Cooperativity in Cellular Biochemical Processes: Noise-Enhanced Sensitivity, Fluctuating Enzyme, Bistability with Nonlinear Feedback, and Other Mechanisms for Sigmoidal Responses

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Abstract

Cooperativity in classical biophysics originates from molecular interactions; nonlinear feedbacks in biochemical networks regulate dynamics inside cells. Using stochastic reaction kinetic theory, we discuss cooperative transitions in cellular biochemical processes at both the macromolecular and the cellular levels. We show that fluctuation-enhanced sensitivity (stochastic focusing) shares an essential feature with the transition in a bistable system. The same theory explains zeroth-order ultrasensitivity with temporal cooperativity. Dynamic cooperativity in fluctuating enzyme (i.e., dynamic disorder), stochastic focusing, and the recently proposed stochastic binary decision all have a shared mechanism: They are generalizations of the hyperbolic response of Michaelis-Menten kinetics $x/(K + x)$, with fluctuating K or stochastic x. Sigmoidal dependence on substrate concentration necessarily yields affinity amplification for competing ligands; both sigmoidal response and affinity amplification exhibit a square law. We suggest two important characteristics in a noise: its multimodal distribution structure and its temporal irreversibility. The former gives rise to self-organized complexity, and the latter contains useful, albeit hidden, free energy that can be utilized for biological functions. There could be structures and energy in biochemical fluctuations.

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INTRODUCTION

Ever since the work of Adair, Monod et al. (59), and Koshland et al. (48) on oxygen binding by hemoglobin and of Schellman (85) and Zimm & Bragg (110) on α-helix formation of polypeptides, the concept of cooperativity has become one of the most important cornerstones of molecular biophysics (33). This concept is now widely used in biology, beyond macromolecular interactions; a special issue of *Nature Chemical Biology* was dedicated to the subject in 2008 (16).

Michaelis-Menten (MM) kinetics: the simplest mathematical model for enzyme kinetics that predicts the rate of steady-state enzyme turnover rate v^{ss} as a hyperbolic function of substrate concentration $[S] : v^{ss} = v_{max}[S]/(K_M + [S])$

Phenomenologically, cooperativity is intimately related to various mathematical expressions known as sigmoidal. It deviates from hyperbolic $ax/(b+x)$, also known as Michaelis-Menten (MM) kinetics and Hill's function. The reason for the central role of $ax/(b+x)$ as noncooperativity lies in the notion of identical, independent subsystems, each having two states, within a system. This is known as Bernoulli trials. For a sequence of N independent, identical, but unfair coins, each with probabilities p and $q = 1 - p$ for heads and tails, respectively, the expected number of heads is $Np/(p+q) = Nz/(1+z)$, where $z = p/q \in [0, \infty)$. Note $ax/(b+x)$ can also be written as $az/(1+z)$ with $z = x/b$.

In chemistry, $z/(1+z)$ is known as the Langmuir-Hill equation. It relates the binding of molecules on a solid surface (macromolecule) to concentration of a medium (ligand) above the surface (macromolecule) at a fixed temperature. A statistical mechanical treatment of this problem based on a binomial distribution follows exactly the probabilistic theory of Jacob Bernoulli, the Swiss who discovered the mathematical constant e in 1683.

Subsystems are no longer independent when there are interactions among them. In physics, strong molecular interactions give rise to phase transitions in macroscopic states of matter (33). Cooperative phenomena are a form of nonlinear behavior; they have long been one of the central issues in structural biology, macromolecular interactions, and cellular regulations. The concept of cooperativity is now considered a paradigm (16).

Open system: classical statistical thermodynamics deals with matters in equilibrium. A cell in homeostasis is sustained by chemical energy input, just as a radio with a battery.

The relation between sigmoidal response curve and allosteric cooperativity is now well understood (32). Sigmoidal response curves also have been observed in many other cellular regulations (47). They are always discussed in contrast to a hyperbolic curve, or MM kinetics:

$$y(x) = \frac{x}{K + x}, \quad (K > 0). \qquad 1.$$

Note this curve has a relative change in the response, y, which is smaller than that in the signal x: $d \ln y / d \ln x \leq 1$; the curvature $d^2 y / dx^2 = -2K/(K + x)^3 < 0$ for all $x \geq 0$. A sigmoidal curve, however, has a region with a positive curvature. For example: $d^2/dx^2(x^2/(K^2 + x^2)) = (2K^2 - 4x^2)/(K^2 + x^2)^3 > 0$ when $x < K/\sqrt{2}$. In fact, it has a square dependence on x for $x \ll K$.

In a small volume such as a cell, signals in terms of biochemical activities fluctuate due to macromolecular copy number fluctuations (87) and/or to macromolecular conformational fluctuations (57). Hence, cellular biochemical signaling is stochastic. With respect to the simple hyperbolic response in Equation 1, a fluctuating enzyme with dynamic disorder has a fluctuating K, and fluctuating copy numbers in a substrate molecule lead to a fluctuating x. In some cases, the fluctuations can lead to the mean response being a sigmoidal function of the mean signal (64, 70, 80). In fact, the distribution of a response can be bimodal while the distribution of the signal has a single maximum (3, 81). Responses in such systems exhibit all-or-none cooperative behavior.

These discoveries have revitalized an interest in noise-induced phenomena (39), which include noise-induced movement in molecular motors (42) and fluctuation-induced oscillation in stochastic resonance (22). Both are emergent phenomena in nonlinear stochastic dynamics of open systems (108). Theories for molecular motors and stochastic resonance have taught us that a fluctuating signal, albeit hidden in a noise, often has both underlying deterministic structures (79) and hidden free energy. These two aspects of a nonequilibrium fluctuation are responsible for many interesting phenomena (81). Feynman had an illuminating discussion on the subject via his famous thermal ratchet (20). Hill (34) has written several books on mesoscopic free energy transductions quantifying the hidden free energy in driven unimolecular fluctuations. We are accustomed to macroscopic organizations and machines performing tasks due to mechanical forces; therefore, realizing the hidden structures and energy in a noise, it is not surprising that noise can lead to mesoscopic organizations and can perform nontrivial biochemical tasks.

With this newfound unifying perspective, this review explores various cooperative phenomena in biophysics. First, we give a coherent account of bistability in chemical kinetics. We assume the readers are familiar with basic chemical kinetics, the law of mass action, and the stochastic approach to chemical kinetics (27, 75). The last is widely known as the Gillespie algorithm; it first appeared as a chemical kinetic theory in Delbrück's work in 1940 (13, 17).

Second, we review equilibrium allosterism, nonequilibrium zeroth-order ultrasensitivity, and non-MM behavior from fluctuating enzyme (dynamic disorder) (58, 70). Fluctuation-induced sensitivity enhancement, also called stochastic focusing, is introduced. Third, we present an in-depth discussion on stochastic focusing, enzyme dynamics disorder, stochastic binary decision and bimodality, and specificity amplification by Hopfield-Ninio kinetic proofreading. Careful readers will notice a transition is accomplished logically from classical biophysical theory of cooperativity to modern systems theory of cellular dynamics based on nonlinear, stochastic biochemical networks with feedbacks. Cooperativity is a form of feedback.

Fourth, the study of stochastic kinetics in mesoscopic biochemical reaction systems naturally leads to a discussion of what noise is in cellular biochemistry. Molecular fluctuations are inherent in each and every biochemical reaction. However, when stochasticity is coupled with nonlinear biochemical reaction networks with feedbacks and chemical driving force(s), nontrivial behaviors emerge. Such behavior can be exploited by a biological organism as a part of its life, thus acquiring a biological function. Finally, the review offers a summary and outlook.

UNISTABILITY AND BISTABLITY IN DETERMINISTIC AND STOCHASTIC DYNAMICS

Some highly cooperative biochemical processes exhibit sharp all-or-none transitions. We now know they are intimately related to the phenomenon of chemical bistability. Because the concept of bistability is not widely taught in elementary biophysics texts, we shall give a brief introduction through a simple example.

Unistability and Bistability in Deterministic Kinetics

One of the most important notions in deterministic nonlinear dynamics is a distinction between unistability and bistability. Let us consider kinetic equations for the following two chemical reaction systems *I* and *II* (75):

$$I : X \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Y; \quad II : X \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Y, \quad X + 2Y \xrightarrow{k_3} 3Y. \qquad 2.$$

According to the law of mass action, the chemical kinetics follow ordinary differential equations (93):

$$I : \frac{dy}{dt} = \mu(1-y) - y; \quad II : \frac{dy}{dt} = y(\mu(1-y)y - 1) + \lambda(1-y), \qquad 3.$$

where $y = [Y]/([X]+[Y])$, $\mu = k_1/k_2$ for system *I* and $\mu = k_3[Y]_{tot}^2/k_2$, $\lambda = k_1/k_2$ for system *II*. **Figure 1** shows the steady state(s) of the two systems as functions of their respective parameter

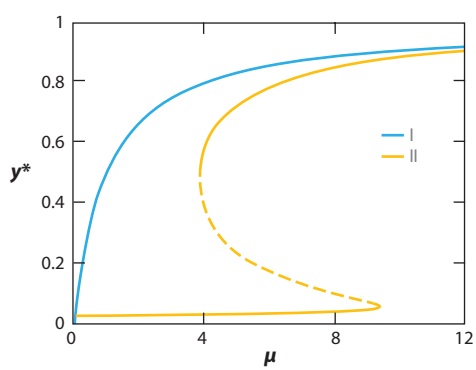

Figure 1

Steady state(s) y^* as functions of μ, according to Equation 3, for systems *I* and *II* in Equation 2. System *I*: $y^* = \mu/(1+\mu)$. System *II* with $\lambda = 0.03$: $y^* \approx \lambda + (\mu - 1)\lambda^2$ for $\mu < 3.88$. For $\mu \in (3.88, 9.39)$, two additional steady states, y_2^* (unstable) and y_3^* (stable), appear. For very small λ, $y_2^*, y_3^* \approx \frac{1}{2} \mp \sqrt{\mu^2 - 4\mu}/(2\mu)$. When $\mu > 9.39$, y_1^* and y_2^* disappear, and $y_3^* \approx 1 - \frac{1}{\mu} - \frac{1-\lambda}{\mu^2}$.

μ. System *I* has a unique steady state $y^* = \mu/(1+\mu)$. For system *II*, when $\mu > 4$, three steady states are the roots of the cubic equation $\mu y^3 - \mu y^2 + (1+\lambda)y - \lambda = 0$.

With increasing μ, e.g., from 0.5 to 5, both systems exhibit a transition from a small to a large steady-state value y^*. In system *I*, y^* changes from 0.33 to 0.83; in system *II*, y^* changes from 0.03 to 0.72. However, if we follow the changing y^* continuously as μ changes, the two systems cannot be more different. System *I* has a single steady state that changes continuously with μ (Equation 5). System *II* shows hysteresis: When μ increases from 0.5 to 10, y^* follows the lower branch and remains at $y_1^* = 0.03$ until $\mu = 9.39$, where y^* jumps upward to 0.87 discontinuously. If μ decreases from 10 to 0.5 with y^* starting on the upper branch, then it will follow the upper branch until $\mu = 3.88$, where y^* jumps downward to 0.03 discontinuously.

Modal value of a random distribution: the most frequently occurring value of a variable

Expected value of a random distribution: the mean of a random variable

We note that transitions of any two individual *X* molecules to *Y* are statistically independent in system *I*. They are not so in system *II*, in which *X* molecules are competing for the *Y* molecules, which act as a catalyst. This is the mechanistic origin of nonlinearity, or cooperativity: When there are more *Y* molecules, the transition of an individual *X* to *Y* is faster, catalyzed by *Y* molecules. Bistability in a chemical or biochemical reaction system is a consequence of strong cooperativity, or positive feedback in the language of network regulations (106). A dynamical system such as system *II* with $\mu \in (3.88, 9.39)$ is called *bistable*: The chemical reaction system inherently has two possible stable steady states, y_1^* and y_3^*. The existence and locations of multiple steady states are emergent phenomena of a nonlinear dynamical system. Which steady state a (macroscopic) system actually adopts depends on its initial condition. In nonlinear dynamical system theory (93), $[0, y_2^*)$ and $(y_2^*, 1]$ are called two basins of attraction associated with y_1^* and y_3^*, respectively.

Unistability and Bistability in Stochastic Kinetics

The biochemical network kinetics in a mesoscopic volume on the order of a cell are stochastic (87, 107). With stochastic fluctuations in the copy numbers of biochemical species in a single cell (104), it is no longer meaningful to consider a steady state, or multiple steady states, as a deterministic chemical composition(s). Rather, a deterministically stable steady state corresponds to a locally most-probable state, i.e., a maximum in a probability distribution. For systems with bistability, the modal values, not the expected value (mean), correspond to the deterministic kinetics.

System *I* in Equation 2 is best described as follows. It contains *N* identical and independent copies of a molecule with two states, *X* and *Y*, with transition rate constants k_1 and k_2 for $X \to Y$ and $Y \to X$, respectively. Each transition of a single molecule is exponentially distributed just as radioactive decay. The steady-state distribution for the number of molecules in state *Y*, n_Y, is therefore binomial:

$$\Pr\{n_Y = \ell\} = \frac{N!}{\ell!(N-\ell)!}\frac{k_1^\ell k_2^{N-\ell}}{(k_1+k_2)^N} = \frac{N!}{\ell!(N-\ell)!}\frac{\mu^\ell}{(1+\mu)^N}. \quad 4.$$

The modal value (i.e., peak) of the distribution, n_Y^*, is between $(N\mu - 1)/(1+\mu)$ and $(N\mu + \mu)/(1+\mu)$. Its expected value is at $\langle n_Y \rangle = N\mu/(1+\mu)$. So for large *N*, n_Y^* and $\langle n_Y \rangle$ are essentially the same. Let $\mu = k_1/k_2$, then one has the fraction of the molecules in state *Y*:

$$y(\mu) = \frac{\langle n_Y \rangle}{N} = \frac{\mu}{1+\mu}. \quad 5.$$

Equations 4 and 5 are the mesoscopic version of the hyperbolic response curve, with the presence of fluctuations. This is the reference against which all cooperativity phenomena are discussed (4, 69). With this in mind (25, 69, 76), we have discussed zeroth-order ultrasensitivity in terms of temporal cooperativity and have shown a critical phenomenon akin to phase transitions in matter (40). One can see a close resemblance between figure 1 of Reference 40 and **Figure 2** below.

