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Cellular Biology in Terms of Stochastic Nonlinear Biochemical Dynamics: Emergent Properties, Isogenetic Variations and Chemical System Inheritability

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Abstract Based on a stochastic, nonlinear, open biochemical reaction system perspective, we present an analytical theory for cellular biochemical processes. The chemical master equation (CME) approach provides a unifying mathematical framework for cellular modeling. We apply this theory to both self-regulating gene networks and phosphorylation-dephosphorylation signaling modules with feedbacks. Two types of bistability are illustrated in mesoscopic biochemical systems: one that has a macroscopic, deterministic counterpart and another that does not. In certain cases, the latter stochastic bistability is shown to be a "ghost" of the extinction phenomenon. We argue the thermal fluctuations inherent in molecular processes do not disappear in mesoscopic cell-sized nonlinear systems; rather they manifest themselves as isogenetic variations on a different time scale. Isogenetic biochemical variations in terms of the stochastic attractors can have extremely long lifetime. Transitions among discrete stochastic attractors spend most of the time in "waiting", exhibit punctuated equilibria. It can be naturally passed to "daughter cells" via a simple growth and division process. The CME system follows a set of nonequilibrium thermodynamic laws that include non-increasing free energy $F(t)$ with external energy drive $Q_{hk} \geq 0$, and total entropy production rate $e_p = -dF/dt + Q_{hk} \geq 0$. In the thermodynamic limit, with a system's size being infinitely large, the nonlinear bistability in the CME exhibits many of the characteristics of macroscopic equilibrium phase transition.

Keywords Biochemistry · Cell biology · Chemical master equation · Evolution · Nonequilibrium · Nonlinear dynamics · Stochastic processes · Thermodynamics

1 Introduction

From an evolutionary biology standpoint, Kirschner and Gerhart have argued that a central task of cellular and organismal biology is to provide phenotypic variations with molecular mechanisms that connect genome to life [1]. Molecular mechanisms demystify the

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biological variations upon which Darwin's natural selection occurs, thus giving the "plausibility of life".

Biochemistry and molecular genetics/genomics are the two foundations of cellular molecular biology [2]. According to the Modern Synthesis School of population genetics [3] and its genomic interpretations [4], the molecular basis of biological variations is coded in the DNA sequence, which is inheritable via Mendelian genetics and Watson-Crick base-pairing. Biochemistry, on the other hand, has long been considered as a deterministic mechanics that executes the instructions in the DNA [5, 6].

Continuous theoretical investigations [7, 8] and recent experimental demonstrations of *stochastic gene expression* in single cells, however, have transformed the genomic monoplay of biological variations [9, 10]. Stochasticity has risen rapidly to prominence in cellular molecular biology [11–13]. Isogenetic biochemical variations are now widely considered as mechanisms for "novelty" in cellular processes ranging from cell differentiation to oncogenesis [14–16].

It is against this backdrop that statistical physics and physical chemistry have a defining role to play in complementing the yet descriptive cellular molecular biology with a first-principle based analytical theory. Stochastic fluctuations in atomic and molecular processes have been the rule in our fields; it is the emergent macroscopic deterministic behavior that begs for explanations. However, as we shall show, there are emergent biochemical variations from stochastic molecular systems with nonlinearity. Stochastic variations do not disappear in mesoscopic molecular systems; they simply emerge on a different, much longer "evolutionary" time scale [17, 18].

We shall also discuss statistical thermodynamics. By thermodynamics, we mean one is interested in a system's organizing properties such as entropy and energy, and their interrelations. It turns out, thermodynamics, at least the isothermal part, is a general mathematical law of any stochastic system endowed with a Markovian dynamics [19]. The concept of "entropy" in the classical thermodynamics was defined empirically via the *quasi-static process*. Therefore, there is a feeling that one can only work with this concept in systems at, or near, equilibrium. As we shall demonstrate, however, that one can introduce a mathematical concept of entropy for *any* stochastic dynamics that follows a Markov process. Therefore, the Gibbs entropy, the relative entropy, and their time derivatives (see (36)–(38)) can all be defined for a system at any give time, near or far from equilibrium, in stationarity or in a transient. When applying the "thermodynamics" to biological systems, the real question is the validity of a stochastic Markovian description of the Nature. If that is valid, so is the application of the thermodynamic and the entropy theory [20].

In the approach we take in the present work, the origin of the stochasticity is due to the "intrinsic noise" of molecular collisions [9]. It is interesting to point out that a distinction between intrinsic and extrinsic noises can be made if one considers *simultaneously* two stochastic trajectories with *different* initial conditions. The former assumes the two with different stochastic realizations, while the latter assumes the two with a same realization. The "extrinsic noise", thus, is more consistent with the *random dynamical systems* (RDS) approach which regards the origin of temporal stochasticity being in a system's parameters [21], while intrinsic noise is more consistent with the *stochastic processes* approach which considers one initial condition at a time. See [22] for an example of applying RDS models in neural biology. While a stochastic differential equation with multiplicative Browian motion can have both interpretations, a birth-and-death process can not have a RDS interpretation.[1]

[1]This statement is not strictly true: In the random time-change Poisson representation of a birth-and-death process, one can have a RDS interpretation. However, this seems to pin the stochasticity entirely on the flow of a global time.

2 Stochastic Chemical Dynamics

Two papers published in 1940 have laid the theoretical foundation of stochastic chemical dynamics that connects statistical physics to cellular biology.

H.A. Kramers' paper "Brownian motion in a field of force and the diffusion model of chemical reactions", which was published in that April [23], has shown us how to compute the rate constant for a discrete, individual chemical reaction in aqueous solution, such as $X + Y \to Z$ or $A \to B$, in terms of atomic coordinates and molecular energy functions. In a nutshell, Kramers' theory connected chemistry to physics by understanding chemical reactions using the mechanics of molecular particles and their interactions. This approach, together with Smoluchowski's earlier work on diffusion-controlled chemical reactions and their later synthesis [24, 25], is now one of the main areas of theoretical chemistry [26].

Figure 1 shows the stochastic transitions between two conformations A and B of a single molecule in terms of an energy function. Kramers' theory predicted that the rate constant follows the Arrhenius' law $k_1 \propto e^{-\Delta G^{\ddagger}/k_B T}$. More importantly, the reaction time is spent in waiting, which is random and exponentially distributed, while the actual transition time is instantaneous. This feature is general for any "barrier crossing" process in stochastic, nonlinear systems. Stochastic trajectories of single molecule conformational transitions under room temperature were not observed experimentally until 1990s [27].

In the same year, the January of 1940, Max Delbrück published an equally groundbreaking paper, though with much less fanfare in the subsequent fifty years [28]. While Kramers' Brownian motion is in the "configuration space", Delbrück's birth-and-death process is in the "copy number space". This theory, now under the name of the *chemical master equation* (CME) and more popular Gillespie algorithm [29, 30], has recently emerged as a main workhorse in computational systems biology [31]. In a nutshell, the CME connected cell biology to chemistry by understanding cellular phenotypes and their evolutions in terms of nonlinear biochemical networks in a mesoscopic reaction volume on the order of hundred femtolitres, the size of a cell (1 femtolitre $= 1\ \mu m^3$).

Kramers' theory and the CME clearly marked two complementary domains of physical chemistry. The former computes the rate constant of a individual chemical reaction based on the molecular structures, energy functions, and the solvent environment, while the latter describes the dynamic behavior of a chemical reaction *system*, assuming that the rate constants are given for each and every reaction within.

