FEATURE ARTICLE

Open-System Nonequilibrium Steady State: Statistical Thermodynamics, Fluctuations, and Chemical Oscillations

Hong Qian

Department of Applied Mathematics, University of Washington, Seattle, Washington 98195

Received: March 24, 2006; In Final Form: May 25, 2006

Gibbsian equilibrium statistical thermodynamics is the theoretical foundation for isothermal, closed chemical, and biochemical reaction systems. This theory, however, is not applicable to most biochemical reactions in living cells, which exhibit a range of interesting phenomena such as free energy transduction, temporal and spatial complexity, and kinetic proofreading. In this article, a nonequilibrium statistical thermodynamic theory based on stochastic kinetics is introduced, mainly through a series of examples: single-molecule enzyme kinetics, nonlinear chemical oscillation, molecular motor, biochemical switch, and specificity amplification. The case studies illustrate an emerging theory for the isothermal nonequilibrium steady state of open systems.

1. Introduction

To traditional chemists, a biological cell is a chemical reaction system as complex as one can imagine. Still, no matter how complex a chemical system is, if it is left alone in a test tube, it gradually approaches a chemical equilibrium. In biology, an equilibrium state is dead; in physics, it is the least organized according to the second law of thermodynamics.[1] There are many important characteristics of an equilibrium. Here we highlight a few. First, there can be no sustained net energy conversion of one form to another; every process in fact has an equally probable reverse process. This is known as the principle of detailed balance.[2] Second, the fluctuating stationary molecular system is reversible in time in a statistical sense. This statement becomes increasingly relevant because single-molecule spectroscopies have become commonplace in physical chemistry. And third, the most celebrated one, the probability for the fluctuating molecular system follows the Gibbs distribution. This last statement, translated to simple chemical terms, means there is a unique equilibrium constant for every chemical reaction in a system, irrespective of how complex the system is.

All the above statements are embodied in the fundamental theory of equilibrium statistical thermodynamics.[3] The subject is introduced to every chemistry undergraduate in a physical chemistry class. But to think about the physical chemistry of a living cell, one realizes that we are dealing with a scenario that is completely different from all that was said above. In fact, the most important thing to a biochemist studying living cells is to maintain a "cell culture".[4] That is, he or she has to regularly change the medium in which cells grow.

If not complexity, then what is the difference between a set of reactions in a test tube and in a living cell? The answer is that the former is in a closed system with chemical isolation, while the latter is open to exchange with its environment, both in chemical energy and in materials. If the exchange with its surroundings is sustained, then an open system usually approaches a steady state that is not an equilibrium. The most distinguished characteristics of a nonequilibrium steady-state (NESS) is that it has nonzero fluxes and nonzero chemical potential gradients in the system. It converts chemical energy into heat.[5−7] Chemical reaction systems in NESS can process information and generate spatial patterns; they are the chemical basis of cellular signal transduction[8,9] and biological morphogenesis.[10−12]

The focus of this article is to present an introductory theory of NESS with fluctuations. A more comprehensive review is forthcoming.[13] We believe that the pedagogically most effective exposition starts with several simple examples, which we shall present in Section 2. In Section 3, we establish the statistical thermodynamics in terms of the Smoluchowski equation that characterizes stochastic dynamics of molecular systems. According to Kramers' theory, the Smoluchowski equation is the theoretical basis of chemical rate equations.[14,15] In Section 4, we review the general theory of chemical reactions in a closed system and show several key results that are pertinent to our discussion. In Section 5, a recently developed application of NESS theory to complex networks of chemical reactions in terms of their stoichiometry is presented. In particular, we establish the analogue of Kirchhoff's current and loop laws for chemical reaction networks. In Section 6, we illustrate three key applications of the theory of NESS to current biology: the kinetic proofreading mechanism for specificity amplification, biochemical switches and their energy expenditure, and motor proteins and their chemomechanics. Section 7 concludes the paper with a discussion.

2. Three Examples

In this section, we give three simple examples of biochemical reaction systems in NESS with increasing complexity. We provide kinetic as well as thermodynamic analyses. While the former is routinely carried out, the latter is not. Through elementary mathematics, these examples illustrate a novel nonequilibrium statistical thermodynamic theory and show how it is applied.

10.1021/jp061858z CCC: $33.50
Published on Web 07/11/2006

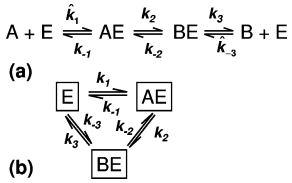

Figure 1. Kinetic scheme of a simple reversible enzyme reaction (a) in which $\hat{k}_1$ and $\hat{k}_{-3}$ are second-order rate constants. From the perspective of a single enzyme molecule, the reaction is unimolecular and cyclic (b). The pseudo-first-order rate constants $k_1 = \hat{k}_1 c_A$ and $k_{-3} = \hat{k}_{-3} c_B$ where c_A and c_B are the concentrations of substrate A and product B. With sustained concentrations of c_A and c_B, the cyclic reaction has a steady-state nonzero cycle flux if and only if $k_1k_2k_3/(k_{-1}k_{-2}k_{-3}) \neq 1$.

2.1. Single Enzyme Kinetics: Cycle Flux and NESS. We first consider the enzyme reaction shown in Figure 1a, in which the rate constants $\hat{k}_1$ and $\hat{k}_{-3}$ are second order. If there is only one enzyme molecule, then from the enzyme perspective, the kinetics are stochastic and cyclic, as shown in Figure 1b, with pseudo-first-order rate constants $k_1 = \hat{k}_1 c_A$ and $k_{-3} = \hat{k}_{-3} c_B$, where c_A and c_B are the concentrations of A and B. In chemical equilibrium, the concentrations of A and B satisfy $c_B/c_A = (\hat{k}_1k_2k_3)/(k_{-1}k_{-2}\hat{k}_{-3})$. That is

$$\frac{k_1k_2k_3}{k_{-1}k_{-2}k_{-3}} = 1 \tag{1}$$

This is the "thermodynamic box" in elementary chemistry, also known as Wegscheider's relation. [In some literature, this relation itself is also called detailed balance. In the framework of chemical rate equations, Wegscheider's relation and detailed balance, i.e., zero flux, in steady state are mathematically equivalent. See refs 16, 17.] However, if the c_A and c_B are maintained at constant levels that are not at chemical equilibrium, as metabolite concentrations are in living cells, then the enzyme reaction is in an open system that approaches a NESS.[5,18] This is the scenario in enzyme kinetics.[19,20]

The rate equation for the probabilities of the states of the single enzyme is a master equation[21]

$$\frac{dP_E(t)}{dt} = -(k_1 + k_{-3})P_E + k_{-1}P_{AE} + k_3P_{BE} \tag{2a}$$

$$\frac{dP_{AE}(t)}{dt} = k_1P_E - (k_{-1} + k_2)P_{AE} + k_{-2}P_{BE} \tag{2b}$$

$$\frac{dP_{BE}(t)}{dt} = k_{-3}P_E + k_2P_{AE} - (k_{-2} + k_3)P_{BE} \tag{2c}$$

Equation 2 differs from the standard rate equation for unimolecular reactions in two important aspects: (i) the P_X (X = E,AE,BE) is a probability, not a concentration, and (ii) the rate constants k's do not satisfy the Wegscheider's relation (eq 1). The equations have conservation of matter, but (ii) implies that the system is open to chemical energy exchange; there is a hidden energy source and sink. One of the important tasks of NESS analysis of biochemical reaction systems is to explicitly identify sources and sinks, usually hidden in pseudo rate constants.

The steady-state probabilities for states E, AE, and BE are easy to compute from eq 2 by setting the time derivative to zero and noting that $P_E + P_{AE} + P_{BE} = 1$ for the total probability. Then, the clockwise steady-state cycle flux in Figure 1b, which is precisely the enzyme turnover rate of A → B in Figure 1a, is $J^{ss} = P_E^{ss}k_1 - P_{AE}^{ss}k_{-1} = P_{AE}^{ss}k_2 - P_{BE}^{ss}k_{-2} = P_{BE}^{ss}k_3 - P_E^{ss}k_{-3}$. That is,[22]

$$J^{ss} = (k_1k_2k_3 - k_{-1}k_{-2}k_{-3})/(k_1k_2 + k_{-1}k_{-3} + k_2k_{-3} + k_2k_3 + k_{-2}k_{-1} + k_3k_{-1} + k_3k_1 + k_{-3}k_{-2} + k_1k_{-2}) \tag{3}$$

If we substitute k_1 and k_{-3} with $\hat{k}_1c_A$ and $\hat{k}_{-3}c_B$, then we recover the celebrated Michaelis−Menten equation for reversible enzyme kinetics:[20,23]

$$J^{ss} = \frac{V^f_{max}c_A/K_{M,A} - V^b_{max}c_B/K_{M,B}}{1 + c_A/K_{M,A} + c_B/K_{M,B}} \tag{4}$$

where $V^f_{max} = k_2k_3/(k_2 + k_3 + k_{-2})$, $V^b_{max} = k_{-1}k_{-2}/(k_2 + k_{-2} + k_{-1})$, $K_{M,A} = (k_2k_3 + k_{-1}k_{-2} + k_3k_{-1})/\hat{k}_1/(k_2 + k_3 + k_{-2})$, and $K_{M,B} = (k_2k_3 + k_{-1}k_{-2} + k_3k_{-1})/\hat{k}_{-3}/(k_2 + k_{-2} + k_{-1})$.

The NESS of the open system, a single enzyme molecule in this case, is driven by the chemical potential difference between the A and B

$$\Delta\mu_{AB} = \mu_A - \mu_B = k_BT\ln\left(\frac{k_1k_2k_3}{k_{-1}k_{-2}k_{-3}}\right) \tag{5}$$

Hence, $J^{ss} = 0$ if and only if $\Delta\mu_{AB} = 0$, i.e., the system is at equilibrium. Otherwise, the nonzero $\Delta\mu$ is the chemical driving force for the flux J^{ss}, a terminology introduced by Onsager.[24] Furthermore, their product, $J^{ss} \times \Delta\mu_{AB}$, is the amount of work one has to do, per unit time, to sustain the NESS by constantly supplying A and removing B from the open system. $J^{ss} \times \Delta\mu_{AB}$ is also the rate of heat dissipation of the chemical reaction into the aqueous solution in NESS.

