THE LIMIT OF MESOSCOPIC ONE-WAY FLUXES IN A NONEQUILIBRIUM CHEMICAL REACTION WITH COMPLEX MECHANISM

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Abstract. Peng et al. (2020) formulated one-way fluxes for a general chemical reaction far from equilibrium, with arbitrary complex mechanisms, multiple intermediates, and internal kinetic cycles. Species are classified into internal $Y'$s and external $X'$s. They defined the limit of mesoscopic one-way fluxes when the volume of the tank reactor tends to infinity as macroscopic one-way fluxes, but a rigorous proof of existence of the limit is still awaiting. In this article, we fill this gap under a mild hypothesis: the Markov chain associated with the chemical master equation has finite states and any two rows in the stoichiometric matrices for the internal species $Y'$s are not identical. In fact, an explicit expression of the limit is obtained.

Keywords: nonequilibrium chemical reaction network, chemical master equation, cycle flux, kinetic one-way fluxes

1. Introduction

Nonlinear and nonequilibrium systems of biochemical reactions occur widely and generally in various living biochemical systems[1, 2, 4], such as the stochastic kinetics of enzyme[5, 3], phosphorylation-dephosphorylation kinetics of proteins[6], Calcium signal dynamics[7], and stochastic state transitions of cell populations [8]. For years, it’s believed that there exists a fundamental relationship between unbalanced kinetic one-way fluxes and thermodynamic chemical driving forces in nonequilibrium chemical reaction systems. But the formula for this fundamental relation and the very definition of kinetic one-way fluxes in nonlinear chemical reaction networks, were missing for a long time. Peng et al. (2020) [9] considered the general chemical reaction network (CRN) in a nonequilibrium steady state (NESS) sustained by a chemostat [10]. They defined the one-way flux of each reaction cycle through the cycle fluxes in the counting space of the corresponding chemical master equation model [11] describing the stochastic kinetics of molecular numbers. Then, they prove the equation

$$\Delta G = -k_B T \ln(\frac{J^+}{J^-}) \quad (1.1)$$

for a general chemical reaction, where $J^+$ and $J^-$ are forward and backward one-way fluxes and $\Delta G$ is the corresponding free energy difference.

In their work, they define the limit of mesoscopic kinetic one-way fluxes dividing $V$ when $V \to \infty$ as macroscopic kinetic one-way fluxes i.e.,

$$\mathcal{J}_V = \lim_{V \to \infty} \frac{w_i}{V}.$$

Here \( w_c \) is the mesoscopic kinetic one-way fluxes defined as the reaction cycle fluxes, and \( V \) is the volume of the tank reactor. However, the existence of \( J_c \) was not very clear.

In this article, we will give a clear expression of the mesoscopic reaction cycle flux \( w_c \) in Peng et al. (2020) [9] based on general mathematical results on cycle fluxes of a Markov process [12][13]. Then, we show existence of the limit of macroscopic kinetic one-way flux and give an explicit expression by rearrangement cycle \( c \).

2. Preliminary

2.1. Chemical Reaction Model and Symbols. In this article, we use the model from Peng et al. (2020) [9] below. A general CRN in a continuously stirred tank reactor consists of a set of species \( X_1, X_2, \ldots, X_N, Y_1, Y_2, \ldots, Y_N \), and a set of \( M \) reactions between them \( R_1, R_2, \ldots, R_M \). Species can be further classified into internal species \( \Xi \), external species \( \Theta \), and intermediary species \( \Theta_i \) as defined in Peng et al. (2020) [9] below. A general CRN in a continuously stirred tank reactor consists of a set of species \( X_1, X_2, \ldots, X_N, Y_1, Y_2, \ldots, Y_N \), and a set of \( M \) reactions between them \( R_1, R_2, \ldots, R_M \). Species can be further classified into internal species \( \Xi \), external species \( \Theta \), and intermediary species \( \Theta_i \) as defined in Peng et al. (2020) [9]. The complete stoichiometric matrix, then, is

\[
\Xi = \begin{bmatrix} \Xi^X \\ \Xi^Y \end{bmatrix}.
\]

The concentrations of all the species \( \Theta \) are clamped at constant levels. All reactions in this system are stochastic, elementary, and reversible, under isothermal and isobaric conditions with fixed volume \( V \).
2.2. The Transition Trajectory of Chemical reactions.