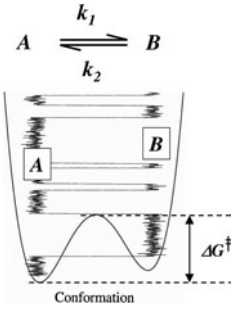

Fig. 1 Kramers 1940 theory connects the chemical reaction kinetics to the stochastic motions crossing an energy barrier. It is shown that the reaction rate constant $k_1 \propto e^{-\Delta G^{\ddagger}/k_B T}$. The stochastic dynamics spends most of the time in "waiting" while the actual transition is instantaneous. The waiting times are random and exponentially distributed. Barrier-crossing is a generic feature of any stochastic, nonlinear dynamical system

2.1 The Chemical Master Equation (CME)

Birth-and-death processes, to which the CME belongs, are a very special class of discrete state, continuous time, Markov processes. The discrete states are non-negative integers forming a lattice $\mathbb{Z}^N$. Consider a system of N chemical species X_i $(i = 1, 2, \ldots, N)$ with M chemical reactions, with the jth chemical reaction being represented by a set of stoichiometric coefficients ν_i^j and μ_i^j (the superscript being reaction and the subscript being species):

$$\nu_1^j X_1 + \nu_2^j X_2 + \cdots + \nu_N^j X_N \xrightarrow{k^j} \mu_1^j X_1 + \mu_2^j X_2 + \cdots + \mu_N^j X_N, \tag{1}$$

in which some of the integers ν's and μ's can be zero $(j = 1, 2, \ldots, M)$. If $\nu_\ell^j = \mu_\ell^j \neq 0$ for a particular ℓ, the corresponding X_ℓ is called a catalyst for the reaction j. If $\mu_\ell^j > \nu_\ell^j > 0$, then X_ℓ is an autocatalyst.

The state of the chemical reaction system at time t is characterized by the set of N integer $\boldsymbol{n}(t) = (n_1(t), n_2(t), \ldots, n_N(t))$, i.e., a grid point on the $\mathbb{Z}^N$ lattice, which specifies the copy number of X_i being $n_i(t)$. The dynamic of the chemical reaction system, thus, is represented by a trajectory in the copy number space $\mathbb{Z}^N$. The stochastic dynamics, according to Lebowitz-Gillespie's algorithm [30, 32], runs as follows: each of the M reactions by itself, say reaction j, can occur at a random time T^j, very much like the radioactive decay, which follows an exponential distribution

$$f_{T^j}(t) = \lambda^j e^{-\lambda^j t}, \tag{2}$$

with the rate

$$\lambda^j = V k^j \prod_{i=1}^{N} \frac{n_i(n_i - 1)\cdots(n_i - \nu_i^j + 1)}{V^{\nu_i^j}}. \tag{3}$$

The parameter V in (3) stands for the volume of the reaction system. It converts molecular copy number n_i to concentration n_i/V for species X_i; all the rate constants in (1) are concentration based. Now with the presence of all M reactions in the system, the first one to occur is at the random time

$$T^* = \min\{T^1, T^2, \ldots, T^M\}, \tag{4}$$

which follows again an exponential distribution

$$f_{T^*}(t) = \lambda^* e^{-\lambda^* t}, \quad \lambda^* = \sum_{j=1}^{M} \lambda^j. \tag{5}$$

The T^* determines the time for the system to move away from the current grid point. The system then moves randomly to one of the M grid points

$$(n_1, n_2, \ldots, n_N) + \left(\mu_1^j - \nu_1^j, \mu_2^j - \nu_2^j, \ldots, \mu_N^j - \nu_N^j\right), \quad j = 1, 2, \ldots, M, \tag{6}$$

with the probability λ^j/λ^*.

The above stochastic dynamics on $\mathbb{Z}^N$ lattice can also be described by a probability distribution $p(n_1, n_2, \ldots, n_N, t)$, which satisfies the chemical master equation (CME):

$$\frac{dp(\boldsymbol{n},t)}{dt} = \sum_{j=1}^{M} \left[\lambda^j(\boldsymbol{n}-\boldsymbol{\mu}^j+\boldsymbol{\nu}^j)p(\boldsymbol{n}-\boldsymbol{\mu}^j+\boldsymbol{\nu}^j) - \lambda^j(\boldsymbol{n})p(\boldsymbol{n})\right] \tag{7}$$

where $\boldsymbol{n} = (n_1, n_2, \ldots, n_N)$, $\boldsymbol{\mu}^j = (\mu_1^j, \mu_2^j, \ldots, \mu_N^j)$, and $\boldsymbol{\nu}^j = (\nu_1^j, \nu_2^j, \ldots, \nu_N^j)$.

Therefore, the CME and the Gillespie algorithm are two different descriptions for the same stochastic dynamical model of chemical reaction systems in a mesoscopic volume, parallel to the diffusion (Fokker-Planck) equation and stochastic differential equation approaches to Brownian motion, developed by Einstein and Langevin respectively in 1905 and 1908.

See [33–35] for several recent reviews on the theory of the CME and [36–38] for its applications to simple nonlinear chemical reaction systems.

2.2 Nonlinear Chemical Dynamics

The parameter V, the volume of the reaction system, is a crucial parameter of the CME. For chemical reaction systems with macroscopic volume and Avogadro's number of molecules, one can introduce the concentration for species X_i, $u_i = n_i/V$. If we let both n_i and $V \to \infty$ in the CME, but keep $n_i/V \to u_i$ finite, then it can be mathematically shown that a set of deterministic, nonlinear kinetic equations arise [35, 39]:

$$\frac{du_i(t)}{dt} = \sum_{j=1}^{M} \left(\mu_i^j - \nu_i^j\right) J^j, \tag{8}$$

in which

$$J^j = k^j u_1^{\nu_1^j} u_2^{\nu_2^j} \cdots u_N^{\nu_N^j} = \lim_{V,\,\boldsymbol{n}\to\infty} \frac{\lambda^j(\boldsymbol{n})}{V}, \tag{9}$$

where $\lambda^j(\boldsymbol{n})$ is given in (3). We note that the system of kinetic equations (9) is nothing but the classic Law of Mass Action for the reaction scheme (1)! Therefore, the CME is not an alternative approach to biochemical kinetics. Rather, the deterministic dynamics based on the Law of Mass Action is the *skeleton* of the CME dynamics. A thorough understanding of any CME, thus, requires a full grasp of the deterministic dynamics from the corresponding nonlinear differential equation.

In fact, studying the CME, (7), together with its deterministic counterpart, (8), side-by-side leads to a series of insights into the nonlinear, stochastic chemical kinetics.

Stationary Distribution First, for most biochemical reaction systems, the CME has a unique stationary probability distribution $p_V^{ss}(\boldsymbol{n})$. Note that this distribution is also a function of the system's volume V. As a function of V the $p_V^{ss}(\boldsymbol{n})$ usually has the general form of the so called WKB (Wentzel-Kramers-Brillouin) expansion:

$$p_V^{ss}(\boldsymbol{n}) = \exp\left(-V\phi_0(\boldsymbol{u}) + \phi_1(\boldsymbol{u}) + \cdots\right), \quad \boldsymbol{u} = \frac{\boldsymbol{n}}{V}, \tag{10}$$

when $V \to \infty$. The function $\phi_0(\boldsymbol{u})$ is independent of V. It can be obtained, if it exists, from

$$\phi_0(\boldsymbol{u}) = \lim_{V\to\infty} -\frac{1}{V} \log p_V^{ss}(V\boldsymbol{u}). \tag{11}$$

One of the most important properties concerning the $\phi_0(\boldsymbol{u})$ is the equation [40]

$$\boldsymbol{b}(\boldsymbol{u}) \cdot \nabla \phi_0(\boldsymbol{u}) = -\left(\nabla \phi_0(\boldsymbol{u})\right)^2, \tag{12}$$

where the $\boldsymbol{b}(\boldsymbol{u}) = d\boldsymbol{u}/dt$ is the right-hand-side of (8). Equation (12) implies that

$$\frac{d}{dt}\phi_0\left(\boldsymbol{u}(t)\right) = \nabla \phi_0(\boldsymbol{u}) \cdot \frac{d\boldsymbol{u}}{dt} = \nabla \phi_0(\boldsymbol{u}) \cdot \boldsymbol{b}(\boldsymbol{u}) \leq 0. \tag{13}$$

In other words, the deterministic, nonlinear chemical dynamics given by (8) follow the downhill of the function $\phi_0(\boldsymbol{u})$. The function $\phi_0(\boldsymbol{u})$ can and should be considered as a *dynamic landscape*. In fact, the stable steady states of (8) are precisely the local minima of $\phi_0(\boldsymbol{u})$.