The inequality $J^{ss} \times \Delta\mu_{AB} \geq 0$, in fact, is a statement of the second law of thermodynamics: with only a single temperature bath T, one can only continuously convert chemical work to heat, but not in reverse. If that were possible, then one would have a chemical perpetual motion machine of the second kind.[25] In what follows, we shall make these ideas more precise. This is the core material of what we call isothermal NESS statistical thermodynamics.

In terms of the dynamic cyclic reaction in Figure 1b, let us introduce Gibbs free energy and Gibbs entropy for the isothermal open system as

$$G(t) = P_E(t)\mu_A(t) + P_{AE}(t)\mu_{AE}(t) + P_{BE}(t)\mu_{BE}(t) \tag{6}$$

$$S(t) = P_E(t)s_E(t) + P_{AE}(t)s_{AE}(t) + P_{BE}(t)s_{BE}(t) \tag{7}$$

where $\mu_X(t) = \mu_X^o + k_BT\ln P_X(t)$, $\mu_X^o = h_X^o - Ts_X^o$, $s_X(t) = s_X^o - k_B\ln P_X(t)$, and X = E, AE, BE. μ_X^o, h_X^o, and s_X^o are the standard state free energy, enthalpy, and entropy, respectively, of species X. $Ts_X(t)$ is the entropic part of $\mu_X(t)$. By the chain rule and eq 2, it is easy to obtain[26,27]

Hong Qian was born in Shanghai, China. He received his B. S. in astrophysics from Peking University in 1982, and his Ph.D. in biochemistry from Washington University in St. Louis in 1989, working with Prof. Elliot Elson on fluorescence correlation cpectroscopy and single-particle tracking. His research interests turned to theoretical biophysical chemistry when he was a postdoctoral fellow with Prof. John Schellman at the University of Oregon and with Prof. John Hopfield at the California Institute of Technology. After a brief stay with the Department of Biomathematics at the UCLA School of Medicine, he joined the University of Washington in 1997 and is now Professor of Applied Mathematics. His current research is in stochastic analysis and statistical physics of biological systems.

$$\frac{dG(t)}{dt} = J_{E\to AE}(\mu_{AE} - \mu_E) + J_{AE\to BE}(\mu_{BE} - \mu_{AE}) + J_{BE\to E}(\mu_E - \mu_{BE}) = -T \times \text{epr} + \text{cmf} \quad (8)$$

and

$$\frac{dS(t)}{dt} = J_{E\to AE}(s_{AE} - s_E) + J_{AE\to BE}(s_{BE} - s_{AB}) + J_{BE\to E}(s_E - s_{BE}) = \text{epr} - \frac{\text{hdr}}{T} \quad (9)$$

In eqs 8 and 9, we introduced three novel thermodynamic quantities: the *entropy production rate*, the *chemical motive force*, and the *heat dissipation rate*. They are time-dependent entropy, work, and heat:

$$T \times \text{epr} \equiv J_{E\to AE}(t)\Delta\mu_{E,AE}(t) + J_{AE\to BE}(t)\Delta\mu_{AE,BE}(t) + J_{BE\to E}(t)\Delta\mu_{BE,E}(t) \quad (10)$$

$$\text{hdr} \equiv J_{E\to AE}(t)\Delta h^o_{E,AE} + J_{AE\to BE}(t)\Delta h^o_{AE,BE} + J_{BE\to E}(t)\Delta h^o_{BE,E} \quad (11)$$

$$\text{cmf} \equiv J_{E\to AE}(t)\mu_A - J_{BE\to E}(t)\mu_B \quad (12)$$

where

$$\Delta\mu_{E,AE}(t) = k_B T \ln \frac{k_1 P_E(t)}{k_{-1} P_{AE}(t)}, \qquad \Delta h^o_{E,AE} = h^o_E - h^o_{AE} \quad (13)$$

and similarly for the $\Delta\mu_{AE,BE}$, $\Delta h^o_{AE,BE}$, $\Delta\mu_{BE,E}$, and $\Delta h^o_{BE,E}$.

The entropy balance eq 9 is the most important equation in the theory of irreversible thermodynamics.[28−30] In the past, this equation was introduced usually from an entropy balance point of view without molecular or kinetic details. The expressions in eqs 10 and 11 provide this abstract thermodynamic equation with a molecular interpretation. As we can see, the epr is always positive, whereas the time-dependent *hdr* and *cmf* can be either positive or negative. In NESS, $dS/dt = dG/dt = 0$ and $J_{E\to AE} = J_{AE\to BE} = J_{BE\to E} = J^{ss}$. Then

$$T \times \text{epr} = \text{hdr} = \text{cmf} = J^{ss} \times \Delta\mu_{AB} \geq 0 \quad (14)$$

That is, in the NESS, the amount of energy input maintaining the system, cmf, is equal to the entropy production rate, which in turn is the amount of the heat dissipated.

For a closed system, the cmf which represents the exchange of chemical energy with the surroundings is zero. Then, eq 8 indicates that the free energy of the system, G, is always decreasing and reaches its steady state with minimal G when epr = 0 and hdr = 0. It is easy to show that, in this case, Wegscheider's relation holds true and G is exactly the equilibrium Gibbs free energy computed from the partition function. The equilibrium statistical thermodynamics is, as it should be, a special case of the nonequilibrium theory.

2.2. Open Linear Chemical System with Number Fluctuations. The enzyme reaction in Figure 1 goes through NESS cycle kinetics, while it exchanges substrates molecules, A and B, with the solvent. However, if we identify the single enzyme as the "open system" and mathematically absorb the concentrations of A and B into the pseudo-first-order rate constants, we obtain a model of the open system that has the conservation of the number of enzyme molecules. Such an open system does not explicitly exchange material with its environment. Rather, it extracts chemical energy from its surrounding that is maintained at constant A and B concentrations with $\Delta\mu_{AB} \neq 0$. The situation is quite different in the cellular metabolic networks in which the number of enzyme molecules itself can fluctuate.

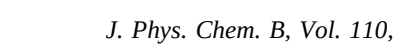

Figure 2. Schematic for a linear reaction system with material exchange with a source A and sink B. In contrast, the NESS in Figure 1b is sustained by chemical energy input, through the breakdown of detailed balance, without explicit material exchange. The most significant difference between these two systems is that the total number of molecules in the former fluctuates, analogous to the grand canonical ensemble in equilibrium statistical mechanics.

Let us consider a sequence of unimolecular chemical transformations in an open system, as shown in Figure 2, in which the upstream and downstream species A and B are maintained at their respective constant level. In this case, the open system is constantly exchanging material with the surroundings through the source A and the sink B; the number of molecules in the system in fact fluctuates. This situation is very similar to that of the grand canonical ensemble in equilibrium statistical mechanics.[3] The difference is that, in the NESS, there is a nonzero flux in the system as well as a chemical potential gradient.

Just as for an equilibrium system in contact with a material reservoir, there is a NESS theory of the grand canonical system.[31] [If one explicitly identifies the A and B in the enzyme example above, then that system could be considered as a NESS version of the semigrand ensemble. See ref 32.] The kinetic equation for the probabilities of having mX and nY molecules in the system in Figure 2, $P(m, n, t)$, satisfies the chemical master equation:[33−35]

$$\begin{aligned}\frac{dP(m,n,t)}{dt} = {} & k_1 n_A(P(m-1,n) - P(m,n)) + \\ & k_{-1}((m+1)P(m+1,n) - mP(m,n)) + \\ & k_2((m+1)P(m+1,n-1) - mP(m,n)) + \\ & k_{-2}((n+1)P(m-1,n+1) - nP(m,n)) + \\ & k_3((n+1)P(m,n+1) - nP(n,m)) + \\ & k_{-3} n_B(P(m,n-1) - P(m,n))\end{aligned} \quad (15)$$

The right-hand side of eq 15 is organized according to the six chemical reactions in Figure 2. n_A and n_B are given numbers of molecules A and B. The stochastic dynamics exhibits a two-dimensional random walk on the (m, n) lattice, representing the numbers of X and Y in the system as functions of time.

Equation 15 is simple enough to have some nice analytical results.[31] Multiplying m on both sides of eq 15, summing over m and n from 0 to ∞, and noting $\sum_{m,n=0}^{\infty} P(m, n) = 1$ is the total probability, $\sum_{m,n=0}^{\infty} mP(m, n, t) = n_X(t)$ is the mean number of X at time t in the system, and similarly, $\sum_{m,n=0}^{\infty} nP(m, n, t) = n_Y(t)$, we have

$$\frac{dn_X}{dt} = k_1 n_A - (k_{-1} + k_2)n_X + k_{-2} n_Y \quad (16a)$$

$$\frac{dn_Y}{dt} = k_2 n_X - (k_{-2} + k_3)n_Y + k_{-3} n_B \quad (16b)$$

which is exactly the standard chemical rate equation for the concentration of X and Y in terms of the law of mass action. Note that, with constant volume V, the number of molecules and the concentrations simply differ by a factor of V. Furthermore, the NESS probability distribution can be solved from eq

15 by setting the time derivative to zero to give

$$P^{ss}(n, m) = \left(\frac{n_X^m}{m!} e^{-n_X}\right)\left(\frac{n_Y^n}{n!} e^{-n_Y}\right) \tag{17}$$

Both the number of X and Y follow the Poisson distribution. This is a generalization of the equilibrium Poisson distribution in the grand canonical ensemble.

