changing environments by allowing individual cells to stochastically switch among multiple phenotypes, thus ensuring that some cells are always prepared for an unforeseen environmental fluctuation. This type of phenotypic switching is stochastic and temporary. Individual cells can switch to an alternative state at a random time, and after some random time, switch back again. Stochastic phenotype switching has been observed in numerous bacterial species. Even without a significant change in environmental conditions, stochastic phenotype switching still exists. As an example, upon encountering nutrient limitation, a minority of *Bacillus subtilis* cells transiently enter the competent state with the capability for DNA uptake from the environment before returning to vegetative growth [18].

To better understand how stochastic phenotype switching occurs, we simulate the time course of the level of protein X in a single cell based on the stochastic model (13). The simulation result (Fig. 3(a)) shows that the cell switches between the low- and high-expressing states at certain random times. The Freidlin-Wentzell theory shows that the escape times $T_L$ and $T_H$ from the low- and high-expressing states approximately follow exponential distributions with time constants $\langle T_L \rangle$ and $\langle T_H \rangle$, respectively. From the derivation in SI, the mean escape times, $\langle T_L \rangle$ and $\langle T_H \rangle$, have the form of

$$
\langle T_L \rangle = \frac{2\pi}{\sqrt{\kappa_L \kappa_M}} \exp \left( \frac{1}{\epsilon} \Delta U_L \right),
\langle T_H \rangle = \frac{2\pi}{\sqrt{\kappa_H \kappa_M}} \exp \left( \frac{1}{\epsilon} \Delta U_H \right),
$$

(6)

where $\Delta U_L = U(a, x_M, y_M) - U(a, x_L, y_L)$ is the potential difference between the saddle and the low-expressing attractor, $\Delta U_H = U(a, x_M, y_M) - U(a, x_H, y_H)$ is the potential difference between the saddle and the high-expressing attractor (Fig. 3(c)), and $\kappa_L$, $\kappa_M$, and $\kappa_H$ are the curvatures of the function $U(a, x, G(x))$ at $x_L$, $x_M$, and $x_H$, respectively. In this way, the dynamic behavior of the stochastic system (13) at the molecular level can be approximated by a Markov chain with two states (Fig. 3(b)) at the cellular level. Equations (6) show that the mean escape time is an exponential function of the potential barrier. The higher the potential barrier, the longer time is needed for a cell to make a state transition.

Using the mean escape times, we easily calculate the steady-state proportion $p_L$ of cells in the low-expressing phenotype and the steady-state proportion $p_H$ of cells in the high-expressing phenotype. According to the ergodic property of the stochastic system, the ratio of $p_H$ and $p_L$ has the form of

$$
\frac{p_H}{p_L} = \frac{\langle T_H \rangle}{\langle T_L \rangle} = \sqrt{\frac{\kappa_L}{\kappa_H}} \exp \left( -\frac{1}{\epsilon} \left( U(a, x_H, y_H) - U(a, x_L, y_L) \right) \right).
$$

(7)

As the noise level $\epsilon$ tends to zero, then either $p_L$ or $p_H$ tends to 1, according to which one of the two potential minima, $U(a, x_L, y_L)$ and $U(a, x_H, y_H)$, is lower. This indicates that if there
Figure 3. **Spontaneous phenotypic switching driven by noise.**  

**a.** Simulation of the time course of the levels of protein X in a single cell. The cell will switch between the low- and high-expressing states at certain random times. Both the escape times from the low- and the high-expressing states approximately follow exponential distributions.  

**b.** The simplified dynamics of the stochastic system (13) as a two-state Markov chain.  

**c.** The steady-state behavior of the stochastic system when the noise level $\epsilon$ is zero. The curve represents the one-dimensional effective potential $U(a, x, G(x))$. All bacterial cells will stay in the state with the lowest potential.  

**d.** The steady-state behavior of the stochastic system when the noise level $\epsilon$ is positive. The accumulation of noise will drive bacterial cells to surmount the potential barrier and switch to the other state.

According to Equation (6), the mean escape times have the time scale of $\exp(1/\epsilon)$. This shows that stochastic phenotype switching is long-term dynamic behavior of the system. If the noise level $\epsilon$ is small, the escape time may be longer than the time of cell division. For a fraction of cells, stochastic phenotype switching may not occur in a single cell cycle, and thus they can pass their phenotypic state to the next generation.

