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Review

Thermodynamics of stoichiometric biochemical networks in living systems far from equilibrium

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Abstract

The principles of thermodynamics apply to both equilibrium and nonequilibrium biochemical systems. The mathematical machinery of the classic thermodynamics, however, mainly applies to systems in equilibrium. We introduce a thermodynamic formalism for the study of metabolic biochemical reaction (open, nonlinear) networks in both time-dependent and time-independent nonequilibrium states. Classical concepts in equilibrium thermodynamics–enthalpy, entropy, and Gibbs free energy of biochemical reaction systems–are generalized to nonequilibrium settings. Chemical motive force, heat dissipation rate, and entropy production (creation) rate, key concepts in nonequilibrium systems, are introduced. Dynamic equations for the thermodynamic quantities are presented in terms of the key observables of a biochemical network: stoichiometric matrix Q, reaction fluxes J, and chemical potentials of species μ without evoking empirical rate laws. Energy conservation and the Second Law are established for steady-state and dynamic biochemical networks. The theory provides the physiochemical basis for analyzing large-scale metabolic networks in living organisms.

Keywords: Biological networks; Cycle kinetics; Mathematical modeling; Metabolism; Systems biology

Contents

1. Introduction

Thermodynamics is one of the branches of physics most directly applied to biochemistry. Concepts such as entropy, enthalpy, and free energy are the cornerstones of understanding various biological processes such as protein folding, protein–DNA interaction, and DNA supercoiling. Yet, a majority of thermodynamic analyses and/or kinetic studies focus only on "non-living" systems. By a non-living system, we mean that if one waits a sufficiently long time compared with its relaxation time, the system approaches a

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thermochemical equilibrium. In contrast, a living system cannot be isolated; it is open to energy and material exchange with its surrounding, accompanied with heat dissipation. After a time sufficiently long compared with its relaxation time, an open system approaches to a *nonequilibrium steady-state* (NESS) [1–4], also known as a stationary nonequilibrium state or dissipative structure.

The best known and most important example of such living biochemical network is the central energy metabolism operating inside a cell. From the perspective of molecular biology, this subject may appear well-understood since the majority of the pathways are elucidated and the enzymes involved extensively characterized [5]. But knowledge of the behavior of individual reactions in test tubes does not directly translate to a comprehensive understanding of the functioning of metabolic network as a whole inside a living cell. In particular, modern biology and medicine demand quantitative predictions on metabolic consequences of gene deletion, attenuation, and over-expression. Analyses like these are the challenges facing what is now called Systems Biology [6,7].

To develop an integrative, open systems view of a metabolic network, one needs to define its boundary. This is an important issue and the different choices one makes lead to different analyses. For example, one may consider the metabolic reactions occurring in the cytosol. Under this definition, the mitochondria will act as a source and a sink for ATP and ADP, respectively. This approach raises the question: how does one model the cytosolic concentrations of ATP and ADP which are in exchange with the mitochondria? A normal cell has an ability to maintain its ATP concentration and phosphorylation potential through various control mechanisms. Hence in modeling an open system with normal ATP supply, one assumes either (i) the ATP and ADP concentrations are held constant, or (ii) the ATP influx and ADP efflux are balanced and relatively constant. These two extreme scenarios will be called concentration clamping and boundary flux injection. In whole cells or whole organisms, the nonequilibrium state is maintained by the boundary fluxes transporting material into and out of the system. Concentration clamping and flux injection are the ultimate "driving forces" under which a network necessarily approaches a NESS, with stationary stochastic fluctuations, or possibly a complex dynamical state with oscillations [8], rather than relaxing back to a chemical equilibrium.

Still, there is currently no a single universal theory of nonequilibrium thermodynamics applicable to biochemical networks. In fact, nonequilibrium statistical thermodynamics remains an active topic of research in the forefront of theoretical physics [9–11]. However, for isothermal systems in NESS, several cogent theories exist that are essentially equivalent [1,2,12,13]. Thus concepts such as entropy and free energy are defined and can be appropriately applied in certain nonequilibrium situations. Prigogine and his colleagues have championed this cause for decades [14]; and Oster et. al. were among the first to introduce network thermodynamics [15]. Yet none of the classic works provides an adequate and complete theory applicable to the analysis of large-scale biochemical systems. In fact, even today, when discussing nonequilibrium problems, biochemists tend to think in terms of transient kinetics and transport phenomena in closed systems instead of open-system NESS thermodynamics. NESS is a state of living matters research into which is becoming the focus of biophysical chemistry and the systems biology of cells.