In the NESS, the auto- and cross-correlations between the number of X and the number of Y are[31]

$$\langle \Delta m(0)\Delta m(t)\rangle = \frac{n_X}{\lambda_1 - \lambda_2}((\lambda_1 + k_{-1} + k_2)\, e^{\lambda_2 t} - (\lambda_2 + k_{-1} + k_2)\, e^{\lambda_1 t}) \tag{18a}$$

$$\langle \Delta n(0)\Delta n(t)\rangle = \frac{n_Y}{\lambda_1 - \lambda_2}((\lambda_1 + k_{-2} + k_3)\, e^{\lambda_2 t} - (\lambda_2 + k_{-2} + k_3)\, e^{\lambda_1 t}) \tag{18b}$$

$$\langle \Delta m(0)\Delta n(t)\rangle = \frac{k_2 n_X}{\lambda_1 - \lambda_2}(e^{\lambda_1 t} - e^{\lambda_2 t}) \tag{18c}$$

$$\langle \Delta n(0)\Delta m(t)\rangle = \frac{k_{-2} n_Y}{\lambda_1 - \lambda_2}(e^{\lambda_1 t} - e^{\lambda_2 t}) \tag{18d}$$

where λ_1 and λ_2 are the two eigenvalues of the kinetic matrix

$$\begin{pmatrix} -k_{-1} - k_2 & k_{-2} \\ k_2 & -k_{-2} - k_3 \end{pmatrix} \tag{19}$$

We see that even though the number of X and Y molecules are uncorrelated at a given time, they are correlated with a delay. Furthermore, the fact that the two cross-correlation functions, eq 18c and d, are different is a distinct characteristic of a NESS. If $k_2 n_X = k_{-2} n_Y$, i.e, X and Y are in equilibrium, then the two cross-correlation functions are the same. Their difference is in fact a measure of irreversibility, i.e., the flux between X and Y in NESS.[36]

Curiously, we have noticed that the difference in cross-correlation functions given in eq 18 has the same mathematical expression as that of a single molecule in a cyclic reaction[36]

$$Z \underset{k_{-1}}{\overset{q_1}{\rightleftharpoons}} X \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Y \underset{q_{-3}}{\overset{k_3}{\rightleftharpoons}} Z \tag{20}$$

where,

$$p_X P_{XY}(t) - p_Y P_{YX}(t) = \frac{k_2 p_X - k_{-2} p_Y}{\lambda'_1 - \lambda'_2}(e^{\lambda'_1 t} - e^{\lambda'_2 t}) \tag{21}$$

p_X and p_Y are the steady-state probabilities, $P_{XY}(t)$ is the probability of the molecule in Y at time t given it is in X at time 0, and similarly, $P_{YX}(t)$ is the probability of the molecule in X at time t given it is in Y at time 0. The $\lambda'_{1,2}$ are the two nonzero eigenvalues of the kinetic system in eq 20. How general this result is is under current investigation.

The open system nonequilibrium thermodynamics in terms of Gibbs entropy and Gibbs free energy can be similarly worked out as in the previous example. See refs.[27,31] for more details.

2.3. Nonlinear Chemical Reaction System with Oscillations. In the previous two examples, we studied open linear chemical reaction systems with only chemical energy exchange with the surroundings and with explicit material exchange. Open biochemical reaction systems in living cells are also highly nonlinear, including bimolecular reactions, dimerizations, and autocatalysis. The nonlinear chemical reactions lead to complex temporal dynamics, including sustained chemical oscillations.[37,11] What is the relation between an oscillatory chemical reaction system and a NESS? We shall address this question through the third example.

Studies have shown that one needs to be more precise about the terminology:[38] in chemical dynamics literature in terms of deterministic kinetic models, a "steady state" is when all the concentrations of species in a system are constant and do not change with time. A periodic chemical oscillation is a different kind of dynamic behavior. Even though the concentrations of the species are not constant in time, the temporal behavior is "stationary" in time. However, the deterministic view of chemical dynamics is only a limiting case of a molecular system when the system is large and thermal fluctuations are negligible. The stochastic, chemical master equation approach[33,34] is a more realistic view of the chemical dynamics of both small and large systems. In stochastic terms, a steady state means a system with stationary temporal behavior with probability distributions being independent of time; there are still fluctuations. In stochastic mathematics, a steady state is a *stationary stochastic process*.[39,40]

We now study a system of nonlinear chemical reactions[41]

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} X, \qquad B \xrightarrow{k_2} Y, \qquad 2X + Y \xrightarrow{k_3} 3X \tag{22}$$

in which the third reaction is autocatalytic. This chemical reaction system has been extensively studied in terms of deterministic dynamics.[12] With appropriate parameters and sustained A and B, it exhibits periodic oscillations in the concentrations of X and Y.

The deterministic dynamics for the open reaction system (eq 22) in terms of the law of mass action follows nonlinear rate equations

$$\frac{dn_X}{dt} = k_1 n_A - k_{-1} n_X + k_3 n_X{}^2 n_Y \tag{23a}$$

$$\frac{dn_Y}{dt} = k_2 n_B - k_3 n_X{}^2 n_Y \tag{23b}$$

where n_X, n_Y, n_A, and n_B are the number of X, Y, A, and B, respectively. We assume there is a constant volume V, and all the rate constants are scaled accordingly. Equation 23 should be compared with eq 16. The difference is that eq 23 is nonlinear, while eq 16 is linear. Parts a and b of Figure 3 show two kinds of dynamic behavior. In Figure 3a, the dynamics has a sustained periodic oscillation over a long time, shown by the solid loop, called the limit cycle of the dynamics. The dashed lines are transient dynamics approaching the limit cycle either from inside or outside of the cycle. In contrast, in Figure 3b, all the dynamics are spiraling into a point, which corresponds to a constant $n_X \approx 20$ and $n_Y \approx 250$, called the fixed point of the dynamics. Even though there is no sustained periodic oscillation, there is still an oscillatory relaxation to the fixed point.

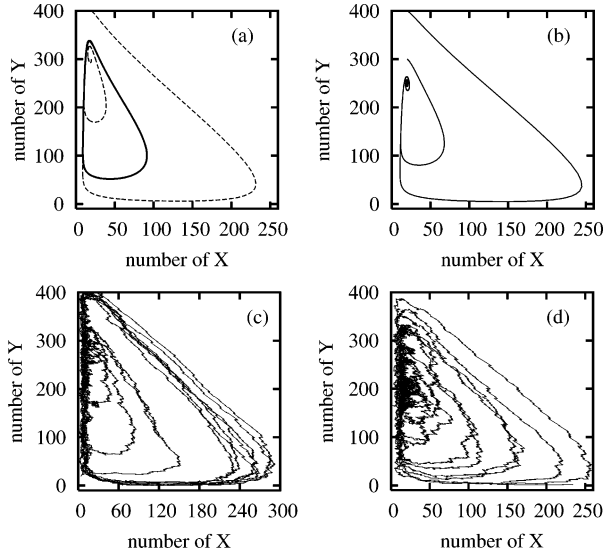

Figure 3. Oscillatory nonlinear chemical reaction system given in eq 22. In terms of the deterministic dynamic model (eq 23), the system can either oscillate periodically when the parameter $a = 0.08$ (a) or approach the fixed concentrations around $n_X = 20$ $n_Y = 250$ when $a = 0.1$ (b). In terms of the stochastic model (eq 24), however, the system exhibits a rotational random walk for both $a = 0.08$ (c) and $a = 0.1$ (d). Parameter $a = (k_1/k_{-1})\sqrt{k_3/k_{-1}}n_A$. Other parameters used in the calculation: $\sqrt{k_2/k_{-1}}n_B = 0.1$ and $\sqrt{k_3/k_{-3}} = 0.01$.

In terms of the chemical master equation, the probability of having mX and nY in the open system satisfies

$$\begin{aligned}\frac{dP(m,n,t)}{dt} = {} & k_1 n_A(P(m-1,n) - P(m,n)) + \\ & k_{-1}((m+1)P(m+1,n) - mP(m,n)) + \\ & k_2 n_B(P(m,n-1) - P(m,n)) + \\ & k_3((m-1)(m-2)(n+1)P(m-1,n+1) - \\ & m(m-1)nP(m,n)) \quad (24)\end{aligned}$$

which should be compared with eq 15. Parts c and d of Figure 3 show two dynamic trajectories according to eq 24 with parameters corresponding to that in Figure 3a and b. As is expected, the system is oscillatory, but with fluctuations. Both parts c and d of Figure 3 in fact exhibit *rotational random walks*, which are the mesoscopic signatures of deterministic chemical oscillations. There is a higher probability for the trajectories moving in a clockwise direction than in a counterclockwise direction. For more discussions, see ref 38.

Because of the nonlinearity, the mathematical relationship between the stochastic model in eq 24 and the deterministic model in eq 23 is no longer as straightforward as in the previous example, between eqs 15 and 16. Their relationship in physical chemistry, however, is just as simple: in the limit of large volume V and a large number of molecules, eq 24 approaches eq 23. The stochastic solution to eq 24 approaches the solution of eq 23 with negligible fluctuations. This was shown mathematically by T. G. Kurtz.[42] Corresponding to the dynamics in parts a and b of Figure 3, the rotational random walk in parts c and d of Figure 3 will have probability distributions peaked over a closed loop and at a single point, respectively.

The stochastic dynamics in Figure 3c exhibits two kinds of temporal fluctuations: one is the temporal complexity, i.e., periodic oscillations exhibited in 3a, and the second is the thermal fluctuations around the periodic oscillation. The latter disappears when a system becomes macroscopically large. We have discovered that[38] increasing energy input into the open system supresses the thermal fluctuation while promoting the temporal complexity. In other words, energy can be used to reduce thermal noise and produce temporal complexity in small chemical reaction systems, making them like machines, a lesson that could have implications in future nanotechnology.

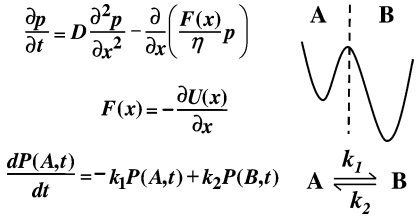

Figure 4. Relation between discrete rate processes and Smoluchowski equations of a particle in a force field. The latter is a more comprehensive description for chemical kinetics with an explicit energy landscape. It has a closer tie to Newtonian mechanics: eq 28 is in fact the Newton's law assuming an overdamped particle in a force field, together with a random collision force.

2.4. Brief Section Summary. The examples in this section show that isothermal open chemical systems can be subjected to rigorous statistical thermodynamic and stochastic kinetic studies. This theory is a natural generalization of equilibrium statistical thermodynamics, which has played a central role in the development of molecular biology through the studies of biological macromolecules, i.e., protein and DNA.[43−45] However, biochemical reaction systems in cellular biology are open systems. Therefore, the theory we illustrated in the three examples and present in the remainder of this article will be useful theoretical tools in studying living biological systems in terms of physical chemistry.