### Importance of the critical state

According to the stochastic system (13), we have seen that at certain ranges of the inducer concentration, the two attractors are separated by a boundary, forming two attraction basins. The saddle of the deterministic system (2) lies exactly on the boundary of the two attraction basins (Fig. 2b) and thus characterizes a critical state between the two steady states of gene expression. This saddle is not targeted in early works since it is rarely observed in experiments and cannot be estimated by simple statistical analysis of gene expression data. However, the identification of the critical state has drawn increasing attention in recent years due to the
following three reasons.

First, the saddle reflects a critical level of gene expression. Recent studies on complex diseases show that any disease progression can be divided into a normal state, a pre-disease state, and a disease state, similar to the low-expressing state, the critical state, and the high-expressing state in our present framework. Once the gene expression of a person is close to the saddle, we have good reasons to believe that this person is in a pre-disease state and is at high risk of disease progression. This shows that the identification of the critical state is closely related to the early diagnosis of complex diseases.

Second, the saddle is the most important point on the boundary of two attraction basins. According to the Freidlin-Wentzell theory, at the moment that the system escapes from an attraction basin, it will cross the boundary around certain points which are the local minimums of the quasipotential on the boundary. To see this more clearly, we simulate the time course of the levels of proteins X and Y in a single cell according to the stochastic model (13). The simulation result (Fig. 2(b)) shows that expression levels of the cell stay around the attractors at most times and cross the boundary of two attraction basins around the saddle, which is a global minimum point of the quasipotential on the boundary.

Third, the saddle characterizes a critical transition state with low resilience and robustness to perturbations. In order to finish stochastic phenotype switching, the system needs to first climb up the potential from one attractor to the saddle, and then climb down the potential from the saddle to another attractor. Before reaching the saddle, the system is driven by stochastic forces. The accumulation of stochastic forces will drive the system climb up the potential against the potential gradient. Thus the system will need a long time reach the saddle. Once the system crosses the saddle, the system will reach another attractor along the potential gradient in a short time. Thus the dynamic features of an individual cell before reaching the saddle and after reaching the saddle are totally different. To be more precise, denote by $T_u$ the time for the system to climb up the potential from one attractor to the saddle and denote by $T_d$ the time for the system to climb down the potential from the saddle to another attractor. The Freidlin-Wentzell theory shows that the ratio between $T_u$ and $T_d$ is of the time scale $\exp(1/\epsilon)$. This suggests that the process of climbing up the potential is much longer than that of climbing down the potential. The fact that $T_u$ is much longer than $T_d$ is consistent with the old saying: diseases come on horseback, but go away on foot.

Identification of the critical state

We have seen that the critical state is extremely important. This then raises the question that whether or not we can detect the critical state in an effective way by using the noisy data of gene expression. Recently, L. Chen and his coworkers developed a method of identifying the leading network in complex diseases by evaluating a kind of network entropy [52]. However, their method does not have a sufficient theoretical support. Inspired by their creative idea, in the present article, we define a characteristic quantity which can be used to detect the critical state effectively. Furthermore, we prove the effectiveness of our method from a theoretical point of view.

We see from the stochastic model (13) that the expression level of the pivotal gene, gene X, has two steady-state levels, $x_L$ and $x_H$, and a critical level, $x_M$. We assume that we have measured the expression levels of the pivotal gene of all cells within a bacterial population at two discrete times, $t$ and $t + h$, where the interval $h$ of two successive measurements is chosen to have the time scale of $1/\epsilon$, which is much shorter than the time scale $\exp(1/\epsilon)$ of stochastic
Figure 4. Identification of the critical expression level. a. The graph of the function $p_H(x)$. The function $p_H(x)$ experiences a critical transition around the critical level $x_M$. With the decrease of the noise level $\epsilon$, the slope of the function at $x_M$ tends to infinity. b. The variance function $D(x)$. The variance $D(x)$ around $x$ is defined to be the variance of the expression level at time $t + h$ conditional on the information of the expression level to be $x$ at time $t$. The line represents the theoretical curve of $D(x)$ and the circles represent the simulation result based on the simulated dynamics of 500,000 cells. The variance function changes slowly around the low level $x_L$ and the high level $x_H$, and experiences a drastic change around the critical level $x_M$. Thus the variance function $D(x)$ provides a clear signal for the position of the critical expression level $x_M$.

phenotype switching. Intuitively, if the expression level of an individual cell is around $x_L$ or $x_H$ at time $t$, the expression level at time $t + h$ should be also around $x_L$ or $x_H$ since the interval $h$ is chosen to be much shorter than the time scale of stochastic phenotype switching. However, if the expression level of an individual cell is around the critical level $x_M$ at time $t$, the expression level at time $t + h$ will become rather chaotic since the critical state has low resilience and robustness to perturbations.