Hill, based on his own extensive work on ion channel kinetics, muscle contraction, and linear aggregation, developed a rather complete theory for systems in isothermal NESS [1,16]. But his approach, which was later shown to be mathematically equivalent to an irreversible Markov model [17], applies only to unimolecular reaction networks. (In physics literature they are known as master equation systems [18]. The theory of complex unimolecular reaction networks is also developed independently in chemical engineering literature [19].) The most important biological processes applicable to Hill's theory are single motor protein kinetics [20,21], protein polymerization [22,23], and active membrane transport and ion pumping, which involve chemomechanical and electrochemical free energy transduction.

In recent years, there has been a growing interest in quantitative modeling of large-scale (whole cell) nonlinear biochemical reaction networks [24–26], for example, in profiling gene product network interactions. Therefore, it is natural that one seeks a NESS thermodynamic theory applicable to such systems [27–29]. The new computational approach to metabolic networks complements new developments in laboratory studies of control and regulation of cellular metabolism. For example, genetic manipulations lead to the identification of essential and non-essential genes. Fluorescence-based optical methods [30], NMR spectroscopy, and mass spectroscopy all hold great potential for providing data on in vivo metabolite concentrations and reaction fluxes in the future.

The basic concepts and ideas in the thermodynamic theory for isothermal NESS systems are closely related to those in equilibrium. In fact, one can view equilibrium as a special case of steady-state with all reaction fluxes $J=0$. Because of this fact, there is a danger for confusion and misconception. The objective of this paper is to provide a simple and clear presentation of this important subject. There is a growing need to clarify and further develop, beyond the NESS regime, the new concepts of thermodynamics applicable to biochemical networks.

In equilibrium thermodynamics, the basic questions are change of free energy, enthalpy and entropy associated with biomolecular processes. For a system in a NESS, important quantities are the rate of work done to maintain a steady-state and the rate of heat dissipation, as well as the rates of increasing entropy and decreasing free energy. To be concrete, we shall discuss in this paper only biochemical

reaction networks with prescribed stoichiometry. We start with a simple example, which requires no more than college freshman chemistry and mathematics, to illustrate the essential ideas and then introduce the general concepts and results in a following section.

2. Basic concepts by a simple example

The simplest biochemical reaction is a unimolecular isomerization between A and B with rate constants k_1 and k_2:

$$\mathrm{A} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{B}. \tag{1}$$

If one leaves such molecules alone in a test tube, a chemical equilibrium will eventually be reached that obeys the well-known relation

$$\frac{[\mathrm{B}]_{\mathrm{eq}}}{[\mathrm{A}]_{\mathrm{eq}}} = \frac{k_1}{k_2} = e^{-\frac{\mu_{\mathrm{B}}^{\mathrm{o}} - \mu_{\mathrm{A}}^{\mathrm{o}}}{k_{\mathrm{B}}T}}. \tag{2}$$

(Here we have used k_{B} rather than gas constant R, which transform the chemical potential and other extensive thermodynamic variables into molar quantities and is more commonly used in biochemistry.) However, to simulate a reaction in a living metabolic network, suppose that someone is standing by the test tube and actively controlling both the concentrations of A and B at prescribed levels of c_{A} and c_{B}. Thus the system is open and the controller has to take out an A molecule whenever the concentration of A is greater than c_{A} and put in an A into the test tube whenever the concentration of A is less than c_{A}; the controller has to do the same for B. Under this setting, the system maintains a steady-state since neither concentration changes with time. This state is not an equilibrium state since the flux is not zero:

$$J = J_+ - J_- \neq 0. \tag{3}$$

Here $J_+ = k_1 c_{\mathrm{A}}$ and $J_- = k_2 c_{\mathrm{B}}$ are the forward and backward fluxes. Furthermore, one can calculate the chemical potential difference between the A and B under this setting. Since[1]

$$\mu_{\mathrm{A}} = \mu_{\mathrm{A}}^{\mathrm{o}} + k_{\mathrm{B}}T \ln c_{\mathrm{A}},$$

$$\mu_{\mathrm{B}} = \mu_{\mathrm{B}}^{\mathrm{o}} + k_{\mathrm{B}}T \ln c_{\mathrm{B}},$$

the chemical potential difference between A and B is

$$\Delta\mu = \mu_{\mathrm{B}} - \mu_{\mathrm{A}} = \mu_{\mathrm{B}}^{\mathrm{o}} - \mu_{\mathrm{A}}^{\mathrm{o}} + k_{\mathrm{B}}T \ln \frac{c_{\mathrm{B}}}{c_{\mathrm{A}}} = k_{\mathrm{B}}T \ln\left(\frac{J_-}{J_+}\right). \tag{4}$$

Most interestingly from Eq. (4), one finds:

$$-J\Delta\mu = k_{\mathrm{B}}T(J_+ - J_-)\ln\left(\frac{J_+}{J_-}\right) \geq 0. \tag{5}$$

The equality of Eq. (5) holds true if an only if $J=\Delta\mu=0$, e.g., if the system is in equilibrium.