On the mesoscopic scale, we focus on molecular numbers in the CRN, which are stochastic. Stochastic vector \( \mathbf{n}^Y(t) = [n_1^Y(t), n_2^Y(t), ..., n_{N_2}^Y(t)] \) is used to denote molecular numbers of the internal species at time \( t \). \( X \)'s species still possess fixed molecular numbers, denoted by \( \mathbf{n}^X(t) = [n_1^X(t), n_2^X(t), ..., n_{N_2}^X(t)] \). The evolution of \( \mathbf{n}^Y(t) \) can be seen as a continuous time Markov chain on a high-dimensional graph (called a counting space).

In the present paper, we assume the Markov chain satisfying the following hypothesis:

**HYPOTHESIS 2.1.** (i) The Markov chain \( \mathbf{n}^Y(t) \) is irreducible with a finite state space \( S \).

(ii) Any two columns in the matrix \( \Xi \) are not identical.

The hypothesis (i) is just for the mathematical simplicity. The hypothesis (ii) is very mild and natural since the existence of two identical columns of the matrix \( \Xi \) means that the two chemical reactions associated with that columns are completely equivalent and can not be distinguished. We would like to point out that in Peng et al. (2020) [9], both the above two hypotheses are assumed implicitly. We will get rid of these hypotheses in a separate paper.

Denote by \( N \) the number of elements of the state space \( S \). Furthermore, denote by \( Q = (q_{ij})_{N \times N} \) the transition rate matrix of the Markov chain. Each state in \( S \), namely each vertex of the graph, has an \( N_2 \)-dimensional coordinate \( \mathbf{n}^Y = [n_1^Y, n_2^Y, ..., n_{N_2}^Y] \). The occurrence of an elementary reaction in \( \{ R_1, ..., R_M \} \) converts state of the system from one vertex to another.

According to the Markov processes theory in [10], the counting space is a scaffold for a Markov process with transition rates

\[
\begin{align*}
    r_{+\ell} (\mathbf{n}^Y; \mathbf{V}) & \triangleq \tilde{k}_{+\ell} (\mathbf{V}) \prod_{j=1}^{N_2} n_j^Y (n_j^Y - 1) \cdots \left( n_j^Y - \Xi_{+\ell, j}^Y + 1 \right) \\
    r_{-\ell} (\mathbf{n}^Y; \mathbf{V}) & \triangleq \tilde{k}_{-\ell} (\mathbf{V}) \prod_{j=1}^{N_2} n_j^Y (n_j^Y - 1) \cdots \left( n_j^Y - \Xi_{-\ell, j}^Y + 1 \right)
\end{align*}
\]

for the forward reaction \( R_{+\ell} \),

\[
\begin{align*}
    r_{-M} (\mathbf{n}^X, \mathbf{n}^Y; \mathbf{V}) & \triangleq \tilde{k}_{-M} \prod_{i=1}^{N_1} n_i^X (n_i^X - 1) \cdots \left( n_i^X - \Xi_{-M}^{i, X} - M \right) \prod_{j=1}^{N_2} n_j^Y (n_j^Y - 1) \cdots \left( n_j^Y - \Xi_{-M}^{j, Y} + 1 \right)
\end{align*}
\]

and

\[
\begin{align*}
    r_{+1} (\mathbf{n}^X, \mathbf{n}^Y; \mathbf{V}) & \triangleq \tilde{k}_{+1} \prod_{i=1}^{N_1} n_i^X (n_i^X - 1) \cdots \left( n_i^X - \Xi_{+1}^{i, X} + 1 \right) \prod_{j=1}^{N_2} n_j^Y (n_j^Y - 1) \cdots \left( n_j^Y - \Xi_{+1}^{j, Y} + 1 \right)
\end{align*}
\]