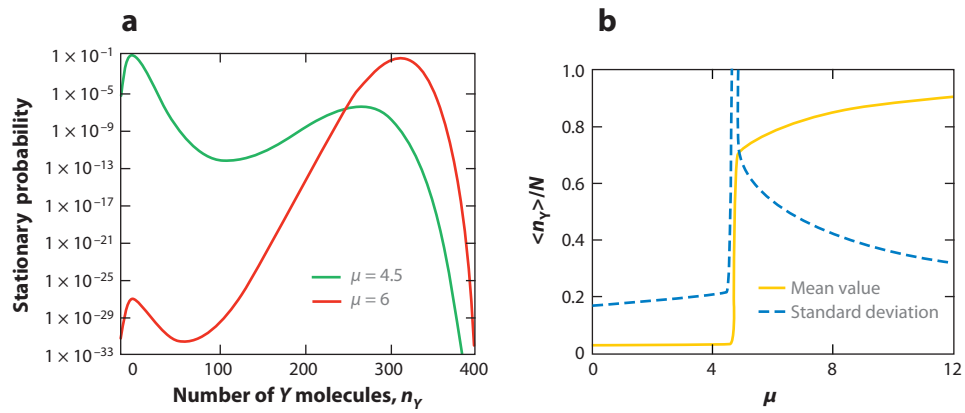

Figure 2

Stochastic kinetics of system *II* (Equation 2) in a small volume: $N = 400$, $\lambda = k_1/k_2 = 0.03$. (*a*) Stationary probability, $\Pr\{n_Y = \ell\}$, for the copy number of Y exhibits bimodality for $\mu = k_3 N^2/(k_2 V^2) = 4.5$ and $\mu = 6$. n_1^* dominates when $\mu = 4.5$ and n_3^* dominates when $\mu = 6$. Noting the logarithmic ordinate, the dominant steady state almost has a probability of 1. (*b*) The mean $\langle n_Y \rangle/N$ and the standard deviation $[(\langle n_Y^2 \rangle - \langle n_Y \rangle^2)/N]^{1/2}$ are shown as functions of μ. Transition in a bistable system is much sharper than hyperbolic transition; it exhibits an almost abrupt jump at some critical value μ^* where the variance reaches a maximum ($\mu^* \approx 4.74$).

One also observes from Equation 4 that its modal value, i.e., the peak of the distribution, increases with the parameter μ, whereas the relative variance $\mathrm{var}[n_Y]/\langle n_Y \rangle^2 = 1/(N\mu)$ decreases with increasing μ. The expected value, i.e., mean of the distribution, follows the modal value. The transition is hyperbolic.

The binomial coefficients in Equation 4, $N!/(\ell!(N-\ell)!)$, represent the proper combinatorial weights for N identical, independent subsystems with two states. Any combinatorial sequence deviating from them implies nonindependence, i.e., interactions and cooperativity, between subunits. For examples, binomial coefficients with $N = 4$ are 1, 4, 6, 4, 1. However, $1, 4\frac{1+cL}{1+L}, 6\frac{1+c^2L}{1+L}, 4\frac{1+c^3L}{1+L}, \frac{1+c^4L}{1+L}$, and $1, 4, 4\eta+2, 4\eta^2, \eta^4, (\eta > 1)$ represent Monod-Wyman-Changeux statistics (48) and Koshland-Némethy-Filmer statistics (59), respectively, for tetrameric hemoglobin oxygen binding (32). Both sequences indicate positive cooperativity.

For N identical subunits, the sequences $1, 1, 1, \ldots, 1, 1$ and $1, 0, 0, \ldots, 0, 1$ also represent cooperativity with corresponding response curves

$$\frac{x - (N+1)x^{N+1} + N x^{N+2}}{N(1-x)(1-x^{N+1})} \quad \text{and} \quad \frac{x^N}{1+x^N}, \qquad 6.$$

and respective Hill's coefficients $(N+2)/3$ and N. Distribution in Equation 4 is associated with binomial coefficient; distribution associated with $1, 1, 1, \ldots, 1, 1$ is truncated geometric $P_n(N, x) = \frac{(1-x)x^n}{1-x^{N+1}}, (n \le N)$. When x increases, $\langle n \rangle$ increases as well, but the distribution $P_n(N, x)$ remains peaked at $n = 0$. This implies that the relative variance increases with x. When x passes a critical value of $x^* = 1$, the peak abruptly jumps to $n = N$. The variance is now decreasing while the $\langle n \rangle$ continues to increase. This describes the celebrated zeroth-order ultrasensitivity (6, 76).

The sequence $1, 0, 0, \ldots, 0, 1$ is widely called all-or-none. It presents a coexistence of two peaks (modal values) in a probability distribution. Transitions in such a system have a different process. Taking system *II* in Equation 2 as an example (**Figure 2**), the three steady states, for each

A BISTABLE STOCHASTIC BIOCHEMICAL SYSTEM

The kinetics of a nonlinear biochemical reaction system with autocatalysis in a mesoscopic volume V,

$$X \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Y, \quad X + 2Y \xrightarrow{k_3} 3Y,$$

can be represented by the stochastic theory of the Delbrück-Gillespie process (54, 71, 73, 75). The number of Y molecules in the reaction system, $n_Y(t)$, is stochastically fluctuating, with forward and backward transition rates between $n_Y = \ell$ and $n_Y = \ell + 1$ being $(k_3\ell(\ell-1)/V^2 + k_1)(N-\ell)$ and $k_2(\ell+1)$, $\ell = 0, 1, \ldots, N-1$. The stationary distribution for the n_Y then is predicted to be

$$\Pr\{n_Y = \ell\} = \frac{C_N N!}{\ell!(N-\ell)!} \prod_{n=0}^{\ell-1} (\hat{\mu} n(n-1) + \lambda),$$

in which N is the total number of Y molecules possible, i.e., $N = V[Y]_{tot}$, C_N is a normalization factor, $\hat{\mu} = k_3/(k_2V^2) = \mu/N^2$, and $\lambda = k_1/k_2$. **Figure 2*a*** shows two bimodal distributions with $\mu = 4.5$ and $\mu = 6$. One can verify that $n_i^* = Vy_i^*$, $i = 1, 3, 2$. When $\mu \notin (3.88, 9.39)$, the distribution is unimodal. The distribution tells us which of the two stable steady states is more probable and by how much. There are two steady states with copy number fluctuations: $n_Y \in [0, n_2^*)$ with mean value $\approx n_1^*$ and $n_Y \in (n_2^*, N]$ with mean value $\approx n_3^*$. Dynamics within each peak region and between two regions are called intra-attractoral and inter-attractoral, respectively.

given $\mu > 4$, in fact correspond to two peaks with a trough in a probability distribution. With an increasing μ, the weight in the peak at x_1^* decreases from 100% to 0, whereas the weight in the peak at x_3^* increases from 0 to 100%. The modal value of the distribution thus changes from x_1^* to x_3^*. In the entire process, the two peak positions move relatively little; it is their relative weight that is changing.

How does one compute the expected value of the distribution given in **Figure 2*a***? This simple question in fact has two different answers, depending on the timescale on which the expected value is computed: There is a great separation of timescales between intra-attractoral and inter-attractoral dynamics. For time shorter than the inter-attractoral dynamics, the expected value depends on the initial conditions as shown in **Figure 1**. For time much longer than the latter, the expected value is a weighted average between the two steady states: $n_Y \in [0, n_2^*)$ and $n_Y \in (n_2^*, N]$; it is unique.

Figure 2*b* shows the expected value of stationary n_Y as a function of μ, with time much longer than the inter-attractoral dynamics, $\langle n_Y \rangle = \sum_{\ell=0}^{N} \ell \Pr\{n_Y = \ell\}$. One observes an almost abrupt transition at a critical value of $\mu^* \approx 4.74$. The variance of the stationary n_Y is dominated not by the fluctuations near each peaks but rather by the large separation between the two peaks. If we neglect the fluctuations within each peak region, and let θ be the relative weight of the two peaks, then we have

$$\langle n_Y \rangle = \frac{n_1^* + \theta n_3^*}{1+\theta}, \quad \mathrm{var}[n_Y] = \frac{\theta}{(1+\theta)^2}(n_1^* - n_3^*)^2. \qquad 7.$$

As a function of θ, Equation 7 seems to be similar to Equation 5. However, **Figure 2*a*** suggests that θ, as a function of μ, changes from 0 to 1 drastically at $\mu = \mu^*$. In fact, the greater N is, the closer $\theta(\mu)$ is to a step function. This leads to the notion of a Maxwell construction in the classical theory of phase transition (23).

Maxwell Construction

Why are the orange curves in **Figure 1** and **Figure 2*b*** so different? Isn't $\langle n_Y \rangle$ in Equation 7 a hyperbolic function? For most cellular biochemical kinetics, the θ in Equation 7 is not a reasonable parameter of the model. As the probability ratio of two emergent steady states of the system, it cannot be experimentally controlled in a simple manner. The following thought experiment illustrates the issues involved.

Consider a biochemical reaction system in a cellular volume V with a bimodal distribution for its key signaling protein Y. Consider a regulatory protein S that can change the relative weights between the two peaks, but not their positions at y_1^* and y_3^*. What does the curve of $\langle n_Y \rangle$ as a function of the number of S, denoted by s, look like?

The answer to this question depends on the volume V and the number of molecules n_Y, even when concentration n_Y/V is given. The relative weight (θ) of the two peaks practically goes to zero for almost all the value of $s < s^*$, and to $\theta \to \infty$ for all the values of $s > s^*$. So, $\langle n_Y \rangle$ in Equation 7 is always at y_1^* when $s < s^*$ and always at y_3^* when $s > s^*$. There is an abrupt transition when $s = s^*$, i.e., $\theta = 1$. This scenario is called the Maxwell construction; Maxwell first introduced a line at the value of s^* (**Figure 1**) when working on gas-to-liquid phase transition in physics (23, 24).

One simple example of this type of transition is a phosphorylation-dephosphorylation cycle with a positive feedback through autocatalytic kinase (23, 24). The sharpness of the transition increases with the number of molecules in the system. At the same time, the timescale for transitions between the two steady states grows to an astronomical magnitude. Therefore, for macroscopic kinetics, the transition between the two steady states ceases to be possible, and the two stable steady states shown in **Figure 1**, with initial value dependence, become meaningful.

One of the most important insights from the stochastic kinetic study is that fluctuations inherent to molecular processes do not disappear in mesoscopic cell-sized nonlinear systems; rather they manifest themselves as biochemical variations on a different timescale. Transitions between these biochemical variants may well be the mechanism for cell differentiation and phenotypic switching (2, 71).

MECHANISMS GENERATING SIGMOIDAL RESPONSES

Classical Theories

The literature on this subject is large; therefore, I give only a brief overview of most of the well-known theories. Interested readers are referred to recent review articles (58, 70, 81) and several excellent monographs (5, 14, 33).

Allosteric cooperativity. The classical allosteric cooperativity that requires interactions between multiple binding sites for ligands is well understood (48, 59). This type of cooperativity is equilibrium physics in nature; its origin is in the molecular interactions between the binding sites via specific protein structural elements that are shaped by evolutionary processes. The interaction energy need not be localized in particular structural groups or through a structural pathway; it can be distributed throughout an entire macromolecule (35). The sharpness of the response is related to the number of interacting sites (5, 32, 33).

Activation with multisteps. Still within the equilibrium binding scenario, this mechanism is less known. If a signal is associated only with the fully bound state of a protein with two (or more)

independent binding sites, then signal $f = \frac{K_1 K_2 x^2}{1+K_1 x+K_2 x+K_1 K_2 x^2}$, where x represents the concentration of the signaling ligand. The curve has a positive curvature at $x = 0$. Furthermore, at the half-saturation point $x_{\frac{1}{2}} = (K_1 + K_2 + \sqrt{(K_1 + K_2)^2 + 4K_1K_2})/(2K_1K_2)$, it has a Hill's coefficient (4)

$$2\left(\frac{d\ln f}{d\ln x}\right)_{f=\frac{1}{2}} = 1 + \frac{1}{1+(K_1+K_2)x_{\frac{1}{2}}}. \qquad 8.$$

It is greater than 1 but always less than $4 - 2\sqrt{2} = 1.17$ (when $K_1 = K_2$). The sharpness of the response is related to the number of steps leading to the function. The Hill's coefficient being less than 1 is often related to $f(x)$ being convex on $x \in [0, x_{\frac{1}{2}}]$.

Nonequilibrium steady state (NESS): a system with a stationary dynamics with time-independent statistics, while continuously converting useful energy into heat

Gunawardena (31) and Wang et al. (102) have discussed in detail this type of biochemical mechanism in cellular regulations. Although the multiple bindings are assumed to be independent, the fact that a cell responds to a signaling protein with different numbers of bound ligand nonlinearly is a form of cooperativity at a higher level. An early example of this is the four-gate model for a potassium channel in Hodgkin-Huxley's dynamic theory of membrane action potential (4).

Zeroth-order ultrasensitivity with temporal cooperativity. This mechanism is based on the driven phosphorylation-dephosphorylation cycle in which the kinase and the phosphatase are highly saturated (6, 28). It was shown to be a nonequilibrium steady (or stationary) state (NESS) mechanism (41, 67, 69) related to a dissipative structure of an open chemical system (60). Although it shares many mathematical similarities with allosterism (33), the physics of the NESS mechanism is fundamentally different from that of the equilibrium mechanisms discussed above.

Qian & Cooper (76) and Ge & Qian (25) have further pinpointed the molecular origin of cooperativity in this system, which they called temporal cooperativity. Copies of the substrates are competing for an enzyme. With the progression of the reaction, the substrate number decreases and the competition lessens. Thus, earlier turnovers help the later turnovers, i.e., the substrates are temporally cooperative. Note that the above argument neglects the competition from the product for the same enzyme. This is precisely the role of the driven system: If the products were equally likely to compete for the enzymes, i.e., the enzymatic reactions were fully reversible, then the temporal cooperativity would disappear (76). Competitive inhibition based on a similar idea was proposed as a mechanism for ultrasensitivity in cellular signaling (46).