The quantity on the left-hand side of Eq. (5) has significance. It is the amount of work done by the controller in order to maintain the NESS. The controller is "pumping" A molecules in and B molecules out (or vice versa). Since the system is not changing, the work done by the controller has to leave the system in the form of heat dissipation—First Law of Thermodynamics. The inequality in Eq. (5) is the Second Law of Thermodynamics: one cannot transform heat into work from a single temperature source.

One should note that the dissipated heat is related to $\Delta\mu$, not enthalpic difference $\Delta h = \frac{\partial(\Delta\mu/T)}{\partial(1/T)}$. The entire reaction is cyclic: the reaction heat $-\Delta h$ from A to B in the test tube is exactly balanced by the reaction heat from B to A carried out by the controller, $-\Delta h$. If the $\Delta\mu>0$ for the A to B in the test tube, then the $\Delta\mu$ for the B to A in the hand of the controller is negative. Hence the external reaction carried out by the controller is not spontaneous, rather it requires active work. The cyclic reaction in NESS balances the work and heat (First Law) and transforms useful energy into entropy in the surrounding (Second Law).

One should also not be confused by the dissipated heat in the NESS, $\Delta\mu$, and the enthalpic difference $\Delta h^{\mathrm{o}} = \frac{\partial(\Delta\mu^{\mathrm{o}}/T)}{\partial(1/T)}$. The latter can be positive or negative, depending on the reaction AB being exothermal or endothermal, respectively. However, the $\Delta\mu$ contains the additional energy dissipation associated with taking a B molecule from a solution with concentration c_{B} and putting an A molecule into a solution with concentration c_{A}.

3. Time-dependent energetics (Thermodynamics) of stoichiometric biochemical networks

We now generalize the above simple example to a network of biochemical reactions characterized by known stoichiometry [32–34]. The system consists of $N+N'$ species among which N' species have their concentrations clamped. There are M internal reactions and M' boundary fluxes, which pump mass into or out of the system. The clamped concentrations and boundary fluxes maintain the system in a NESS. When $M'=N'=0$, the system is closed and its only steady-state is thermodynamic equilibrium irrespective of the complexity of the reaction networks.

[1] The validity of introducing chemical potentials for biochemical species inside living cells is questionable when dealing with signaling molecules with only a few copy numbers and due to molecular crowding. Hence the main application of the present approach is to cellular metabolic networks and concentrations should be understood as activities when necessary [31].

The stoichiometry of this set of reactions can be mathematically represented by the $(N+N')\times(M+M')$ incidence matrix Q (the stoichiometric matrix is usually represented by S, but in this paper S is reserved for the entropy of the system).

$$Q=\left(\begin{array}{ccc|c} \ddots & \vdots & & \vdots \\ \cdots & \widetilde{Q}_{NM} & \cdots & \overline{Q}_{NM'} \\ & \vdots & \ddots & \vdots \\ \hline \cdots & \widehat{Q}_{N'M} & \cdots & 0 \end{array}\right)$$

The lower-right 0 block indicates that there should be no boundary flux to or from a clamped species (see Appendix A). The matrix Q is the starting point of many approaches to modeling biochemical networks, including flux balance analysis (FBA) [35–37]. If there are no clamped metabolites then $\hat{Q}$=0. The matrix $\tilde{Q}$ contains only the dynamic species and internal fluxes.

Let c_i be the concentration of species i, where i=1, 2,..., N indexes the N dynamic biochemical species in the system and i=N+1,..., $N+N'$ indexes the N' clamped concentrations. Then,

$$\mu_i=\mu_i^{\circ}+k_{\mathrm{B}}T\ln\left(\frac{c_i}{c_{\mathrm{t}}}\right) \tag{6}$$

is the chemical potential for species i. $c_{\mathrm{t}}=\sum_{\ell=1}^{N+N'}C_\ell$ is the total concentration of all species. The quantity μ° is further decomposed into $\mu_i^{\circ}=h_i^{\circ}-Ts_i^{\circ}$ in terms of partial molar enthalpy and entropy. The *total* enthalpy, entropy, and free energy of the system are

$$H=\sum_{i=1}^{N+N'}c_ih_i^{\circ}, \tag{7}$$

$$G=\sum_{i=1}^{N+N'}c_i\mu_i, \tag{8}$$

$$S=\sum_{i=1}^{N+N'}c_i\left(s_i^{\circ}-k_{\mathrm{B}}\ln\frac{c_i}{c_{\mathrm{t}}}\right)=\frac{H-G}{T}. \tag{9}$$

