Nonequilibrium Thermodynamics and Nonlinear Kinetics in a Cellular Signaling Switch

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We develop a rigorous nonequilibrium thermodynamics for an open system of nonlinear biochemical reactions responsible for cell signal processing. We show that the quality of the biological switch consisting of a phosphorylation-dephosphorylation cycle, such as those in protein kinase cascade, is controlled by the available intracellular free energy from the adenosine triphosphate (ATP) hydrolysis *in vivo:* $\Delta G = k_B T \ln([\mathrm{ATP}]/K_{\mathrm{eq}}[\mathrm{ADP}])$, where K_{eq} is the equilibrium constant. The model reveals the correlation between the performance of the switch and the level of ΔG. The result demonstrates the importance of nonequilibrium thermodynamics in analyzing biological information processing, provides its energetic cost, establishes an interplay between signal transduction and energy metabolism in cells, and suggests a biological function for phosphoenergetics in the ubiquitous phosphorylation signaling.

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Signal transduction, the informational processing in biological cells, is increasingly described as functional units (modules) in quantitative terms: An era of quantifying the signaling processes in terms of physiochemical principles is emerging [1]. Biological switches, one large class of such processes inside living cells, is based on an open system of nonlinear biochemical reactions operating under nonequilibrium steady state (NESS) [2]. While the requirements for both *nonlinearity* and *nonequilibrium* are intuitively obvious [3,4], quantitative aspects of such a system have never been studied, in particular, the interaction between the nonlinear nature of biochemical reactions and the nonequilibrium condition of the system. Nonlinear switches exhibit sharp transitions with ultrasensitivity [5], bistability, and hysteresis [6]. We propose a *thermodynamically consistent* modeling for a well-known phosphorylation-dephosphorylation switch with feedback [7]. Focusing on the free energy utilization of the system, we carry out a rigorous thermodynamic analysis and mathematically demonstrate a direct modulation of the performance of the switch by the available free energy. The mesoscopic biological devices are subjected to similar design considerations as for electronics and future nanotechnology: amplification, robustness, and noise tolerance.

The basic form of the present model for a phosphorylation-dephosphorylation cycle (PdPC) kinetic was first proposed in [8]. Reference [5] identified the origin of the switching behavior and introduced the concept of zero-order ultrasensitivity. Reference [9] pointed out the reversibility of both enzymes. The discovery of switchlike behavior in *in vivo* mitogen-activated protein kinase (MAPK) signaling led to a feedback step [7]. All the previous models emphasize the nonlinear kinetics, but the thermodynamics is inconsistent since irreversible enzyme reactions are assumed. Thus the free energy associated with each reaction is infinite. Introducing the small, but nevertheless nonzero, reverse reaction rate does not add more free parameters into a model since the forward and backward rates k_+ and k_- are constrained by $-RT\ln(k_+/k_-) = 7.3$ kcal/mol at pH 7 [10]. Reference [11] studied the enzyme kinetic model of [5] with thermodynamic consistency. The present model (Fig. 1) is the thermodynamically consistent version ($k_{-2}, k_{-3} \neq 0$) of [7] which shows a wide range of interesting nonlinear behavior [12]. There is a fundamental difference between the standard ultrasensitive switch [5,11] and the current bistable one with hysteresis. The former has a single steady state continuous change with the amount of stimulus, monotonically. The latter, however, has two stable steady states in coexistence, separated by a unstable saddle. In response to the amount of stimulus, there is a discontinuous jump from one state to another, i.e., hysteresis.

Using this model, one is able to address the open-system nonequilibrium thermodynamics (heat dissipation and entropy production) and nonlinear dynamics (bifurcation) in the mathematical model. Our computation shows that the availability of free energy is crucial to the integrity of signaling processed in mesoscopic systems; it gives an explicit formula for calculating the NESS heat dissipation rate. This finding supports a recent claim [13] that the accuracy of a biological timer [14] is controlled by the energy available in the phosphorylation reaction.