Slowly fluctuating enzyme and dynamic cooperativity. The term dynamic cooperativity has a long history in enzymology. It is intimately related to several other terms such as hysteretic behavior (1, 21), mnemonic enzymes (82), and energy relay (37). The phenomena were well documented as non-MM behavior (57, 58): The steady-state turnover rate has a sigmoidal dependence on the substrate concentration. Again, this is a driven NESS phenomenon of an open chemical system (70, 72). Cooperativity stems from competition between two pathways, one fast and one slow, for catalysis. The partition between the two pathways is modulated by the concentration of the substrate, which biases toward the faster pathway (70). Hence, with increasing substrate concentration, the partition increases the probability as well as speeds up the rate of the faster pathway. The enzyme has a certain kind of memory (mnemonic) (37, 82). An attempt to synthesize all the classical ideas has been carried out in Reference 72. The author proposed a new concept, cyclic conformational modification, and pointed out its implications to cellular signal regulations.

Dynamic cooperativity is intimately related to the phenomenon of kinetic proofreading (36, 61, 68). Both exhibit a square law (70): A square dependence on the substrate concentration

occurs in the former and a square dependence on the binding constant occurs in the latter. Note that in a mathematical representation, increasing ligand concentration is equivalent to increasing binding constant. Therefore, a sigmoidal dependence on ligand concentration necessarily leads to a specificity beyond the ratio of binding affinities.

Fluctuation-Induced Sensitivity and Stochastic Focusing

Stochastic focusing (SF) is a recently discovered mechanism for sigmoidal response (7, 64). The intriguing feature of this mechanism is rooted in stochastic fluctuation, while the corresponding deterministic biochemical system only exhibits hyperbolic behavior. The functioning of SF requires the breakdown of detailed balance (7), implying it also has a NESS origin. Although different in their appearance, SF shares certain underlying principles with dynamic cooperativity. Specifically, SF considers the response curve $1/(1+s/K_1)$ with a fluctuating s, the concentration of an inhibitor or repressor. K_1 is a binding parameter. In a fluctuating enzyme with dynamic cooperativity, one considers the turnover kinetics $v_{max}/(1+K_2/[S])$ with a fluctuating K_2. $[S]$ is the substrate concentration. Therefore, the plot of $1/(1+s/K_1)$ against $\log(s)$ and the plot of $v_{max}/(1+K_2/[S])$ against $\log[S]$ have a simple symmetry! Again, in a mathematical representation, fluctuations in ligand concentration differ little from fluctuations in binding affinity.

SF also shares certain features with the recently discussed phenomenon stochastic binary decision (SBD), in which one is concerned with $1/(1+e^b)$ with a fluctuating b. For example, $b=-\Delta G/k_BT$ could represent fluctuations in free energy (3, 81). Indeed, both SF and SBD can exhibit noise-induced bistability when the signal fluctuation is sufficiently slower than response time (38, 81).

FLUCTUATION-INDUCED COOPERATIVITY, SENSITIVITY, AND BISTABILITY

Fluctuation-enhanced (or induced) sensitivity, i.e., stochastic focusing (SF) (7, 64, 65), is a rich molecular regulatory phenomenon that provides insights into a wide range of related cellular biochemical phenomena. In this section, we present a thorough analysis of SF.

We consider a biomolecular signal in terms of the concentration s of a molecular inhibitor. Assuming slow fluctuations in s and rapid response to the signal, one can express the steady-state level of the response q to the slowing varying s:

$$q=\frac{1}{1+s/K}. \qquad 9.$$

Equation 9 is the canonical form for studying SF; note that $1-q=s/(K+s)$ is Equation 1. The inhibitor could be a repressor in transcriptional regulation (63, 64).

Copy Number Fluctuations and Deviations from Poisson Distribution in a Chemical System

To investigate $q(s)$ with fluctuating signal, the first question is the distribution for s in Equation 9. Poisson distribution for molecular number fluctuations can be derived from equilibrium with Gibbs' grand canonical ensemble theory. It can also be derived from a simple mechanistic Delbrück-Gillespie model with a constant synthesis rate and a first-order degradation rate (4, 63). Another widely considered distribution for fluctuating numbers is negative binomial, or

Pólya distribution. It can be obtained if the degradation of X follows the standard MM kinetics (64).

Whereas Poisson distribution can be verified for equilibrium number fluctuations of a grand canonical system, the negative binomial distribution is a consequence of a driven, open-chemical system (see **Supplemental Section 1**; follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**). In Reference 64, truncated geometric

COPY NUMBER FLUCTUATIONS AND POISSON AND PÓLYA DISTRIBUTIONS

Consider a cell-sized biochemical kinetic system, with volume V, that consists of a protein X with constant rate of biosynthesis, J, and first-order degradation process with rate constant q:

$$\text{constant source} \xrightarrow{J} X \xrightarrow{q} \text{degradated}.$$

In terms of the Delbrück-Gillespie stochastic model, the system's transition rate from $\{n_X = \ell\}$ to $\{n_X = \ell + 1\}$ is J and the rate from $\{n_X = \ell + 1\}$ to $\{n_X = \ell\}$ is $q(n_X + 1)$. Then the stationary probability mass function (pmf) for the copy number n_X is a Poisson distribution ($n_X \geq 0$):

$$\Pr\{n_X = \ell\} = \frac{1}{\ell!}\left(\frac{J}{q}\right)^{\ell} e^{-J/q},$$

with an expected value of $\langle n_X \rangle = J/q$.

If the degradation process is catalyzed by an enzyme with very few copies according to the MM mechanism, then the system's transition rate from $\{n_X = \ell + 1\}$ to $\{n_X = \ell\}$ becomes $v_{max}(\ell + 1)/(K_M V + \ell + 1)$ where v_{max} and K_M are the MM parameters for the enzyme. Note that when $(\ell + 1)/V \ll K_M$, the reaction is first order; when $(\ell + 1)/V \gg K_M$, it is zeroth order. The stationary pmf for n_X then follows Pólya distribution (negative binomial):

$$\Pr\{n_X = \ell\} = \frac{\Gamma(\ell + r)}{\ell!\Gamma(r)} p^{\ell}(1 - p)^{r},$$

where $r = K_M V + 1$ and $p = J/v_{max}$. $v_{max} > J$ is necessary for the system to reach a stationary state.

VARIANCES OF NUMBER FLUCTUATIONS IN LINEAR AND NONLINEAR BIOCHEMICAL REACTIONS

The copy number N of a chemical species in exchange with a material reservoir at a constant chemical potential, in a dilute solution, fluctuates following a Poisson distribution: $P_N(n) = \mu^n e^{-\mu}/n!$. Poisson distribution has $\langle N \rangle = \langle (\Delta N)^2 \rangle = \mu$. Therefore we have an interesting relation

$$\langle (\Delta N)^2 \rangle = \left(\frac{1}{\langle N \rangle}\right)^{-1}. \qquad 1.$$

For a unimolecular, linear reaction $A \rightleftharpoons B$ with equilibrium constant K_{eq} in a dilute solution, the copy numbers N_A and N_B fluctuate following binomial distributions, with $p = 1/(1 + K_{eq})$ and $N_A + N_B = N$. We thus have $\langle N_A \rangle = Np$ and $\langle (\Delta N_A)^2 \rangle = Np(1 - p)$. Therefore,

$$\langle (\Delta N_A)^2 \rangle = \langle (\Delta N_B)^2 \rangle = \frac{\langle N_A \rangle \langle N_B \rangle}{\langle N_A \rangle + \langle N_B \rangle} = \left(\frac{1}{\langle N_A \rangle} + \frac{1}{\langle N_B \rangle}\right)^{-1}. \qquad 2.$$

(Continued)

We see that if $\langle N_B \rangle \gg \langle N_A \rangle$, then Equation 2 is reduced to Equation 1: B can be considered as a quasi-static chemical buffer.

Now for a nonlinear chemical reaction $A + B \rightleftharpoons C$ with association constant K_{eq}, such as biochemical binding $E + S \rightleftharpoons ES$, the Delbrück-Gillespie theory predicts

$$\frac{\Pr\{N_C = \ell + 1\}}{\Pr\{N_C = \ell\}} = \frac{K_{eq}(M - \ell)(N - \ell)}{V(\ell + 1)},$$

in which $N_A + N_C = M$ and $N_B + N_C = N$ are constants. Then,

$$\langle(\Delta N_A)^2\rangle^{-1} = \langle(\Delta N_B)^2\rangle^{-1} = \langle(\Delta N_C)^2\rangle^{-1} = \frac{1}{\langle N_A\rangle} + \frac{1}{\langle N_B\rangle} + \frac{1}{\langle N_C\rangle}. \quad 3.$$

We see that if $N \ll M$, for example, B and C are free E and bound ES, then $\langle N_A \rangle \gg \langle N_B \rangle, \langle N_C \rangle$ and Equation 3 is reduced to Equation 2 for a pseudo-first-order reaction $E \rightleftharpoons ES$.

The deterministic kinetics based on the law of mass action for the above three reactions are, respectively,

$$\frac{dc(t)}{dt} = J - kc, \quad c = \frac{N}{V}, \quad \mu = \frac{VJ}{k};$$

$$\frac{dc_A(t)}{dt} = k_-(c_t - c_A) - k_+ c_A, \quad c_A = \frac{N_A}{V}, \quad c_t = \frac{N}{V}, \quad p = \frac{k_+}{k_+ + k_-}; \quad \text{and}$$

$$\frac{dc(t)}{dt} = k_+(c_a - c)(c_b - c) - k_- c, \quad c = \frac{N_C}{V}, \quad c_{a,b} = \frac{M, N}{V}, \quad K_{eq} = \frac{k_+}{k_-}.$$

It is easy to verify that the steady-state concentrations obtained from these kinetic equations correspond to the modal values in the respective probability distributions.

distribution is also used as a possible probability mass function for copy number fluctuations. Truncated geometric distribution arises in zeroth-order ultrasensitivity (6, 76).

To experimentally measure copy number fluctuations, or more generally concentration fluctuations, fluorescence correlation spectroscopy is one of the most feasible biophysical methods (77, 83). Although single-molecule techniques have an ultimate signal-to-noise characteristic of fluctuation measurements, in principle, they do not provide nonlinear information on biochemical reaction systems.

Explaining SF by the EFAZ Mechanism

In this section, we explain how, as a mechanism, the end-effect at zero, or extinction effect at zero (EFAZ), leads to fluctuation-enhanced sensitivity. Similar mechanisms are responsible for several other stochastic effects: Keizer's paradox (95), zeroth-order ultrasensitivity (6, 76), and stochastic bimodality (8) (see below). EFAZ also shares certain features with the sharp transition in bistable systems.

When the modal value and expected value of a fluctuation distribution are far apart, it implies the fluctuation is severely non-normal, and then nontrivial phenomena due to statistics can occur. To illustrate this, let us first consider Equation 9 with a fluctuating s that follows the distribution

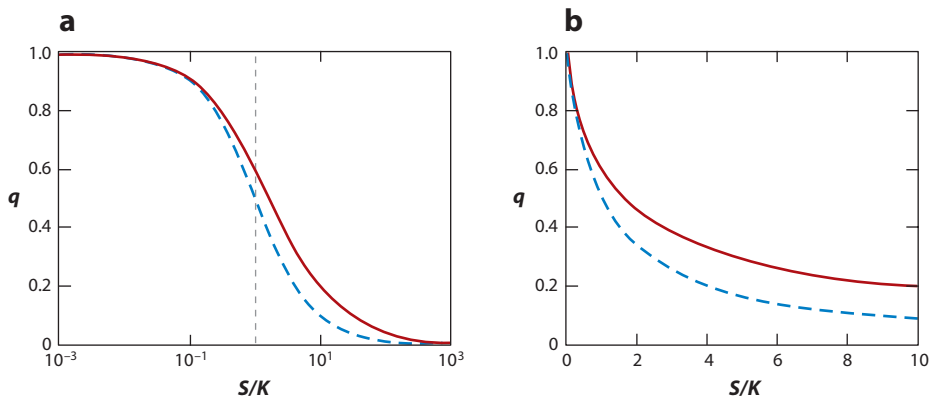

Figure 3

A comparison between the $q(s)$ as a function of s (*blue dashed line*, Equation 9) and the mean $\bar{q}(\langle s \rangle)$ as a function of the mean $\langle s \rangle$ (*red line*, Equation 10), with the distribution for s being $P_s(x) = \mu e^{-\mu x}$ with $\langle s \rangle = 1/\mu$. (*a*) Semilog plot. (*b*) Linear plot.

$P_s(x) = \mu e^{-\mu x}$. Note that with decreasing μ, the expected value, $\langle s \rangle = 1/\mu$, and the modal value, which remains at zero, become farther apart. Therefore, as a function of the $\langle s \rangle$, the mean response

$$\bar{q}(\langle s \rangle) = \int_0^\infty \frac{\mu e^{-\mu x}}{1 + x/K} dx = \int_0^\infty \frac{e^{-y}}{1 + y\langle s \rangle/K} dy. \qquad 10.$$

Figure 3 shows a comparison between Equations 9 and 10.

As we have stated, exponential function is a rather special distribution whose expected value increases with $1/\mu$ while its modal value stays at $s = 0$. In cellular biochemistry, fluctuations in an inhibitory signal s are usually related to the activity of a signaling protein. It has to be a non-negative random variable. Therefore, when the distribution $P_s(s)$ has a very small expected value, it usually peaks at $s = 0$. For example, a Poisson distribution has its peak located at $n = 0$ until its expected value is greater than 1.