In terms of the stoichiometric matrix, the conservation of mass is expressed as

$$\frac{\mathrm{d}c_i}{\mathrm{d}t}=\sum_{j=1}^{M+M'}Q_{ij}J_j, \tag{10}$$

for internal species i=1, 2,..., N where J_j is the flux in the jth reaction. The chemical potential difference for the internal reaction j, $1\le j\le M$ is

$$\Delta\mu_j=\sum_{i=1}^{N+N'}\mu_iQ_{ij}. \tag{11}$$

Some straightforward calculations (see Appendix A) show that there is a dynamic equation for entropy:

$$T\frac{\mathrm{d}S}{\mathrm{d}t}=epr-hdr, \tag{12}$$

where epr is the entropy production (creation) rate, more precisely the entropic contribution to the rate of free energy degradation, which is never negative (Eq. (25)) and hdr is the heat dissipation rate. In the isothermal biochemical reaction network, the entropy of the system changes either due to entropy created in the system or the heat leaving the system. This is a well-known result in the irreversible thermodynamics [38,39] in which epr and hdr are usually expressed as $T\frac{\mathrm{d}_iS}{\mathrm{d}t}$ and $T\frac{\mathrm{d}_eS}{\mathrm{d}t}$, or $T\frac{\mathrm{d}S_{\mathrm{rev}}}{\mathrm{d}t}$ and $T\frac{\mathrm{d}S_{\mathrm{cr}}}{\mathrm{d}t}$. We choose to use epr and hdr instead of the traditional notations $\dot{S}_i$ and $\dot{S}_e$ for the entropy production (creation) and heat dissipation rates to emphasize that they are source and sink term, not time derivatives [12]. Similarly we have a dynamic equation for enthalpy:

$$\frac{\mathrm{d}H}{\mathrm{d}t}=-hdr+cmf, \tag{13}$$

where cmf is the chemical motive force determined by either flux injection or concentration clamping, or both at the system's boundary. For a closed system in which heat becomes enthalpy, cmf=0. Eq. (13) is a statement of energy conservation. Finally, we have a dynamic equation for Gibbs free energy:

$$\frac{\mathrm{d}G}{\mathrm{d}t}=cmf-epr. \tag{14}$$

Eq. (14) is a free energy balance equation. It is widely known that free energy is not conserved quantity: epr is constantly degrading the free energy. In a nonequilibrium steady-state,

$$\frac{\mathrm{d}G}{\mathrm{d}t}=\frac{\mathrm{d}H}{\mathrm{d}t}=\frac{\mathrm{d}S}{\mathrm{d}t}=0.$$

Therefore,

$$cmf=hdr=epr\ge 0. \tag{15}$$

The first equality is conservation of energy, the second equality is the isothermal Clausius equality, and the last inequality is the Second Law of Thermodynamics. As far as we know, Eqs. (13) and (14) are not in the standard textbooks on nonequilibrium thermodynamics.

4. Kirchhoff's laws for biochemical networks

The thermodynamic formulae given in the previous section can be thought of as Kirchhoff's laws for biochemical networks, in analogy to those for electric circuits [27,28]. For a biochemical network in NESS with given

stoichiometric matrix Q, we expect the metabolic fluxes inside a cell satisfies

$$Q\mathbf{J} = 0 \tag{16}$$

for flux balance (mass conservation) where $\mathbf{J}$ is a vector listing all of the J_j. Eq. (16) is Kirchhoff's current law. The NESS $\mathbf{J}$ consists of internal reaction loops $\mathbf{v}$, obtained from $\tilde{Q}\mathbf{v}=0$, and throughput fluxes. For each internal loop $\mathbf{v}$ (see Appendix A):

$$\sum_{j=1}^{M}\left(\Delta\mu_j - \Delta\pi_j^{\mathrm{ext}}\right)v_j = 0, \tag{17}$$

where

$$\Delta\pi_j^{\mathrm{ext}} = \sum_{i=N+1}^{N+N'}\mu_i Q_{ij} \tag{18}$$

is the force driving the internal reaction j, $0\le j\le M$, via concentration clamping—i.e., $\Delta\pi_j^{\mathrm{ext}}$ is a chemical "battery". This equality is the Kirchhoff's loop law, reflecting energy conservation. Finally, for each reaction in the NESS system, as in Eq. (5), $\Delta\mu_\ell = k_{\mathrm{B}}T\ln\left(J_{\ell,-}/J_{\ell,+}\right)$ and $J_\ell = J_{\ell,+} - J_{\ell,-}$. Hence $-\Delta\mu_\ell J_\ell \ge 0$. This inequality is the Second Law of Thermodynamics.