Maxwell Construction Many nonlinear dynamical systems have multiple, locally stable steady states. We will see one of such examples in self-regulating gene network in Sect. 3.1. A deterministic dynamical system approaches one of its stable steady states (or attractors) and then stays there forever. Which attractor it goes to depends on the initial condition of the dynamical system. This behavior gives rise to the concept of "basin of attraction". This is the picture one obtains from studying chemical dynamics based on (8).

Is one attractor more "important" than another? This question can not be answered under strictly deterministic dynamics. However, for a dynamical system with stochasticity, different attractors can have different probabilities. In this sense, one attractor can be more "stable" then another—While jumping among different attractors, the system spends *totally* more time in a more stable attractor. This is an insight the CME offers that does not exist in the deterministic kinetics.

Now let us consider again $p_V^{ss}(\boldsymbol{n})$. Let us assume it has several peaks corresponding to the stable steady states of the deterministic dynamics. What is its limit when $V \to \infty$? Noting that $p_V^{ss}(\boldsymbol{n})$ is always normalized, and $p_V^{ss}(\boldsymbol{n}) \sim \exp(-V\phi(\boldsymbol{u}))$, we see that the entire distribution converges to the global minimum of $\phi_0(\boldsymbol{u})$ with probability 1. Even though a $\phi_0(\boldsymbol{u})$ can have many local minima, in the limit of $V \to \infty$, all their probabilities are infinitesimal but one!

All these other local minima are called *metastable*. If a stochastic dynamics start from within one of the basins of metastable states, if the V are very large, it will take an extremely long time, $\sim e^{\alpha V}$ $(\alpha > 0)$ to exit. This is consistent with the result from the deterministic dynamics. The "infinite time" in the deterministic dynamics is meant to be much shorter than these extremely long exit times.

Applying the above discussions to a bistable system which undergoes saddle-node bifurcation:

$$\frac{du}{dt} = b(u, \theta) \tag{14}$$

where θ is a parameter. The steady state(s) $u^*(\theta)$ of the system is obtained from solving $b(u, \theta) = 0$. The solid line in Fig. 2, the S-shaped curve, is called bifurcation diagram. For the middle range of the θ value, the system has three steady states, the top and bottom ones are stable, while the middle one is unstable. The corresponding stochastic dynamics gives its stationary distribution and corresponding $\phi_0(u, \theta)$ which is also shown in Fig. 2. The peaks and troughs match the steady states $u^*(\theta)$. For each θ, in the limit of $V \to \infty$, the stationary probability $p_V^{ss}(u)$ converges to the global minima of $\phi_0(u, \theta)$. Therefore, the stochastic dynamics selects only one of the two minima of $\phi_0(u)$: When $\theta < \theta^*$, the lowest branch and when $\theta > \theta^*$, the uppermost branch. The dashed vertical line at θ^* is known as Maxwell construction [41].

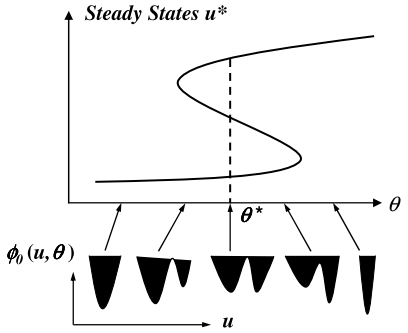

Fig. 2 The S-shaped curve $u^*(\theta)$ is known as *bifurcation diagram* for saddle-node bifurcation. It shows the steady states of a deterministic chemical dynamical system $du/dt = b(u, \theta)$ changing with parameter θ. The system is bistable for the middle ranged value of θ. The corresponding CME gives dynamic landscape $\phi_0(u, \theta)$, with its peak(s) and trough match the u^*. In the limit of $V \to \infty$, the stationary distribution of the stochastic dynamics has probability 1 located at the global minimum of $\phi_0(u)$. Hence when $\theta < \theta^*$, it is located at the lowest branch; and when $\theta > \theta^*$, it is located at the uppermost branch. There is a *discontinuity* at $\theta = \theta^*$. The *vertical dashed line* is known as Maxwell construction

Competition Between Large System Size and Long Time The stochastic CME clearly shows that there are two very different time scales in the nonlinear chemical dynamics with multistability. The two time scales are well separated by the exit times from one attractor to another. In the time scale much shorter than this, the deterministic chemical kinetics rules. However, in the time scale much longer than this, the system stochastically jumps among the multiple attractors, as a set of discrete states. The dynamics on this time scale is again stochastic. The exit time of an attractor depends exponentially on the system's volume V, $e^{\alpha V}$ ($\alpha > 0$). Hence, the larger a reaction system, the longer one has to wait to observe the stochastic jumps.

Mathematically, for a chemical reaction system with bistability, exchanging the two limits

$$\lim_{V\to\infty}\lim_{t\to\infty} p_V(\boldsymbol{n}, t) \neq \lim_{t\to\infty}\lim_{V\to\infty} p_V(\boldsymbol{n}, t). \tag{15}$$

The left-hand-side gives probability 1 at the global minimum of $\phi_0(\boldsymbol{u})$ independent of the initial condition; the right-hand-side goes to different local minima of $\phi_0(\boldsymbol{u})$ depending on the initial condition. The inequality in (15) is the origin of T. Kurtz's convergence theorem for only finite time [39], as well as the so called Keizer's paradox [36, 37].

To Kirschner and Gerhart's thesis, the most important insight from the nonlinear biochemical dynamics is that on an "evolutionary" long time scale, even a simple chemical reaction system can exhibit stochastic variations. These variations and the stochastic dynamics among them, though deeply rooted in the random fluctuations of molecular reactions as understood by Kramers, are mesoscopic, or even macroscopic, emergent properties of nonlinear interacting molecular species. Biological variations need not be solely from DNA sequences; it could come also from *isogenetic* nonlinear biochemical reaction systems.

There is a crucial conceptual issue to be resolved: From a thermodynamic standpoint, how can the above mentioned chemical variations, i.e., diversity, be maintained? Should not all chemical systems approach to equilibrium in the long-time limit?

Indeed, all the above discussed mutistable systems are open chemical systems with *sustained* external chemical driving force. Therefore, they do not approach to an equilibrium but rather their respective nonequilibrium steady states [42, 43]. In fact, if one eliminates

all the chemical driving force on a system, then the CME predicts an equilibrium steady state in the long-time limit. The $p_V^{ss}(\boldsymbol{n})$ in this case is a simple, Gaussian-like distribution; all chemical reactions satisfy the *principle of detailed balance* [44]. The net flux is zero in each and every reaction in the system.

2.3 Nonequilibrium Steady State (NESS)

The mathematical theory of nonequilibrium steady state in stochastic dynamical systems represented by master equations and Markov processes has only been established recently [45]. The physics, however, has a long and diverse histories which can be traced back to H. Haken, T.L. Hill, J. Keizer, M.J. Klein, M. Lax, J.L. Lebowitz, G. Nicolis, I. Prigogine, J. Ross, to name a few among the many pioneers [46–53]. See [42, 43, 54] for some recent applications to chemical and biochemical systems.

Conceptually, there are four kinds of mesoscopic chemical kinetic systems: (i) stationary systems in chemical equilibrium with equilibrium fluctuations; (ii) systems with time-dependent transient relaxation to equilibrium; (iii) stationary open chemical systems which are sustained out-of-equilibrium by a sustained chemical driving force; and (iv) systems with time-dependent transient relaxation toward the (iii). Nonequilibrium steady state (NESS), or stationary nonequilibrium state, the (iii), is the most appropriate chemical dynamic model for a living cell under homeostasis [55].

Equilibrium Stochastic Dynamics and Time Reversibility It is now well understood that the stochastic fluctuations in an equilibrium, as a function of time, is *time reversible*. All statistical properties of a forward stochastic trajectory can not be distinguished from its time reversal. In fact, any sequence of events that occur will have equal probability to occur in reverse—thus nothing can be accomplished in an equilibrium dynamics. There is no energy conversion from one form to another; or transport materials from one place to another.