Model and analysis.—The model assumes three distinct pathways between A, the inactive form of a signaling protein, and biologically active A^*, the phosphorylated form of the protein (Fig. 1). Pathways 0, I, and II are the basal level activity, kinase catalyzed stimulus, and phosphatase catalyzed inhibition, respectively. All three reactions are reversible with respective forward and backward rate constants k_+ and k_-. In a chemical equilibrium we have the obligatory detailed-balance relations

$$\frac{[A^*]_{\mathrm{eq}}}{[A]_{\mathrm{eq}}} = \frac{k_1}{k_{-1}} = \frac{k_2[\mathrm{ATP}]_{\mathrm{eq}}}{k_{-2}[\mathrm{ADP}]_{\mathrm{eq}}} = \frac{k_3}{k_{-3}} = \sigma, \qquad (1)$$

where $[X]$ is the concentration of X. (1) assumes that the orthophosphate concentration is constant and absorbed into rate constants k_{-1} and k_{-3}. Inside living cells, the

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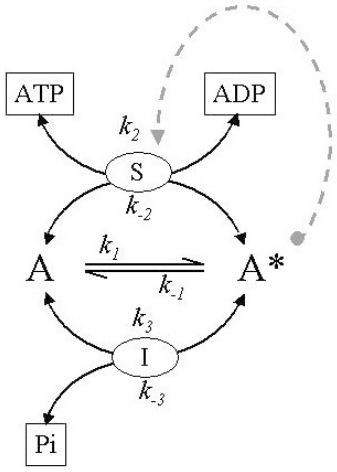

FIG. 1. The kinetic scheme for a typical PdPC in cell signaling. A is a signaling molecule in its inactive form; A^* is the phosphorylated, active form. Several pathways exist between A and A^*. Pathway I involves a biologically specific kinase for the phosphorylation, i.e., $A + \mathrm{ATP} \rightleftharpoons A^* + \mathrm{ADP}$; pathway II is the dephosphorylation due to a specific phosphatase: $A^* \rightleftharpoons A + \mathrm{Pi}$. Hence II is not the reverse reaction of I; they are different biochemical reactions. Pathway 0 represents all the background, nonspecific kinases, and phosphatases. As a signaling module, the kinase acts as a stimulus (S) and the phosphatase acts as an inhibitor (I) for the PdPC. The dashed line represents a positive feedback from A^* to the kinase activity [7].

concentrations of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) are not at chemical equilibrium. In fact, $\gamma = (k_2[\mathrm{ATP}])/(\sigma k_{-2}[\mathrm{ADP}]$ is around 10^{10}. Thus there is a phosphorylation potential of $\Delta G = k_B T \ln\gamma = 13.8$ kcal/mol being applied to the open system. In terms of these three pathways, the kinetic equation for the activation of A can be written as

$$\frac{d[A^*]}{dt} = J_{+1} - J_{-1} + J_{+2} - J_{-2} + J_{+3} - J_{-3} \qquad (2)$$

in which the fluxes in the respective reactions are

$$\begin{aligned} J_{+1} - J_{-1} &= k_1(a - [A^*]) - k_{-1}[A^*], \\ J_{+2} - J_{-2} &= k_2[\mathrm{ATP}](a - [A^*])[S][A^*]^2 - k_{-2}[\mathrm{ADP}] \\ &\quad \times [A^*][S][A^*]^2, \\ J_{+3} - J_{-3} &= k_3(a - [A^*])[I] - k_{-3}[A^*][I], \end{aligned} \qquad (3)$$

where $a = [A] + [A^*]$ is the total concentration of the signaling protein and $[S]$ and $[I]$ are the concentrations of the kinase as a stimulus and phosphatase as an inhibitor, respectively. An enzyme necessarily catalyzes both forward and backward steps of a reaction equally. Following [7] we have assumed an ultrasensitive, second-order sigmoidal feedback of A^* on S (the dashed line in Fig. 1): the kinase involved is assumed to be activated by binding two A^* molecules [15].