We see that even with no knowledge of kinetic rate constants or kinetic mechanisms, the steady-state is uniquely determined as the equilibrium state when all the $\Delta\pi^{\mathrm{ext}}=0$. Since $\Delta\boldsymbol{\mu}\cdot\mathbf{J}=0$, and for each reaction in the loop $\Delta\mu_j J_j\le 0$, it is necessary that $J_j=\Delta\mu_l=0$ for all j.

The set of thermodynamic constraints (Eq. (17) and the second law) can significantly reduce the thermodynamically feasible NESS flux $\mathbf{J}$ and potential $\boldsymbol{\mu}$. Knowledge of the value of $\boldsymbol{\mu}^{\mathrm{o}}$ for a species k, will provide insight into the concentration for that species in NESS: $c_k = \mathrm{e}^{(\mu_k-\mu_k^{\mathrm{o}})/k_{\mathrm{B}}T}$. This approach, generally known as constraint-based biochemical network analysis, has already yielded significant insights into the systems biology of cells [26–29, 35–37].

5. Relation to concentration fluctuations

Fluxes and concentrations are the most important observables for an open biochemical network. Concentrations together with the standard-state chemical potential μ^{o} yields the nonequilibrium chemical potential. The theory we present relates the thermodynamics of the open system to the structure (Q) and the variables ($\mathbf{J}$ and $\boldsymbol{\mu}$) of the biochemical network.

Systems near but not at equilibrium are well understood in Onsager's theory of linear irreversibility [40]. In this regime, the fluxes and chemical potentials are linearly related. Using the simple example from Eq. (4), at equilibrium $J=J_+^{\mathrm{eq}}-J_-^{\mathrm{eq}}=0$. Near equilibrium $J\neq 0$, while $J\ll J_+^{\mathrm{eq}}$ and J_-^{eq}, one has

$$\Delta\mu = k_{\mathrm{B}}T\ln\left(\frac{J_-}{J_+}\right) = k_{\mathrm{B}}T\ln\left(1-\frac{J}{J_+}\right) \approx -\frac{k_{\mathrm{B}}T}{J_+^{\mathrm{eq}}}J. \tag{19}$$

$\Delta\mu$ and J are not linearly related in general, and in our theory. The phrase "far from equilibrium" is used to describe the nonlinear regime. Eq. (19) also indicates that the unidirectional flux, J_+^{eq} is in fact the linear Onsager coefficient between the force $\Delta\mu$ and the flux J [41], also called biochemical conductance [27].

Concentrations of biochemical species in living cells are fluctuating. Spectroscopic measurements of such fluctuations are now possible. Interestingly, the concentration fluctuations can also be included in the present theory. More specifically, one is able to predict the rates of concentration fluctuations with known Q, $\mathbf{J}$ and $\boldsymbol{\mu}$. We shall briefly illustrate this idea. A complete treatment of the problem will be published elsewhere. Again, for the simple example in Eq. (4) we have at equilibrium

$$J_+^{\mathrm{eq}} = J_-^{\mathrm{eq}} = k_1 c_{\mathrm{A}} = k_2 c_{\mathrm{B}} = \frac{k_1+k_2}{\dfrac{1}{c_{\mathrm{A}}}+\dfrac{1}{c_{\mathrm{B}}}} = \frac{\langle\Delta c_{\mathrm{A}}\Delta c_{\mathrm{A}}\rangle}{\tau}. \tag{20}$$

The significance of the right-hand side is that $(1=c_{\mathrm{A}}+1/c_{\mathrm{B}})^{-1}$ is in fact the equilibrium fluctuations in the concentrations of A and B [42]. And the $\tau=(k_1+k_2)^{-1}$ is the fluctuation relaxation time. Hence Eqs. (19) and (20) together state that the biochemical conductance is intimately related to the rate of concentration fluctuations.

More generally away from equilibrium, according to Keizer [2], if we use matrix H to denote the linear relaxation kinetics of concentrations back to a NESS: $\mathrm{d}(\delta c_i)/\mathrm{d}t = -\sum_j H_{ij}\delta c_j$ and $\sigma_{ij}=\langle\Delta c_i\Delta c_j\rangle$ to denote the NESS concentration fluctuations, then the rates of concentration fluctuations [2]

$$\gamma = H\sigma + \sigma H^T, \tag{21}$$

which is a positive-definite matrix

$$\begin{aligned}\gamma_{ij} &= \sum_{k=1}^{M} Q_{ik}Q_{jk}\left(J_{k,+}+J_{k,-}\right)\\ &= \sum_{k=1}^{M} Q_{ik}Q_{jk}\left(\frac{1+\mathrm{e}^{\Delta\mu_k/k_{\mathrm{B}}T}}{1-\mathrm{e}^{\Delta\mu_k/k_{\mathrm{B}}T}}\right)J_k.\end{aligned} \tag{22}$$

It can be constructed from the Q, $\mathbf{J}$ and $\boldsymbol{\mu}$. Eq. (20) is a special case of this general result. We see that in the linear regime, $\gamma_{ij}=2\sum_k Q_{ik}Q_{jk}J_{k,+}^{\mathrm{eq}}$ which is what

known as conductance matrix of an electrical (linear) network.