Therefore, when the expected value of the signaling protein $\langle s \rangle$ is less than a certain critical value s^*, $\langle s \rangle$ increases but the distribution continues peaking at zero. In this regime, the mean response $\bar{q}$ will be

$$\bar{q} = \left\langle \frac{1}{1 + s/K} \right\rangle > \frac{1}{1 + \langle s \rangle/K}, \quad \text{small } \langle s \rangle. \qquad 11.$$

On the other hand, when the expected value of the signaling protein is greater than the critical value s^*, the peak location (modal value) starts to increase with the expected value while the relative variance decreases, such as in Poisson distribution. Then in this regime

$$\bar{q} = \left\langle \frac{1}{1 + s/K} \right\rangle \approx \frac{1}{1 + \langle s \rangle/K}, \quad \text{large } \langle s \rangle. \qquad 12.$$

Thus, combining the two regimes in Equations 11 and 12, the transition from 1 to 0 in the mean response, $\bar{q}(\langle s \rangle)$, will be steeper than $1/(1 + s/K)$.

In terms of the EFAZ mechanism, the critical value s^* can be determined as the expected value of a distribution $P_s(s)$ whose $P_s(0) = P_s(1)$. Paulsson et al. (64) considered four distributions: (*a*) Poisson, (*b*) binomial, (*c*) Pólya, and (*d*) truncated geometric. Each has its critical expected value: (*a*) $s^* = 1$; (*b*) $s^* = 100/101$ when $N = 100$; (*c*) $s^* = 11$ when $p = 10/11$; and (*d*) $s^* = 500$

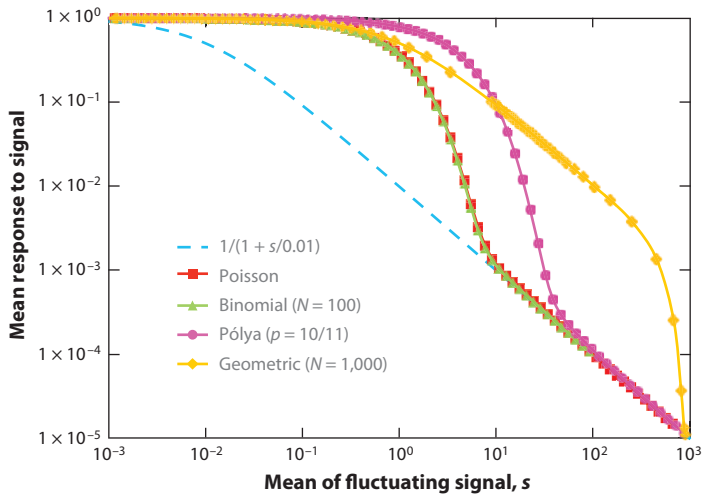

Figure 4

The end-effect at zero (EFAZ) mechanism explains the origin of fluctuation-enhanced sensitivity. The mean response with fluctuating s, $\bar{q} = \langle 1/(1+s/K)\rangle$, has a sharper transition than $1/(1+\langle s\rangle/K)$ does. Results for four distributions are as follows: Poisson; binomial with $N = 100$ and $\langle s\rangle = Np$; Pólya with $p = 10/11$ and $\langle s\rangle = rp/(1-p)$; and truncated geometric with $N = 1{,}000$. The modal values of the distributions depart from $n = 0$ when $s^* = 1{,}100/101{,}11$ and 500, correspondingly. They agree with the sharp transitions shown. All computations use $K = 0.01$. The turquoise dashed line is a hyperbolic curve used for comparison. Parameters are taken from Reference 64.

with $N = 1{,}000$. **Figure 4** convincingly shows that all $\bar{q}(\langle s\rangle)$ sharply decrease at the respective critical s^*. See **Supplemental Section 2** for more discussions.

SF with Possible Bimodality (Bistability)

SF in fact can lead to bimodal distribution for q. We give two examples through which we demonstrate that SBD proposed in Reference 3 is a mechanism intimately related to SF.

Bimodality in $q(s) = 1/(1+s/K)$ with fluctuating s. One can in fact compute the probability density function for q, $P_q(q)$, on the basis of the probability density function for s, $P_s(x)$:

$$P_q(q) = (K/q^2)P_s(K/q - K), \qquad 13.$$

where $0 \le q \le 1$. **Figure 5** shows that for $P_s(x) = \frac{\sqrt{a}}{2}(a+x)^{-3/2}$ $(x \ge 0)$, the distribution for q is bimodal.

In general, we note that the distribution $P_q(q)$ has a maximum at $q = 1$ if $P_s'(0) < -2P_s(0)/K$. On the other hand, the distribution also has a maximum at $q = 0$ if $\lim_{x\to\infty} d\ln P_s(x)/d\ln x > -2$. This condition indicates that the distribution of $P_s(x)$ has to have a fat tail $\sim x^{-2}$ for the bimodality to occur. While this is theoretically interesting, it is not realistic for most cellular processes: Note that the mean value of s does not exist for such fat-tailed distribution.

Bimodality in $y(z) = 1/(1+e^z)$ with fluctuating z. The bimodality in Equation 13 is similar to the SBD problem in References 3 and 81, described as follows. Consider $y = 1/(1+e^z)$ and $1-y$ represent the probabilities of a binary decision. Let z follow a Gaussian distribution with mean μ

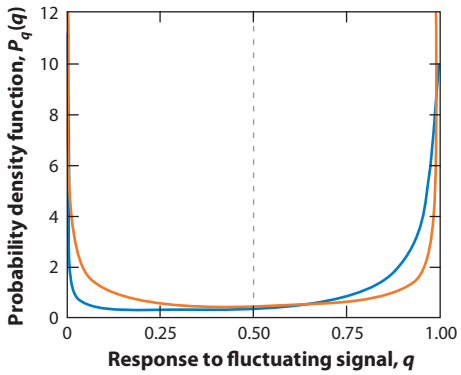

Figure 5

Bimodal distribution for the response $P_q(q)$ with fluctuating signal implies the response is all or none, i.e., either 0% or 100% (3, 81). Blue curve: $q = 1/(1 + s/K)$, where s follows $P_s(x) = \sqrt{a}(a + x)^{-3/2}/2$ with $a/K = 0.05$. In fact, the distribution is bimodal for any $a/K < 3/4$ (see text). Orange curve: $q = 1/(1 + e^z)$, where z follows a Gaussian distribution with mean $\mu = 0$ and variance $\sigma^2 = 10$.

and variance σ^2. Then $x = e^z$ follows a lognormal distribution, and y can also exhibit bimodality:

$$f_y(y) = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left\{-\frac{[\ln(1-y) - \ln y - \mu]^2}{2\sigma^2}\right\}\left(\frac{1}{y(1-y)}\right), \qquad 14.$$

where $y \in [0, 1]$. **Figure 5** shows an example with $\mu = 0$ and $\sigma^2 = 10$.

Stochastic Bimodality and Bistability

We have seen that a macroscopic nonlinear bistable biochemical reaction system corresponds to a stationary bimodal distribution when the same reaction is in a mesoscopic volume. The two peaks are separated by a trough, a unstable steady state, forming two basins of attraction.

For a macroscopic chemical reaction system with one dynamic species, its concentration $x(t)$ follows a differential equation according to the law of mass action: $dx(t)/dt = b(x) - d(x) = r(x)$, where $b(x)$ and $d(x)$ are the formation (birth) and degradation (death) rates. **Figure 6*a*** shows an example with bistability. The same chemical reaction system in a mesoscopic volume V will exhibit concentration fluctuations. Its stationary probability density function for the concentration, $p_x(x, V)$, can be written as $e^{-V\phi(x,V)}$ (4, 71, 73, 75):

$$p_x(x, V) = e^{-V\phi(x,V)}, \quad \text{where} \quad \phi(x, \infty) = -\int_0^x \ln\left(\frac{b(z)}{d(z)}\right) dz. \qquad 15.$$

We see that the minima and maxima of $\phi(x, \infty)$ are where $b(x) = d(x)$, i.e., $r(x) = 0$. In fact, the minima and maxima correspond to the stable and unstable steady states of $dx/dt = r(x)$, one to one, as shown in **Figure 6**. The two basins of attraction, $[0, x_2^*)$ and (x_2^*, ∞), correspond to two wells in the function $\phi(x, \infty)$ (**Figure 6*a***).

What happens if deterministic kinetics has only two, not three, steady states, one stable and one unstable, as shown in **Figure 6*b***? In this case, the deterministic dynamics is not bistable; in fact, it exhibits the phenomenon of extinction: $x_1^* = 0$. However, the corresponding mesoscopic kinetics has a $\phi(x, V)$, as shown in **Figure 6*b***. It has an additional minimum at $x = 0$ for finite V. This leads to a stochastic bistability, or bimodality, that has no deterministic counterpart

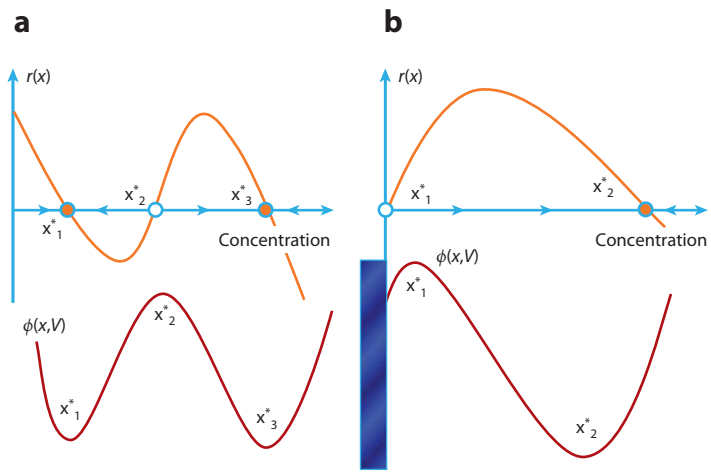

Figure 6

The steady states of nonlinear kinetics can be determined by $dx/dt = r(x) = 0$, the roots of $r(x)$, as illustrated by the orange curves. The blue arrows on the axis represent the direction in which a concentration changes with time. Filled and open circles represent stable and unstable steady states, respectively. In a mesoscopic volume V, the same chemical reaction exhibits concentration (or copy number) fluctuation $p_x(x, V)$. The red curves are $\phi(x, V) = -(1/V)\ln p_x(x, V)$. Noting the minus sign, a maximum (minimum) of $p_x(x, V)$ corresponds to a minimum (maximum) of $\phi(x, V)$. A basin of attraction corresponds to a well in the ϕ function. (*a*) A biochemical reaction system with nonlinear bistability. (*b*) A biochemical reaction system with stochastic bistability: In a bulk solution with $V = \infty$, $x = 0$ is an unstable steady state (i.e., extinction in the language of population dynamics), as shown by the orange curve. However, for small V, there is a region left of the peak of $\phi(x, V)$ (*red curve*). This constitutes another peak in the probability density function $p_x(x, V) = e^{-V\phi(x,v)}$. The dark blue rectangle indicates that x cannot be negative.

(8, 84). In the limit of $V \to \infty$, $\phi(x, V) \to \phi(x, \infty)$, which has the maximum at $x = 0$, as given in Equation 15.

Therefore, there are two types of bistability. With increasing volume V, stochastic bistability disappears whereas bistability due to nonlinear feedbacks emerges. The lifetime of the former decreases with V whereas the lifetime of the latter increases with V. If one makes an analogue between temperature and $1/V$, then stochastic bistability has an entropic barrier and nonlinear bistability has an enthalpic barrier.

Copy Number Distribution in Cell and Stochastic Single Gene Expression

There is now a sizable literature on this subject (see a review on earlier work in Reference 63). Widely observed stochastic transcriptional and translational bursting (29, 107) has been interpreted by various stochastic kinetic models. At the conceptual level, Hornos et al. (38) obtained an analytical solution to a model for a self-regulating gene that consists of a binary gene activation and the copy number for a protein as the gene product. The protein is a repressor for its own gene expression; hence negative feedback contributes a nonlinear term to the kinetics model. Kepler & Elston (44) and Walczak et al. (99) also solved a self-regulating gene model with positive feedback. The gene product, in a dimer form, activates transcription. Both models can predict bistability, i.e., bimodal distributions in the copy number of the protein. The bimodality/bistability can be interpreted as two different isogenetic phenotypes of a biochemical cell. Walczak et al. and Shi & Qian (89) also studied the kinetics of transitions between the two attractors.

In terms of classical kinetics, these models can be written in the form of a pair of ordinary differential equations (ODEs) according to the law of mass action:

$$\frac{dx}{dt} = h(y)(1-x) - fx, \quad \frac{dy}{dt} = (g_0(1-x) + g_1 x) - ky, \qquad 16.$$

where x is concentration of the DNA with bound transcription factor(s) (TFs), and y is the concentration of the TF. One is particularly interested in the three cases of $h(y) = h_o y^\chi$, with $\chi = 0, 1, 2$, corresponding to no self-regulation ($\chi = 0$), feedback with a monomer, and a dimer ($\chi = 1, 2$), respectively. Furthermore, $g_0 < g_1$ means the TF is an activator, and $g_0 > g_1$ means the TF is a repressor. f is the dissociation rate between DNA and the TF; k is the rate constant for protein degradation.

The simplest case of $\chi = 0$ serves as a control: There is a unique steady state for the ODEs in Equation 16. The corresponding stochastic Delbrück-Gillespie model, assuming a single copy of DNA in a volume V, yields a Poissonian stationary distribution for the protein copy number with mean value

$$\frac{fg_0 + hg_1}{(f+h)k} V. \qquad 17.$$

The ODEs in Equation 16, as a kinetic equation for a macroscopic system, correspond to a collection of homogenized cell-free extract that contains a large amount of DNA. A valid comparison between deterministic chemical kinetics and its stochastic counterpart is made when both have the same concentration, i.e., concentration = copy number/volume for a stochastic model. With such a comparison, one can mathematically show that a steady-state concentration of a chemical species, obtained from a system of ODEs, corresponds to the peak of the distribution for the concentration fluctuation in a small system (4, 54, 71, 73, 75). See **Supplemental Section 3** for more discussions.

