Thermodynamic Limit of a Nonequilibrium Steady State: Maxwell-Type Construction for a Bistable Biochemical System

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We show that the thermodynamic limit of a bistable phosphorylation-dephosphorylation cycle has a selection rule for the ''more stable'' macroscopic steady state. The analysis is akin to the Maxwell construction. Based on the chemical master equation approach, it is shown that, except at a critical point, bistability disappears in the stochastic model when fluctuation is sufficiently low but unneglectable. Onsager's Gaussian fluctuation theory applies to the unique macroscopic steady state. With an initial state in the basin of attraction of the ''less stable'' steady state, the deterministic dynamics obtained by the law of mass action is a metastable phenomenon. Stability and robustness in cell biology are emergent stochastic concepts.

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The statistical physics of a living cell requires a theory for an open molecular system with a chemical driving force and free energy dissipation [1]. Such a system is capable of reaching a self-organizing state to which many biological functions are attributed. The state has been widely known as a nonequilibrium steady state (NESS) following Klein's concise terminology [2]. Two of the most exciting recent developments in statistical physics are concerned precisely with the NESS: the fluctuation theorem studies the novel entropy production characteristics of a NESS [3]; and the one-dimensional exclusion process deals with highly nontrivial phase behavior [4].

However, the concept of NESS requires further refinements. This is the objective of this Letter. From a statistical mechanics perspective, a NESS is a fluctuating, stochastic, and stationary process. It has a stationary probability distribution as well as correlation functions [5]. For a wide class of physical and biological systems, this state is unique [6]. However, from a macroscopic perspective, an open, driven system can have multiple steady states. In fact, the dynamics can be more complex that include oscillations and spatial-temporal chaos [7].

''Macroscopic'' studies of living cell biochemistry are usually based on deterministic nonlinear differential equations according to the law of mass action [8]. Currently, it is generally accepted that a bistability in the deterministic dynamics corresponds to a bimodal probability density function in the stochastic approach [9,10]. With the increasing size of chemical reaction systems [11], there is a separation of time scale: the transition rates between the two macroscopic states become infinitesimal $\sim e^{-\alpha V}$, where V is the systems volume and α is a positive constant. See [10] for a detailed exposition.

On the other hand, there is a well-developed, phenomenological fluctuation theory of NESS in statistical physics, pioneered by Onsager and Machlup, Lax, and Keizer,

among many others [12]. One of the most important conclusions from this classic NESS fluctuation theory is multivariate Gaussian fluctuations around a NESS. This result essentially conforms with Einstein's equilibrium fluctuation theory.
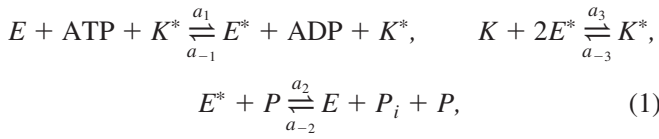
In this Letter, we shall use a concrete example to provide insights into this seeming paradox between the current view of NESS fluctuation, with multiple macroscopic steady states, and the classic Einstein-Onsager-Lax-Keizer (EOLK) Gaussian theory. Using the chemical master equation (CME) as the tool and a bistable system from current biochemical literature, we show that as $V \rightarrow \infty$, a Maxwell-like construction is necessary. Such a construction effectively singles out one unique state (or an attractor in the case of more complex dynamics) in the thermodynamic limit. It is around this state that the EOLK theory applies. Our analysis confirms the claim that the information necessary for the Maxwell-type construction is not present in the deterministic differential equation model of the system [13]; one requires building a mesoscopic, mechanistic model with stochasticity in order to gain the required information. Our conclusion is that, when dealing with biochemical reaction systems, one needs to differentiate the thermodynamic limit of a mesoscopic system and the differential equations based on the law of mass action. The latter follows Kurtz's theorem [14,15]; the thermodynamic limit, however, has to augment the Maxwell construction, based on which the EOLK fluctuation theory applies.