Denote by $x(t)$ is the expression level of gene $X$ at time $t$. We notice that $x(t)$ is a random variable whose value can differ significantly between two individual cells. The above discussion illuminates us to define the variance $D(x)$ around $x$ as the variance of $x(t + h)$ conditional on the information of $x(t) = x$. More precisely, we define the variance $D(x)$ around $x$ as

$$D(x) = \text{Variance}(x(t + h) | x(t) = x),$$

(8)

where $\text{Variance}(u) = \langle (u - \langle u \rangle)^2 \rangle$ is the variance of $u$. From the above intuitive discussion, the variance $D(x_L)$ around $x_L$ and the variance $D(x_H)$ around $x_H$ should be small since the distribution of $x(t + h)$ will be rather concentrated if $x(t)$ is around $x_L$ or $x_H$. Moreover, the variance $D(x_M)$ around the critical level $x_M$ should be large since the distribution of $x(t + h)$ will be rather scattered if $x(t)$ is around $x_M$. This suggests that we may detect the critical level $x_M$ by seeking the maximum point of the variance function $D(x)$.

We next validate our intuition from the theoretical point of view. From the derivation in $SI$, the variance function $D(x)$ has the form of

$$D(x) \doteq (x_H - x_L)^2 p_L(x)p_H(x) + \epsilon \left( \frac{p_L(x)}{\kappa_L} + \frac{p_H(x)}{\kappa_H} \right),$$

(9)

where

$$p_H(x) = \frac{\int_{x_L}^{x} \exp \left( \frac{1}{\epsilon} U(a,y,G(y)) \right) dy}{\int_{x_L}^{x} \exp \left( \frac{1}{\epsilon} U(a,y,G(y)) \right) dy},$$

(10)
and $p_L(x) = 1 - p_H(x)$. We denote the maximum point of $D(x)$ by $x^*$. Then we easily see from Equation (63) that

$$p_H(x^*) = \frac{1}{2} + \frac{(\kappa_L - \kappa_H)\epsilon}{2(x_H - x_L)^2\kappa_L\kappa_H},$$

(11)

To find the specific location of the maximum point $x^*$, we depict the graph of the function $p_H(x)$ in Fig. 4(a), from which we see that the shape of $p_H(x)$ is an sigmoidal curve with a critical transition around $x_M$, and that with the decrease of the noise level $\epsilon$, the slope of the function $p_H(x)$ at $x_M$ tends to infinity. Combining this fact and Equation (11), we clearly see that

$$\lim_{\epsilon \to 0} x^* = x_M,$$

(12)

which shows that when the noise level $\epsilon$ is small, the maximum point of the variance function $D(x)$ is almost attained at the critical level $x_M$. This suggests that we can detect the critical level $x_M$ by seeking the maximum point of the variance function $D(x)$.

A natural and important question is that whether or not we can estimate the function $D(x)$ around $x$ by using the noisy data of gene expression. The answer is of course affirmative. Recent developments in fluorescent proteins, flow cytometry, and single-molecule assays allow us to measure the gene expression of all cells within a bacterial population at a series of times $t_1, \cdots, t_m$ with interval $h$. We assume that the experimental data of the $n$-th cell have the form of $x(n, t_1), \cdots, x(n, t_m)$, where $x(n, t_i)$ is the expression level of the $n$-th cell at time $t_i$. If $x(n, t_i)$ is in a given small neighborhood of $x$, we pick out this datum, whereas if $x(n, t_i)$ is not in a given small neighborhood of $x$, we throw way this datum. By evaluating the sample variance of those data which are picked out, we can obtain a good estimation of the variance $D(x)$ around $x$.

To validate the effectiveness the above method, we simulate the dynamics of 500,000 cells based on the stochastic model (13) and estimate the variance function $D(x)$ at several discrete levels of $x$ (circles in Fig. 4(b)), from which we see that the simulation results coincide perfectly with the theoretical results (line in Fig. 4(b)). Moreover, we notice that the variance function $D(x)$ changes drastically around the critical level $x_M$, whereas there is no significant change in $D(x)$ around $x_L$ and $x_H$. This shows that our method is effective in detecting the critical state through seeking the maximum point of the variance function $D(x)$.