Here, \( \tilde{k}_{+\ell} \) are called the forward and backward macroscopic rate constants in the stochastic model, and \( k_{+\ell} = \tilde{k}_{+\ell} V^{\nu_{+\ell} - 1} \) are mesoscopic rate constants, where \( n_{+\ell} \) is the summation of stoichiometric numbers of reactants in the reaction \( R_{+\ell} \)

\[
\begin{align*}
    n_{+\ell} &= \sum_{j=1}^{N_2} \Xi_{+\ell, j}^Y Y_j, \ell = 2, 3, ..., M \\
    n_{-\ell} &= \sum_{j=1}^{N_2} \Xi_{-\ell, j}^Y Y_j, \ell = 1, 2, ..., M - 1
\end{align*}
\]
and
\[ n_{+1} = \sum_{j=1}^{N_2} \Xi_{j+1}^+ Y_j + \sum_{i=1}^{N_1} \Xi_{i+1} X_i, \]
\[ n_{-M} = \sum_{j=1}^{N_2} \Xi_{-M}^+ Y_j + \sum_{i=1}^{N_1} \Xi_{i-M} X_i. \]

In our model, the reaction coefficients decide all the step size along the transition trajectory. When hypothesis (2.1 ii) satisfied, the phenomenon that two different reactions in \( R_1, ..., R_M \) leading to same transferring in the state space \( S \) will be removed. Then, the transition rate of the Markov chain is either,
\[ q_{av}(n^y + \Xi^1_y; n^x; V), (2 \leq \ell \leq M), \]
\[ q_{av}(n^y - \Xi^1_y; n^z; V), (1 \leq \ell \leq M - 1), \]

or
\[ q_{av}(n^y + \Xi^1_y) = r_{+1}(n^x, n^y; V) \]
\[ q_{av}(n^y - \Xi^1_y) = r_{-m}(n^x, n^y; V). \]

Then the chemical master equation (CME) describing the evolution of the probability
\[ p_V(n^y, t) = \text{Prob}(n^Y(t) = n^y) \]
is
\[ \frac{\partial p_V(n^y, t)}{\partial t} = \sum_{\ell=2}^{M-1} \left\{ r_{+\ell}(n^y - \Xi^\ell y; V) p_V(n^y - \Xi^\ell y, t) - [r_{+\ell}(n^y; V) + r_{-\ell}(n^z; V)] p_V(n^y, t) \right. \]
\[ + r_{-\ell}(n^y + \Xi^\ell y; V) p_V(n^y + \Xi^\ell y, t) \}
\[ \left. - [r_{+1}(n^x, n^y; V) + r_{-1}(n^z; V)] p_V(n^x, t) + r_{+1}(n^x + \Xi^Y y; V) p_V(n^x + \Xi^Y y, t) \right\} \]
\[ + \sum_{\ell=M}^{M+1} \left\{ r_{+\ell}(n^y - \Xi^\ell y; V) p_V(n^y - \Xi^\ell y, t) - [r_{+\ell}(n^y; V) + r_{-\ell}(n^z; V)] p_V(n^y, t) \right. \]
\[ + r_{-\ell}(n^y + \Xi^\ell y; V) p_V(n^y + \Xi^\ell y, t) \}.

2.3. Cycle Flux.

A directed cycle in the counting space is a slice of path with the same origin and destination in which no other states overlap. It can be expressed in this way: \( c = [y_1, y_2, ..., y_s] \) with some integer \( s \) where \( y_1, y_2, ..., y_s \in S \) are successive states in the path which are different from each other. The next state of \( y_s \) in the path is exactly \( y_1 \), forming a single closed loop.

We denote an ordered sequence of distinct points \( y_1, y_2, ..., y_s \) by \( [y_1, y_2, ..., y_s] \). The set \([S]\) is all the finite ordered sequences \([y_1, y_2, ..., y_s]\) with \( s \neq 1 \). For any \( i \) with indices modulo \( k \), we identify \([y_1, y_2, ..., y_s]\) and \([y_i, y_{i+1}, ..., y_{i+k-1}]\) as the same directed cycle.