6. Summary

Starting with Eq. (10) which reflects the mass conservation in a stoichiometric chemical reaction network and the concept of chemical potential of ideal solutions (Eq. (6)), we have derived three balance equations, Eqs. (12), (13), and (14), describing the time evolution of energetics of the open, isothermal biochemical network in terms of its entropy, enthalpy, and Gibbs free energy. These equations for nonequilibrium thermodynamics are straightforward to interpret. Eq. (13) states that the change in the enthalpy of the system is balanced by the work done to the system minus the dissipated heat. This is clearly the law of energy conservation. The First Law, however, does not differentiate "high grade" energy from "low grade" energy. When input work is transformed into dissipated heat, entropy is created. Eq. (12) states that the change in entropy of the system is balanced by the entropy production minus the expulsion of the low grade energy-heat.

A few words on the existing literature are in order. It was known to Gibbs [43] that the chemical equilibrium is directly related to the minimization of the Gibbs free energy function Eq. (8). Later, the relation between the Gibbs free energy and the rate equations based on the mass-action law was extensively studied [44] as well as the Gibbs free energy minimization in terms of a general network theory [45]. In the theory of the present paper, a closed system has cmf=0 in Eq. (14) since all π^{ext}=0. The dynamics thus follows $dG/dt=-epr\le 0$ in Eq. (14) since epr is always non-negative (Eq. (25)). The entire network approaches to the minimum of G, with zero hdr (Eq. (13)) and epr=0 (Eq. (12)).[2] Finally, all of the J_j=0 (Eq. (25)).

For biochemical networks in living systems, sustained energy input means cmf>0. Therefore the system approaches a NESS. The significance of these new equations in study systems biology of cells remains to be explored.

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[2] In fact, one can show that the Gibbs free energy is mathematically a global Lyapunov function for the concentration dynamics.

Appendix A. Mathematical formalism for nonequilibrium thermodynamics

The dynamic equation for species i due to conservation of mass is

$$\frac{dc_i}{dt} = \sum_{j=1}^{M} Q_{ij}J_j + \phi_i^{\text{ext}} \tag{23}$$

where the Q_{ij}'s are stoichiometric coefficients, J_j is the flux in the jth reaction, and ϕ_i^{ext} (see below) represents a constant external boundary flux that does not change with the concentration of any species in the system. The J_j's however are functions of the concentrations of the participating species (e.g., via the law of mass action). If c_k is clamped at a value c_k^*, the dc_k/dt equation is eliminated from the system of equations, and the $J_j(\ldots, c_k,\ldots)$ as functions of c_k will be fixed at $J_j(\ldots, c_k^*,\ldots)$.

The standard-state chemical potentials for species i–Eq. (6)–are decomposed into $\mu_i^{\circ}=h_i^{\circ}-Ts_i^{\circ}$ in terms of partial molar enthalpy and entropy. The total enthalpy, entropy, and free energy of the system are given in Eqs. (7) (8) (9). We denote $\phi_i^{ext}=\sum_{j=M+1}^{M+M'} Q_{ij}J_j (1\le i\le N)$ as the external injection flux into internal species i. For clamped species $i>N$, we denote $-\sum_{j=1}^{M} Q_{ij}J_j=\phi_i^{\text{ext}}$ so that $dc_i/dt\equiv 0$; this is the flux required from the external in order to keep the constant concentration for i species. The dynamic equation for entropy is expressed as,

$$\begin{aligned} T\frac{dS}{dt} &= T\sum_{i=1}^{N}\frac{dc_i}{dt}\left(s_i^{\circ}-k_B\ln\frac{c_i}{c_t}\right) = T\sum_{i=1}^{N}\sum_{j=1}^{M+M'} \\ &\quad\times Q_{ij}J_j\left(s_i^{\circ}-k_B\ln\frac{c_i}{c_t}\right) = \sum_{i=1}^{N}\sum_{j=1}^{M+M'} \\ &\quad\times Q_{ij}J_j\left(h_i^{\circ}-\mu_i\right) = epr - hdr \end{aligned} \tag{24}$$