Several papers have addressed related issues in the past. We choose to revisit these important and fundamental issues in NESS due to the recent resurgent interests in the CME and its applications to cellular biochemistry. In addition to [13], Keizer developed Maxwell-type constructions for multiple nonequilibrium steady states [16]. While the present Letter shares a similar idea, the previous ap-

proach was based on the diffusion approximation to a CME, an approach that can fail to represent correctly the mesoscopic steady state [17], now known as Keizer's paradox [10].

The analysis performed here is for a particular example of a biochemical cycle, but what we show is general for nonlinear, driven chemical reaction systems with multistability.

Phosphorylation-dephosphorylation cycle and the CME.—Biochemical information processing inside cells uses a canonical reaction system called the phosphorylation-dephosphorylation cycle [15]. We consider the Ferrell's kinetic model for the phosphorylation-dephosphorylation cycle [18], which includes a positive feedback step, and its reversible extension first studied in [19]:

$$E + ATP + K^* \underset{a_{-1}}{\overset{a_1}{\rightleftharpoons}} E^* + ADP + K^*, \qquad K + 2E^* \underset{a_{-3}}{\overset{a_3}{\rightleftharpoons}} K^*,$$

$$E^* + P \underset{a_{-2}}{\overset{a_2}{\rightleftharpoons}} E + P_i + P, \qquad (1)$$

in which E and E^* are inactive and active forms of a signaling protein. K and P are enzymes, kinase, and phosphatase, that catalyze the phosphorylation and dephosphorylation, respectively. K and K^* are active and inactive forms of the kinase. The chemical reaction of ATP hydrolysis $ATP \rightleftharpoons ADP + P_i$ provides the chemical driving force of the reaction. In fact the free energy from the reaction is $\Delta G = k_B T \ln\{a_1 a_2[ATP]/(a_{-1}a_{-2}[ADP][P_i])\}$. In a cell, K and P, ATP, ADP, and P_i are all at constant concentrations, and $[E] + [E^*] = e_{tot}$. $[z]$ denotes the concentration of the species z.

The system in (1) exhibits bistability according to a deterministic analysis based on the law of mass action [18]. It has been further shown that the bistability is distinctly a driven phenomenon that requires a sufficient large free energy dissipation [19]. Here we consider its mesoscopic stochastic model in terms of the CME [15].

We shall denote $k_1 = a_1 a_3[ATP]/a_{-3}$, $k_{-1} = a_{-1}a_3[ADP]/a_{-3}$, $k_2 = a_2[P]$, and $k_{-2} = a_{-2}[P_i][P]$. Following the previous treatment [15,18,19], we assume the reversible binding $K + 2E^* \rightleftharpoons K^*$ is rapid. The model thus is simplified into $E \rightleftharpoons E^*$ with forward and backward rates $R^+(x) = (k_1[K]x^2 + k_{-2})(e_{tot} - x)$, $R^-(x) = (k_2 + k_{-1}[K]x^2)x$, and $x(t) = [E^*](t)$. The energy parameter from ATP hydrolysis $\gamma = \exp(\Delta G/k_B T) = k_1 k_2/(k_{-1}k_{-2})$. $\gamma = 1$ is equivalent to a nondriven system which reaches a unique equilibrium steady state. In fact, the equilibrium probability distribution for the number of E^* is binomial.