Detailed Balance At the chemical reaction level, all reactions are going forward and backward with equal likelihood. The net flux within each and every reaction is zero. This is the principle of detailed balance [44]. One immediately sees that any chemical kinetic scheme that assumes irreversible reactions is incompatible with an equilibrium steady state. In fact, the rate constants of a kinetic scheme for an equilibrium reaction system, or a system approaching to an equilibrium, have to satisfy the Wegscheider cycle condition [44, 56]. In the CME formulation, the Wegscheider cycle condition becomes the Kolmogorov cycle condition for reversible Markov processes [45]: each and every cycle in the $\mathbb{Z}^N$ satisfies the detailed balance. In order to distinguish the subtle difference, we have termed the former *chemical detailed balance* and the latter *mathematical detailed balance* [37].

Gardiner [57] has shown that for chemical reaction systems with chemical detailed balance, not only its CME has mathematical detailed balance, hence its stationary distribution is solvable, the stationary distribution is also a multivariate Poissonian conditioned on the conservation laws among molecular species. It has a single peak.

Cycle Flux in NESS Any kinetic model that contains irreversible chemical reactions, therefore, implicitly assumes an chemical driving force. In fact, the force is assumed to be infinite. To have a firm thermodynamic basis, it is advised that one finds out explicitly the source(s) and sink(s) of the chemical driving force(s) applied to a biochemical system in cellular biochemical modeling. For example, in the stochastic models for motor proteins, the driving force is from the hydrolysis of ATP $\rightarrow$ ADP + Pi. Their concentrations are assumed to be constant in motor protein kinetics.

With the presence of a sustained chemical driving force(s), the stochastic dynamics according to a CME approaches not to an equilibrium, but to a NESS. When a system is in a NESS, since all the probabilities are no longer changing with time, and since the system is not detailed balanced, there must be balanced *cycle fluxes*. This is a simple consequence of Kirchhoff's Law. The cycle flux and the landscape $\phi_o(\boldsymbol{u})$ in a chemical NESS are like the current and voltage in an electrical circuit with battery. They provide complementary information on a "living" chemical system [58, 59].

NESS on Different Time Scales The term "nonequilibrium steady state" deserves further clarification. It seems to have two different meanings in systems with multiple attractors. On the time scale shorter than the jumping times between the attractors, a NESS corresponds to a single, average chemical composition with a multivariate Gaussian-like concentration (or number) fluctuations. In this sense, a nonlinear chemical system can have multiple steady states. The long-time fate of the system depends on its initial condition. They are the attractors of deterministic dynamics.

However, on the time scale much longer than the slowest exit time between the attractors, the term NESS takes another, completely different, meaning. Here a NESS has a stationary distribution which peaks at every attractors with appropriate weights. The chemical system jumps continuously among the multiple attractors with ergodicity. In this case, a system has a unique NESS which has the stationary distribution as the solution to the CME, the $p_V^{ss}(\boldsymbol{u})$.

We should mention that for a chemical reaction system with individual reaction rates on the order of milli- and micro-seconds, and with a couple of thousand copies of molecules, the exit time of an attractor can easily be as long as 10^{11} years. That is an eternity! However, if the number of molecules is reduced to a few hundreds, then the time is only on the order of hours.

2.4 Nonequilibrium Phase Transition in the Bistable CME

Multiple steady states, or attractors, and bifurcations upon parameter changes are the essence of deterministic nonlinear dynamical systems. Since the 1970s, it has long been argued that cellular and physiological states of biological systems should be understood in terms of the concept of attractors in nonlinear dynamics [14, 60–63]. The CME approach to the reaction dynamics of mesoscopic biochemical systems adds a significant mathematical rigor to this still elusive idea. In particular, the interplay between nonlinearity and stochasticity seems to provide a deeper understanding of the complexity of real biological systems. The concepts such as "barrier crossing" and "nonlinear bifurcation" are unified in the CME theory.

In a recent study of the CME of an open, driven biochemical system, the phosphorylation-dephosphorylation cycle with feedback (see Sect. 3.2), it has been shown that [17] the bistable system exhibits all the characteristics of equilibrium phase transitions extensively studied in statistical physics. This includes the Maxwell construction for discontinuity in the thermodynamic ($V \to \infty$) limit, the Lee-Yang theory of a zero of the partition function being the origin of non-analyticity, and the terminal critical point in a phase diagram matching the cusp in nonlinear saddle-node bifurcation. These findings seem to suggest that isogenetic variations in biochemical systems are intimately related to phase transition.

3 Self-Regulating Genes and Phosphorylation-Dephosphorylation Cycles with Feedback

We now turn our attention to two concrete biochemical reaction systems, one is the self-regulating gene network (see (16)) and the other is the phosphorylation-dephosphorylation

signaling cycle (see (23)). As we shall show, even though they are considered completely different biochemical entities, their biochemical kinetics are essentially identical. Hence, their analysis based on deterministic mass-action kinetics and stochastic CME will be carried out in parallel.

3.1 Self-regulating Gene Networks

Self-regulating gene networks have been extensively studied in recent years [64–66]. These systems can be described in terms of a biochemical kinetic scheme that consists of biosynthesis and degradation of a transcription factor (TF), as well as the TF binding to the DNA regulatory sequence of its own gene:

$$\text{transcription factor binding:} \quad DNA + \chi\ TF \underset{f}{\overset{h}{\rightleftharpoons}} DNA \cdot (TF)_\chi, \tag{16a}$$

$$\text{TF biosynthesis:} \quad DNA \xrightarrow{g_0} TF, \quad DNA \cdot (TF)_\chi \xrightarrow{g_1} TF, \tag{16b}$$

$$\text{TF degradation:} \quad TF \xrightarrow{k} . \tag{16c}$$

Hornos *et al.* [65] considered $g_1 < g_0$ with $\chi = 1$, i.e., the TF in monomeric form is a repressor for its own gene expression. Walczak *et al.* [66] studied $g_1 > g_0$ with $\chi = 2$. The gene product in this case, in dimeric form, is its own transcription enhancer.

The corresponding macroscopic kinetics of gene regulations with feedback, in terms of the deterministic Law of Mass Action, can be written in ordinary differential equations as

$$\frac{dx}{dt} = hy^\chi(1-x) - fx, \qquad \frac{dy}{dt} = (g_0(1-x) + g_1 x) - ky, \tag{17}$$

where x is the fraction of the DNA with TF bound ($\chi = 1, 2$ for monomer and dimer, respectively), y is the concentration of the TF, the gene product. Figure 3 shows that system (17) can have unique steady state as well as bistability.

To solve the steady state(s), we introduce nondimensionalization. The two equations in (17) are then simplified

$$\frac{dx}{d\tau} = \omega\left[\theta z^\chi(1-x) - x\right], \qquad \frac{dz}{d\tau} = g + (1-g)x - z. \tag{18}$$

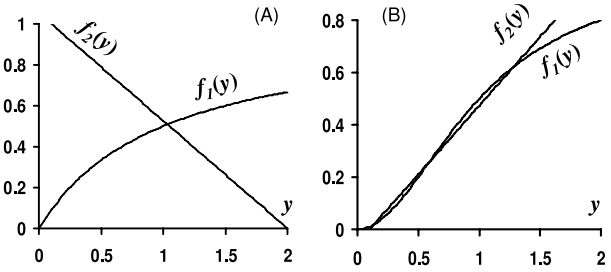

Fig. 3 The null clines (isoclines) of the system in (17) for negative feedback case (**A**) and positive feedback case (**B**), showing unique steady state and bistability, respectively. For the negative feedback case, $g_0 > g_1$ and $\chi = 1$. The two null clines are $x = f_1(y) = y/(K_d + y)$ and $x = f_2(y) = (g_0 - ky)/(g_0 - g_1)$, where $K_d = f/h$. For the positive feedback case, $g_1 > g_0$ and $\chi = 2$. The two null clines are $f_1(y) = y^2/(K_d + y^2)$ and $f_2(y) = (ky - g_0)/(g_1 - g_0)$. Parameters used in computations for (**A**): $K_d = 1$, $k = 0.5$, $g_0 = 1$, $g_1 = 0.05$, $\chi = 1$; and for (**B**) $K_d = 1$, $k = 0.5$, $g_0 = 0.05$, $g_1 = 1$, $\chi = 2$