We note again that Equation 17 is a hyperbolic function of (h/f) if $g_0 = 0$ or, in the form of Equation 9, if $g_1 = 0$. To look into this further, we adopt the model studied by Hornos et al. (38): A single gene can be either in state 1 with the TF bound or in state 0 with the TF unbound. The production rates for the corresponding protein, the TF repressor, are g_1 and $g_0 (\gg g_1)$ and with same degradation rate constant k. The gene state switches from 0 to 1 with rate nh, where n is the copy number of the repressor, and from 1 to 0 with rate f. We note that this mathematical model is intimately related to the motor protein ratchet model, also known as coupled diffusion (81, 89), and to a version of the fluctuating enzyme model (66, 80).

Within this kinetic framework, if the on-and-off gene fluctuations are rapid, then the probability of the gene being on is

$$p_{1|n} = \frac{1}{1 + n/K_d}, \qquad 18.$$

where $K_d = f/h$. If the copy number fluctuation of the repressor is regulated by the gene, i.e., a *cis* regulation, then the copy number fluctuation distribution

$$\frac{\Pr\{repressor\,\# = n+1)\}}{\Pr\{repressor\,\# = n\}} = \frac{K_d \mu_1 + n\mu_0}{(K_d + n)(n+1)}, \qquad 19.$$

in which $\mu_0 = g_0 V/k$ and $\mu_1 = g_1 V/k$. This distribution is not Poissonian; its generating function is a hypergeometric function ${}_1F_1(K_d\mu_1/\mu_0; K_d; \mu_0 z)$. It has only a single peak n^*, as long as $\mu_1 > \mu_0$, which satisfies $(n^*)^2 + (K_d + 1 - \mu_0)n^* - K_d(\mu_1 - 1) = 0$. This equation is essentially the same equation obtained by Shea & Ackers (88) in their pioneering work (38): $(x^*)^2 + (\tilde{K}_d - \tilde{\mu}_0)x^* - \tilde{K}_d\tilde{\mu}_1 = 0$, where $x^* = n^*/V$, $\tilde{K}_d = K_d/V$, and $\tilde{\mu}_j = \mu_j/V$ are bulk biochemical quantities. In the limit of rapid protein-DNA binding and unbinding, the stochastic

self-regulating gene model recovers the deterministic behavior with equilibrium binding between TF protein and DNA (88).

On the other hand, still in the limit of rapid TF protein-DNA binding and unbinding but the transcription is for an independent gene, i.e., a trans-regulation with $\chi = 0$, the problem is precisely that of SF: The level of TF is the signal and the level of gene product is the response. However, with the left-hand side of Equation 18 itself being a probability of a single gene, what is the meaning of its distribution, as discussed in above? This question is addressed below.

SIGNAL AND NOISE IN MESOSCOPIC BIOCHEMICAL DYNAMICS

Probability Distribution of a Probability

We have discussed the probability distribution for the response q in Equation 9 when the signal s is fluctuating. In Equation 18, which has a similar expression, the left-hand side $p_{1|n}$ is the probability of a single gene with a TF bound in a cell, when the number of TF molecules is n. If n is fluctuating, so is $p_{1|n}$. But what does this mean? The answer to this question is closely related, in a fundamental way, to the issue of dynamic disorder in single-molecule enzymology (57, 58, 81, 105). In fact, it gives insights into the important question "What is noise?" and the invaluable perspective of multiple timescales.

Consider two games of a coin toss: In the first game, one is tossing a fair coin, which has 50/50 chance of landing on heads or tails. In the second game, two coins are involved; one coin has 10 to 90 odds of landing on heads and the other coin has 90 to 10 odds of landing on heads. For the second game, a hidden agent is switching the two coins randomly with equal probabilities. Are the two games the same?

The answer depends very much on how often the hidden agent randomly switches the coins. If he does it every time, then the two games will have identical outcomes—the difference cannot be measured. However, if he does it only rarely, then the two games will be different, even though the final tally for a very long run will give exactly 50/50 for heads and tails. The issue concerns adiabatic and nonadiabatic, in the terminology of Hornos et al. (38). In single-molecule enzymology, the slow conformational fluctuation of an enzyme is the hidden agent that randomly switches the affinity of the enzyme for its substrate K_M or turnover rate constant k_{cat}.

With a slow hidden agent, the second game in fact fluctuates between two rather deterministic modes: the head-dominant mode and the tail-dominant mode. This is the meaning of the bimodal distribution in **Figure 5** for p_1 in Equation 18. The emergence of the bimodal distribution should be considered as a self-organization in a highly nonlinear mesoscopic system, rather than simply treated as large fluctuations. See Reference 79 for a more extensive discussion of this viewpoint, and see References 49 and 81 for a philosophical commentary on the relationship between bistability and the notion of complexity in mesoscopic systems.

What Is Noise?

With all the discourse on noise-induced phenomena (39) in recent years in relation to the functions of cellular biochemical regulations, it is timely to ask the naive question, What is noise?

The answer(s) to this question is far from simple. Let us again use a radio as an analogue (50). Using a voltage meter to measure the electrical potential of a node in an opened radio with its power on, one is likely to observe a fluctuating voltage $V(t)$. Over a period of few minutes, the fluctuating $V(t)$ is stationary. If the person is also listening to the speaker, then a correlation

between the signal and the broadcasted music leads to the conclusion that "it is a signal." If the speaker emits only static, then "it is noise." But if the speaker is turned off, how does one know?

Measurements of copy number (or concentration) fluctuations of biological molecules inside a living cell face a similar problem. In fact, one man's signal is another man's noise. A clear correlation between biochemical activity and certain biological functions, therefore, is often the most valuable information.

At room temperature, thermal molecular fluctuation is inevitable. This is the physical origin of intrinsic noise in chemical and biochemical reactions in small systems. However, when this molecular thermal noise is coupled with a nonlinear, driven, open biochemical reaction system, nontrivial behavior emerges and such behavior can be exploited by biological organisms or bioengineering. This is one of the origins of the current fascination with stochastic, nonlinear systems in regards to a growing list of fluctuation-related phenomena: noise-induced transition and Brownian ratchet (11, 42), stochastic resonance (22, 108), fluctuation-enhanced sensitivity (7, 64, 109), noise suppression by noise (65, 97), stochastic bifurcation (8, 81, 84), and noise-induced stabilization (94), to name a few.

More fundamentally in terms of thermodynamics (69, 74), almost all above-mentioned phenomena are nonequilibrium-driven processes with free energy input (11, 34) and should be considered self-organized emergent phenomena with dissipation (60). Nonlinearity, nonequilibrium, and stochasticity are three key elements (78). Their interplay, we suggest, is the essence of complexity in a mesoscopic world of living cells (49, 79, 81).

Concerning a fluctuating, stationary time course, two issues require further elaboration: its structure and its energy. The structure might be hidden in the multimodality of stationary distribution, and the energy resides in the temporal irreversibility (52, 77). Stochastic dynamics sustained under a nonzero chemical potential contains a certain amount of energy that can be utilized to perform chemical work (26, 41, 108).

We use a simple example to illustrate the issue of signal versus noise in a mesoscopic chemical system. This nonlinear reaction system contains two species X and Y in an open environment (79, 96):

$$B \rightarrow Y, \quad Y + 2X \rightarrow 3X, \quad X \rightleftharpoons A, \qquad 20.$$

with chemical potential difference between A and B, $\mu_B - \mu_A \neq 0$. **Figure 7*a,d*** shows the copy number fluctuations of $n_X(t)$ and $n_Y(t)$. A reasonable explanation of this stationary stochastic data is that both $n_X(t)$ and $n_Y(t)$ have a mean value and a variance, both of which are constant over time. They are noise. But in fact, in **Figure 7*b,e*** they are expected macroscopic dynamics of the reaction system in Equation 20, when fluctuations diminish. $n_X(t)$ and $n_Y(t)$ are periodic oscillations. Aided by this realization, a more insightful description of the data in **Figure 7*a,d*** would be a noisy periodic oscillation with a time-dependent mean value and a time-dependent variance, as shown in panels c and f of **Figure 7**, respectively. It is a periodic signal!

Structure in fluctuations. In biophysical measurements of fluctuations, limited by the amount of data, one often computes only statistical quantities such as mean, variance, and time-correlation function. A histogram is a statistical estimation of the entire probability distribution function. A distribution deviating from unimodality cannot be detected from its mean and variance alone. Bimodality or multimodality in a fluctuating system, and a ring-shaped or a Mexican sombrero-like distribution for a pair of fluctuating concentrations, are emergent structures in stochastic dynamics. To characterize such structures, Wang et al. (101) proposed a potential and flux landscape to account for both of the global features in nonequilibrium stationary dynamics. There is a growing

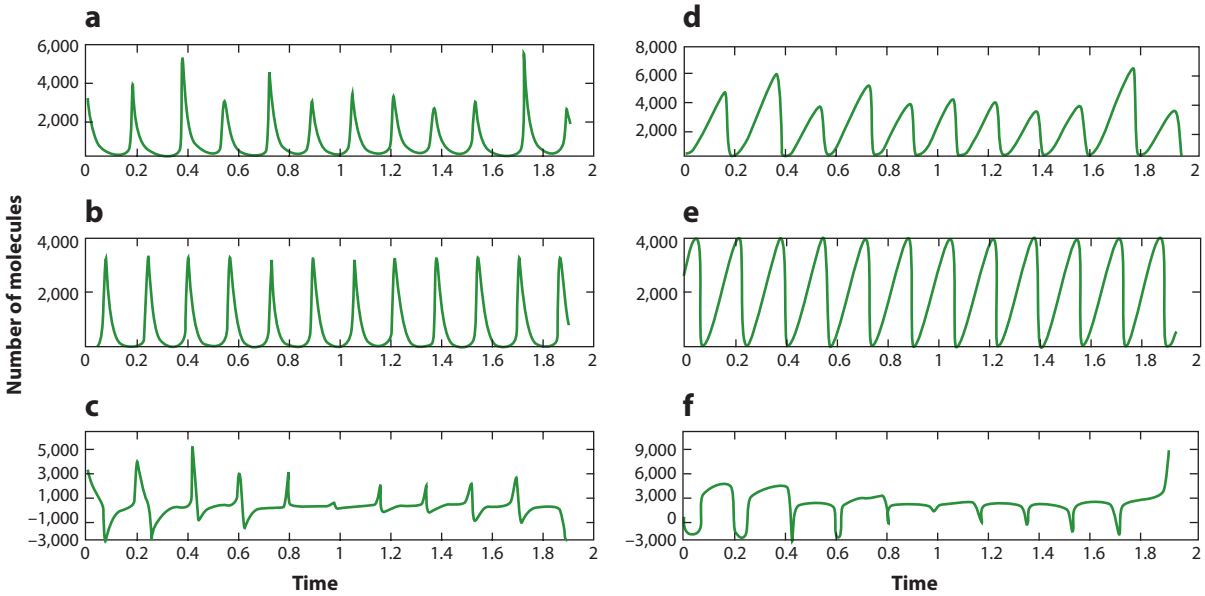

Figure 7

Nonlinear chemical oscillation in a reaction system (Equation 20) in a mesoscopic volume exhibiting fluctuating $n_X(t)$ and $n_Y(t)$, shown in panels *a* and *d*. The corresponding deterministic dynamics in a bulk solution is a periodic chemical oscillation as shown in panels *b* and *e*. The differences between panels *a* and *b* and panels *d* and *e* are shown in panels *c* and *f*. Without knowing the macroscopic behavior, a reasonable description of panels *a* and *d* would be noise. But with the realization of the corresponding macroscopic behavior, panels *a* and *d* should be considered as signals with temporal complexity (79).

awareness of the importance of the stationary distribution of a biochemical reaction system as a landscape (10, 71, 100).

Energy in a noise. Equilibrium concentration fluctuation as a function of time is symmetric with respect to time reversal (41, 69). Time-irreversible stationary concentration fluctuation without detailed balance implies a chemical driving force is at play (77). Turning this statement around, any time-irreversible stationary noise contains a certain amount of energy that can be utilized. This idea is at the heart of the Brownian ratchet theory. By utilizing chemical energy, a molecular system need not obey Boltzmann's law: An insight from Hopfield-Ninio kinetic proofreading is that a state with higher internal energy could have greater probability. (An even earlier work by Overhauser on nuclear spin polarization provided the possibility for inverted nuclear spin population due to microwave irradiation.) Although the term kinetic proofreading has been widely taught in general molecular biology, the deep insight into the necessity of energy expenditure, unfortunately, is often lost. In fact, it is sometime misinterpreted, to the great dismay of Ninio (62), in terms of a macromolecular structural mechanism, which it could not be.

Stochastic Nonlinear Kinetics of Prokaryotic and Eukaryotic Cellular Systems

For a concrete biological function, sensitivity and specificity are aspects of a biochemical response to a molecular signal(s). One of the extensively studied systems of stimulus-response coupling in cellular biochemistry is the two-component signaling system: how prokaryotic organisms sense and respond to changes in their environment (30, 92). Kierzek et al. (45) developed a stochastic

model and observed both all-or-none and graded responses depending on model parameters. See **Supplemental Section 4** for more discussions on the two-component system.

Stochastic, nonlinear kinetic models have been developed for various cellular and subcellular biochemical systems. In addition to the two-component signaling, Miller et al. (56) studied the bistability of a stochastic CaMKII (calmodulin-dependent protein kinase II) switch in single neuronal cells. Dodd et al. (15) studied epigenetic cell memory based on nucleosome modification. Cao et al. (10) studied the heritability and robustness of the lysogenic state of λ-phage. Skupin et al. (90) showed how cellular calcium signaling arises from single-channel protein fluctuations.

An all-or-none bistability is also observed in lymphoid cells (12). Interestingly, Smith (91) had proposed earlier a quantal theory for immunity that shares some of the key features of multistability. Cağatay et al. (9) suggested that *Bacillus subtilis* discriminates different biochemical circuits on the basis of their stochastic fluctuations (86) and demonstrated a stochastic switch between its two fates: sporulation and competence. Kar et al. (43) studied the eukaryotic cell cycle and explored the role of fluctuations from biochemical signaling as well as stochastic variations from unequal cell division. Wang et al. (100) characterized cell cycle stability and robustness in budding yeast in terms of a landscape theory. Levine & Hwa (51) studied general theory of stochastic fluctuations in metabolic pathways.