The deterministic kinetic model based on the law of mass action is

$$\frac{dx}{dt} = R^+(x) - R^-(x) = r(x; \theta, \epsilon)$$

$$= k_2\{\theta x^2[(e_{tot} - x) - \epsilon x] + [\mu(e_{tot} - x) - x]\}, \quad (2)$$
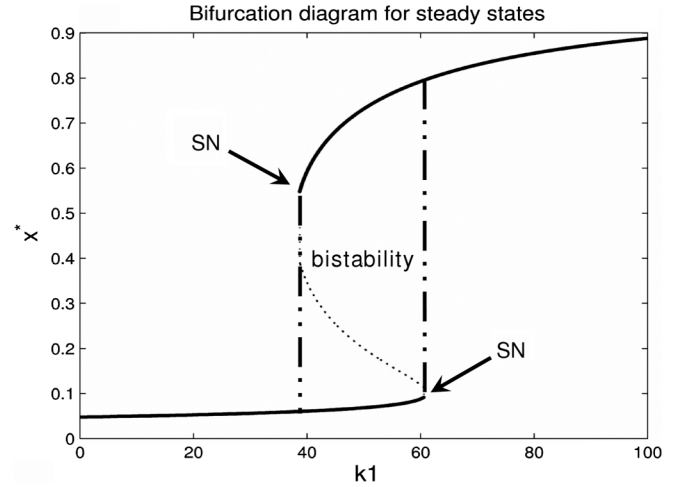

FIG. 1.   Bifurcation diagram of the steady states of the reaction system in Eqs. (1) and (2), x^*, as a function of the parameter k_1. SN denotes saddle-node bifurcation. Other parameters in the calculation: $[K] = 1$, $e_{tot} = 1$, $k_{-1} = 0.01$, $k_2 = 10$, $k_{-2} = 0.5$.

in which the three parameters $\theta = k_1[K]/k_2$ represents the ratio of the activity of the kinase to that of the phosphatase; $\epsilon = k_{-1}/k_1$ represents the ADP to ATP concentration ratio, and $\mu = k_{-2}/k_2$ represents the strength of phosphorolysis. In a living cell, both μ and ϵ are small; hence $\gamma = 1/(\mu\epsilon) \gg 1$.

The fixed points of Eq. (3) are the solution to $R^+(x) = R^-(x)$. Their stability is determined by the $\frac{d}{dx} \times [R^+(x) - R^-(x)]$. For some parameter ranges, Eq. (3) exhibits saddle-node bifurcations [18,19], as shown in Fig. 1. One obtains the parameter region for the bistability from simultaneously solving $r(z) = 0$ and $\frac{dr(z)}{dz} = 0$, which gives the boundary of the region of bistability, with a cusp, in (θ, ϵ) space (in terms of z as a parametric curve):

$$\theta = \frac{2(1 + \mu)}{z e_{tot}} - \frac{3\mu}{z^2}, \qquad \epsilon = \frac{2\mu e_{tot}^2 - (\mu + 1)z e_{tot}}{3\mu e_{tot}z - 2(\mu + 1)z^2} - 1. \quad (3)$$

For the stochastic model in terms of the CME, one is interested in the number of E^*, X, rather than its concentration. X takes non-negative integer values and is related to $x = X/V$ where V is the system's volume. While $X(t)$ is stochastic, its probability, $P(X, t)$, satisfies the CME [15]:

$$\frac{\partial P(X, t)}{\partial t} = VR^+\left(\frac{X - 1}{V}\right)P(X - 1, t) + VR^-\left(\frac{X + 1}{V}\right)$$

$$\times P(X + 1, t) - V\left[R^+\left(\frac{X}{V}\right) + R^-\left(\frac{X}{V}\right)\right]P(X, t).$$

Its stationary solution gives the probability distribution in the NESS:

$$P^{ss}(X) = P_s(0)\prod_{i=1}^{X} \frac{R^+(\frac{i-1}{V})}{R^-(\frac{i}{V})}. \quad (4)$$

Maxwell-type construction and stochastic bifurcation.— When V is large, by the Euler-MacLaurin summation formula,

$$P^{ss}(xV) \propto Ae^{-V\phi(x)}, \qquad (5)$$

where

$$\phi(x) = -\int^x \log\left[\frac{R^+(y)}{R^-(y)}\right]dy$$

$$= e_{tot}\ln(e_{tot} - x) - x\ln\left[\frac{(e_{tot} - x)(\theta x^2 + \mu)}{x(\theta\epsilon x^2 + 1)}\right]$$

$$- 2\sqrt{\frac{\mu}{\theta}}\arctan\left(\sqrt{\frac{\theta}{\mu}}x\right) + \frac{2}{\sqrt{\theta\epsilon}}\arctan\sqrt{\theta\epsilon}x. \quad (6)$$