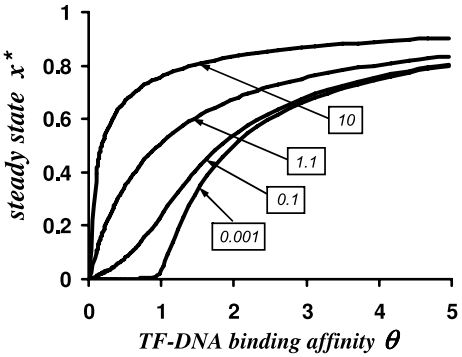

Fig. 4 Self-regulating gene network with $\chi = 1$. The steady state fraction of gene with TF bound, x^*, increases with the binding affinity parameter $\theta = (g_1/k)K_d^{-1}$ where $K_d = f/h$. The four curves are for $g = g_0/g_1 = 0.001, 0.1, 1.1, 10$ where g_0 and g_1 are the TF biosynthesis rates in the absence and presence of TF binding to DNA. $g > 1$ corresponds to the TF being a repressor, and $g < 1$ corresponds to the TF being an enhancer. For strong enhancer with very small $g = 0.001$, the TF induced gene expression is highly cooperative, exhibiting delayed onset

where

$$z = \frac{k}{g_1}y, \qquad \tau = kt, \qquad \theta = \frac{h}{f}\left(\frac{g_1}{k}\right)^{\chi}, \qquad \omega = \frac{f}{k}, \qquad g = \frac{g_0}{g_1}. \tag{19}$$

The three cases of $\chi = 0, 1, 2$ yield, respectively, hyperbolic gene activation, delayed onset (with possible transcritical bifurcation when $g = 0$, at $\theta = 1$), and bistability with saddle-node bifurcation.

$\chi = 0$ There is only a single steady state. The fraction of activated gene is $x^* = \theta/(1+\theta)$ which has a hyperbolic dependence on θ. In the meantime the gene product $z^* = (g + \theta)/(1+\theta)$.

$\chi = 1$ The quadratic equation $(1-g)x^2 - (1 - 2g - 1/\theta)x - g = 0$ has a unique root $x^* \in (0, 1]$ for $\theta \geq 0$:

$$x^* = \frac{(1 - 2g - \frac{1}{\theta}) + \sqrt{(1 - 2g - \frac{1}{\theta})^2 + 4g(1-g)}}{2(1-g)}. \tag{20}$$

Figure 4 shows that x^* increases from 0 to 1 when θ increases from 0 to ∞. When $g = 1$, there is no self-regulation and $x^* = \theta/(1+\theta)$ which has the standard hyperbolic shape. When $g = 0$, i.e., the TF is a strong enhancer, $x^* = 0$ for $\theta \leq 1$ and $x^* = 1 - 1/\theta$ for $\theta \geq 1$. There is a transcritical bifurcation at $\theta = 1$. This type of response is called delayed onset.

$\chi = 2$ For the repressor case with negative feedback, i.e., $g > 1$ in (18), there is no bistability because the null cline for $dx/dt = 0$ is an increasing function $z = (x/(1-x)/\theta)^{1/2}$ while the null cline for $dz/dt = 0$ is a decreasing function $z = g - (g-1)x$. For the case of positive feedback with $g < 1$, the system (18) can have three steady states in the positive quadrant, two stable and one unstable. Figure 3 shows the qualitatively different arrangements of the null clines for the two cases.

The condition for the positive feedback case to have bistability is when (θ, g) is in the cusp region bound by the parametric curve $(\frac{1}{z(2-3z)}, \frac{z(1-2z)}{2-3z})$ as shown in Fig. 5.

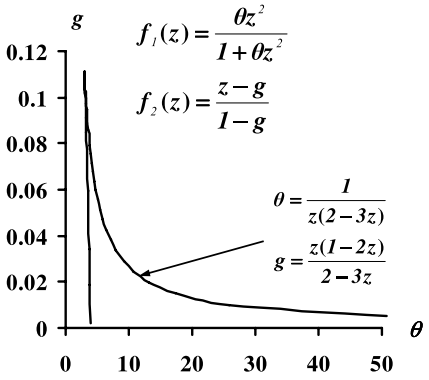

Fig. 5 The $f_1(y)$ and $f_2(y)$ in Fig. 3B has three intersection, corresponding to three steady states for the ODE system (bistability). The parameter region for the bistability typically has a "cusp", known as *cusp catastrophe*. If we let $z = ky/g_1$, then the two null clines in Fig. 3B become the $f_1(z)$ and $f_2(z)$ with $\theta = (hg_1^2/(fk^2)$ and $g = g_0/g_1$. To obtain the cusp region, we solve simultaneously $f_1(z) = f_2(z)$ and $f_1'(z) = f_2'(z)$. This yields the (θ, g) parametrically in terms of z

Adiabatic and Non-adiabatic Limits If $\omega \gg 1$ in (18), then the FT binding to DNA is much faster than its own biosynthesis and degradation. This is known as the *adiabatic limit* [65, 66]. In this case, one can first solve the quasi-steady-state for $dx/d\tau = 0$ to obtain $x = \theta z^\chi/(1 + \theta z^\chi)$. Then the system of equations is reduced to a single one:

$$\frac{dz}{d\tau} = g + \frac{\theta(1-g)z^\chi}{1+\theta z^\chi} - z. \tag{21}$$

On the other hand, if $\omega \ll 1$, then the FT binding to DNA is much slower than its own biosynthesis and degradation. This is known as the *non-adiabatic scenario* [65, 66]. In this case, one can solve the quasi-steady-state for $dz/d\tau = 0$ to obtain $z = g + (1-g)x$. Then again the system in (18) is reduced:

$$\frac{dx}{d\tau} = \omega\left\{\theta\left[g + (1-g)x\right]^\chi (1-x) - x\right\}. \tag{22}$$

Strong Enhancer with $g = 0$ The steady state of the above kinetic system with $g = 0$ has been extensively studied in the context of phosphorylation-dephosphorylation cycles with feedback [17, 41, 67–69]. We now introduce this biochemical signaling system which is kinetically almost isomorphic to the self-regulating gene network.

We have assumed in (16) a cooperative binding of two copies of TF to the DNA in the case of $\chi = 2$. It is important to point out that the strong nonlinearity required in the bistable behavior is not from the cooperativity *per se*. Rather, it is from the fact that only the doubly occupied DNA is functional. In this case, the response function is sigmoidal even for completely independent binding: $\frac{x^2}{1+2x+x^2}$. This response function is in sharp contrast to the *fraction of binding*, $\frac{1\cdot 2x+2\cdot x^2}{2\cdot(1+2x+x^2)}$, which is hyperbolic $\frac{x}{1+x}$. See [70, 71] for recent experimental and theoretical studies on the consequences of nonlinearity from time delay and cooperative binding.

3.2 Phosphorylation-Dephosphorylation Cycles (PdPC) with Feedback

Phosphorylation-dephosphorylation cycles (PdPC) are biochemical regulatory systems in cell signaling. They consist of a substrate protein E which can be phosphorylated to become E^*, catalyzed by a protein kinase K, and protein phosphatase P. In many cases, the kinase itself can be regulated via binding to the E^*; thus the feedback is in the form of autocatalysis. For more discussions on concrete biological examples see the Fig. 1 of [69].

The system can be described in terms of biochemical kinetic scheme

$$\text{kinase regulation via binding of } E^*\text{:} \quad K + \chi\ E^* \underset{f}{\overset{h}{\rightleftharpoons}} K^\dagger, \tag{23a}$$

$$\text{protein phosphorylation:} \quad E + K \xrightarrow{g_0} E^* + K, \quad E + K^\dagger \xrightarrow{g_1} E^* + K^\dagger, \tag{23b}$$

$$\text{protein dephosphorylation:} \quad E^* + P \xrightarrow{\widehat{k}} E + P. \tag{23c}$$

The deterministic kinetic equations for this class of models, according to the Law of Mass Action, is

$$\frac{dx}{dt} = hy^\chi(1-x) - fx, \qquad \frac{dy}{dt} = (g_0(1-x) + g_1 x)(y_t - y) - ky, \tag{24}$$

where x is the fraction of the kinase in the $K^\dagger$ form, y is the concentration of phosphorylated E^*, $k = \widehat{k}[P]$, and y_t is the total concentration of E and E^*. Comparing (24) to (17), we see that the two systems of equations are essentially the same except the former contains an extra term $(y_t - y)$ on the right-hand-side of dy/dt. When $k \gg g_0, g_1$, the former is reduced to the latter.