Another interesting system is cell fate switching in *Xenopus* oocytes (18, 106). Even though several studies on highly simplified caricatures of this system have already appeared (19, 24, 78), a full stochastic kinetic model that predicts a distribution for the switching time remains to be developed.

SUMMARY AND OUTLOOK

Regulation is a key notion in cellular molecular biology. To carry out regulations in molecular terms, different parts of a biological macromolecule have to communicate via molecular interactions. This gives rise to allosteric cooperativity. Hyperbolic response is usually a consequence of a system containing identical, independent subsystems with two states. Cooperativity leads to nonlinear behavior; it often exhibits sigmoidal non-MM responses. Understanding the mechanisms for cooperativity in terms of structures and thermodynamics has been a central theme of molecular biophysics, from allosterism to protein folding. Enzyme kinetics operating inside a living cell is in a NESS; biochemical regulations also can be achieved by dynamic cooperativity (1, 21, 37).

Regulations in cellular biochemical processes involve a network of signaling molecules with nonlinear reactions. Feedback is a widely used term that represents a related concept such as cooperativity to a macromolecule: Ultrasensitivity corresponds to positive cooperativity, and adaptation could be related to negative cooperativity (47, 55). Hence, it is not surprising that sensitivity in a response is also discussed with respect to hyperbolic function and Hill's coefficient. This review aims to provide both of these phenomena in a unified theoretical framework in terms of stochastic nonlinear biochemical kinetics.

Nonlinearity is not a term widely used in molecular biophysics. However, in a cellular biochemical reaction network, nonlinear reactions lead to a wide range of new cooperative phenomena, among which the most important is bistability in deterministic kinetics and its corresponding bimodal distribution in a mesoscopic volume. Bistability leads to abrupt transitions. The Hill's coefficients for such a transition are proportional to the number of molecules in a system. In the macroscopic limit, the transition is truly discontinuous, similar to a first-order phase-transition.

In terms of the hyperbolic, or MM function $y = x/(K + x)$, SF considers $1 - y = 1/(1 + x/K)$ with a fluctuating x, and dynamic cooperativity of fluctuating enzyme considers a fluctuating K. Both lead to non-MM, sigmoidal behaviors. For some particular forms of fluctuating x or K, y

can be bimodal, producing a purely SBD (3). A sigmoidal (e.g., square) dependence on substrate concentration x in a fluctuating enzyme also leads to a specificity amplification with respect to competing ligands with square law, similar to Hopfield-Ninio kinetic proofreading (70). Enhanced sensitivities to variations in substrate concentration (sigmoidal response) and to affinity (kinetic proofreading) are complementary.

Cellular biochemical processes have always been treated as deterministic machines in terms of sequence of events (98). Rapid development of single-molecule and single-cell biophysics (53, 104) has brought stochastic dynamics to the forefront of cell biology. See References 54, 71, 73, and 75 for recent reviews of the Delbrück-Gillespie process approach to cellular biochemical systems. As a successor of molecular biophysics that focused on macromolecular structure, equilibrium statistical thermodynamics, and relaxation kinetics, chemical biophysics (4) studies cellular biochemical systems in terms of reaction networks, NESS thermodynamics, and nonlinear stochastic kinetics. It is an analytical tool for a systems approach to cell biology (103). Nonlinearity, nonequilibrium, and stochasticity are three key elements of dynamics at the cellular and subcellular levels. Their interplay is the essence of complexity in a mesoscopic world of living cells (49).

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LITERATURE CITED

1. Ainslie GR, Shill JP, Neet KE. 1972. Transients and cooperativity: a slow transition model for relating transients and cooperative kinetics of enzymes. *J. Biol. Chem.* 247:7088–96
2. **Ao P, Galas D, Hood L, Zhu X. 2008. Cancer as robust intrinsic state of endogenous molecular-cellular network shaped by evolution. *Med. Hypoth.* 70:678–84**
3. Artyomov MN, Das J, Kardar M, Chakraborty AK. 2007. Purely stochastic binary decisions in cell signaling models without underlying deterministic bistabilities. *Proc. Natl. Acad. Sci. USA* 104:18958–63
4. Beard DA, Qian H. 2008. *Chemical Biophysics: Quantitative Analysis of Cellular Systems*. Cambridge, UK: Cambridge Univ. Press
5. Ben-Naim A. 2010. *Cooperativity and Regulation in Biochemical Processes*. New York: Kluwer Academic
6. Berg OG, Paulsson J, Ehrenberg M. 2000. Fluctuations and quality of control in biological cells: zero-order ultrasensitivity reinvestigated. *Biophys. J.* 79:1228–36
7. **Berg OG, Paulsson J, Ehrenberg M. 2000. Fluctuations in repressor control: thermodynamic constraints on stochastic focusing. *Biophys. J.* 79:2944–53**
8. Bishop LM, Qian H. 2010. Stochastic bistability and bifurcation in a mesoscopic signaling system with autocatalytic kinase. *Biophys. J.* 98:1–11
9. Cağatay T, Turcotte M, Elowitz MB, Garcia-Ojalvo J, Süel GM. 2009. Architecture-dependent noise discriminates functionally analogous differentiation circuits. *Cell* 139:512–22
10. Cao Y, Lu H-M, Liang J. 2010. Probability landscape of heritable and robust epigenetic state of lysogeny in phage lambda. *Proc. Natl. Acad. Sci. USA* 107:18445–50

2. Suggests that cancerous states might already exist in a normal cell, analogous to the denatured state of a protein under native conditions.

7. One of the original papers that proposed the mechanism of stochastic focusing (see also Reference 64).

11. Chen Y-D. 1987. Asymmetry and external noise-induced free energy transduction. *Proc. Natl. Acad. Sci. USA* 84:729–33
12. Das J, Ho M, Zikherman J, Govern C, Yang M, et al. 2009. Digital signaling and hysteresis characterize Ras activation in lymphoid cells. *Cell* 136:337–51
13. Delbrück M. 1940. Statistical fluctuations in autocatalytic reactions. *J. Chem. Phys.* 8:120–24
14. Di Cera E. 2005. *Thermodynamic Theory of Site-Specific Binding Processes in Biological Macromolecules*. Cambridge, UK: Cambridge Univ. Press
15. Dodd IB, Micheelsen MA, Sneppen K, Thon G. 2007. Theoretical analysis of epigenetic cell memory by nucleosome modification. *Cell* 129:813–22
16. Editorial. 2008. Capturing cooperativity. *Nat. Chem. Biol.* 4:433–47
17. Érdi P, Tóth J. 1989. *Mathematical Methods of Chemical Reactions: Theory and Applications of Deterministic and Stochastic Models*. Manchester, UK: Manchester Univ. Press
18. Ferrell JE, Machleder EM. 1998. The biochemical basis of an all-or-none cell fate switch in *Xenopus* oocytes. *Science* 280:895–98
19. Ferrell JE, Xiong W. 2001. Bistability in cell signaling: how to make continuous processes discontinuous, and reversible processes irreversible. *Chaos* 11:227–36
20. Feynman RP, Leighton RB, Sands M. 1963. Ratchet and pawl. In *The Feynman Lectures on Physics*, Vol. 1, pp. 46-1–46-9. Reading, MA: Addison-Wesley
21. Frieden C. 1970. Kinetic aspects of regulation of metabolic processes: the hysteretic enzyme concept. *J. Biol. Chem.* 245:5788–99
22. Gammaitoni L, Hänggi P, Jung P, Marchesoni F. 1998. Stochastic resonance. *Rev. Mod. Phys.* 70:223–87
23. Ge H, Qian H. 2009. Thermodynamic limit of a nonequilibrium steady-state: Maxwell-type construction for a bistable biochemical system. *Phys. Rev. Lett.* 103:148103
24. Ge H, Qian H. 2011. Nonequilibrium phase transition in mesoscopic biochemical systems: from stochastic to nonlinear dynamics and beyond. *J. R. Soc. Interf.* 8:107–16
25. Ge H, Qian M. 2008. Sensitivity amplification in the phosphorylation-dephosphorylation cycle: nonequilibrium steady states, chemical master equation and temporal cooperativity. *J. Chem. Phys.* 129:015104
26. Ge H, Qian M, Qian H. 2012. Stochastic theory of nonequilibrium steady states. Part II: applications in chemical biophysics. *Phys. Rep.* 510:87–118
27. Gillespie DT. 2007. Stochastic simulation of chemical kinetics. *Annu. Rev. Phys. Chem.* 58:35–55
28. **Goldbeter A, Koshland DE. 1981. An amplified sensitivity arising from covalent modification in biological systems. *Proc. Natl. Acad. Sci. USA* 78:6840–44**
29. Golding I, Cox EC. 2006. Protein synthesis molecule by molecule. *Genome Biol.* 7:212–13
30. Goulian M. 2010. Two-component signaling circuit structure and properties. *Curr. Opin. Microbiol.* 13:184–89
31. Gunawardena J. 2005. Multisite protein phosphorylation makes a good threshold but can be a poor switch. *Proc. Natl. Acad. Sci. USA* 102:14617–22
32. Hammes GG. 1982. *Enzyme Catalysis and Regulation*. New York: Academic
33. **Hill TL. 1985. *Cooperativity Theory in Biochemistry*. New York: Springer-Verlag**
34. Hill TL. 1989. *Free Energy Transduction and Biochemical Cycle Kinetics*. New York: Springer-Verlag
35. Hopfield JJ. 1973. Relation between structure, cooperativity and spectra in a model of hemoglobin action. *J. Mol. Biol.* 77:207–22
36. Hopfield JJ. 1974. Kinetic proofreading: a new mechanism for reducing errors in biosynthetic processes requiring high specificity. *Proc. Natl. Acad. Sci. USA* 71:4135–39
37. Hopfield JJ. 1980. The energy relay: a proofreading scheme based on dynamic cooperativity and lacking all characteristic symptoms of kinetic proofreading in DNA replication and protein synthesis. *Proc. Natl. Acad. Sci. USA* 77:5248–52
38. Hornos JEM, Schultz D, Innocentini GC, Wang J, Walczak AM, et al. 2005. Self-regulating gene: an exact solution. *Phys. Rev. E.* 72:051907
39. Horsthemke W, Lefever R. 1984. *Noise-Induced Transitions: Theory, Applications in Physics, Chemistry, and Biology*. Springer Ser. Synergetics, Vol. 15. Berlin: Springer-Verlag
40. Huang Q, Qian H. 2011. The dynamics of zeroth-order ultrasensitivity: a critical phenomenon in cell biology. *Discr. Cont. Dyn. Sys. S* 4:1457–64

28. Proposes the mechanism of zeroth-order ultrasensitivity.

33. A underappreciated, lucid monograph on theoretical treatment of cooperative phenomena in biochemistry with the rigor of statistical physics (see also Reference 5).

41. Jiang D-Q, Qian M, Qian M-P. 2004. *Mathematical Theory of Nonequilibrium Steady States. Lect. Notes Math.*, Vol. 1833. New York: Springer
42. Jülicher F, Ajdari A, Prost J. 1997. Modeling molecular motors. *Rev. Mod. Phys.* 69:1269–81
43. Kar S, Baumann WT, Paul MR, Tyson JJ. 2009. Exploring the roles of noise in the eukaryotic cell cycle. *Proc. Natl. Acad. Sci. USA* 106:6471–76
44. Kepler TB, Elston TC. 2001. Stochasticity in transcriptional regulation: origins, consequences, and mathematical representations. *Biophys. J.* 81:3116–36
45. Kierzek AM, Zhou L, Wanner BL. 2010. Stochastic kinetic model of two component system signalling reveals all-or-none, graded and mixed mode stochastic switching responses. *Mol. Biosyst.* 6:531–42
46. Kim SY, Ferrell JE. 2007. Substrate competition as a source of ultrasensitivity in the inactivation of Wee1. *Cell* 128:1133–45
47. Koshland DE, Goldbeter A, Stock JB. 1982. Amplification and adaptation in regulatory and sensory systems. *Science* 217:220–25
48. Koshland DE, Némethy G, Filmer D. 1966. Comparison of experimental binding data and theoretical models in proteins containing subunits. *Biochemistry* 5:365–85
49. Laughlin RB, Pines D, Schmalian J, Stojković BP, Wolynes PG. 2000. The middle way. *Proc. Natl. Acad. Sci. USA* 97:32–37
50. Lazebnik Y. 2002. Can a biologist fix a radio? Or, what I learned while studying apoptosis. *Cancer Cell* 2:179–82
51. Levine E, Hwa T. 2007. Stochastic fluctuations in metabolic pathways. *Proc. Natl. Acad. Sci. USA* 104:9224–29
52. Lewis GN. 1930. The symmetry of time in physics. *Science* 71:569–77
53. Li G-W, Xie XS. 2011. Central dogma at the single-molecule level in living cells. *Nature* 475:308–15
54. Liang J, Qian H. 2010. Computational cellular dynamics based on the chemical master equation: a challenge for understanding complexity (survey). *J. Comput. Sci. Technol.* 25:154–68
55. Mello BA, Tu Y. 2003. Perfect and near-perfect adaptation in a model of bacterial chemotaxis. *Biophys. J.* 84:2943–56
56. Miller P, Zhabotinsky AM, Lisman JE, Wang X-J. 2005. The stability of a stochastic CaMKII switch: dependence on the number of enzyme molecules and protein turnover. *PLoS Biol.* 3:e107
57. Min W, Gopich IV, English BP, Kou SC, Xie XS, Szabo A. 2006. When does the Michaelis-Menten equation hold for fluctuating enzymes? *J. Phys. Chem. B* 110:20093–97
58. Min W, Jiang L, Xie XS. 2010. Complex kinetics of fluctuating enzymes: phase diagram characterization of a minimal kinetic scheme. *Chem. Asian J.* 5:1129–38
59. Monod J, Wyman J, Changeux J-P. 1965. On the nature of allosteric transitions: a plausible model. *J. Mol. Biol.* 12:88–118
60. Nicolis G, Prigogine I. 1977. *Self-Organization in Nonequilibrium Systems: From Dissipative Structures to Order Through Fluctuations.* New York: Wiley
61. **Ninio J. 1975. Kinetic amplification of enzyme discrimination. *Biochimie* 57:587–95**
62. Ninio J. 2005. Contributions to the kinetic theory of accuracy. **http://www.lps.ens.fr/~ninio/Accuracy-kinetic.html**
63. **Paulsson J. 2005. Models of stochastic gene expression. *Phys. Life Rev.* 2:157–75**
64. Paulsson J, Berg OG, Ehrenberg M. 2000. Stochastic focusing: fluctuation-enhanced sensitivity of intracellular regulation. *Proc. Natl. Acad. Sci. USA* 97:7148–53
65. Paulsson J, Ehrenberg M. 2000. Random signal fluctuation can reduce random fluctuations in regulated components of chemical regulatory networks. *Phys. Rev. Lett.* 84:5447–50
66. Qian H. 2002. Equations for stochastic macromolecular mechanics of single proteins: equilibrium fluctuations, transient kinetics and nonequilibrium steady-state. *J. Phys. Chem. B* 106:2065–73
67. Qian H. 2003. Thermodynamic and kinetic analysis of sensitivity amplification in biological signal transduction. *Biophys. Chem.* 105:585–93
68. Qian H. 2006. Reducing intrinsic biochemical noise in cells and its thermodynamic limit. *J. Mol. Biol.* 362:387–92
69. Qian H. 2007. Phosphorylation energy hypothesis: open chemical systems and their biological functions. *Annu. Rev. Phys. Chem.* 58:113–42