With the kinetic equations given in (2) and (3), one can analytically determine the fixed point of the reaction system [16] and yield a relation between the stimulus $[S]$ and the steady state $[A^*]_{\mathrm{ss}}$ at a given level of $[I]$:

$$[S] = \frac{(k_{-1} + k_{-3}[I])((1+\sigma)[A^*]_{\mathrm{ss}} - \sigma a)}{\tilde{k}_{-2}[A^*]_{\mathrm{ss}}^2(\sigma\gamma a - (1+\sigma\gamma)[A^*]_{\mathrm{ss}})}, \qquad (4)$$

where σ and γ are defined above and $\tilde{k}_{-2} = k_{-2}[\mathrm{ADP}]$. Similarly, we have

$$[I] = \frac{\tilde{k}_{-2}[S](1+\sigma\gamma)[A^*]_{\mathrm{ss}}^3 - \tilde{k}_{-2}\sigma\gamma a[S][A^*]_{\mathrm{ss}}^2 + k_{-1}(1+\sigma)[A^*]_{\mathrm{ss}} - k_{-1}\sigma a}{k_{-3}\sigma a - k_{-3}(1+\sigma)[A^*]_{\mathrm{ss}}}. \qquad (5)$$

We identify a fundamental difference between a chemical equilibrium ($\Delta G = 0$) and a NESS [17]: When the ATP and ADP concentrations are in equilibrium [Eq. (1)], the kinetic system eventually reaches a unique equilibrium state. However, when the ATP and ADP concentrations are sustained at the level with nonzero phosphorylation potential $\Delta G > 0$ as inside a living cell, the kinetic system approaches to a NESS (can be an even more complex oscillation [18]). One of the most important features of the latter is the possible coexistence of multiple steady states, which allows a switchlike behavior with abrupt transition [7].

Equilibrium analysis.—To be thermodynamically realistic, we show that when $\Delta G = 0$ ($\gamma = 1$) there will be only one steady state which is independent of the stimulus S and inhibitor I. Solving the steady-state solution of (2) and from Eqs. (4) or (5), we can show that when $\gamma = 1$, i.e., the available free energy $\Delta G = k_B T \ln\gamma = 0$, the above relations can always be simplified into

$$[A^*] = \frac{\sigma a}{1+\sigma}, \qquad (6)$$

independent of $[S]$ and $[I]$. This is the only physically meaningful solution; all other mathematical solutions involve nonphysical negative concentrations [see Fig. 2(a)]. Therefore, there is a unique equilibrium steady state independent of the stimulus and inhibitor when $\gamma = 1$. A switch cannot function without available free energy driving the PdPC away from equilibrium.

Effect of positive ΔG.—Figure 2(b) shows that to be a reasonable switch the ΔG has to be greater than a threshold value [Eq. (10)]. In other words, the free energy source has to be sufficiently large. In Fig. 2(c), with increasing stimulus, the system starts with an NESS with A dominant and, passing through a region in which the A and A^* can coexist in NESS, then becomes A^* dominant. Similarly in Fig. 2(b), increasing inhibition causes the system starting with A^* and becoming A dominant after the coexistence region. In both Figs. 2(b) and 2(c), there is hysteresis [4,7].

The conditions for bifurcation are

$$\frac{-9(1+\sigma\gamma)+\gamma(1+\sigma)}{2[\gamma^2-3(1+\sigma)z]}z=\frac{\gamma\pm\sqrt{\gamma^2-3(1+\sigma)z}}{3} \tag{7}$$

with

$$z=(1+\sigma\gamma)(\sigma a)^2\frac{k_{-1}+k_{-3}[I]}{\tilde{k}_{-2}[S]}. \tag{8}$$

One can solve the quadratic Eq. (7) for z:

$$(1+\sigma)^3z^2+[\tfrac{3}{4}(2\sigma\gamma-\gamma+3)^2-\gamma^2(1+\sigma)^2]z+\gamma^3(1+\sigma\gamma)=0. \tag{9}$$

The condition for (7) having two positive roots yields the minimal required free energy for the switch:

$$\gamma>\frac{3\sqrt{3}}{(\sqrt{3}+2)-2(\sqrt{3}-1)\sigma}. \tag{10}$$

In the limit of $\tilde{k}_2\gg\tilde{k}_{-2}$ and $k_3\ll k_{-3}$ [7], (7) is simplified with only one positive root for z:

$$[I]=\frac{2\sqrt{3}-3}{3k_{-3}}\gamma a^2\tilde{k}_{-2}[S]-\frac{k_{-1}}{k_{-3}}. \tag{11}$$