Next, we describe the cycle flux of a continuous time Markov chain, please refer to \([12, 13, 14]\) for details.

**Definition 2.2.** Suppose \( \xi \) is a continuous time Markov chain. When Hypothesis (2.1(i)) is satisfied, set
\[ T_0(\omega) = 0 \]
\[ T_1(\omega) = \inf\{ t > 0 : \xi_t(\omega) \neq \xi_0(\omega) \} \]
\[ T_{r+}(\omega) = \inf\{ t > T_r(\omega) : \xi_t(\omega) \neq \xi_{T_r(\omega)}(\omega) \}, \forall r \in \mathbb{N} \]
where \( \mathbb{N} \) is the set of natural numbers. Define
\[ m_t(\omega) = \sup\{ m \geq 0 : T_m(\omega) \neq t \} \]
For such a cycle \( c = [y_1, y_2, \ldots, y_s] \in [S] \),

(i) The number of the cycle \( c \) up to time \( t \) along the sample path \( \{\xi_m(\omega)\}_{m \neq 0} \) is

\[
w_{c,t} = \sum_{l=1}^{m_{l}(\omega)} \mathbb{1}_{c_{l-1} = (\omega) \cdot \eta_l = (\eta_l(\omega) \cdot y_k, y_{k+1}, \ldots, y_{k+s-1})} (\omega)
\]

where \( \mathbb{1}_A(\cdot) \) is the indicator of the set \( A \) and the sums \( k + 1, k + 2, \ldots, k + s - 1 \) are understood to be modulo \( s \).

(ii) The limit

\[
\lim_{t \to \infty} \frac{w_{c,t}}{t}
\]

exists. It’s called the cycle flux for \( c \) and denoted by \( \omega_c \). Moreover, it can be expressed more precisely:

\[
\omega_c = (-1)^{s-1} q_{y_1 y_2} q_{y_2 y_3} \cdots q_{y_s y_1} \frac{|Q(\{y_1, y_2, \ldots, y_s\})|}{\sum_{j \in S | Q(\{j\})|}},
\]

where \( |Q(H)| \) is the determinant of \( Q \) with rows and columns indexed by \( H \) and \( H^c \) is the complement of \( H \).

2.4. Reaction Cycle and Reaction Cycle Flux.

Definition 2.3 (Peng et al. [9]). Denote \( C_\infty \) as the set of all cycles in the counting space. For any cycle \( c = [y_1, y_2, \ldots, y_s] \in C_\infty \), the net numbers of occurrence of all the reactions in \( c \) satisfies the following linear equation:

\[
\Xi Y \tilde{c} = 0.
\]

(i) A map \( \phi : C_\infty \rightarrow \mathbb{R}^M \) is given by

\[
\phi(c) = \tilde{c} = (c_1, c_2, \ldots, c_M),
\]

where \( c_\ell, 1 \leq \ell \leq M, \) is the number of occurrence of \( \ell \)-th forward reaction minus the number of occurrence backward reaction in the cycle \( c \).

(ii) The cycle flux for \( \tilde{c} \) is defined as:

\[
\omega_{\tilde{c}} = \sum_{\phi(c) = \tilde{c}} \omega_c,
\]

which is the averaged frequency \( \tilde{c} \) occurs in the CME model.

3. Main Results and their Proofs

In this section, we show the existence of the macroscopic reaction cycle flux \( J_c \), i.e. the existence of the limit of mesoscopic reaction flux

\[
\lim_{V \to \infty} \frac{1}{V} \sum_{\phi(c) = \tilde{c}} w_c.
\]

Lemma 3.1 gives a more clear presentation of the mesoscopic reaction flux \( w_c \).