**Discussion**

Given the small size of a cell and the small copy numbers of participating macromolecules, cellular processes during gene expression are inherently stochastic [50]. In this article, we established a unified nonlinear stochastic model of multistable bacterial systems at the molecular level and presented a mathematical explanation of phenotypic heterogeneity, stochastic phenotype switching, and bet-hedging within isogenic bacterial populations. We also used our stochastic model to provide an answer to the important question of detecting the critical state by using the noisy data of gene expression. We defined a characteristic quantity called the variance function. This quantity can be estimated by using the gene expression data and can be used to identify the critical state effectively. All results in the present article are expected to give a clearer picture about phenotypic heterogeneity within isogenic bacterial populations.

In our recent study of the antibiotic resistance of *Escherichia coli* (unpublished work), we find that the one-dimensional expression data of the hydrolase gene does not have a multimodal distribution, but the high-dimensional expression data of a group of stress-related genes have an
apparent multimodal distribution. This phenomenon is described in this article. Our simulation results show that although the expression data of a group of genes are distributed within multiple attraction basins in the high-dimensional phase space, their marginal distribution may overlap to a large extent so that it is impossible to observe multimodal distributions if we only focus on one-dimensional expression data of a specific gene. In our future work, we shall further apply the general theory discussed in this article to the specific problem of the antibiotic resistance of Escherichia coli.

Although different multistable systems have different gene regulatory networks, the mathematical structure behind them are exactly the same. Biological systems with multistability are ubiquitous in nature. Some fundamental cellular processes, such as decision-making processes in cell cycle progression [54], cell differentiation [55–57], and apoptosis [58, 59], display multistable features. In addition, multistability is also involved in disease progression, which can be divided into a normal state, a pre-disease state, and a disease state [60, 61]. We hope that the stochastic approach discussed in this article can become a fundamental and effective tool in analyzing biological systems with multistability and the related new phenomena and new questions.

Methods

The parameters used in drawing the figures in the main text are chosen as $\alpha = 1$, $\gamma = 0.8$, $K = 0.05$, $\delta = 0$, and $n = 6$. The inducer concentration $a$ and the noise level $\epsilon$ are chosen different when drawing different figures.

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Appendix A: Derivation of the steady-state probability density

In the main text, we establish a stochastic model describing the dynamics of the levels of proteins X and Y. The model can be represented by the following two-dimensional system of stochastic differential equations (SDE):

\[
\begin{align*}
\dot{x} &= -\alpha(x - F(a,y)) + \sqrt{2}\epsilon\xi_x, \\
\dot{y} &= -\beta(y - G(x)) + \sqrt{2}\eta\xi_y.
\end{align*}
\]  

(13)

We denote the steady-state probability density of the stochastic system by \( p_s(a, x, y) \). To calculate the expression of \( p_s(a, x, y) \), we make an assumption that the dynamics of the variable \( y \) is much faster than that of the variable \( x \). In other words, we assume that \( \alpha \ll \beta \). Under this assumption, we see that \( x \) is a slow variable and \( y \) is a fast variable. In order to study the dynamics of the fast variable \( y \), we can think that the slow variable \( x \) is frozen. Thus the steady-state probability density \( p_s(a, x, y) \) of the stochastic system \((13)\) can be represented as

\[
p_s(a, x, y) = p_s(y | a, x) p_s(a, x),
\]

(14)

where \( p_s(y | a, x) \) is the steady-state probability density of the fast variable \( y \) given \( a \) and \( x \), and \( p_s(a, x) \) is the steady-state probability density of the slow variable \( x \).

Note that the dynamics of the fast variable \( y \) described by the second equation of Equation \((13)\) is an Ornstein-Uhlenbeck (OU) process if we froze the slow variable \( x \). Thus the steady-state distribution
of $y$ given $a$ and $x$ is frozen follows the normal distribution $N(G(x), \eta/\beta)$ with probability density

$$p_s(y|a, x) = \sqrt{\frac{\beta}{2\pi\eta}} \exp\left(-\frac{\beta}{2\eta} (y - G(x))^2\right).$$  \hspace{1cm} (15)$$

In order to study the dynamics of the slow variable $x$, we can think that the fast variable $y$ is always in a quasi-steady state. This indicates that the fast variable $y$ in the first equation of Equation (13) can be averaged out as

$$\dot{x} = \int_R \left(-\alpha (x - F(a, y)) + \sqrt{2\epsilon} \xi_x\right) p_s(y|a, x) dy.$$ \hspace{1cm} (16)