If the reaction $K + E^* \rightleftharpoons K^\dagger$ is fast, $y_t^\chi \ll f/h = K_d$, and $g_0 = 0$, then one has a quasi-steady-state for $dx/dt = 0$ and a simplified equation for $u = y/y_t$,

$$\frac{du}{d\tau} = \sigma u^\chi(1-u) - u, \tag{25}$$

in which u represents the fraction of phosphorylated E^*, $\tau = kt$, and $\sigma = g_1 h y_t^\chi / fk$ represents the ratio of activities of a kinase to that of a phosphatase.

When $\chi = 0$, the steady state u^* is a hyperbolic function of σ: $u^* = \sigma/(1+\sigma)$. When $\chi = 1$, $u^* = 0$ for $\sigma \le 1$ and $1 - 1/\sigma$ for $\sigma \ge 1$, exhibiting delayed onset. When $\chi = 2$, $u_1^* = 0$ is always stable, and when $\sigma \ge 4$, a second stable steady state $u_2^* = (\sigma + \sqrt{\sigma^2 - 4\sigma})/(2\sigma)$ appears. u_1^* and u_2^* are separated by an unstable $u_3^* = (\sigma - \sqrt{\sigma^2 - 4\sigma})/(2\sigma)$. See Fig. 6.

The open, driven chemical nature of the PdPC with bistability has been studied in [68]. It was shown that if the free energy from ATP hydrolysis is below a critical value (this can be

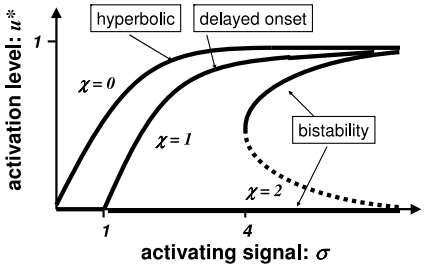

Fig. 6 Steady state(s) of phosphorylation-dephosphorylation cycle (PdPC) with positive feedback, described by the model in (25). $\chi = 0, 1, 2$ represent no-, monomeric, and dimeric activations of the kinase, respectively. Find the equations for the three curves in the text

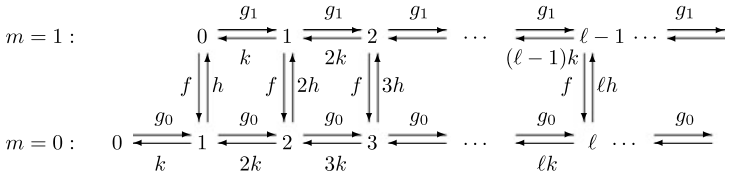

Fig. 7 $m = 0$ and $m = 1$ represent the unbound and bound state of the single copy of DNA (gene). ℓ denote the copy of free TF. Monomeric TF binds DNA with on-rate constant h and off-rate constant f. Binding reduces the copy number of free TF by 1 ($\chi = 1$). TF biosynthesis rate is g_1 and g_0 when the gene is bound and unbound, respectively. TF degradation rate is k

accomplished by either decreasing ATP concentration or increasing ADP/Pi concentrations), then the bistability disappears all together. Biochemical variations can only be maintained with an energy expenditure and free energy dissipation.

3.3 Stochastic Dynamics According to the CME

The theory in Sect. 2.2 indicates that for systems with nonlinear, deterministic bistability, the CME will have its stationary distribution with two peaks located precisely at the two fixed points; there are two stochastic attractors.

However, it comes as a surprise that the CME of a self-regulating gene network, with a monomeric repressor ($g_0 > g_1$, $\chi = 1$), also exhibits bimodal stationary distribution [64, 65]. This is not expected from (17), as illustrated in Figs. 3A and 4. In a similar vein, it has also been discovered that PdPC with feedback, even for $\chi = 1$ and $g_0 = 0$, can have bimodality [69, 72]. This phenomenon has been called *noise-induced bistability*.

It turns out, this type of stochastic bistability (to be distinguished from the nonlinear bistability) is a small copy number effect. In fact, for the self-regulating gene network in a single cell, there is only one copy of the DNA [64, 65]! In the PdPC system studied, the copy number is also small, about 30 [69]. The stochastic bistability is intimately related to the extinction phenomenon [69, 73].

Stochastic Bistability with Slow Fluctuations Stochastic bistability can be best understood in the single-molecule context with slow fluctuations [73, 74]. Consider the CME for kinetic scheme in (16) and assuming only a single copy of the DNA, then one has the detailed kinetics on a lattice, $m = 0, 1$ and $\ell = 0, 1, 2, \ldots$, in Fig. 7.

If the rates h and f are sufficiently smaller than g's and k, then one has a quasi-stationary Poisson distribution along each line in Fig. 7:

$$p(\ell|m=0) = \frac{1}{\ell!}\left(\frac{g_0}{k}\right)^{\ell} e^{-g_0/k}, \qquad p(\ell|m=1) = \frac{1}{\ell!}\left(\frac{g_1}{k}\right)^{\ell} e^{-g_1/k}. \tag{26}$$

The transition rate from $m = 0$ to $m = 1$ is given as the average

$$\overline{h} = h\sum_{\ell=0}^{\infty} \ell\ p(\ell|m=0) = \frac{hg_0}{k}. \tag{27}$$

Then the stationary distribution is

$$p^{ss}(\ell, m) = p(\ell|m)p(m) = \begin{cases} \frac{fk}{fk+hg_0}\frac{1}{\ell!}\left(\frac{g_0}{k}\right)^{\ell} e^{-g_0/k}, & m = 0; \\ \frac{hg_0}{fk+hg_0}\frac{1}{\ell!}\left(\frac{g_1}{k}\right)^{\ell} e^{-g_1/k}, & m = 1. \end{cases} \tag{28}$$

Therefore, if the peak on the $m = 1$ line, g_1/k, and the peak on the $m = 0$ line, g_0/k, are well separated, then the marginal distribution $p^{ss}(\ell)$ will have two peaks. This is the stochastic bistability due to slow, non-adiabatic gene regulation.

Adiabatic Limit We now consider the case when the rates h and f are much greater than g's and k [65, 74]. We can obtain a quasi-stationary distribution between $m = 0$ and $m = 1$ for each and every ℓ. We shall now denote the *total copy number* of TF by ℓ, so the labels along the two lines in Fig. 7 match:

$$p(m = 0|\ell) = \frac{f}{\ell h + f}, \qquad p(m = 1|\ell) = \frac{\ell h}{\ell h + f}. \tag{29}$$

Then the kinetics are simplified into a 1-dimensional birth-and-death process with birth and death rates

$$b_\ell = \frac{f g_0 + \ell h g_1}{f + \ell h}, \qquad d_{\ell+1} = \frac{(f + \ell h)(\ell + 1)k}{f + (\ell + 1)h}. \tag{30}$$

The marginal stationary distribution for the copy number of total TF is

$$p^{ss}(\ell) = C \prod_{i=1}^{\ell} \frac{b_{i-1}}{d_i} = C \prod_{i=1}^{\ell} \frac{[f g_0 + (i-1)h g_1][f + ih]}{i[f + (i-1)h]^2 k}, \tag{31}$$

where C is a normalization factor. We note that

$$b_\ell - d_\ell = \frac{f g_0 + \ell(h g_1 - f k + h k) - \ell^2 h k}{f + \ell h}. \tag{32}$$

The numerator of (32) is only a quadratic function of ℓ. When $\ell = 0$ it is positive and when $\ell = \pm\infty$ it is negative. Therefore, it has only a single zero for positive ℓ. The distribution $p^{ss}(\ell)$ in (31) can only have a single peak for $\ell > 0$. This result agrees with that from the deterministic kinetics. However, because of the discrete nature of ℓ, the $p^{ss}(\ell)$ can also peak at $\ell = 0$ [69]. The condition for this is $p^{ss}(0)/p^{ss}(1) = d_1/b_0 > 1$. That is,

$$\left(\frac{f}{f + h}\right)\frac{k}{g_0} > 1. \tag{33}$$

In fact, if $g_0 = 0$, then the system has an absorbing state at $\ell = m = 0$. Therefore, the stochastic bistability is the "ghost" of the extinction phenomenon.