Lemma 3.1.

\[
\omega_{\tilde{c}} = \sum_{s=2}^{N} \sum_{c \in [y_1, y_2, \ldots, y_s]} \left( w_c \cdot \prod_{l=1}^{M} \mathbb{1}_{\sum_{h=1}^{s-1} (1_{(y_{h+1} = y_h + x_l^c)}) - 1_{(y_{h+1} = y_h - x_l^c)}) = c_l} \right)
\]
Proof. First, we pick out the cycle satisfying the map \( \phi(c) = \tilde{c} \) in the set \([S]\) with different cycle length. Next, for any cycle \( c = [y_1, y_2, \ldots, y_s] \in [S] \), the number of occurrence of \( \ell \)-th forward reaction is \( \sum_{h=1}^{s} \mathbb{1}_{(y_{h+1} = y_h + \mathbb{E}^Y)} \), and the number of occurrence of \( \ell \)-th backward reaction is \( \sum_{h=1}^{s} \mathbb{1}_{(y_{h+1} = y_h - \mathbb{E}^Y)} \), \( \ell = 1, 2, \ldots, M \). Thus, the net number of occurrence of \( \ell \)-th reaction in \( c \) is

\[
\sum_{h=1}^{s} (\mathbb{1}_{(y_{h+1} = y_h + \mathbb{E}^Y)} - \mathbb{1}_{(y_{h+1} = y_h - \mathbb{E}^Y)}).
\]

When all sums are equal to corresponding \( c_{\ell, \ell} \), \( \ell = 1, 2, \ldots, M \), it’s obvious that the net numbers of occurrence of all the reactions in \( c \) form a reaction cycle \( \tilde{c} = (c_1, c_2, ..., c_M) \).

Lemma 3.2. Take \( V \to \infty \), all reaction rates become

\[
\begin{align*}
r_{+\ell} &= V \left( k_{+\ell} n_{+\ell} \prod_{j=1}^{N_2} (\beta_j)^{\Xi^Y_j} + O(V^{-1}) \right), \quad \ell = 2, 3, \ldots, M \\
r_{-\ell} &= V \left( k_{-\ell} n_{-\ell} \prod_{j=1}^{N_2} (\beta_j)^{\Xi^Y_j} + O(V^{-1}) \right), \quad \ell = 1, 2, \ldots, M - 1
\end{align*}
\]

or

\[
\begin{align*}
r_{+1} &= V \left( k_{+1} n_{+1} \prod_{i=1}^{N_1} (\alpha_i)^{\Xi^X_i} \prod_{j=1}^{N_2} (\beta_j)^{\Xi^Y_j} + O(V^{-1}) \right) \\
r_{-M} &= V \left( k_{-M} n_{-M} \prod_{i=1}^{N_1} (\alpha_i)^{\Xi^X_i} \prod_{j=1}^{N_2} (\beta_j)^{\Xi^Y_j-M} + O(V^{-1}) \right)
\end{align*}
\]

Please refer to Kurtz [16] for this lemma. We give a proof for the readers’ convenience.

Proof. Let \( N_A \) be the Avogadro’s number. First, notice that

\[
k_{+\ell} = \tilde{k}_{+\ell} V^{n_{+\ell}-1},
\]

and then rewrite Eq. (2.3)-(2.6) in terms of concentrations

\[
\beta_i = n_i^Y / (V N_A).
\]

Notice \( n_{+\ell} = \sum_{j=1}^{N_2} \Xi^Y_{+\ell,j} \), then

\[
r_{+\ell} (\beta; V) = \frac{k_{+\ell}}{(V N_A)^{n_{+\ell}}} \sum_{j=1}^{N_2} \beta_j (V N_A) (\beta_j (V N_A) - 1) \cdots (\beta_j (V N_A) - \Xi^Y_{+\ell} - 1)
\]

\[
= k_{+\ell} \cdot \{(V N_A) \prod_{j=1}^{N_2} (\beta_j)^{\Xi^Y_{+\ell,j}} - (\prod_{j=1}^{N_2} (\beta_j)^{\Xi^Y_{+\ell,j} - 1})(\sum_{\mu=1}^{\Xi^Y_{+\ell} - 1} \mu) + (V N_A)^{-1} \prod_{j=1}^{N_2} (\beta_j)^{\Xi^Y_{+\ell,j} - 2} \}
\]

\[
\cdot \sum_{\mu>0} (\mu \rho) + \cdots + (-1)^{\Xi^Y_{+\ell}} (V N_A)^{-(\sum_{j=1}^{N_2} \Xi^Y_{+\ell,j} - 1)} \prod_{\mu=1}^{\Xi^Y_{+\ell} - 1} \mu
\]

for the forward reaction \( R_{+\ell} \), where \( \ell = 2, 3, \ldots, M \).