Since the noisy level $\eta$ is small, by the Laplace method, Equation (13) can be further approximated as

$$\dot{x} = \sqrt{\frac{\beta}{2\pi\eta}} \int_R \left(-\alpha (x - F(a, G(x))) + \sqrt{2\epsilon} \xi_x\right) \exp\left(-\frac{\beta}{2\eta} (y - G(x))^2\right) dy\hspace{1cm} (17)$$

This shows that the dynamics of the slow variable $x$ can be approximated by the following one-dimensional Langevin equation:

$$\dot{x} = -\alpha (x - F(a, G(x))) + \sqrt{2\epsilon} \xi_x = -\partial_x H(a, x) + \sqrt{2\epsilon} \xi_x,$$ \hspace{1cm} (18)

where

$$H(a, x) = \alpha \int_0^x (u - F(a, G(u))) du.$$ \hspace{1cm} (19)

Let $p(a, x, t)$ denote the probability density of the slow variable $x$ at time $t$. Then the dynamics of $p(a, x, t)$ is governed by the following Fokker-Planck equation:

$$\partial_t p = -\partial_x (\partial_x U p - \epsilon \partial_x p).$$ \hspace{1cm} (20)

By solving the Fokker-Planck equation, the steady-state probability density $p_s(a, x)$ of the slow variable $x$ has the form of

$$p_s(a, x) = \frac{1}{Z_x} \exp\left(-\frac{1}{\epsilon} U(a, x)\right),$$ \hspace{1cm} (21)

where $Z_x$ is a normalization constant.

Combining Equations (15) and (23), we obtain the steady-state probability density $p_s(a, x, y)$ in the phase space:

$$p_s(a, x, y) = \frac{1}{Z} \exp\left(-\frac{1}{\epsilon} U(a, x, y)\right),$$ \hspace{1cm} (22)

where $Z$ is a normalization constant and $U(a, x, y)$ is a potential (landscape) defined as

$$U(a, x, y) = \frac{\epsilon \beta}{2\eta} (y - G(x))^2 + \alpha \int_0^x (u - F(a, G(u))) du.$$ \hspace{1cm} (23)

**Appendix B: Derivation of the mean escape times**

The approach here to derive the mean escape times is very similar to the reaction rate theory established by Kramers [62]. Readers familiar with the reaction rate theory can skip this part. We only discuss the mean escape time $\langle T_L \rangle$ for the OFF state, that is, the mean time needed for a cell to switch from the OFF state to the ON state. The derivation of the mean escape time $\langle T_H \rangle$ for the ON state is totally the same. Denote by $X_t$ the level of protein X at time $t$. For any $x$, we define

$$\tau_x = \inf\{t \geq 0 : X_t = x\}.$$ \hspace{1cm} (24)
Note that $\tau_x$ represents the time needed for the protein level to arrive at $x$ for the first time. Mathematically, the time $\tau_x$ is a random time and is named as the first-passage time for $x$. Under this notation, the mean escape time $\langle T_L \rangle$ for the OFF state should be

$$\langle T_L \rangle = E_{xL} \tau_{xH} = E(\tau_{xH} | X_0 = x_L),$$

(25)

that is, the mean time needed for $X_t$ which starts from $x_L$ to arrive at $x_H$ for the first time.

We rewrite our one-dimensional model in Ito’s differential form as

$$dX_t = -U'(X_t)dt + \sqrt{2}\epsilon dW_t,$$

(26)

where $U'(x) = \partial_x U(a, x)$. According to the celebrated Ito’s formula in the theory of SDE, for any function $f$ whose second derivative is continuous, we have

$$df(X_t) = f'(X_t)dX_t + \frac{1}{2}f''(X_t)dX_t^2$$

$$= f'(X_t)(-U'(X_t)dt + \sqrt{2}\epsilon dW_t) + \frac{1}{2}f''(X_t)2\epsilon dt$$

(27)

We rewrite the above equation as the integration form as

$$f(X_t) - f(X_0) = \int_0^t (\epsilon f''(X_s) - U'(X_s)f'(X_s))ds + \sqrt{2}\epsilon \int_0^t f'(X_s)dW_s.$$  

(28)

This shows that

$$f(X_{t\wedge \tau_{xH}}) - f(X_0) = \int_0^{t\wedge \tau_{xH}} (\epsilon f'' - U'f') (X_s)ds$$

(29)

is a martingale. Taking expectation in the above equation and letting $t \to \infty$, we obtain

$$E_{xL} f(X_{\tau_{xH}}) - f(x_L) = E_{xL} \int_0^{\tau_{xH}} (\epsilon f'' - U'f') (X_s)ds.$$  

(30)

If we can find a function $f$ so that

$$\epsilon f'' - U'f' \equiv 1,$$

(31)

then the above equation reduces to

$$E_{xL} \tau_{xH} = E_{xL} f(X_{\tau_{xH}}) - f(x_L) = f(x_H) - f(x_L).$$  

(32)

Thus the remaining question is to solve the second-order ODE (31).