Mathematically, we note that the $\chi = 1$ case has a quadratic nonlinearity and the $\chi = 2$ case has a cubic nonlinearity. This distinction, leading to stochastic bistability and nonlinear bistability respectively, has been discussed in the context of PdPC with feedback in [69].

Transition Rate Volume Dependence as an Indicator for Bistability Mechanism How can one determine whether the bistability in a mesoscopic system is stochastic in nature or nonlinear in nature? We suggest that the volume-dependence of exit rates can be used as an indicator. By increasing volume and numbers of molecules but keeping their concentrations invariant, stochastic bistability disappears while nonlinear bistability intensifies. As a function of the system's size, the two types of bistability behave differently in a fundamental way.

In thermodynamics, one investigates the mechanism of chemical and biochemical reactions (e.g., protein folding) by measuring reaction rates as functions of temperature and

plotting the so-called Arrhenius plot with *activation enthalpy*. The widely practiced approach does not mean one is interested in a chemical reaction in high temperature or low temperature, *per se*. Rather it is understood that *temperature dependence* provides insights into the mechanism of a reaction: Is it entropic or enthalpic driven?

An analogue exists for the case of *volume dependence* of the transition rates between two stochastic attractors: The rates increase for nonlinear bistability but decrease for stochastic bistability when the volume decreases.

4 Nonequilibrium Statistical Thermodynamics

We now turn our attention to thermodynamics. Afterall, the initial motivation of statistical physics is to understand thermodynamics from a molecular perspective in terms of the theory of probability. We now have a probabilistic, stochastic description of the dynamics of open, driven biochemical reaction systems. Is there an overarching nonequilibrium thermodynamics?

The answer is "yes". Recently, it becomes known that there is a completely statistical thermodynamics for Markovian dynamics based on a master equation [19, 75–79]. Thermodynamics, it turns out, is a general mathematical law of any Markovian dynamics. The thermodynamics of molecular systems discovered in thermal physics is simply one special example.

The Mathematical Theory of Thermodynamics Let us consider a master equation

$$\frac{dp_i}{dt} = \sum_{j=1}^{N} \left(p_j q_{ji} - p_i q_{ij} \right). \tag{34}$$

As we have discussed, one needs to assume that $q_{ij} \neq 0$ iff $q_{ji} \neq 0$ for any i, j in order to be able to study thermodynamics. For simplicity, we further assume the Markovian system is irreducible. Hence, it has a unique, positive stationary distribution we shall denote by $\{\pi_i\}$:

$$\sum_{j=1}^{N} \left(\pi_j q_{ji} - \pi_i q_{ij} \right) = 0, \quad \pi_i > 0. \tag{35}$$

Two thermodynamic quantities will be investigated [19]: The Gibbs entropy

$$S(t) = -\sum_{i=1}^{N} p_i(t) \ln p_i(t), \tag{36}$$

and the Gibbs free energy

$$F(t) = \sum_{i=1}^{N} p_i(t) \ln \left(\frac{p_i(t)}{\pi_i} \right). \tag{37}$$

Applying the chain rule, one has

$$\frac{dS(t)}{dt} = e_p - h_d, \qquad \frac{dF(t)}{dt} = -f_d, \tag{38}$$

where the entropy production rate e_p, heat dissipation rate h_d, and free energy dissipation rate f_d are

$$e_p = \frac{1}{2}\sum_{i,j}\left(p_i q_{ij} - p_j q_{ji}\right)\ln\left(\frac{p_i q_{ij}}{p_j q_{ji}}\right), \tag{39}$$

$$h_d = \frac{1}{2}\sum_{i,j}\left(p_i q_{ij} - p_j q_{ji}\right)\ln\left(\frac{q_{ij}}{q_{ji}}\right), \tag{40}$$

$$f_d = \frac{1}{2}\sum_{i,j}\left(p_i q_{ij} - p_j q_{ji}\right)\ln\left(\frac{p_i \pi_j}{p_j \pi_i}\right). \tag{41}$$

One can mathematically show that

$$S(t) \geq 0, \qquad F(t) \geq 0, \qquad e_p(t) \geq 0, \quad \text{and} \quad f_d(t) \geq 0. \tag{42}$$

More importantly, one can define the so called *house keeping heat*, originally introduced by Oono and Paniconi [80] in a phenomenological NESS thermodynamics, to quantify the driving force applied to the system:

$$Q_{hk} = e_p - f_d = \frac{1}{2}\sum_{i,j}\left(p_i q_{ij} - p_j q_{ji}\right)\ln\left(\frac{\pi_i q_{ij}}{\pi_j q_{ji}}\right) \geq 0. \tag{43}$$

It is also non-negative.

The interpretations of these inequalities are clear: Since the h_d can be positive and negative, there is no guarantee that $dS/dt \geq 0$. As it was clear to Gibbs, for canonical systems it is not the entropy that always increases, but it is the free energy that always decrease: $dF/dt \leq 0$.

Furthermore, we have the decomposition of the entropy production rate

$$e_p = f_d + Q_{hk}, \quad \text{in which } f_d \geq 0 \text{ and } Q_{hk} \geq 0. \tag{44}$$

Now we see that the total time irreversibility, which is characterized by the entropy production rate, e_p [45], really comes from two different origins: The f_d characterizes the spontaneous relaxation (or organization) to a system's stationarity. $f_d = 0$ when a system reaches its stationary π_i. This irreversibility is Boltzmann's original thesis. However, Q_{hk} characterizes irreversibility due to sustained driving, or energy pumping, of the system out-of-equilibrium. There is a continuous dissipation even in NESS. As we have discussed in Sect. 2.3, this driving force is characterized by the breakdown of detailed balance: $\pi q_{ij} \neq \pi_j q_{ji}$. When there is no external driving force, $Q_{hk} = 0$. This irreversibility has long been Prigogine's thesis [49]. For systems without detailed balance, spontaneous approaching to stationary distribution π_i is a form of *self-organization* [49, 51, 63].

Systems with Detailed Balance For systems with detailed balance, which is the subject of classic statistical mechanics, we have the free energy dissipation precisely equal to entropy production rate: $f_d = e_p$. In fact, we see that in this case, $\ln \pi_i = -E_i$ is the energy of the state i ($k_B T = 1$). Then

$$F = \sum_{i=1}^{N} E_i \; p_i - S = \langle E \rangle - S. \tag{45}$$

We have recovered the fundamental equation of classical thermodynamics. Furthermore, we see that if $\pi_i =$ constant, i.e., the system's equilibrium has an *equal probability a priori*, then the $S(t) = -F(t)+$constant, and $dS/dt = -dF/dt \geq 0$. This is in fact Boltzmann's statistical mechanics for isolated system with microcanonical ensemble. In this case, the Second Law states "entropy never decreases".

The Energy of a Stochastic System The foregoing theory seems to suggest that for any stochastic dynamical system, with or without detailed balance, one can define a "statistical energy" as $E_i = -\ln \pi_i$. Combined with the discussion on $\phi_0(\boldsymbol{u})$ from Sect. 2.2, it seems to us that Boltzmann's law, $p_i = \exp(-E_i/k_B T)$, might be understood backward and used as a way to introduce a new form of energy: The energy of stochastic systems [75].

Taking the CME as an example. If one takes $\phi_o(\boldsymbol{u})$ as the energy function, and takes V as $1/(k_B T)$, then one has a "partition function"

$$Z(V) = \int d\boldsymbol{u}\; e^{-V\phi_0(\boldsymbol{u})}. \tag{46}$$

One can in fact develop an entire system of "volumodynamics". It will be interesting to see whether this line of inquiry leads to any new insights for analyzing the CME or nonequilibrium thermodynamics [81].

5 Summary

There are implications to cellular biological systems from the stochastic, nonlinear chemical dynamics perspective. But at the onset, we shall first stress that the CME theory we have discussed assumes a spatially homogeneous chemical reaction system. This is certainly not true for a real biological cell. The CME is a highly idealized model, just as the Ising model extensively studied in statistical mechanics. While the Ising model and related interacting particle systems emphasize spatial aspect of a molecular system, the CME emphasizes compositional heterogeneity in biological systems.