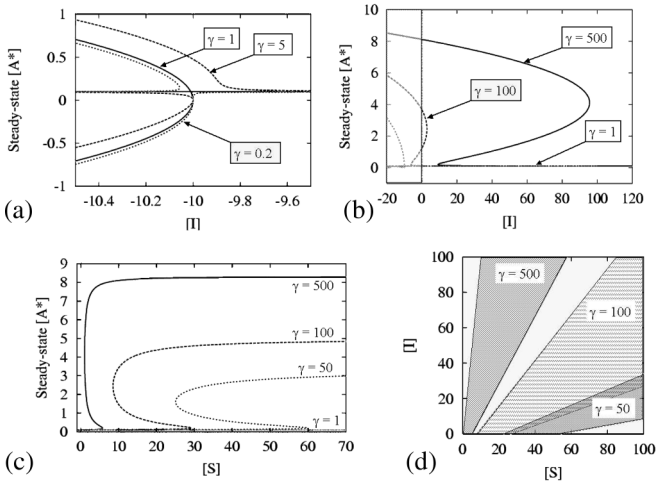

FIG. 2. The steady state of the kinetics in Fig. 1 as a function of phosphorylation potential $\Delta G=k_BT\ln\gamma$, stimulus strength $[S]$, and inhibitor strength $[I]$. All computations are done with the parameter set $a=10$, which sets the scale for the unit for all concentrations. $\sigma=0.01$, $k_{-1}=10$, $\tilde{k}_{-2}=0.1$, $k_{-3}=1$. (a),(b) $[S]=10$. (c) $[I]=1.0$. (a) For $\gamma=1$, no matter what are the positive $[S]$ and $[I]$, there is only one stable steady state: $[A^*]_{ss}=\sigma a/(1+\sigma)$ as expected for a simple chemical equilibrium. The unphysical negative $[I]$ region is shown to illustrate that the closed system ($\gamma=1$) is structurally unstable mathematically. When $\gamma>1$, multiple steady states appear. For example, in (b) when $[S]=10$ and $\gamma>100$, there are either one steady state, or three steady states. (d) shows the parameter region in which there are three steady states, which represents the hysteresis.

Therefore, one of the bifurcation points disappears and the on state can never be turned off. See more discussions in [7].

The robustness of the PdPC switch.—Having the mathematical model for the PdPC biochemical switch enables one to quantitatively characterize the robustness of the signaling process. We introduce the fault safety factor for the robustness for a switch. Figure 2 shows the hysteresis in the transitions from A to A^* and back. It has been suggested that the biological functions of the hysteresis are (i) to generate persistent activation [19] as well as (ii) to secure the transition and to prevent ''chattering'' [7]: It decreases the likelihood of a system near a threshold repeatedly switching back and forward due to noises. In Fig. 2(d), the hysteresis region is shown for a different amount of available ΔG. One can quantitatively define the relative width of the hysteresis zone, i.e., the fraction of the fluctuations in $[S]$ (or $[I]$) the switch can tolerate in Fig. 2(d). Let s^+ and s^- be the upper and lower bounds of the $[S]$ region for a given $[I]$. Then following (11) and in terms of the two roots of (9), z_+ and z_-, we have

$$\frac{s_+-s_-}{s_++s_-}=\frac{z_+-z_-}{z_++z_-}. \tag{12}$$

The fault safety factor is shown in Fig. 3. For the parameters used in the computation, there is a minimal phosphorylation potential of 2.0 kcal/mol for the biological switch.