Recall that the steady-state distribution of our one-dimensional model is

$$p_s(a, x) = \frac{1}{Z_x} e^{-\frac{1}{\epsilon} U(a, x)}.$$  

(33)

Thus we obtain that

$$p_s' = -\frac{1}{\epsilon} U' p_s.$$  

(34)

Multiplying Equation (31) by $p_s$, we obtain that

$$\epsilon f'' p_s - U'f'p_s = p_s.$$  

(35)

Applying Equation (34), we obtain that

$$\epsilon (f' p_s)' = \epsilon f'' p_s + \epsilon f' p_s' = p_s.$$  

(36)

Integrating the above equation, we obtain that

$$f'(y)p_s(y) = \frac{1}{\epsilon} \int_y^\infty p_s(z)dz.$$  

(37)

Thus

$$f'(y) = \frac{1}{\epsilon p_s(y)} \int_y^\infty p_s(z)dz.$$  

(38)
Integrating the above equation, we obtain that

\[ f(x) - f(x_L) = \frac{1}{\epsilon} \int_{x_L}^{x} \frac{1}{p_s(y)} dy \int_{-\infty}^{y} p_s(z) dz. \] (39)

By Equation (32), we obtain that

\[ E_{x_L} \tau_{xH} = f(x_H) - f(x_L) = \frac{1}{\epsilon} \int_{x_L}^{x_H} \frac{1}{p_s(y)} dy \int_{-\infty}^{y} p_s(z) dz \]

\[ = \frac{1}{\epsilon} \int_{x_L}^{x_H} e^{\frac{1}{\epsilon} U(a,y)} dy \int_{-\infty}^{y} e^{-\frac{1}{\epsilon} U(a,z)} dz. \] (40)

Applying the Laplace method, we obtain that

\[ E_{x_L} \tau_{xH} = \frac{1}{\epsilon} \int_{x_L}^{x_H} e^{\frac{1}{\epsilon} U(a,y)} dy \int_{-\infty}^{y} e^{-\frac{1}{\epsilon} U(a,z)} dz \]

\[ = \frac{1}{\epsilon} \sqrt{\frac{2\pi}{\kappa_L}} e^{\frac{1}{\epsilon} U(a,x_M)} \sqrt{\frac{2\pi}{\kappa_L}} e^{-\frac{1}{\epsilon} U(a,x_L)} \]

\[ = \frac{2\pi}{\sqrt{\kappa_L \kappa_M}} e^{\frac{1}{\epsilon} (U(a,x_M) - U(a,x_L))}, \] (41)

where \( \kappa_L = \partial_{a}^2(a, x_L) \) and \( \kappa_M = -\partial_{a}^2(a, x_M) \) are the curvatures of \( U(a, x) \) at \( x_L \) and \( x_M \), respectively. Thus we finally obtain that

\[ \langle T_L \rangle = E_{x_L} \tau_{xH} = \frac{2\pi}{\sqrt{\kappa_L \kappa_M}} e^{\frac{1}{\epsilon} (U(a,x_M) - U(a,x_L))}. \] (42)

Similarly, we can obtain that

\[ \langle T_H \rangle = E_{x_H} \tau_{xL} = \frac{2\pi}{\sqrt{\kappa_H \kappa_M}} e^{\frac{1}{\epsilon} (U(a,x_M) - U(a,x_H))}, \] (43)

where \( \kappa_H = \partial_{a}^2(a, x_H) \) is the curvature of \( U(a, x) \) at \( x_H \). Thus the transition rate from the OFF state to the ON state is

\[ k_{LH} = 1/\langle T_L \rangle = \frac{\sqrt{\kappa_H \kappa_M}}{2\pi} e^{\frac{1}{\epsilon} (U(a,x_L) - U(a,x_M))}, \] (44)

and the transition rate from the OFF state to the ON state is

\[ k_{HL} = 1/\langle T_H \rangle = \frac{\sqrt{\kappa_H \kappa_M}}{2\pi} e^{\frac{1}{\epsilon} (U(a,x_H) - U(a,x_M))}. \] (45)