Since the 1950s, following the discovery of the DNA double helix, applying the principle of equilibrium statistical thermodynamics to biological systems has been the central theme of biophysical chemistry,[46−48] which has led to the ultimate genomic revolution. The simple examples in this section suggest that statistical thermodynamics also has a role in studying more complex living biological systems and processes. In particular, the relationship between thermodynamics and energy and signal transduction in cells can be subjected to a rigorous physiochemical analysis.

3. Brownian Dynamics and Nonequilibrium Statistical Thermodynamics

The kinetic rate equations have long been used to model molecular fluctuations. It might come as a surprise to some that these same equations have a hidden statistical thermodynamic structure. This turns out to not be an accident. From the pioneering work of Kramers, we know that the rate equations, i.e., discrete-state Markov jump processes, have a continuous-coordinates, energy-based representation in terms of the Smoluchowski equation and barrier crossing (Figure 4).[14,15,49] In this section, we trace the origin of the statistical thermodynamics, more precisely the conservation of energy, to the Smoluchowski equation of Brownian motion of an overdamped particle in a force field:

$$\frac{\partial p(x,t)}{\partial t} = D\frac{\partial^2 p(x,t)}{\partial x^2} + \frac{1}{\eta}\frac{\partial}{\partial x}\left(\frac{dU(x)}{dx}p(x,t)\right) \quad (25)$$

together with the Einstein's relation $D\eta = k_BT$. In eq 25, $p(x,t)$ is the probability density of the particle at x at time t, and $F(x) = -dU(x)/dx$ is a force field. This equation is well-known

and widely used since the work of Chandrasekhar, Ornstein, and Uhlenbeck.[50] What is not widely appreciated, however, is that it contains the law of energy conservation. To show this, let us consider the steady state of eq 25:

$$D\frac{\mathrm{d}p(x,t)}{\mathrm{d}x}+\frac{1}{\eta}\frac{\mathrm{d}U(x)}{\mathrm{d}x}p(x,t)=-J \tag{26}$$

which can be rewritten, using the Einstein relation, as

$$U(x)+k_{\mathrm{B}}T\ln P(x)=-\int \eta\frac{J}{p(x)}\mathrm{d}x \tag{27}$$

This equation can be interpreted as energy conservation. We note that J/p is the particle velocity, $\eta(J/p)\,\mathrm{d}x$ is the amount of heat dissipated for the particle moving through distance dx in a fluid with friction η, and we identify $U(x)+k_{\mathrm{B}}T\ln P(x)=\mu(x)$ as the chemical potential. Hence, conservation of energy in NESS is built in the mathematics of eq 25.

It is instructive to point out that there is another equivalent formulation for eq 25 in terms of the Langevin equation[51]

$$\eta\frac{\mathrm{d}X_{\mathrm{t}}}{\mathrm{d}t}=F(X_{\mathrm{t}})+\sqrt{2k_{\mathrm{B}}T\eta}\,\xi(t) \tag{28}$$

where X_{t} is a stochastic trajectory of the particle, $\xi(t)$ is the Brownian white noise, $\langle\xi(t)\rangle=0$, and $\langle\Delta\xi(0)\Delta\xi(t)\rangle=\delta(t)$, representing the random collisions between the particle and solvent molecules. Equation 28 is really Newton's equation $(m\mathrm{d}^2/\mathrm{d}t^2)X_t+(\eta\mathrm{d}/\mathrm{d}t)X_t=F(X_t)+\sqrt{2k_{\mathrm{B}}T\eta}\xi(t)$ assuming overdamping $m\approx 0$. Therefore, it is not surprising that eq 25 contains energy conservation. What was not known until recent years is that one can further introduce all the important thermodynamic quantities mathematically based on eq 28[52−54]

$$\mathrm{d}W_{\mathrm{t}}=F(X_{\mathrm{t}})\circ\mathrm{d}X_{\mathrm{t}} \tag{29}$$

$$\Psi_t=-k_{\mathrm{B}}\ln p(X_t,t) \tag{30}$$

$$\Pi_t=F(X_t)-k_{\mathrm{B}}T\frac{\mathrm{d}}{\mathrm{d}x}\ln p(X_t,t) \tag{31}$$

$$\mathrm{d}Q_t^{\mathrm{irr}}=\Pi_t\circ\mathrm{d}X_t \tag{32}$$

These four equations are interpreted as follows.

Equation 29: The work performed by a "particle" in a phase space, dW, equals the force times the displacement. The multiplication symbol "$\circ$" indicates the stochastic integration is in the Stratonovich sense.[51] In a closed system, $F=-\mathrm{d}U/\mathrm{d}x$. Then, $\mathrm{d}W=-\mathrm{d}U$.

Equation 30: The instantaneous entropy, Ψ, is the logarithm of the probability distribution. $1/p$ is the thermodynamic probability of Boltzmann.

Equation 31: Onsager's thermodynamic force, Π, consists of a mechanical force and an entropic force. In a closed system, Onsager's force is the gradient of the free energy:[55] $-U-k_{\mathrm{B}}T\ln p$. It is zero in the equilibrium steady state. Hence, $p\propto e^{-\mathrm{U}/k_{\mathrm{B}}T}$.

Equation 32: The irreversible heat dissipation of the particle, dQ^{irr}, equals the Onsager's force times the displacement. In a closed system, the equilibrium steady-state has $\mathrm{d}Q^{\mathrm{irr}}=0$.

From these stochastic quantities, nonequilibrium thermodynamic eqs 8 and 9 can be obtained, not only on average but also along stochastic trajectories.[53,56] Furthermore, two surprising equalities in terms of the log-mean-exponential of Q_t^{irr} in NESS can be derived:[54]

$$\ln\langle e^{-Q^{\mathrm{irr}}_t/k_{\mathrm{B}}T}\rangle=0 \tag{33}$$

$$\ln\langle\delta(x-X_t)\,e^{-Q^{\mathrm{irr}}_t/k_{\mathrm{B}}T}\rangle=\ln p^{\mathrm{ness}}(x) \tag{34}$$

both are valid for any t, where the average $\langle\cdots\rangle$ is carried out with all possible X_{t} that starts with the steady-state distribution. These two equalities are intimately related to Jarzynski's equality.[57−60] While Jarzynski's equality applies to closed systems with time-dependent Hamiltonian, eqs 33 and 34 apply to NESS. The significance of these two equalities is that the Q_t^{irr} is well-known to be a path-dependent quantity; the heat is not a state function in thermodynamics. In fact, dissipation $\langle Q_t^{\mathrm{irr}}\rangle$ increases with time without bound in a NESS. But via the log-mean-exponential, Q_t^{irr} is related to a function of state, the entropy. In classic thermodynamics, entropy is defined through infinite slow quasistationary processes. The Jarzynski equality-like relation in eq 34 circumvents this difficulty and enables one to define entropy (and free energy) through processes with finite speed. And more importantly, these results seem generalizable beyond equilibrium to NESS.

Equations 27−34 furnish the Smoluchowski eq 25 with a rich thermodynamic structure. While this is a nonorthodox approach, it follows the true spirit of the statistical mechanics: relating mechanical energy of molecular systems and probabilities of mesoscopic systems. In a complementary approach, J. M. Rubí and co-workers have shown that one can derive the stochastic description for open chemical and physical systems precisely in terms of the Smoluchowski equation based on the principles of nonequilibrium thermodynamics.[61]

4. Closed Chemical Systems: Detailed Balance, Time Reversibility, and the Fluctuation−Dissipation Relation

There is much to be learned about open systems. But to truly understand open systems and the NESS, one needs to first thoroughly understand closed systems and the equilibrium state. In this section, we discuss some of the not-widely-known results about closed systems. In terms of master equations and/or Brownian dynamics, two key concepts in connection to closed systems are Wegscheider's relation and Boltzmann's law.

4.1. Law of Mass Action and Deterministic Kinetics. Let us start our discussion with a simple chemical reaction in terms of the law of mass action,

$$\mathrm{A}+\mathrm{B}\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}\mathrm{C}+\mathrm{D} \tag{35}$$

If we denote the forward and reverse reaction fluxes as $J_+=k_1[\mathrm{A}][\mathrm{B}]$ and $J_-=k_{-1}[\mathrm{C}][\mathrm{D}]$, then we have the net flux J and chemical potential $\Delta\mu$

$$J=J_+-J_-,\qquad \Delta\mu=k_{\mathrm{B}}T\ln(J_+/J_-) \tag{36}$$

We see that then $J\neq 0$, $\Delta\mu\neq 0$. As we have pointed out earlier, the product $J\times\Delta\mu$ is the amount of entropy produced per unit time; it characterizes energy dissipation. Because a closed system cannot continuously dissipate energy indefinitely, $J\to 0$ and $\Delta\mu\to 0$ in the limit of $t\to\infty$. That is, a closed system can only approach a chemical equilibrium with zero flux in each reaction.

Zero flux means that each forward reaction is balanced by the reverse reaction. This is known as detailed balance in chemical equilibrium.[2] One then immediately obtains the ratio of equilibrium concentrations in terms of the rate constants (i.e., the equilibrium constant):

$$\frac{[C]^{eq}[D]^{eq}}{[A]^{eq}[B]^{eq}} = \frac{k_1}{k_{-1}} \tag{37}$$

More interestingly, one can introduce Gibbs free energy of the closed system:

$$G = [A]\mu_A + [B]\mu_B + [C]\mu_C + [D]\mu_D$$
$$= k_B T\left([A]\ln\frac{[A]}{[A]^{eq}} + [B]\ln\frac{[B]}{[B]^{eq}} + [C]\ln\frac{[C]}{[C]^{eq}} + [D]\ln\frac{[D]}{[D]^{eq}}\right) \tag{38}$$

where concentrations [A], [B], [C], and [D] are all functions of time. With the rate equations based on the law of mass action and aid from detailed balance, one can show that

$$\frac{dG}{dt} = -J \times \Delta\mu \le 0 \tag{39}$$

Therefore, a closed isothermal system is necessarily and spontaneously decreasing its Gibbs free energy until it reaches its equilibrium with minimal Gibbs free energy. It can be shown that, as a function of all the concentrations, G in eq 38 is convex. Hence, according to the a mathematical theorem due to A. M. Lyapunov,[62] the equilibrium state is unique and globally attractive.