The significance of the CME is its richness, depth, and sophistication. It endows a full range of dynamics from the stochastic mesoscopic scale to the deterministic macroscopic scale, and beyond. It provides insights into the nature of "thermodynamic limit".

Furthermore, it gives rigorous distinction between closed systems that approach to equilibrium and open, driven systems that exhibit spontaneous self-organization. It also allows for studying the relationship between stochastic dynamics and statistical thermodynamics. In terms of the CME, investigations into elusive ideas such as "energy cost that sustains complexity (diversity) measured by entropy" becomes possible.

Last, but not the least, the CME offers an understanding of the interaction between nonlinearity and stochasticity in dynamics. There is no doubt that these two elements are central to many biological processes.

5.1 Emergent Properties of Stochastic Nonlinear Systems

Emergent properties are central to any complex systems and processes [74, 82]. In nonlinear dynamics, emergent properties manifest themselves as the existence and locations of multiple attractors with fixed points, periodic oscillations, or chaotic motions. Simple dynamics are associated with gradient systems in which the attractors are known *a priori* and are determined *locally*; every step of the way, the system is closer to the final destiny. A non-gradient system has no such certainty.

A stochastic CME with detailed balance has its dynamics essentially following a gradient of a function. In protein folding dynamics, the energy landscape is the function. In any study of protein folding dynamics, an energy landscape is always known *a priori*; and its gradient field is the *cause* of the dynamics.

The $\phi_0(\boldsymbol{u})$ as a dynamic landscape, however, has a different characteristics. First, it is non-local. One requires the dynamics to move over the entire possible space many times in order to establish it. The $\phi_0(\boldsymbol{u})$ is a *consequence* of the dynamics. It is only known *retrospectively*.

Therefore, in this perspective, the very existence of multistability, and the average time required to move from one attractor to another, are all emergent properties. They are the results of the complex dynamics of an open biochemical system as a whole under a particular given environment condition.

Dynamics as a Sequence of Punctuated Equilibria Dynamics of nonlinear, stochastic systems with multiple attractors possess certain universal features. As we have said in the beginning, stochastic dynamics jumping among stochastic attractors spend most of the time in waiting. On an evolutionary time scale, the process exhibits a sequence of *punctuated equilibria* (see Fig. 1).

Upon a perturbation, a system's initial response is always a relaxation back to the fixed point with nearly deterministic dynamics. This occurs rapidly. The system then settles at the bottom of the attractor with Gaussian fluctuations. Such a state is often mistaken as an equilibrium. Then in a much longer time scale, the rare event of barrier-crossing leads the system to another attractor (see Fig. 8).

Protein folding kinetics is well-known to have this generic characteristic: A folded protein when immersed in a denaturing solvent, first becomes a "dry molten globule" then unfolds via a thermal activated process; a unfolded protein when immersed in a native solvent, first becomes a "wet molten globule" then folds by thermal activation [83]. These molten globules are folded protein in a denaturant and unfolded protein under a native condition, respectively.

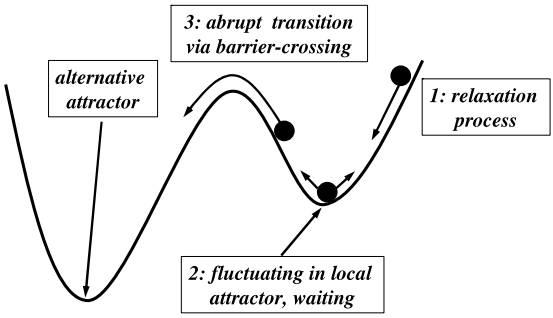

Fig. 8 A schematics showing the generic features of a nonlinear, stochastic dynamical system with multiple attractors under perturbation: Immediately after the perturbation, the system is likely residing at the slope of an attractor. Then (*1*) relaxation occurs and the system returns to its local steady state. At local steady state (*2*), the system fluctuates and spends the time in waiting until a rare event of barrier-crossing (*3*). The rare event only occurs in the evolution time scale; and when it occurs, the actual transition is nearly instantaneous. A rare event usually has an exponential waiting time and the process is "memoryless". This means it occurs without any indication

Other much more complex biological processes seem also to exhibit this feature. For example, the three Es of cancer immunoediting articulate that an immune system counteracts tumor growth with three main phases: elimination, equilibrium, and escape [84].

5.2 Isogenetic Variations of Biochemical Dynamics

Two cells with identical genomes are called *isogenetic*. With exactly same chemical environment, two isogenetic cells can have very different chemical compositions represented by different attractors of the nonlinear biochemical dynamics. Note that by the same chemical environment, we mean a sustained chemical gradient of certain nutrients and their metabolites. With the same chemical potential, however, the two cells can have different nutrient influx. Applying this idea specifically to the biochemical network responsible for gene transcriptional regulation, one can easily understand the origin of isogenetic variations in gene expression [60, 85, 86].

There are growing experimental observations on the multiple steady states of a cell population. The multiple-state nature is most convincing when a cell population is in the middle of a transition: Two peaks with comparable size rather than one can be observed. For example, in Xenopus oocyte maturation induced by hormone progesterone, it had been known that progesterone treatment leads to an increase in the phosphorylation of mitogen-activated protein kinase (MAPK). Ferrell and Machleder [87] have shown, however, that the level of MAPK phosphorylation in an isogenetic Xenopus oocyte population has a bimodal distribution. Furthermore, the relative heights of the two peaks change but their locations are invariant with the increasing progesterone. This is the hallmark of a bistable (also known as two-state, and all-or-none) system under external perturbation.

Similarly, Buckmaster *et al.* [88], using Raman spectrum as an indicator for DNA fragmentation, observed a shift in a bimodal distribution during the apoptosis of DAOY cell line (human brain tumor medulloblatoma) induced by etoposide, a topoisomerase II inhibitor.

Also in cell line U2OS, a human osteosarcoma, Xu *et al.* [89] observed a bimodal distribution in the intensity of fluorescein labeled FITC-Annexin V, a protein that preferentially binds to negatively charged phosphatidylserine (PS). Cell apoptosis involves changes on its surface with the exposure of PS. Upon irradiation, which induced DNA damage and apoptosis in U2OS cells, Xu *et al.* reported a shift in the relative heights of the two peaks. The shift is intensified with the presence of PDCD5 (programed cell death 5) protein, which is known to facilitate apoptosis. Again, the apoptotic process changes the heights of the two peaks without changing their locations.

Cancer cells are well known to be genomically very unstable and heterogeneous; it is not known to us whether these tumor cell lines are truly isogenetic. Still, assuming somatic mutations are rare, these observations strongly suggest nonlinear biochemical multistability in tumor cells.

It is interesting to note that in the 1970s, the field of protein folding had gone through a similar stage in demonstrating the two-state nature of protein folding kinetics [90]. The history of protein science can shed some light on the current development of cellular dynamics.

5.3 Inheritability of Nonlinear Chemical Attractors

DNA in terms of Watson-Crick base-pairing has been considered the only mechanism for inheritability. However, a biochemical system residing in a nonlinear attractor can also be "inherited" via cell growth and division. The CME predicts that the concentrations of the biochemical species are invariant, not their copy numbers. Therefore, if a cell has an autonomous mechanism for increasing its aqueous volume, all the copy numbers will follow

by keeping the concentrations at the steady state. This process is self-organizing and robust. Cell division also maintains the concentrations for both daughter cells. By this mechanism, two isogenetic cells in different biochemical attractors that far apart will go through "growth and division" with their respective chemical compositions inherited.

Finally, we shall also emphasize that the existence and locations of the stochastic attractors of a nonlinear biochemical system are dependent upon the environmental biochemical conditions. Therefore, "mutations" occur upon "environmental" changes in the chemical context. This possibility provides further insights into the debate on spontaneous versus adaptive mutations at the cellular biochemical systems level [91–93]. Still, whether and how such "feedback loops" in cellular evolution leading to genomic innovation is the next stage of the "plausibility of life".

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