Summary and discussions.—A rigorous thermodynamics is established for a cellular signal transduction process in terms of open, nonlinear biochemical reaction systems. Quantitative analysis shows that the energy derived from nucleotide hydrolysis is essential for the functioning of the molecular process. It has been established in the past [10] that a living organism requires an energy input for three major purposes: mechanical movements, molecular and ion transports, and biosynthesis. Our present result suggests a fourth energy sink: the high-energy phosphoanhydride bond is the physicochemical origin of prevailing protein phosphorylations and guanosine triphosphate (GTP) hydrolysis in biological signal transduction. Intracellular signaling networks are essentially made of modules of PdPCs and GTPases [13]. Therefore, our results suggest a unifying principle in quantifying signal transduction in terms of energetics. Energy cost of kinetic proofreading in biosynthesis has been studied in the past [20] and new structural and dynamic information is now available [21], yet a thermodynamic consistent analysis on energy cost remains to be developed. The current work provides an example for computing the energy cost of sending biological information. The relationship between information processing in biology and in man-made machine may be compared [22].

The present work suggests to us the following *phosphorylation energy hypothesis* with respect to cell informational processing: Protein phosphorylation is not merely a chemical signal in terms of a structural tag. Energy derived from

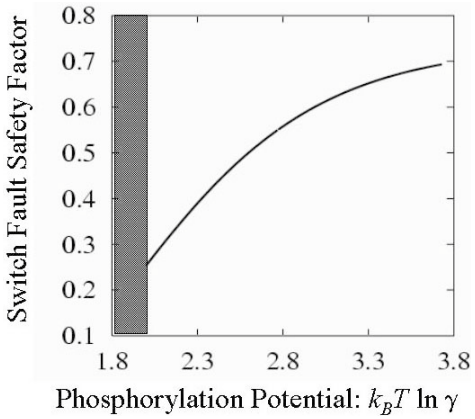

FIG. 3. The fault safety factor as a function of the available cellular energy ΔG, computed under the same condition as in Fig. 2. The minimal ΔG for the switching behavior under this set of conditions is 2 kcal/mol. The fault safety factor, defined as the fractional fluctuations in $[S]$ or $[I]$ the system can tolerate when near the threshold, increases with the energy supply.

the hydrolysis reaction in a living cell is used to ensure the sharpness and accuracy of noisy cellular signaling processes.

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[12] There are two types of graphic representations for biochemical networks. One is transformational based on chemical conversions and one is informational in which the regulations are depicted. The solid lines in Fig. 1 are the first kind and the dotted line is the second. Strictly speaking, however, a regulation is a biochemical species acting on a reaction, not another species. In the language of Angeli *et al.* [6], our model is a system of two positive regulations: S_2 catalyzes $A \rightleftharpoons A^*$, and $S_0 + 2A^* \overset{\kappa}{\rightleftharpoons} S_2$, where S_0 and S_2 are two forms of S. If $\kappa[A^*]^2 \ll 1$ and the reaction is rapid, then $[S_2] = \kappa[S][A^*]^2$ and the intermediate S_2 becomes implicit. The approximation, which can be made mathematically rigorous, preserves the thermodynamic consistency.

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[15] Being more precise, the mechanism in (3) differs from the zero-order ultrasensitivity originally proposed by [5] which requires a saturation of kinase by A and phosphatase by A^*. In (3) both enzymes are assumed in the nonsaturated linear regime (first order). The ultrasensitivity in this case is derived from the feedback, which represents the cascade behavior of the MAPK pathway. This type of ultrasensitivity could be more selective against nonspecific cross-talks. See [13] for more discussion.

[16] Nondimensionalizing (2) gives $\frac{dx}{dt} = a_3x^3 + a_2x^2 + a_1x + a_0 = f(x)$ in which $x = [A^*]$ and $a_3 = -\tilde{k}_{-2}[S] \times (1 + \sigma\gamma)$, $a_2 = \tilde{k}_{-2}[S]\sigma\gamma a$, $a_1 = -(k_{-1} + k_{-3}[I]) \times (1 + \sigma)$, $a_0 = (k_{-1} + k_{-3}[I])\sigma a$. The conditions for bifurcation are $f(x) = f'(x) = 0$ [4] with $x > 0$, which yield

$$\frac{9a_0a_3 - a_1a_2}{2(a_2^2 - 3a_1a_3)} = \frac{-a_2 \pm \sqrt{a_2^2 - 3a_1a_3}}{3a_3}.$$

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