where entropy production rate and heat dissipation rate

$$\begin{aligned} epr &= -\sum_{i=1}^{N+N'}\sum_{j=1}^{M}\mu_i Q_{ij}J_j \\ &= -\sum_{j=1}^{M}\Delta\mu_j J_j \ge 0, \end{aligned} \tag{25}$$

$$hdr = -\sum_{i=1}^{N}\sum_{j=1}^{M+M'} h_i^{\circ}Q_{ij}J_j + \sum_{i=1}^{N}\mu_i\phi_i^{\text{ext}} - \sum_{j=1}^{M}\Delta\pi_j^{\text{ext}}J_j \tag{26}$$

$$= -\sum_{i=1}^{N+N'}\sum_{j=1}^{M} h_i^{\circ}Q_{ij}J_j - T\sum_{i=1}^{N+N'} s_i\phi_i^{\text{ext}}. \tag{27}$$

Similarly, there is a dynamic equation for enthalpy

$$\frac{dH}{dt} = \sum_{i=1}^{N}\frac{dc_i}{dt}h_i^{\circ} = -hdr + cmf, \tag{28}$$

where the chemical motive force is determined by either flux injection or concentration clamping, or both:

$$cmf = \sum_{i=1}^{N+N'} \mu_i \phi_i^{\text{ext}}. \tag{29}$$

Finally, there is a dynamic equation for Gibbs free energy

$$\frac{\mathrm{d}G}{\mathrm{d}t} = \frac{\mathrm{d}(H - TS)}{\mathrm{d}t} = cmf - epr. \tag{30}$$

For any vector $\mathbf{v}$ satisfying $\tilde{Q}\mathbf{v}=0$, i.e., $\mathbf{v}$ is in the null space of matrix $\tilde{Q}$, representing an internal reaction loop in the biochemical network, we have:

$$0 = \sum_{i=1}^{N} \sum_{j=1}^{M} \mu_i Q_{ij} v_j = \sum_{j=1}^{M} \left(\Delta\mu_j - \Delta\pi_j^{\text{ext}} \right) v_j, \tag{31}$$

where

$$\Delta\pi_j^{\text{ext}} = \sum_{i=N+1}^{N+N'} \mu_i Q_{ij} \tag{32}$$

is the driving force to reaction j due to concentration clamping. Eq. (31) is Kirchhoff's loop law for biochemical networks.

References

[1] T.L. Hill, Free Energy Transduction in Biology: The Steady-State Kinetic and Thermodynamic Formalism, Academic Press, New York, 1974.

[2] J.E. Keizer, Nonequilibrium thermodynamics and the stability of states far from equilibrium, Acc. Chem. Res. 12 (1979) 243–249.

[3] D.P. Ruelle, Extending the definition of entropy to nonequilibrium steady states, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 3054–3058.

[4] D.-Q. Jiang, M. Qian, M.-P. Qian, Mathematical Theory of Nonequilibrium Steady States, LNM, vol. 1833, Springer, New York, 2004.

[5] L. Stryer, Biochemistry, 2nd ed., W.H. Freeman, San Francisco, 1981.

[6] L. Chong, L.B. Ray, Whole-istic biology, Science 295 (2002) 1661.

[7] H.V. Westerhoff, B.Ø. Palsson, The evolution of molecular biology into systems biology, Nat. Biotechnol. 22 (2004) 1249–1252.

[8] H. Qian, S. Saffarian, E.L. Elson, Concentration fluctuations in a mesoscopic oscillating chemical reaction system, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 10376–10381.

[9] T. Hatano, S.-i. Sasa, Steady-state thermodynamics of Langevin systems, Phys. Rev. Lett. 86 (2001) 3463–3466.

[10] J.-P. Eckmann, Non-equilibrium steady-state, Proc. Int. Congr. Math. 3 (2002) 409–418.

[11] E.H. Trepagnier, C. Jarzynski, F. Ritort, G.E. Crooks, C.J. Bustamante, J. Liphardt, Experimental test of Hatano and Sasa's non-equilibrium steady-state equality, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 15038–15041.

[12] H. Qian, Entropy production and excess entropy in nonequilibrium steady-state of single macromolecules, Phys. Rev., E 65 (2002) 021111.

[13] H. Qian, M. Qian, X. Tang, Thermodynamics of the general diffusion process: time-reversibility entropy production, J. Stat. Phys. 107 (2002) 1129–1141.

[14] G. Nicolis, I. Prigogine, Self-Organization in Nonequilibrium Systems, Wiley Intersci., New York, 1977.

[15] G.F. Oster, A.S. Perelson, A. Katchalsky, Network thermodynamics: the analysis of biophysical systems, Q. Rev. Biophys. 6 (1973) 1–134.

[16] T.L. Hill, Free Energy Transduction and Biochemical Cycle Kinetics, Spinger-Verlag, New York, 1989.