Equation 38 can be generalized to any closed chemical system with many species. Then the right-hand side of eq 39 will be the sum of $-J_i \times \Delta\mu_i$ for all the reactions in the system.

With the uniqueness of the equilibrium concentrations, one can immediately show Wegscheider's relation among all the rate constants in a reaction loop, as well as Boltzmann's law $[X]^{eq} \propto e^{-\mu_X^o/k_B T}$, where X is any species in the closed system.

4.2. Equilibrium Fluctuations and Time Reversibility. With Wegscheider's relation connecting the rate constants in every reaction loop, the stochastic dynamics of a chemical reaction system, in terms of the chemical master equation, has several distinct characteristics. The proof of these results can be mathematically involved,[63] but their physiochemical meanings are in fact very clear. First, the equilibrium fluctuations are time reversible. That is, in the statistical sense, there is no difference between time traces of equilibrium fluctuations recorded forward or backward in time. This equilibrium property leads to several symmetric relations in the time correlation functions of equilibrium fluctuations.[36]

Second, the linear relaxation kinetics near an equilibrium cannot have complex eigenvalues. Neither can the time correlation function of equilibrium fluctuations.[22] The linear relaxation kinetics is of course intimately related to the time correlation function according to Onsager's theory of linear irreversibility and the Green−Kubo theorem.[24,64,65]

Third, the equilibrium probability distribution has a single maximum that corresponds to the unique deterministic equilibrium. In the limit of a large system, i.e., the thermodynamic limit, the distribution is Gaussian and Einstein's fluctuation theory applies.[66,67]

These closed-system characteristics immediately imply that only open systems can produce sustained, interesting behavior (or a transient phenomenon, in which case, there is an approximate open subsystem within the closed system) such as sustained chemical oscillation, spontaneous spatial pattern formation, free energy transduction, chemical bistability,[9] and stochastic resonance,[68] to name a few. All these fascinating problems deserve a close investigation with a nonequilibrium thermodynamic perspective.

4.3. Diffusion Dynamics and the Fluctuation−Dissipation Relation. Since the work of Kramers,[14] the diffusion dynamic approach to chemical systems has become a mjor component of chemical physics. Polymer dynamic theory,[69] electron transfer theory,[70] and the energy landscape theory of proteins[71] are several successful examples.

There is a dual relationship between the master-equation models and the N-dimensional diffusion equation:

$$\frac{\partial p(\mathbf{x}, t)}{\partial t} = \nabla\cdot(D(\mathbf{x})\nabla p(\mathbf{x}, t) - V(\mathbf{x})p(\mathbf{x}, t)) \tag{40}$$

where $D(\mathbf{x})$ is a diffusion tensor, and $V(\mathbf{x})$ is a velocity field, which is related to a force field $F(\mathbf{x})$ by a frictional coefficient matrix, $\eta(\mathbf{x})V(\mathbf{x}) = F(\mathbf{x})$. On one hand, a discrete transition A → B can be understood as an energy barrier crossing problem with sufficient barrier height (Figure 4), $F(x) = -\nabla U(\mathbf{x})$, and Einstein's relation $D(\mathbf{x})\eta(\mathbf{x}) = k_B T$. In this connection, Wegscheider's relation in master equations corresponds to diffusion in an energy landscape, and the breakdown of Wegscheider's relation corresponds to diffusion in a nonconservative force field, i.e, $\nabla \times F(\mathbf{x}) \neq 0$. It can be shown mathematically that $D^{-1}(\mathbf{x})V(\mathbf{x}) = -\nabla\phi(\mathbf{x})$, i.e., it is a conservative force field with a potential function (known as "the potential condition"[72]), is a sufficient and necessary condition for the stationary fluctuations to be time reversible,[63] and the equilibrium probability density $p^{eq}(\mathbf{x}) \propto e^{-\phi(\mathbf{x})}$. In this respect, the diffusion model is a more mechanistic, energy-based approach to chemical reactions. The fluctuation−dissipation relation, of which Einstein's relation is a special case, and the potential condition, are constraints to ensure diffusion models are consistent with necessary closed-system characteristics.

On the other hand, diffusion equations such as eq 40 are also an approximation of the chemical master equation in the limit of a large system.[6,42,73] The stochastic dynamics in Figure 3c and d can be approximately represented by eq 40. In this connection, the velocity field $V(\mathbf{x})$ in eq 40 defines the deterministic rate equation $(d/dt)\mathbf{x} = V(\mathbf{x})$ according to the law of mass action.[42] The diffusion term $D(\mathbf{x})$ provides the fluctuations. Keizer has developed an extensive thermodynamic theory based on this approach;[67] for recent work, see refs 74, 75.

5. Open Chemical Systems: Network Thermodynamics, Kirchhoff's Laws, and Stoichiometric Network Theory

There is a great demand from biochemistry for a network approach to complex, open chemical reaction systems. Electrical circuit theory has been a cornerstone of electric networks and electronic devices. Is there a similar network theory for chemical systems? Katchalsky and his colleagues were among the first to raise the question and studied network thermodynamics.[76] Extending the NESS theory we discussed above, we were able to develop a network thermodynamics cast in terms of the stoichiometry of a chemical system and the two celebrated Kirchhoff laws.[27,77−79]

The network structure of a chemical reaction system is captured in the stoichiometric matrix. Let there be M species and N reactions in the system, then the stoichiometric numbers can be systematically tabulated in a $M \times N$ matrix $\mathbf{S}$.[80] In a chemical steady state, the fluxes in all the reactions are balanced to maintain constant concentrations of all species. The fundamental law of conservation of mass leads to steady-state fluxes, $\mathbf{J} = (J_1, J_2, ..., J_N)^T$, satisfying

$$\mathbf{SJ} = \mathbf{b} \tag{41}$$

where **b** is the vector of input fluxes that transport material in to and out of the system. This is the chemical analogue of Kirchhoff's current law. Any flux vector **J** satisfying eq 41 satisfies the flux balance in NESS and is a balanced reaction "loop". As an example, consider the simple network in Figure 1a. The stoichiometric matrix is:

$$\mathbf{S} = \begin{matrix} \text{A} \\ \text{B} \\ \text{E} \\ \text{AE} \\ \text{BE} \end{matrix} \begin{bmatrix} -1 & <0 & 0 \\ 0 & 0 & +1 \\ -1 & 0 & +1 \\ +1 & -1 & 0 \\ 0 & +1 & -1 \end{bmatrix} \tag{42}$$

Because species A is transported into the system at a rate b_A and species B is transported out at the rate b_B, the mass-balance equation $\mathbf{SJ} = \mathbf{b}$ can be expressed as

$$\begin{bmatrix} -1 & 0 & 0 \\ 0 & 0 & +1 \\ -1 & 0 & +1 \\ +1 & -1 & 0 \\ 0 & +1 & -1 \end{bmatrix} \begin{bmatrix} J_1 \\ J_2 \\ J_3 \end{bmatrix} = \begin{bmatrix} -b_A \\ +b_B \\ 0 \\ 0 \\ 0 \end{bmatrix} \tag{43}$$

Now, let $\mu = (\mu_A, \mu_B, \mu_E, \mu_{AB}, \mu_{BE})$, in a vector form, denote the chemical potential of all the species. Then $\mu\mathbf{S} = \Delta\mu$ is the chemical potential differences for all the reactions, and

$$\Delta\mu\mathbf{J} = \mu\mathbf{b} \tag{44}$$

if **J** is a balanced reaction loop. Then eq 44 is the chemical analogue of Kirchhoff's loop law, and it is a statement about energy conservation: the left-hand side of the equation is heat dissipation, and the right-hand side is the amount of chemical energy input. For the example in eq 43, this is an alternative of eq 5:

$$\Delta\mu_1 + \Delta\mu_2 + \Delta\mu_3 = \Delta\mu_{AB} \tag{45}$$

because, in the NESS, $J^{ss} = b_A = b_B$.

Finally, in addition to mass and energy conservation, the second law for isothermal NESS can be stated as $-J_i \times \Delta\mu_i \geq 0$ for each reaction in the network.

6. Back to Simple Examples: Biological Applications

We now apply the above theory to several biological problems. In particular, we shall focus on the significant role of energy input in open biochemical systems and its biological functions. These examples reinforce the notion that many biochemical systems with important biological functions consume energy, and there is an intimate correlation between the amount of energy available and the performance of the functions.

6.1. Kinetic Proofreading Mechanism for Specificity Amplification. Kinetic proofreading in biosynthesis is one of the most telling examples of how biological systems utilize energy to overcome limited specificity.[81,82] By specificity, we mean the ability of a protein E to select between two substrates A and B of different affinities. Let us assume that the association constant for E + A $\rightleftharpoons$ EA is K_A and for E + B $\rightleftharpoons$ EB is K_B, $K_A > K_B$. Then with equal amounts of total A and B molecules, the concentration ratio between EA and EB complexes, in an equilibrium, is simply K_A/K_B. This is dictated by Boltzmann's law.

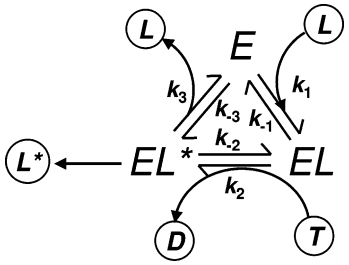

Figure 5. Protein E and substrate L associate in a NESS. The kinetic cycle is driven by the T $\rightarrow$ D reaction representing GTP hydrolysis inside living cells. The L* is the form to be incorporated into biosynthesis, but we assume that the synthesis rate is sufficiently low to be neglected. Assuming that there are two possible ligands, A and B, with different affinities to E, one is interested in the ratio [EA*]/[EB*]. All of the rate constants shown in the figure are first order or pseudo-first order. That is: $k_1 = \hat{k}_1[\text{L}]$, $k_{-3} = \hat{k}_{-3}[\text{L}]$, $k_2 = \hat{k}_2[\text{T}]$, and $k_{-2} = \hat{k}_{-2}[\text{D}]$. Because of the presence of T and D, $k_1k_2k_3/(k_{-1}k_{-2}k_{-3}) \gg 1$.