We note that

$$\frac{d\phi(x)}{dx} = -\log[R^+(x)/R^-(x)]$$

$$= -\ln\frac{(e_{tot} - x)(\theta x^2 + \mu)}{x(\theta\epsilon x^2 + 1)};$$

hence the two stable fixed points of Eq. (3) correspond to the two minima of $\phi(x)$, and the unstable one corresponds to a maximum. In fact, for each steady state x^*,

$$\phi''(x^*) = \frac{1}{x^*}\left[\frac{d\log\frac{R^-(x)}{R^+(x)}}{d\log x}\right]_{x=x^*}, \qquad (7)$$

which has the same sign as $d(R^- - R^+)(x^*)/dx$. x^* is stable if $\phi''(x^*) > 0$, and unstable otherwise. Near a stable x^*

$$\phi(x) = \phi(x^*) + \frac{\phi''(x^*)}{2}(x - x^*)^2 + \cdots. \qquad (8)$$

The Gaussian variance of $P^{ss}(x)$ is $[V\phi''(x^*)]^{-1}$ which tends to zero when V tends to infinity.

The square bracket term in (7) is called elasticity [20] due to its analogue to classical mechanics. Near the "more stable" fixed point of Eq. (3), for a system with large V, a Gaussian, linear approximation is warranted. This is the classic theory of EOLK [12]. The key insight of this theory is the so-called fluctuation-dissipation theorem for the NESS, a consequence of the Markovian Gaussian process. It provides a relationship among the linear relaxation kinetic matrix, the noise amplitude, and the covariance matrix of the Gaussian process. In a similar spirit, Berg, Paulsson, and Ehrenberg have put forward a linear noise approximation [20]. Reference [21] has further illustrated that Gaussian characteristics are not necessarily only related to equilibrium fluctuations. Rather, they are determined by linear dynamics near a steady state [22].

To our current discussion, the most important feature of Eq. (5) is that the function $\phi(x)$ is independent of V, provided that V is sufficiently large. Therefore, even though $\phi(x)$ exhibits bistability which corresponds closely to Eq. (3), when $V \to \infty$, only one of the two stable fixed points is relevant in the thermodynamic limit, and it is the one with smaller $\phi(x)$. A Maxwell-like construction, therefore, is necessary at the critical k_1 when $\phi(x_1^*) = \phi(x_2^*)$. See Fig. 2.
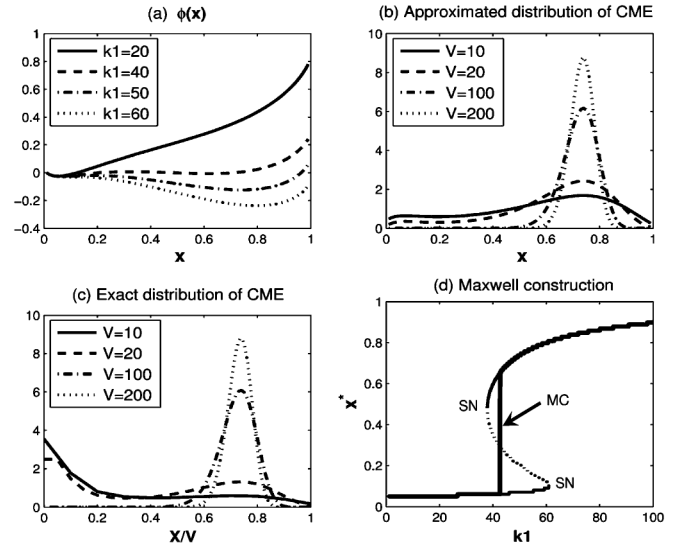

FIG. 2. Stationary distribution of CME and Maxwell construction. (a) The shape of $\phi(x)$ varies with the parameter k_1; (b) approximated stationary distribution of CME according to Eq. (5) varies with the volume with parameter value $k_1 = 50$; (c) exact stationary distribution of CME according to Eq. (4) varies with the volume with parameter value $k_1 = 50$; (d) the saddle-node (SN) bifurcation diagram for steady states and the Maxwell construction (MC). Other parameters used in the calculation: $[K] = 1$, $e_{tot} = 1$, $k_{-1} = 0.01$, $k_2 = 10$, $k_{-2} = 0.5$.