61. One of the two original papers that proposed the theory of kinetic proofreading (see also Reference 36).

63. A comprehensive review of the theory of stochastic gene expression.

70. Qian H. 2008. Cooperativity and specificity in enzyme kinetics: a single-molecule time-based perspective. *Biophys. J.* 95:10–17
71. Qian H. 2010. Cellular biology in terms of stochastic nonlinear biochemical dynamics: emergent properties, isogenetic variations and chemical system inheritability. *J. Stat. Phys.* 141:990–1013
72. Qian H. 2010. Cyclic conformational modification of an enzyme: serial engagement, energy relay, hysteretic enzyme, and Fischer's hypothesis. *J. Phys. Chem. B* 114:16105–11
73. Qian H. 2011. Nonlinear stochastic dynamics of mesoscopic homogeneous biochemical reactions systems - an analytical theory. *Nonlinearity* 24:R19–49
74. Qian H, Beard DA. 2005. Thermodynamics of stoichiometric biochemical networks in living systems far from equilibrium. *Biophys. Chem.* 114:213–20
75. Qian H, Bishop LM. 2010. The chemical master equation approach to nonequilibrium steady-state of open biochemical systems: linear single-molecule enzyme kinetics and nonlinear biochemical reaction networks. *Int. J. Mol. Sci.* 11:3472–500
76. Qian H, Cooper JA. 2008. Temporal cooperativity and sensitivity amplification in biological signal transduction. *Biochemistry* 47:2211–20
77. Qian H, Elson EL. 2004. Fluorescence correlation spectroscopy with high-order and dual-color correlation to probe nonequilibrium steady states. *Proc. Natl. Acad. Sci. USA* 101:2828–33
78. Qian H, Reluga TC. 2005. Nonequilibrium thermodynamics and nonlinear kinetics in a cellular signaling switch. *Phys. Rev. Lett.* 94:028101
79. Qian H, Saffarian S, Elson EL. 2002. Concentration fluctuations in a mesoscopic oscillating chemical reaction system. *Proc. Natl. Acad. Sci. USA* 99:10376–81
80. Qian H, Shi P-Z. 2009. Fluctuating enzyme and its biological functions: positive cooperativity without multiple states. *J. Phys. Chem. B* 113:2225–30
81. Qian H, Shi P-Z, Xing J. 2009. Stochastic bifurcation, slow fluctuations, and bistability as an origin of biochemical complexity. *Phys. Chem. Chem. Phys.* 11:4861–70
82. Ricard J, Cornish-Bowden A. 1987. Cooperative and allosteric enzymes: 20 years on. *Eur. J. Biochem.* 166:255–72
83. Rigler R, Elson EL, eds. 2001. *Fluorescence Correlation Spectroscopy: Theory and Applications.* Springer Ser. Chem. Phys., Vol. 65. New York: Springer
84. Samoilov M, Plyasunov S, Arkin AP. 2005. Stochastic amplification and signaling in enzymatic futile cycles through noise-induced bistability with oscillations. *Proc. Natl. Acad. Sci. USA* 102:2310–15
85. Schellman JA. 1958. The factors affecting the stability of hydrogen-bonded polypeptide structures in solution. *J. Phys. Chem.* 62:1485–94
86. Schultz D, Wolynes PG, Ben Jacob E, Onuchic JN. 2009. Deciding fate in adverse times: sporulation and competence in *Bacillus subtilis*. *Proc. Natl. Acad. Sci. USA* 106:21027–34
87. Shahrezaei V, Swain PS. 2008. The stochastic nature of biochemical networks. *Curr. Opin. Biotechnol.* 19:369–74
88. Shea MA, Ackers GK. 1985. The OR control system of bacteriophage lambda. A physical-chemical model for gene regulation. *J. Mol. Biol.* 181:211–30
89. Shi P-Z, Qian H. 2011. A perturbation analysis of rate theory of self-regulating genes and signaling networks. *J. Chem. Phys.* 134:065104
90. Skupin A, Kettenmann H, Falcke M. 2010. Calcium signals driven by single channel noise. *PLoS Comp. Biol.* 6:e1000870
91. Smith KA. 2006. The quantal theory of immunity. *Cell Res.* 16:11–19
92. Stock AM, Robinson VL, Goudreau PN. 2000. Two-component signal transduction. *Annu. Rev. Biochem.* 69:183–215
93. Strogatz SH. 1994. *Nonlinear Dynamics and Chaos: With Applications to Physics, Biology, Chemistry, and Engineering*. Boston: Westview Press
94. Turcotte M, Garcia-Ojalvo J, Süel GM. 2008. A genetic timer through noise-induced stabilization of an unstable state. *Proc. Natl. Acad. Sci. USA* 105:15732–37
95. Vellela M, Qian H. 2007. A quasistationary analysis of a stochastic chemical reaction: Keizer's paradox. *Bull. Math. Biol.* 69:1727–46

72. Synthesizes four different mechanisms in cellular regulations on the basis of enzyme conformational dynamics in an open chemical system, and proposes the new concept of cyclic conformational modification.

82. A historical review of the cooperativity in enzyme kinetics combining both theoretical and experimental perspectives.

88. This classical paper on lambda phage biology contains thermodynamic and kinetic modeling and has become the standard reference for modeling stochastic gene expression in recent years.

96. Vellela M, Qian H. 2010. On Poincaré-Hill cycle map of rotational random walk: locating stochastic limit cycle in reversible Schnakenberg model. *Proc. R. Sci. A.* 466:771–88
97. Vilar JMG, Rubi JM. 2001. Noise suppression by noise. *Phys. Rev. Lett.* 86:950–53
98. von Hippel PH. 2007. From "simple" DNA-protein interactions to the macromolecular machines of gene expression. *Annu. Rev. Biophys. Biomol. Struct.* 36:79–105
99. Walczak AM, Onuchic JN, Wolynes PG. 2005. Absolute rate theories of epigenetic stability. *Proc. Natl. Acad. Sci. USA* 102:18926–31
100. Wang J, Li C, Wang E. 2010. Potential and flux landscapes quantify the stability and robustness of budding yeast cell cycle network. *Proc. Natl. Acad. Sci. USA* 107:8195–200
101. Wang J, Xu L, Wang E. 2008. Potential landscape and flux framework of nonequilibrium networks: robustness, dissipation, and coherence of biochemical oscillations. *Proc. Natl. Acad. Sci. USA* 105:12271–76
102. Wang L, Nie Q, Enciso G. 2010. Non-essential sites improve phosphorylation switch. *Biophys. J.* 99:L41–43
103. Westerhoff HV, Palsson BO. 2004. The evolution of molecular biology into systems biology. *Nat. Biotechnol.* 22:1249–52
104. Xie XS, Choi PJ, Li G-W, Lee NK, Lia G. 2008. Single-molecule approach to molecular biology in living bacterial cells. *Annu. Rev. Biophys.* 37:417–44
105. Xie XS, Lu HP. 1999. Single-molecule enzymology. *J. Biol. Chem.* 274:15967–70
106. Xiong W, Ferrell JE. 2003. A positive-feedback-based bistable 'memory module' that governs a cell fate decision. *Nature* 426:460–65
107. Yu J, Xiao J, Ren X, Lao K, Xie XS. 2006. Probing gene expression in live *E. coli* cells: one molecule at a time. *Science* 311:1600–3
108. Zhang X-J, Qian H, Qian M. 2012. Stochastic theory of nonequilibrium steady states and its applications. Part I. *Phys. Rep.* 510:1–86
109. Zhu L, Frenkel D, Bolhuis PG. 2011. Role of fluctuations in ligand binding cooperativity of membrane receptors. *Phys. Rev. Lett.* 106:168103
110. Zimm BH, Bragg JK. 1959. Theory of the phase transition between helix and random coil in polypeptide chains. *J. Chem. Phys.* 31:526–31

108. A comprehensive two-part review of the modern mathematical theory of NESS together with applications to molecular motor, stochastic resonance, and a variety of biophysical systems problems (see also References 26 and 41).

Supplemental Materials for *Cooperativity in Cellular Biochemical Processes: Noise-Enhanced Sensitivity, Fluctuating Enzyme, Bistability with Nonlinear Feedback, and Other Mechanisms for Sigmoidal Responses*

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S1 Poisson and Pólya distributions

The Gibbs' grand canonical ensemble is absolute for equilibrium fluctuations of particle numbers. While Poisson distribution can be derived for equilibrium number fluctuations of a grand canonical system, the negative binomial distribution has to be a consequence of a driven, open-chemical system.

To show this, one needs to realize that a fundamental physical principle of enzyme catalysis is that an enzyme has to catalyze both forward and backward steps of a reversible chemical reaction. Hence, a more complete, thermodynamically correct kinetic scheme for biosynthesis and degradation with Michaelis-Menten kinetics is

$$A \underset{q_-}{\overset{q_+}{\rightleftharpoons}} X, \;\; E + X \rightleftharpoons EX \rightleftharpoons E + B, \tag{S1}$$

in which A and B represent a source and a sink with constant chemical potentials. If we denote the parameters for reversible enzyme by forward and reversed Michaelis constants $\tilde{K}_M^f$, $\tilde{K}_M^r$, and forward and reversed maximal velocities v_{max}^f and v_{max}^r, then the stationary distribution

$$\frac{\Pr\{n_X = \ell + 1\}}{\Pr\{n_X = \ell\}} = \frac{q_+ n_A + \frac{v_{max}^r n_B / K_M^r}{1 + (\ell+1)/K_M^f + n_B/K_M^r}}{q_-(\ell+1) + \frac{v_{max}^f (\ell+1)/K_M^f}{1 + (\ell+1)/K_M^f + n_B/K_M^r}}, \tag{S2}$$

in which $K_M^{f,r} = \tilde{K}_M^{f,r} V$. There is a chemical potential difference between the source for X biosynthesis and the sink for its degradation

$$\Delta G = k_B T \ln \gamma, \quad \gamma = \frac{n_A q_+ \left(v_{max}^f / K_M^f \right)}{q_- \left(v_{max}^r / K_M^r \right) n_B}. \tag{S3}$$

Thus in the limit of vanishing n_B, the enzyme catalyzed kinetics becomes irreversible:

$$\begin{aligned} \frac{\Pr\{n_X = \ell + 1\}}{\Pr\{n_X = \ell\}} &= \left(\frac{q_+ n_A}{q_-(\ell+1)} \right) \frac{1 + \frac{1}{\gamma} \frac{v_{max}^f / (K_M^f q_-)}{1 + (\ell+1)/K_M^f + n_B / K_M^r}}{1 + \frac{v_{max}^f / (K_M^f q_-)}{1 + (\ell+1)/K_M^f + n_B / K_M^r}} \\ &\approx \left(\frac{q_+ n_A}{q_-(\ell+1)} \right) \frac{1 + \frac{1}{\gamma} \frac{v_{max}^f / q_-}{K_M^f + (\ell+1)}}{1 + \frac{v_{max}^f / q_-}{K_M^f + (\ell+1)}}. \end{aligned} \tag{S4}$$

Eq. S4 is novel. It allows one to exam the role of nonequilibrium thermodynamics in copy number fluctuations in a living biochemical system such as a cell. We see that if $\gamma = 1$, then the distribution in Eq. S4 becomes Poissonian with $\langle n_X \rangle = (q_+ n_A / q_-)$! Eq. S4 is not reduced to negative binomial if only $\gamma = \infty$: There is a "competition" between first-order q_- and v_{max}^f. If q_- is further negligibly small, $q_- \ll v_{max}^f / (K_M^f + \ell + 1)$, then the distribution indeed becomes negative binomial.

S2 Stochastic focusing, bistability and EFAZ mechanism

The end-effect at zero (EFAZ) mechanism contains an important element of bistability (all-or-none): In an authentic, canonical bistable system, the distribution of s, $P_s(s)$, has two peaks, one near $s = 0$ and another near $s = s_{max}$. With the increasing of the mean signal $\langle s \rangle$, the peak probability near $s = 0$ decreases while the one near s_{max} increases. The locations of the two peaks move very little. The EFAZ effect we described, therefore, shares a part of the mechanism for a sharp transition in a bimodal (bistable) system. The same mechanism is also at work in zeroth-order ultrasensitivity.

Fig. S1 however, shows that the mean responses with fluctuating signal, while has an enhanced sensitivity, did not yield a sigmoidal shape. In fact, it is easy to verify, from Eq. 11, that for small s,

$$\overline{q}(s) = \sum_{j=0}^{\infty} \left(-\frac{s}{K} \right)^j \int_0^{\infty} y^n e^{-y} dy = 1 - \frac{s}{K} + \frac{2s^2}{K^2} + \cdots \tag{S5}$$

That is, $1 - \overline{q}(s)$ has a negative curvature at $s = 0$, not positive.