However, if the protein−substrate complexes are not in equilibrium but in a NESS, the ratio of [EA] and [EB] can be significantly greater than K_A/K_B. Biological processes inside living cells, in fact, use this strategy to amplify specificity and thus improve the accuracy of biochemical processes. For example, protein synthesis on ribosomes according to RNA templates can incorporate wrong amino acids just by chance. In a closed system, this chance is dictated by the specificity of the association between a codon and its corresponding tRNA. However, by operating in a NESS, a living cell can increase the fidelity of the biochemical processes, as we shall show.

The kinetic proofreading mechanism can be cast as a model for specificities of two ligands, A and B, binding to an enzyme. We choose this system rather than the traditional protein synthesis to illustrate the general principle.

We consider the kinetic scheme in Figure 5, in which the substrate L can be either A or B. The system is open, driven by the hydrolysis of GTP to GDP: T $\rightarrow$ D. To simplify the problem, let us further assume that A and B are structurally very similar, hence they have same $\hat{k}_1$, $\hat{k}_{\pm 2}$, and $\hat{k}_{-3}$. The different affinity comes from $k_{-1}^A/k_{-1}^B = k_3^A/k_3^B = K_B/K_A < 1$.

The steady-state probabilities for EL* and E are readily solved:

$$\frac{P_{EL^*}}{P_E} = \frac{k_1k_2 + k_{-1}^x k_{-3} + k_2k_{-3}}{k_2k_3^x + k_{-2}k_{-1}^x + k_3^x k_{-1}^x} \tag{46}$$

where k_{-1}^x and k_3^x, with superscript "x", are dependent on the substrate type, A or B. To obtain the strongest dependence of P_{EL^*} on substrate types, we have:

$$k_{-1}^x k_{-3} \ll k_1k_2 \text{ or } k_2k_{-3}$$

$$k_3^x k_{-1}^x \gg k_{-2}k_{-1}^x \text{ and } k_2k_3^x$$

Then $k_1k_2 \gg k_{-1}^x k_{-3}$ and $k_3^x \gg k_{-2}$, leading to a necessary condition

$$\frac{k_1k_2k_3^x}{k_{-1}^x k_{-2}k_{-3}} \gg 1 \tag{47}$$

for $P_{EL^*}/P_E \approx k_2(k_1 + k_{-3})/(k_3^x k_{-1}^x)$. That is,

$$\frac{P^{A}_{EL*}}{P^{B}_{EL*}} = \frac{k^{B}_{3}\, k^{B}_{-1}}{k^{A}_{3}\, k^{A}_{-1}} = \left(\frac{K_A}{K_B}\right)^2 \tag{48}$$

In other words, energy consumption is necessary for the P_{EL*} to have increased specificity for substrates with different affinities.

The idea that energy can be used to suppress noise is quite general. Recall the simple example in Section 2.3. One can show that, with increasing amounts of energy "pumped" into the system, the reaction is driven to exchange stochastic fluctuations (noise) with temporal complexity, i.e, deterministic oscillation or even chaos.

6.2. Biochemical Switch and Its Energy Requirement. A central theme in molecular cell biology is the biochemistry of cellular signal transduction. Successive events occur inside a living cell through a sequence of enzyme or protein activations. The inactive form of a signaling protein usually has no biological function, but after activation, its biological activity is "turned on" and it functions as a trigger for the activation of its "down stream" signaling target(s). Such a signaling cascade is most often illustrated by the kinetic scheme shown in Figure 6a, and each one of these activation steps is often called a switch.

Let us focus on the switching on and off between the B and B* state of a protein. This is usually accomplished in a cell by the phosphorylation of B to become phosphorylated B*. The A in Figure 6a in this case will be an enzyme, a protein kinase that catalyzes the phosphorylation of protein B. Let k_1 and k_{-1} be the two second-order rate constants for the phosphorylation reaction. The B* is returned to B by a dephosphorylation reaction, represented by k_2 and k_{-2} in Figure 6a.[83]

To be a switch, the chemical reactions between B and B* have to satisfy some requirements. In the absence of A*, the system has to be essentially in the B form. Hence, $k_2 \gg k_{-2}$. When there is a sufficient amount of A*, i.e., A is turned on, the B* should be dominant. Hence $(k_1[A^*] + k_{-2}) \gg (k_{-1}[A^*] + k_2)$. One can easily deduce that the two requirements lead to $\gamma \equiv k_1k_2/(k_{-1}k_{-2}) \gg 1$. In other words, The cyclic reaction of B $\rightarrow$ B* $\rightarrow$ B has to have chemical energy input.

Biochemists usually neglect the k_{-1} and k_{-2} because they are small. However, by incorporating them into the kinetic scheme in Figure 6a, one can quantitatively address the issue of energy consumption and the quality of the switch. Let us define the amplitude of the switch, AOS, as

$$\mathrm{AOS} = \left(\frac{[B^*]}{[B]+[B^*]}\right)_{[A^*]=\infty} - \left(\frac{[B^*]}{[B]+[B^*]}\right)_{[A^*]=0} \tag{49}$$

then we have

$$\mathrm{AOS} = \frac{k_1}{k_1 + k_{-1}} - \frac{k_{-2}}{k_2 + k_{-2}}$$

$$= \frac{\gamma - 1}{(k_1/k_{-1} + 1)(k_2/k_{-2} + 1)}$$

$$\leq \frac{\gamma - 1}{(\gamma + 2\sqrt{\gamma} + 1)} \tag{50}$$

$$= \frac{\sqrt{\gamma} - 1}{(\sqrt{\gamma} + 1)} = \tanh\left(\frac{\Delta\mu}{4k_BT}\right) \tag{51}$$

where we have introduced the chemical driving force in the cyclic reaction $\Delta\mu = k_BT \ln \gamma$, and the inequality in eq 50 means the AOS is in the best case scenario with the given amount of energy $\Delta\mu$ (Figure 6b).

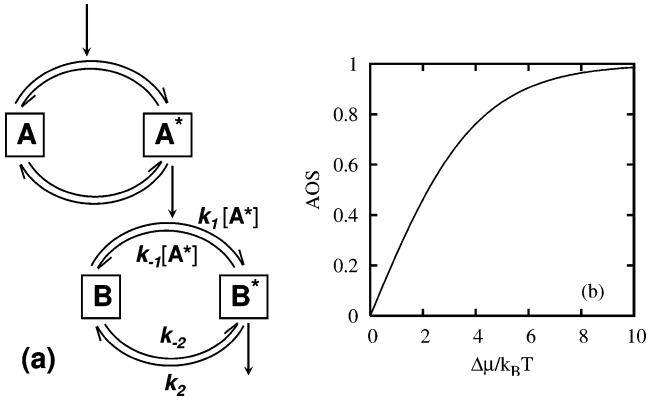

Figure 6. (a) Signal transduction cascade in cell biology: The activation of protein A to become A*, via chemical phosphorylation, leads to its catalysis of the activation, i.e., turning on, of protein B to become B*. Such a cascade is often illustrated by the downward arrow, indicating that A* activates the reaction. There are two distinct chemical reactions, both reversible, between the A and A*: the phosphorylation reaction involving ATP and ADP (convex arcs) and the dephosphorylation reaction involving π (concave arcs). Kinetic schemes such as these are widely studied in biology. (b) The turning on of B to B* can be quantified by the amplitude of switching (AOS), which is a function of the energy available to the open chemical system, $\Delta\mu = k_BT \ln(k_1k_2/(k_{-1}k_{-2}))$. In the best-case scenario, it is simply $\mathrm{AOS} = \tanh(\Delta\mu/(4k_BT))$.

Biochemically, the AOS is the magnitude of the signal. Hence, eq 51 shows that, based on physical chemistry, the signal magnitude is limited by the amount of energy dissipated in the biochemical reactions. If there is no energy dissipation, there is no switch. The ATP hydrolysis cycle coupled to the switch is not futile: it powers the biochemical switch.

6.3. Molecular Motor and Free Energy Transduction. One of the important functions of many biological organisms is to convert chemical energy into mechanical work with high efficiency. Such functions are often carried out by a single protein molecule. This gives rise to the field of molecular motors, which explores the physical, physiochemical, and biological aspects of energy transduction on a mesoscopic scale. For a comprehensive coverage of the subject, see ref 84.

Interestingly, a three-state chemical kinetic model, such as that in Section 2.1 and Section 6.1, can also be used to illustrate the key concepts of the theory of motor proteins.[85,86] More general treatment can be found in ref 87.

A kinetic scheme of a single motor protein is shown in Figure 7a, in which the transitions of the motor between states B and C is accompanied with ATP hydrolysis: B + T $\rightleftharpoons$ C + D with pseudo-first-order rate constants $k_2 = \hat{k}_2[T]$ and $k_{-2} = \hat{k}_{-2}[D]$. The conformational transitions between C and A is accompanied with a step of translocation of the motor protein along its periodic track, with spacing d, as shown in Figure 7b.

The kinetic rate equation for the motor protein is

$$\frac{dP_A(n)}{dt} = k_3P_C(n-1) - (k_{-3} + k_1)P_A(n) + k_{-1}P_B(n) \tag{52a}$$

$$\frac{dP_B(n)}{dt} = k_1P_A(n) - (k_{-1} + k_2)P_B(n) + k_{-2}P_C(n) \tag{52b}$$

$$\frac{dP_C(n)}{dt} = k_2P_B(n) - (k_{-2} + k_3)P_C(n) + k_{-3}P_A(n+1) \tag{52c}$$

where $P_X(n)$ is the probability of the motor protein in its internal

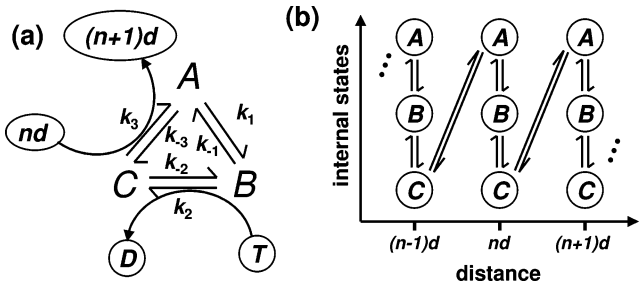

Figure 7. Three-state chemical model for a single motor protein. (a) The cyclic internal conformational transition of the protein. The kinetic cycle is driven by the hydrolysis of ATP to ADP, T → D. (b) The translocation of the motor protein along its linear, periodic track is coupled to the conformational transition between C and A. All of the rate constants are first-order or pseudo-first order: $k_2 = k_2^o[\mathrm{T}]$, $k_{-2} = k_{-2}^o[\mathrm{D}]$.

state X, (X = A, B, C), and at the same time at position n along its track.