It should be emphasized that bistability in the CME is really a nonequilibrium phenomenon. The metastability, however, can exist even when the white noise is "additive." An example is a diffusive particle restricted in a bistable potential with vanishing diffusivity [23].

Discontinuity of stochastic entropy production and first-order phase transition.—A biochemical NESS is sustained by a continuous input of chemical energy which is converted to dissipated heat. The entropy production rate (EPR) can be computed [10]:

$$\frac{\text{EPR}}{V} \approx \frac{\triangle G}{\int_0^\infty e^{-V\phi(x)}dx}\left(J_1^{ss}\int_{x_1^*-\epsilon}^{x_1^*+\epsilon} e^{-V\phi(x)}dx\right.$$

$$\left. + J_2^{ss}\int_{x_2^*-\epsilon}^{x_2^*+\epsilon} e^{-V\phi(x)}dx\right). \qquad (9)$$

When V tends to infinity, EPR/V tends to $\triangle GJ_1^{ss}$ if $\phi(x_1^*) < \phi(x_2^*)$, and tends to $\triangle GJ_2^{ss}$ if $\phi(x_2^*) < \phi(x_1^*)$. Since $J_1^{ss} \neq J_2^{ss}$, the EPR/V is discontinuous at the critical situation when $\phi(x_1^*) = \phi(x_2^*)$.

In classical equilibrium phase transition theory, a first-order phase transition has a discontinuity in the first derivative of the free energy, and a second-order phase transition has a discontinuity in the second derivative. According to this classification, the present (nonequilibrium) phase transition can be considered as first order.

Summary.—A state of a biological cell, called a functional cellular attractor [10], should be dynamically stable against various minor perturbations which are inevitable in

living systems. Thus, it is often thought that "noise" added to the biological models only provides moderate refinements to the behaviors otherwise predicted by the classical, deterministic description. The present Letter, however, shows something deeper: the relative stability and robustness of the phosphorylation-dephosphorylation module cannot be properly inferred without an explicit consideration of the intrinsic noise in the model. In cellular biology, it is incorrect to model biological stability and robustness in terms of deterministic trajectories or sizes of basins of attractors from a deterministic model. Biological stability and robustness are stochastic concepts. Hence the presence of noise not only leads to corrections to the deterministic analysis but may give rise to emergent behaviors.

The CME has now been recognized as a fundamental mathematical theory for mesoscopic chemical and biochemical reaction systems in a small, spatially homogeneous volume [15]. Its large volume limit recovers the law of mass action kinetics [14]. However, the deterministic differential equations, while defining various attractors, provide no information on the relative probabilities between them [13]. Furthermore, in the thermodynamic limit only one of the attractors will be dominant with probability 1. The Maxwell-type construction, thus, enters the CME and becomes an integral part of a more complete theory. The biochemically interesting emergent dynamics from a CME, thus, is not the deterministic differential equations, but rather a stochastic jump process within a set of discrete states defined by the deterministic attractors. This is distinctly a mesoscopic [24] driven system phenomenon: when the volume is too large, the time of escaping an attractor is practically infinite. Thus, the complex dynamics disappears. When there is no chemical driving force, i.e., $\gamma = 1$, the multistability disappears [10]. Near a given attractor which is a deterministic fixed point, the EOLK phenomenological Gaussian fluctuation theory applies [12]. Furthermore, the macroscopic driven system in NESS can behave like an equilibrium system with a (nongradient) potential [25].

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