Although the transition curves in Fig. 4 and Fig. S1 are not sigmoidal, are they more "cooperative" than a hyperbolic function? How to address this question? Motivated by the results in Fig. 2B, let us consider the relative variance of the response q with fluctuating signal. Note that for a Bernoulli trial with mean $z/(1+z)$, the variance

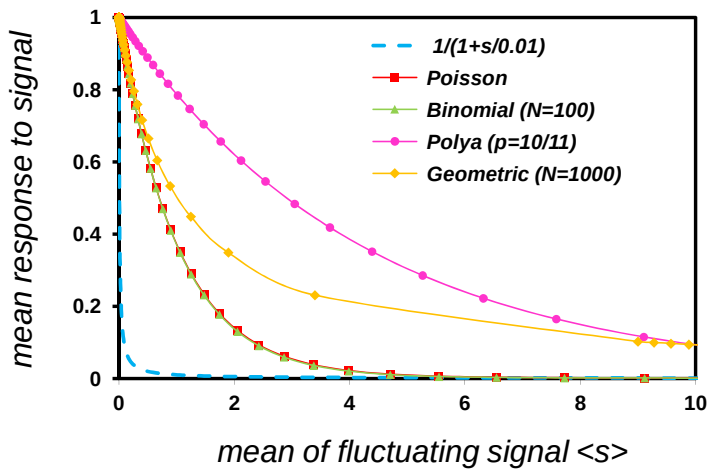

Figure S1: The linear plot of Fig. 4 in the main text. The mechanism of "end-effect at zero" (EFAZ) explains the origin of fluctuation enhanced sensitivity: The mean response with fluctuating s in SF, $\overline{q} = \langle 1/(1+s/K)\rangle$, has a sharper transition when compared with $1/(1+\langle s\rangle/K)$. Results from four distributions are shown: Poisson, binomial with $N = 100$ and $\langle s\rangle = Np$, Polya with $p = 10/11$ and $\langle s\rangle = rp/(1-p)$, truncated geometric distribution with $N = 1000$. The peak positions of the distributions depart from $n = 0$ when $s^* = 1, 100/101, 11$ and 500, correspondingly. They agree with the sharp transitions shown. All computations use $K = 0.01$.

is $z/(1+z)^2$. Hence the relative variance is $1/z$, which monotonically decreases with z. This is in sharp contrast to a highly cooperative transition in which relative variance is maximum at transition mid-point.

Fig. S2 shows SF with Poisson fluctuating signal indeed has a maximum in the relative variance, while for non-fluctuating $1/(1+s/K)$, the relative variance s/K is monotonic.

S3 Kinetic model for gene expression: mRNA and protein copy numbers

Delbrück-Gillespie stochastic kinetic model for gene expression corresponding to the differential equations in Eq. (18) predicts the stationary distributions for the protein copy number being bimodal for both $\chi = 1, g_0 > g_1$ (Hornos *et al.*, 2005) and $\chi = 2, g_0 < g_1$ (Kepler and Elston, 2001; Walczak *et al.*, 2005). From the ODEs in Eq. (18), however, the bistability disappears in the first model since $h(y)$ is a monotonic increasing function. The second model has $g_1 > g_0$ and $h(y) = h_o y^2$. It can be shown

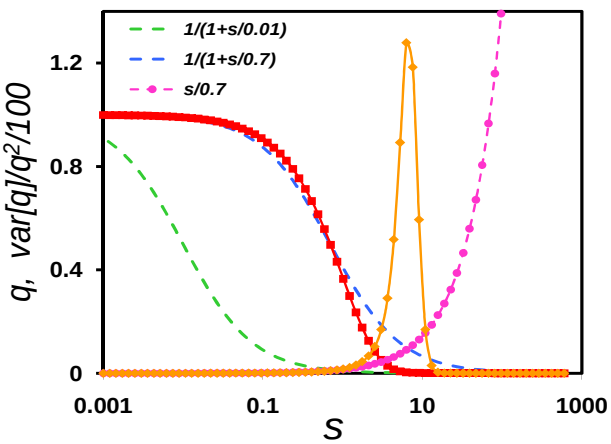

Figure S2: Green and blue dased lines are $q = 1/(1 + s/K)$ withe $K = 0.01$ and $K = 0.7$, respectively. Red line is the mean $\overline{q} = \langle 1/(1 + s/0.01)\rangle$ with fluctuating s following Poisson distribution. Compared with the green dashed line, there is a sensitivity amplification. Furthermore, compared with the blue dashed line, which has a same mid-transition point at $s = 0.71$, the $\overline{q}(s)$ is indeed sharper. The pink and orange curves are the relative variance corresponding to blue and red curves (re-scaled with a factor of 0.01.) The pink curve represents relative variance in the standard hyperbolic response. Orange curve indicates that SF indeed exhibits certain "cooperative" characteristics.

that the system of Eq. (18), when parameters

$$\left(\frac{k}{g_0}\sqrt{f/h_o}, \frac{g_1}{g_0}\right) \tag{S6}$$

is in the region bound by the parametric curve

$$\left\{\frac{2}{z(1-z^2)}, \frac{1+3z^2}{z^2(1-z^2)} \,\middle|\, 0 \le z \le 1\right\}, \tag{S7}$$

has three steady states in the positive quadrant, two stable and one unstable. In fact, in the macroscopic limit, a Maxwell construction emerges. Mathematically, we note that the first model has a quadratic nonlinearity (when $h(y) \propto y$) and the second model has a cubic nonlinearity. The kinetic system of self-regulating gene is nearly equivalent to the kinetic system of phosphorylation-dephosphorylation signaling networks with feedbacks (Bishop and Qian, 2010; Shi and Qian, 2011). More recently, Shahrezaei *et al.* (2008) further studied a kinetic model with binary gene activation, together with copy numbers for both the mRNA and the proteins, but without feedback.

Shahrezaei and Swain (2008) studied a kinetic model with binary gene activation, together with copy numbers for both the mRNA and the proteins, but without feedback.

This is a linear system very much similar to the Goodwin's deterministic equation for Central Dogma, first proposed in 1965 (Murray, 2007):

$$\frac{dx}{dt} = k_0(1-x) - k_1 x,\ \frac{dy}{dt} = v_0 x - d_0 y,\ \frac{dz}{dt} = v_1 y - d_1 z, \tag{S8}$$

where x, y, z are concentrations of the activated gene, mRNA, and protein, respectively. This is a further development of the two-variable, no-feedback model studied by Kepler and Elston (2001). The generating function for the stationary distribution is predicted to be in the form of hypergeometric functions ${}_2F_1(-,-;-;-)$. Stochastic Delbrück-Gillespie approach to Goodwin's model has also been studied in connection to oscillatory testosterone levels in blood (Heuett and Qian, 2006).

S4 Two-component signaling system

One of the most extensively studied systems of stimulus-response coupling mechanism in cellular biochemistry is the two-component system, which has been found widely in prokaryotic organisms sensing and responding to changes in their environmental conditions (Goulian, 2010; Stock *et al.*, 2000).

Two-component systems consist of a trans-membrane histidine kinase whose extracellular domain senses environmental stimulus, usually in the form of hormone-like ligands, and signals a change in the levels of expression of certain genes, via a second intracellular *response regulator*. The system is called "two-component" because the sensing histidine kinase and the response regulator are two proteins, in contrast to one-component system such as the membrane tyrosin kinase signaling with homodimers and trans-autophosphorylation (Schlessinger, 1986; Cooper and Qian, 2008).

In these systems, the sequential biochemical events are as follows: Ligand binding leads to the transfer of a phosphoryl group from an ATP to a histidine residue in a histidine kinase, which in turn catalyses the transfer of the phosphate on the phosphorylated histidine to aspartic acid residues on the response regulator. The phosphorylation of the response regulator yields a conformation change which activates target gene expression. One example is *E. coli*'s EnvZ/OmpR osmoregulation system that controls the differential expression of the outer membrane porin proteins OmpF and OmpC.

S5 Additional references

[1] Adair GS. 1925. The hemoglobin system: VI. The oxygen dissociation curve of hemoglobin. *J. Biol. Chem.* 63:529–545

[2] Alberts B. 1998. The cell as a collection of protein machines: Preparing the next generation of molecular biologists. *Cell* 92:291–294

[3] Alon U. 2006. *An Introduction to Systems Biology: Design Principles of Biological Circuits*. New York: Chapman and Hall/CRC

[4] Ao P. 2008. Emerging of stochastic dynamical equalities and steady state thermodynamics from Darwinian dynamics. *Comm. Theoret. Phys.* 49:1073–1090

[5] Ao P. 2009. Global view of bionetwork dynamics: Adaptive landscape. *J. Genet. Genom.* 36:63–73

[6] Ao P, Kwon C, Qian H. 2007. On the existence of potential landscape in the evolution of complex systems. *Complexity* 12:19–27

[7] Artyomov MN, Mathur M, Samoilov MS, Chakraborty AK. 2009. Stochastic bimodalities in deterministically monostable reversible chemical networks due to network topology reduction. *J. Chem. Phys.* 131:195103

[8] Astumian RD. 1997. Thermodynamics and kinetics of a Brownian motor. *Science* 276:917–922

[9] Colquhoun D. 2006. The quantitative analysis of drug-receptor interactions: A short history. *Trends Pharm. Sci.* 27:149-157

[10] Cooper JA, Qian H. 2008. A mechanism for Src kinase-dependent signaling by noncatalytic receptors (New Concepts). *Biochem.* 47:56815688

[11] Elson EL, Webb WW. 1975. Concentration correlation spectroscopy: A new biophysical probe based on occupation number fluctuations. *Annu. Rev. Biophys. Bioengr.* 4:311–334

[12] Ferrell JE. 2009. Q & A: Cooperativity. *BMC J. Biol.* 8:53

[13] Formaneck M, Ma L, Cui Q. 2006. Reconciling the "old" and "new" views of protein allostery: A molecular simulation study of chemotaxis Y protein (CheY). *Proteins*, 63:846–867

[14] Heuett WJ, Qian H. 2006. A stochastic model of oscillatory blood testosterone levels. *Bulle. Math. Biol.* 68:1383–1399

[15] Hill AV. 1910. The possible effects of the aggregation of the molecules of hæmoglobin on its dissociation curves. *J. Physiol.* 40(Suppl.):iv–vii

[16] Hill TL. 1977. *Free Energy Transduction in Biology: The Steady-State Kinetic and Thermodynamic Formalism*. New York: Academic Press

[17] Kim YC, Furchtgott LA, Hummer G. 2009. Biological proton pumping in an oscillating electric field. *Phys. Rev. Lett.* 103:268102

[18] Koshland DE, Hamadani K. 2002. Proteomics and models for enzyme cooperativity. *J. Biol. Chem.* 277:46841–46844

[19] Langmuir I. 1916. The constitution and fundamental properties of solids and liquids. Part I. Solids. *J. Am. Chem. Soc.* 38:2221–2295

[20] Lewis GN. 1925. A new principle of equilibrium. *Proc. Natl. Acad. Sci. USA* 11:179–183

[21] Meadows D. 2008. *Thinking in Systems: A Primer*. New York: Chelsea Green Publishing

[22] Miller CA, Beard DA. 2008. The effects of reversibility and noise on stochastic phosphorylation cycles and cascades. *Biophys. J.* 95:2183–2192

[23] Murray JD. 2007. *Mathematical Biology I: An Introduction.* New York: Springer. 3rd ed

[24] Qian H. 2000. The mathematical theory of molecular motor movement and chemomechanical energy transduction. *J. Math. Chem.* 27:219–234

[25] Qian H. 2005. Cycle kinetics, steady-state thermodynamics and motors – A paradigm for living matter physics. *J. Phys. Cond. Matt.* 17:S3783–S3794

[26] Qian H. 2006. Open-system nonequilibrium steady-state: Statistical thermodynamics, fluctuations and chemical oscillations (Feature Article). *J. Phys. Chem. B* 110:15063–15074

[27] Qian H, Chan SI. 1999. Hydrogen exchange kinetics of proteins in denaturants: A generalized two-process model. *J. Mole. Biol.* 286:607–616

[28] Schlessinger J. 1986. Allosteric regulation of the epidermal growth factor receptor kinase. *J. Cell. Biol.* 103:2067–2072

[29] Schrödinger E. 1944. *What is Life?* UK: Cambridge Univ. Press

[30] Shahrezaei V, Ollivier JF, Swain PS. 2008. Colored extrinsic fluctuations and stochastic gene expression. *Mol. Syst. Biol.* 4:196

[31] Shi P-Z, Qian H. 2010. Irreversible stochastic processes, coupled diffusions and systems biochemistry. In *Frontiers in Computational and Systems Biology*, eds. J-F Feng, W-J Fu, F-Z Sun, pp. 175–201. New York: Springer-Verlag

[32] Taylor HM, Karlin S. 1998. *An Introduction to Stochastic Modeling*. New York: Academic Press. 3rd ed

[33] Tsong TY, Astumian RD. 1988. Electroconformational coupling: How membrane-bound ATPase transduces energy from dynamic electric fields. *Annu. Rev. Physiol.* 50:273–290

[34] van Kampen NG. 2007. *Stochastic Processes in Physics and Chemistry*. Amsterdam: North-Holland. 3rd ed

[35] Wyman J, Gill SJ. 1990. *Binding and Linkage: Functional Chemistry of Biological Macromolecules*. Mill Vally, CA: Univ. Sci. Books

[36] Xie XS, Yu J, Yang WY. 2006. Living cells as test tubes. *Science* 312:228–230

[37] Zhang Y, Ge H, Qian H. 2010. van't Hoff-Arrhenius analysis of mesoscopic and macroscopic dynamics of simple biochemical systems: Stochastic vs. nonlinear bistabilities. Ithaca, NY: Cornell Univ. Library. http://arxiv.org/abs/1011.2554

[38] Zhou H-X. 2010. From induced fit to conformational selection: A continuum of binding mechanism controlled by the timescale of conformational transitions. *Biophys. J.* 98:L15–L17