The steady-state velocity of the motor moving along its track can be solved from eq 52 with periodic boundary conditions $P_A(n+1) = P_A(n)$ and $P_C(n) = P_C(n-1)$. The NESS flux times the distance d is the steady-state motor velocity: $V = J^{ss} \times d$. The flux J^{ss} is already obtained in eq 3. In the biophysical measurements of motor proteins, one is often interested in the time distribution(s) for motor stepping. To address this, instead of calculating the cycle flux J^{ss}, one considers the forward and backward cycle kinetics in Figure 7a from A_n to A_{n+1} or A_{n-1}. This is accomplished by reformulate the cyclic kinetic problem into a first-passage problem:[88,89]

$$\mathrm{A}_{n-1} \xleftarrow{k_{-1}} \mathrm{B}_{n-1} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{C}_{n-1} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{A}_n \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{B}_n \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{C}_n \xrightarrow{k_3} \mathrm{A}_{n+1} \quad (53)$$

We can compute the time τ to complete a cycle, forward or backward and respective probabilities, p_+ and p_-. The first-passage time problem has been extensively studied.[90,89] The mean of the cycle time is

$$\langle\tau\rangle = (k_1k_2 + k_{-1}k_{-3} + k_2k_{-3} + k_2k_3 + k_{-2}k_{-1} + k_3k_{-1} + k_3k_1 + k_{-3}k_{-2} + k_1k_{-2})/(k_1k_2k_3 + k_{-1}k_{-2}k_{-3}) \quad (54)$$

and the forward and backward probabilities are

$$p_+ = \frac{k_1k_2k_3}{k_1k_2k_3 + k_{-1}k_{-2}k_{-3}} \quad (55)$$

$$p_- = \frac{k_{-1}k_{-2}k_{-3}}{k_1k_2k_3 + k_{-1}k_{-2}k_{-3}} \quad (56)$$

Hence we have $J^{ss} = (p_+ - p_-)/\langle\tau\rangle$, and the motor translational velocity

$$V = \frac{d}{\langle\tau\rangle}(p_+ - p_-) \quad (57)$$

With some more elaborate algebra, one can also compute the variance of τ, σ_τ^2,[90] and then the dispersion in the motor translational motion[88]

$$D = \frac{d^2}{2\langle\tau\rangle}\left(1 - (p_+ - p_-)^2\left(1 - \frac{\sigma_\tau^2}{\langle\tau\rangle^2}\right)\right) \quad (58)$$

So far, all the discussions are on chemical kinetics. To make a connection to the mechanics of motor protein, one realizes

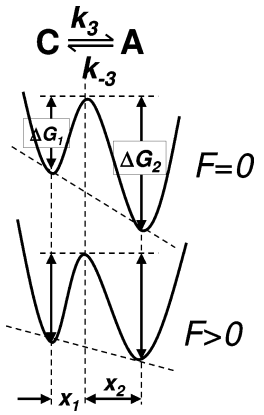

Figure 8. A schematic illustrating how rate constants $k_{\pm3}$ change as a function of a resistant force F. The upper panel is the free energy function, $G(x)$, for $F = 0$, and the lower panel is for $F > 0$, which tilts the energy function leftward: $G(x, F) = G(x, 0) + Fx$. The rate constants $k_{\pm3}$ are related to the transition state energy barrier height: $k_3(F) = \kappa_3\, e^{-\Delta G_1(F)/k_BT}$ and $k_{-3}(F) = \kappa_{-3}\, e^{-\Delta G_2(F)/k_BT}$, where $\Delta G_1(F) = \Delta G_1(0) + Fx_1$, $\Delta G_2(F) = \Delta G_2(0) - Fx_2$, $x_1 + x_2 = d$, $x_1 = \theta d$, $x_2 = (1-\theta)d$, and $\Delta G_2(F) - \Delta G_1(F) = -Fd$. The $\kappa_{\pm3}$ are related to the $k_{\pm3}^o$ in the text: $k_3^o = \kappa_3\, e^{-\Delta G_1(0)}$ and $k_{-3}^o = \kappa_{-3}\, e^{-\Delta G_2(0)}$. This leads to eq 59.

that the rate constants k_3 and k_{-3} have to be a function of external resistant force F, if it is applied. That is,[14,91]

$$k_3(F) = k_3^o\, e^{-Fd\theta/k_BT}, \qquad k_{-3}(F) = k_{-3}^o\, e^{Fd(1-\theta)/k_BT} \quad (59)$$

in which θ is a parameter related to the position of the transition state between C and A (Figure 8), known as the splitting parameter,[92] $k_{\pm3}^o$ are the rate constants in the absence of the resistant force. Substituting $k_{\pm3}(F)$ into eq 57 and recalling $\Delta\mu_{TD} = k_BT \ln(k_1k_2k_3^o/k_{-1}k_{-2}k_{-3}^o)$, one can obtain the motor velocity V as a function of F and $\Delta\mu$.[85] In particular, $V = 0$ when $F = \Delta\mu_{TD}/d$, known as the motor stalling force. Furthermore, we have the conservation of energy

$$k_BT \ln \frac{k_1k_2k_3}{k_{-1}k_{-2}k_{-3}} = \Delta\mu_{TD} - Fd \quad (60)$$

The left-hand side is the heat dissipation, and the two terms on the right-hand side are the chemical energy input and work done against the external force, respectively. With the balance of energy, efficiency can be rigorously defined.[93]

It is gratifying to see that a simple three-state cycle kinetic model, with the breakdown of detailed balance, can provide insights for so many different biochemical processes. This is a testimony of the importance and relevance of open-system NESS in modeling living biochemical systems. With the increasing complexity of realistic biochemical systems, the modeling will become more involved. But the central ideas seem to be contained in the simple model.

7. Discussion

There is no doubt that chemistry is the basis of many cellular phenomena and processes. Cellular and molecular biology are now moving toward a systems understanding of biochemical reaction networks in their living environment. One of the current challenges to theoretical chemistry is to develop a more complete statistical thermodynamic theory for biochemical systems that carry out a range of important biological functions such as signal transduction and gene regulation. Such a theory necessarily has to address the nature of an open system and its NESS.

Statistical mechanics is the theoretical foundation for molecular systems. It is one of the central components of physical chemistry of equilibrium. The general theoretical framework for chemical kinetics and dynamics is the law of mass action for large, macroscopic systems and Kramers' theory for microscopic reaction in aqueous solution (condensed phase). These two areas are now among the main subjects of theoretical chemistry. Between them is the chemical master equation approach to stochastic chemical reactions in open systems. This is precisely what is needed for biochemical studies of cells. The theory for stochastic chemical reaction systems was initially developed in the 1960s,[33] and it has been recently popularized by Gillespie's work.[34,94] While the algorithms for sampling stochastic processes have improved greatly in their efficiencies and have been applied widely to interesting biological systems[95] in recent years, the physical chemistry of the theory is largely lost in the mathematics.

For the chemical reaction systems in biology, one of the most distinct aspects is their open exchange with their environments, either in chemical energy (semigrand ensemble) or in material (grand canonical ensemble). As we have shown in this paper, such a system tends to a fluctuating steady state with constant energy input and dissipation. Even though it is time invariant, it is not an equilibrium. Following refs 96, 97, we call this NESS. The nonlinear chemical oscillations, extensively studied since the birth of the Oregonator,[37] is a limiting behavior of certain NESSs with rotational random walk when a system is sufficiently large.

It is well-known that Newton's dynamics equation contains an expression for the mechanical energy conservation.[98] What is the relation between the chemical rate equations, be they the macroscopic ones with the law of mass action, or the mesoscopic ones in terms of chemical master equations, and the laws of thermodynamics? This question has led our research to develop a rate-equation-based statistical thermodynamics. A surprising result from our study, as illustrated in this paper, is that the Smoluchowski−Kramers approach to chemical dynamics, together with Einstein's relation, encompasses a thermodynamics theory. By thermodynamic, we mean that, in addition to how conformations change with time, the theory also gives how thermodynamic quantities, e.g., entropy, energy, heat dissipation, and Onsager's force, change with time. This adds the richness to the rate theory of conformational dynamics and fluctuations. The resulting equations, in fact, can be applied to open biochemical systems in living cells.

Acknowledgment. I thank P. Ao, D. A. Beard, E. L. Elson, M. E. Fisher, C. Jarzynski, A.B. Kolomeisky, Q. Ouyang, R. Phillips, J. M. Rubí, A. Szabo, Z.-G. Wang, and X. S. Xie for collaborations and helpful discussions. I thank W. J. Heuett for reading the manuscript. I particularly thank Professors J. B. Bassingthwaighte, J. J. Hopfield, and J. D. Murray for continuously encouraging me to pursue the research reported in this article.

References and Notes

(1) Schrödinger, E. *What is Life?* Cambridge University Press: Cambridge, 1992.

(2) Lewis, G. N. *Proc. Natl. Acad. Sci. U.S.A.* **1925**, *11*, 179.

(3) Hill, T. L. *An Introduction to Statistical Thermodynamics*; Dover: New York, 1960.

(4) Helgason, C. D.; Miller, C. L. *Basic Cell Culture Protocols*, 3rd ed.; Humana Press: Totowa, NJ, 2004.

(5) Hill, T. L. *Free Energy Transduction in Biology*; Academic Press: New York, 1977.