Thus we can derive the steady-state proportion \( p_L \) of cells in the OFF state and the steady-state proportion \( p_H \) of cells in the ON state as

\[ p_L = \frac{k_{HL}}{k_{LH} + k_{HL}} = \frac{\sqrt{\frac{\pi}{\kappa_L}} e^{-\frac{1}{\epsilon} U(a,x_L)}}{\frac{\pi}{\kappa_L} e^{-\frac{1}{\epsilon} U(a,x_L)} + \frac{\pi}{\kappa_H} e^{-\frac{1}{\epsilon} U(a,x_H)}}, \] (46)

and

\[ p_H = \frac{k_{LH}}{k_{LH} + k_{HL}} = \frac{\sqrt{\frac{\pi}{\kappa_H}} e^{-\frac{1}{\epsilon} U(a,x_H)}}{\frac{\pi}{\kappa_L} e^{-\frac{1}{\epsilon} U(a,x_L)} + \frac{\pi}{\kappa_H} e^{-\frac{1}{\epsilon} U(a,x_H)}}. \] (47)

**Appendix C: Derivation of the conditional variance**

According to the main text, the conditional variance \( D(x) \) is defined as

\[ D(x) = Var(X_h | X_0 = x) = E_x (X_h - E_x X_h)^2. \] (48)

To calculate \( D(x) \), we have to first gain some knowledge about the distribution of \( X_h \). To this end, we define a random time

\[ \tau = \tau_{xL} \wedge \tau_{xH} = \min\{\tau_{xL}, \tau_{xH}\}. \] (49)
Clearly, \( \tau \) represents the time needed for the protein level to arrive at either the low level \( x_L \) or the high level \( x_H \) for the first time. We can split \( X_h \) as

\[
X_h = X_\tau + (X_h - X_\tau).
\]

Thus we have

\[
D(x) = Var_x(X_\tau) + Var_x(X_h - X_\tau) + Cov_x(X_\tau, X_h - X_\tau).
\]

We define

\[
p_L(x) = P_x(X_\tau = x_L)
\]
and

\[
p_H(x) = P_x(X_\tau = x_H).
\]

Obviously, \( p_L(x) + p_H(x) = 1 \). Note that \( X_\tau \) is a random variable which equals to \( x_L \) with probability \( p_L(x) \) and equals to \( x_H \) with probability \( p_H(x) \). Thus

\[
Var_x(X_\tau) = E_x((X_\tau - E_x X_\tau)^2 | X_\tau = x_L) p_L(x) + E_x((X_\tau - E_x X_\tau)^2 | X_\tau = x_H) p_H(x)
\]

\[
= (x_L - E_x X_\tau)^2 p_L(x) + (x_H - E_x X_\tau)^2 p_H(x).
\]

Note that

\[
E_x X_\tau = x_L p_L(x) + x_H p_H(x).
\]

Thus we obtain that

\[
Var_x(X_\tau) = ((x_L - x_H) p_H(x))^2 p_L(x) + ((x_H - x_L) p_L(x))^2 p_H(x)
\]

\[
= (x_L - x_H)^2 p_L(x) p_H(x) + (x_H - x_L)^2 p_L(x) p_H(x).
\]

Note further that once the protein level arrives at the low level \( x_L \) or the high level \( x_H \), it will maintain around \( x_L \) or \( x_H \) before phenotypic switches. In this case, \( U'(x) \) can be first-order approximated by

\[
U'(x) = U'(x_L) + \kappa_L (x - x_L) = \kappa_L (x - x_L)
\]

or

\[
U'(x) = U'(x_H) + \kappa_H (x - x_H) = \kappa_H (x - x_H).
\]

Thus conditional on \( \{X_\tau = x_L\} \), the one-dimensional model can be approximated by an OU process

\[
\dot{x} = -\kappa_L (x - x_L) + \sqrt{2\epsilon} \xi_x,
\]

whose steady-state distribution is the normal distribution \( N(x_L, \epsilon/\kappa_L) \), and conditional on \( \{X_\tau = x_H\} \), the one-dimensional model can be approximated by another OU process

\[
\dot{x} = -\kappa_H (x - x_H) + \sqrt{2\epsilon} \xi_x,
\]

whose steady-state distribution is another normal distribution \( N(x_H, \epsilon/\kappa_H) \). Since we have assumed that \( \tau \ll h \ll (T_L \cap T_H) \), we can use the steady-state distribution of the OU process to approximate the distribution of \( X_h - X_\tau \). Thus we have

\[
Var_x(X_h - X_\tau) = Var_x(X_h - X_\tau | X_\tau = x_L) p_L(x) + Var_x(X_h - X_\tau | X_\tau = x_H) p_H(x)
\]

\[
\leq \frac{\epsilon}{\kappa_L} p_L(x) + \frac{\epsilon}{\kappa_L} p_H(x)
\]

\[
= \epsilon \left( \frac{p_L(x)}{\kappa_L} + \frac{p_H(x)}{\kappa_L} \right).
\]