[17] C. Qian, M. Qian, M.-P. Qian, Markov chain as a model of Hill's theory on circulation, Sci. Sin. 24 (1981) 1431–1448.

[18] J. Schnakenberg, Network theory of microscopic and macroscopic behavior of master equation systems, Rev. Mod. Phys. 48 (1976) 571–585.

[19] J. Wei, C.D. Prater, The structure and analysis of complex reaction systems, Adv. Catal. 13 (1962) 203–392.

[20] H. Qian, The mathematical theory of molecular motor movement and chemomechanical energy transduction, J. Math. Chem. 27 (2000) 219–234.

[21] H. Qian, Equations for stochastic macromolecular mechanics of single proteins: equilibrium fluctuations, transient kinetics and nonequilibrium steady-state, J. Phys. Chem. 106 (2002) 2065–2073.

[22] T.L. Hill, Linear Aggregation Theory in Cell Biology, Springer-Verlag, New York, 1987.

[23] H. Qian, A stochastic analysis of a Brownian ratchet model for actin-based motility, Mech. Chem. Biosys. (2004) (in press).

[24] J.E. Bailey, Toward a science of metabolic engineering, Science 252 (1991) 1668–1674.

[25] G. Stephanopoulos, Metabolic engineering, Curr. Opin. Biotechnol. 5 (1994) 196–200.

[26] C.H. Schilling, S. Schuster, P.Ø. Palsson, R. Heinrich, Metabolic pathway analysis: basic concepts and scientific applications in post-genomic era, Biotechnol. Prog. 15 (1999) 296–303.

[27] D.A. Beard, S.-D. Liang, H. Qian, Energy balance for analysis of complex metabolic networks, Biophys. J. 83 (2002) 79–86.

[28] H. Qian, D.A. Beard, S.-D. Liang, Stoichiometric network theory for nonequilibrium biochemical systems, Eur. J. Biochem. 270 (2003) 415–421.

[29] D.A. Beard, E. Babson, E. Curtis, H. Qian, Thermodynamic constraints for biochemical networks, J. Theor. Biol. 228 (2004) 327–333.

[30] H. Qian, E.L. Elson, Fluorescence correlation spectroscopy with high-order and dual-color correlation to probe nonequilibrium steady-states, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 2828–2833.

[31] V.A. Parsegian, R.P. Rand, D.C. Rau, Osmotic stress, crowding, preferential hydration, and binding: a comparison of perspectives, Proc. Nat. Acad. Sci. U. S. A. 97 (2000) 3987–3992.

[32] P.H. Sellers, Stoichiometric equations are mathematical equations, SIAM J. Appl. Math. 15 (1967) 637–639.

[33] B.L. Clarke, Stoichiometric network analysis, Cell Biophys. 12 (1988) 237–253.

[34] J. Ross, M.O. Vald, Nonlinear kinetics and new approaches to complex reaction mechanism, Annu. Rev. Phys. Chem. 50 (1999) 51–78.

[35] J.S. Edwards, B.Ø. Palsson, How will bioinformatics influence metabolic engineering? Biotechnol. Bioeng. 58 (1998) 162–169.

[36] J.S. Edwards, B.Ø. Palsson, The *E. coli* MG1655 in silico metabolic genotype: its definition, characteristics, and capability, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 5528–5533.

[37] D.A. Beard, H. Qian, Thermodynamic-based computational profiling of cellular regulatory control in hepatocyte metabolism, Am. J. Physiol., Endocrinol. Metabol. (2004) (in press).

[38] S.R. de Groot, Thermodynamics of Irreversible Processes, North-Holland, Amsterdam, 1951.

[39] I. Prigogine, Introduction to Thermodynamics of Irreversible Processes, Intersceince, New York, 1961.

[40] H.V. Westerhoff, K. van Dam, Thermodynamics and Control of Biological Free-Energy Transduction, Elsevier, Amsterdam, 1987.
[41] T.L. Hill, The linear Onsager coefficients for biochemical kinetic diagrams as equilibrium one-way cycle fluxes, Nature 299 (1982) 84–86.
[42] I. Prigogine, Fluctuations in chemical equilibrium, Physica 16 (1950) 134–135.
[43] J.W. Gibbs, The Scientific Papers of J. Willard Gibbs, vol. I, Dover, New York, 1961.
[44] N.Z. Shapiro, L.S. Shapley, Mass action laws and the Gibbs free energy function, SIAM J. Appl. Math. 13 (1965) 353–375.
[45] G.F. Oster, C.A. Desoer, Tellegen's theorem and thermodynamic inequalities, J. Theor. Biol. 32 (1971) 219–241.