(6) Keizer, J. *Statistical Thermodynamics of Nonequilibrium Processes*; Springer-Verlag: New York 1987.

(7) Qian, H. *J. Phys.: Condens. Matter* **2005**, *17*, S3783.

(8) Westerhoff, H. V.; Palsson, B. Ø. *Nat. Biotechnol.* **2004**, *22*, 1249.

(9) Qian, H.; Reluga, T. C. *Phys. Rev. Lett.* **2005**, *94*, 028101.

(10) Nicolis, G.; Prigogine, I. *Self-Organization in Nonequilibrium Systems*; John-Wiley & Sons: New York, 1977.

(11) Epstein, I. R.; Pojman, J. A. *An Introduction to Nonlinear Chemical Dynamics: Oscillations, Waves, Patterns, and Chaos*; Oxford University Press: New York, 1998.

(12) Murray, J. D. *Mathematical Biology, II: Spatial Models*, 3rd ed.; Springer: New York, 2003.

(13) Qian, H. *Annu. Rev. Phys. Chem.* **2007**, manuscript in preparation.

(14) Kramers, H. A. *Physica* **1940**, *7*, 284.

(15) Hänggi, P.; Talkner, P.; Borkovec, M. *Rev. Mod. Phys.* **1990**, *62*, 251.

(16) Wegscheider, R. *Z. Phys. Chem.* **1902**, *39*, 257.

(17) Schuster, S.; Schuster, R. *J. Math. Chem.* **1989**, *3*, 25.

(18) Wyman, J. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 3983.

(19) Fersht, A. *Enyzme Structure and Mechanism*, 2nd ed.; W. H. Freeman: New York, 1985.

(20) Segel, I. H. *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems*; Wiley: New York, 1993.

(21) Schnakenberg, J. *Rev. Mod. Phys.* **1976**, *48*, 571.

(22) Qian, H.; Elson, E. L. *Biophys. Chem.* **2002**, *101*, 565.

(23) Kou, S. C.; Cherayil, B. J.; Min, W.; English, B. P.; Xie, X. S. *J. Phys. Chem. B* **2005**, *109*, 19068.

(24) Onsager, L. *Phys. Rev.* **1931**, *37*, 405.

(25) Fermi, E. *Thermodynamics*; Dover: New York, 1956.

(26) Qian, H. *J. Phys. Chem. B* **2002**, *106*, 2065.

(27) Qian, H.; Beard, D. A. *Biophys. Chem.* **2005**, *114*, 213.

(28) de Groot, S. R. *Thermodynamics of Irreversible Processes*; North-Holland: Amsterdam, 1951.

(29) de Groot, S. R.; Mazur, P. *Nonequilibrium Thermodynamics*; North-Holland: Amsterdam, 1962.

(30) Prigogine, I. *Introduction to Nonequilibrium Thermodynamics*; Wiley-Interscience: New York, 1962.

(31) Heuett, W. J.; Qian, H. *J. Chem. Phys.* **2006**, *124*, 044110.

(32) Alberty, R.; Oppenheim, I. *J. Chem. Phys.* **1989**, *91*, 1824.

(33) McQuarrie, D. A. *J. Appl. Probl.* **1967**, *4*, 413.

(34) Gillespie, D. *J. Comput. Phys.* **1976**, *22*, 403.

(35) Érdi, P.; Tóth, J. *Mathematical Methods of Chemical Reactions: Theory and Applications of Deterministic and Stochastic Models*; Manchester University Press: Manchester, UK, 1989.

(36) Qian, H.; Elson, E. L. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 2828.

(37) Noyes, R. M.; Field, R. J. *Annu. Rev. Phys. Chem.* **1974**, *25*, 95.

(38) Qian, H.; Saffarian, S.; Elson, E. L. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 10376.

(39) Taylor, H. M.; Karlin, S. *An Introduction to Stochastic Modeling*, 3rd ed.; Academic Press: New York, 1998.

(40) Allen, L. J. S. *An Introduction to Stochastic Processes with Applications to Biology*; Pearson Education: Upper Saddle River, NJ, 2003.

(41) Schnakenberg, J. *J. Theor. Biol.* **1979**, *81*, 389.

(42) Kurtz, T. G. *J. Chem. Phys.* **1972**, *57*, 2976.

(43) Edsall, J. T.; Wyman, J. *Biophysical Chemistry*; Academic Press: New York, 1958.

(44) Tanford, C. 1961 *Physical Chemistry of Macromolecules*; John Wiley: New York, 1961.

(45) Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry*; W. H. Freeman: San Francisco, 1980.

(46) Eisenberg, H. *Biophys. Chem.* **2002**, *98*, 3.

(47) Eisenberg, H. *Biophys. Chem.* **2002**, *100*, 33.

(48) Eisenberg, H. *Biophys. Chem.* **2002**, *101*, 15.

(49) Qian, H. *Protein Sci.* **2002**, *11*, 1.

(50) Wax, N. *Selected Papers on Noise and Stochastic Processes*; Dover: New York, 1954.

(51) van Kampen, N. G. *Stochastic Processes in Physics and Chemistry*; North-Holland: Amsterdam, 1992.

(52) Sekimoto, K. *J. Phys. Soc. Jpn.* **1997**, *66*, 1234.

(53) Qian, H. *Phys. Rev. E* **2002**, *65*, 016102.

(54) Qian, H. *J. Phys. Chem. B* **2005**, *109*, 23624.

(55) Qian, H. *Phys. Rev. E* **2001**, *63*, 042103.

(56) Seifert, U. *Phys. Rev. Lett.* **2005**, *95*, 040602.

(57) Jarzynski, C. *Phys. Rev. Lett.* **1997**, *78*, 2690.

(58) Crooks, G. *Phys. Rev. E* **1999**, *60*, 2721.

(59) Hummer, G.; Szabo, A. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3658.

(60) Hatano, T.; Sasa, S.-I. *Phys. Rev. Lett.* **2001**, *86*, 3463.

(61) Reguera, D.; Rubí, J. M.; Vilar, J. M. G. *J. Phys. Chem. B* **2005**, *109*, 21502.

(62) Perko, L. *Differential Equations and Dynamical Systems*, 3rd ed.; Springer: New York, 2001.

(63) Jiang D.-Q.; Qian, M.; Qian, M.-P. *Mathematical Theory of Nonequilibrium Steady State*; Springer: New York, 2004; LNM Vol. 1833.
(64) Elson, E. L.; Webb, W. W. *Annu. Rev. Biphys. Bioeng.* **1975**, *4*, 311.
(65) Kubo, R. *Science* **1986**, *233*, 330.
(66) Landau, L. D.; Lifshitz, E. M. *Statistical Physics*, 3rd ed.; Pergamon Press: New York, 1980; Part 1, Course of Theoretical Physics, Vol. 5.
(67) Keizer, J. *Acc. Chem. Res.* **1979**, *12*, 243.
(68) Gammaitoni, L.; Hänggi, P.; Jung, P.; Marchesoni, F. *Rev. Mod. Phys.* **1998**, *70*, 223.
(69) Doi, M.; Edwards, S. F. *The Theory of Polymer Dynamics*; Clarendon: Oxford, 1986.
(70) Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265.
(71) Wolynes, P. G.; Onuchic, J. N.; Thirumalai, D. *Science* **1995**, *267*, 1619.
(72) Gardiner, C. W. *Handbook of Stochastic Methods for Physics, Chemistry, and the Natural Sciences*, 3rd ed.; Springer: New York, 2004.
(73) Hänggi, P.; Grabert, H.; Talkner, P.; Thomas, H. *Phys. Rev. A* **1984**, *29*, 371.
(74) Qian, H. *Proc. R. Soc. London, Ser. A* **2001**, *457*, 1645.
(75) Kwon, C.-L.; Ao, P.; Thouless, D. J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 13029.
(76) Oster, G. F.; Perelson, A. S.; Katchalsky, A. *Q. Rev. Biophys.* **1973**, *6*, 1.
(77) Beard, D. A.; Liang, S.-D.; Qian, H. *Biophys. J.* **2002**, *83*, 79.
(78) Qian, H.; Beard, D. A.; Liang, S.-D. *Eur. J. Biochem.* **2003**, *270*, 415.
(79) Deard, D. A.; Babson, E.; Curtis, E.; Qian, H. *J. Theor. Biol.* **2004**, *228*, 327.
(80) Clarke, B. L. *Adv. Chem. Phys.* **1980**, *43*, 1.
(81) Hopfield, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 4135.
(82) Ninio, J. *Biochimie* **1975**, *57*, 587.
(83) Qian, H. *Biophys. Chem.* **2003**, *105*, 585.
(84) Klafter, J.; Urbakh, M. *J. Phys.: Condens. Matter* **2005**, *17*, S3660.
(85) Qian, H. *Biophys. Chem.* **1997**, *67*, 263.
(86) Qian, H. *Biophys. Chem.* **2000**, *83*, 35.
(87) Fisher, M. E.; Kolomeisky, A. B. *Physica A* **1999**, *274*, 241.
(88) Kolomeisky, A. B.; Fisher, M. E. *J. Chem. Phys.* **2000**, *113*, 10867.
(89) Kolomeisky, A. B.; Stukalin, E. B.; Popov, A. A. *Phys. Rev. E* **2005**, *71*, 031902.
(90) Weiss, G. H. *Adv. Chem. Phys.* **1966**, *13*, 1.
(91) Shapiro, B. E.; Qian, H. *Biophys. Chem.* **1997**, *67*, 211.
(92) Hill, T. L. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 5613.
(93) Qian, H. *Phys. Rev. E* **2004**, *69*, 012901.
(94) Arkin, A.; Ross, J.; McAdams, H. H. *Genetics* **1998**, *149*, 1633.
(95) Turner, T. E.; Schnell, S.; Burrage, K. *Comput. Biol. Chem.* **2004**, *28*, 165.
(96) Klein, M. J. *Phys. Rev.* **1955**, *97*, 1446.
(97) Lax, M. *Rev. Mod. Phys.* **1960**, *32*, 25.
(98) Goldstein, H. *Classical Mechanics*; Addison-Wesley: Reading, MA, 1980.