Moreover, by the above OU approximation,

\[
Cov_x(X_\tau, X_h - X_\tau) = E_x(X_\tau - E_x X_\tau)(X_h - X_\tau - E_x(X_h - X_\tau))
\]

\[
= E_x(X_\tau - E_x X_\tau)(X_h - X_\tau - E_x(X_h - X_\tau))|X_\tau
\]

\[
\leq 0.
\]
Combining Equations (56), (61), and (62), the conditional variance \( \mathcal{D}(x) \) can be represented as
\[
\mathcal{D}(x) = (x_H - x_L)^2 p_L(x) p_H(x) + \epsilon \left( \frac{p_L(x)}{\kappa_L} + \frac{p_H(x)}{\kappa_L} \right). \tag{63}
\]
Thus the remaining question is to calculate \( p_L(x) \) and \( p_H(x) \).

By Equation (28), we see that
\[
f(X_t \wedge \tau) - f(X_0) - \int_0^{t \wedge \tau} (\epsilon f'' - U' f')(X_s) ds \tag{64}
\]
is a martingale. Taking expectation in the above equation and letting \( t \to \infty \), we obtain that
\[
E_x f(X_{\tau}) - f(x) = E_x \int_0^{\tau} (\epsilon f'' - U' f')(X_s) ds. \tag{65}
\]
If we can find a function \( f \) so that
\[
\epsilon f'' - U' f' \equiv 0, \tag{66}
\]
then the above equation reduces to
\[
E_x f(X_{\tau}) = f(x). \tag{67}
\]
In other words,
\[
f(x) = f(x_L) p_L(x) + f(x_H) p_H(x). \tag{68}
\]
Noting that \( p_L(x) + p_H(x) = 1 \), we obtain that
\[
p_H(x) = \frac{f(x) - f(x_L)}{f(x_H) - f(x_L)}. \tag{69}
\]
We now solve the second-order ODE (66). Multiplying Equation (66) by \( p_s \), we obtain that
\[
\epsilon f'' p_s - U' f' p_s = 0. \tag{70}
\]
Applying Equation (34), we obtain that
\[
\epsilon (f' p_s)' = \epsilon f'' p_s + \epsilon f' p_s' = 0. \tag{71}
\]
Thus we can choose \( f \) so that
\[
f'(y) p_s(y) \equiv 1. \tag{72}
\]
Thus we have
\[
f'(y) = \frac{1}{p_s(y)}. \tag{73}
\]
Integrating the above equation, we obtain that
\[
f(x) - f(x_L) = \int_{x_L}^x \frac{1}{p_s(y)} dy. \tag{74}
\]
Inserting this result into Equation (69), we obtain that
\[
p_H(x) = \frac{\int_{x_L}^x \frac{1}{p_s(y)} dy}{\int_{x_L}^{x_H} \frac{1}{p_s(y)} dy} = \frac{\int_{x_L}^x e^{-\int_{x_H}^y U(a,y) dy}}{\int_{x_L}^{x_H} e^{-\int_{x_H}^y U(a,y) dy}}. \tag{75}
\]

From Equation (63), we easily see that \( \mathcal{D}(x) \) attains its maximum at a point \( x^* \) satisfying
\[
p_H(x^*) = \frac{1}{2} + \frac{(\kappa_L - \kappa_H) \epsilon}{2(x_H - x_L)^2 \kappa_L \kappa_H}. \tag{76}
\]
Applying the Laplace method, we see that
\[
p_H(x) \to \begin{cases} 0 & \text{if } x < x_M; \\ 1 & \text{if } x > x_M, \end{cases} \tag{77}
\]
as \( \epsilon \to 0 \). Thus the maximum point \( x^* \) of \( \mathcal{D}(x) \) tends to \( x_M \) as \( \epsilon \to 0 \), that is,
\[
\lim_{\epsilon \to 0} x^* = x_M. \tag{78}
\]