When \( V \to \infty \), the above formula become

\[
r_{+\ell} = V \left( N_A k_{+\ell} \prod_{j=1}^{N_2} (\beta_j)^{\Xi^Y_{+\ell,j}} + O(V^{-1}) \right), \quad \ell = 2, 3, \ldots, M.
\]
In the same vein, we have
\[
r_{+\ell} = V \left( N_A k_{+\ell} \prod_{j=1}^{N_2} (\beta_j)^{s_{+1,j}^\ell} + O(V^{-1}) \right), \quad \ell = 1, 2, \cdots, M - 1.
\]

For the other two transition rates, notice
\[
n_{+1} = \sum_{i=1}^{N_1} \Xi_{+1,i} + \sum_{j=1}^{N_2} \Xi_{+1,j}^+
\]
and
\[
n_{-M} = \sum_{i=1}^{N_1} \Xi_{-M,i} + \sum_{j=1}^{N_2} \Xi_{-M,j}^-
\]
then,
\[
r_{+1}(\alpha, \beta; V) = k_{+1}(V N_A) \prod_{i=1}^{N_1} (\alpha_i - \frac{1}{V N_A}) \cdots \left( \alpha_i - \frac{\Xi_{+1,i}^X - 1}{V N_A} \right) \prod_{j=1}^{N_2} \left( \beta_j - \frac{1}{V N_A} \right) \cdots \left( \beta_j - \frac{\Xi_{+1,j}^Y - 1}{V N_A} \right)
\]
When \( V \to \infty \), the above formula become
\[
r_{+1} = V \left( N_A k_{+1} \prod_{i=1}^{N_1} (\alpha_i)^{s_{+1,i}^X} \prod_{j=1}^{N_2} \beta_j^{s_{+1,j}^Y} + O(V^{-1}) \right).
\]
In the same vein, we have
\[
r_{-M} = V \left( N_A k_{-M} \prod_{i=1}^{N_1} (\alpha_i)^{s_{-M,i}^X} \prod_{j=1}^{N_2} \beta_j^{s_{-M,j}^Y} + O(V^{-1}) \right).
\]

Notice that transition rates in lemma 3.2 not only rely on which reaction occurs but also rely on which state the reaction occurs. To represent the transition rate precisely, we define two new reaction cycle similarly to the map in definition 3.3.

**Definition 3.3.** For the cycle \( c = [y_1, y_2, \cdots, y_s] \in \mathcal{C}_\infty \),
(i) define a map \( \phi^+: \mathcal{C}_\infty \to \mathbb{R}^M \) as
\[
\phi^+(c) = \bar{c}^+ = (c_1^+, c_2^+, \cdots, c_M^+)^t,
\]
where \( c_\ell^+, 1 \leq \ell \leq M \) is the number of the occurrence of \( \ell \)-th forward reaction in cycle \( c \). That is to say, \( \bar{c}^+ \) is the unique forward reaction cycle associated with \( c \).
(ii) define a map \( \phi^-: \mathcal{C}_\infty \to \mathbb{R}^M \) as
\[
\phi^-(c) = \bar{c}^- = (c_1^-, c_2^-, \cdots, c_M^-)^t,
\]
where \( c_\ell^-, 1 \leq \ell \leq M \) is the number of the occurrence of \( \ell \)-th backward reaction in cycle \( c \). That is to say, \( \bar{c}^- \) is the unique backward reaction cycle associated with \( c \).
Remark 3.4. It is easy to check that the following relationships between

\[ \phi^+(c) = \hat{c}^+ = (c_1^+, c_2^+, \ldots, c_M^+) \quad \text{and} \quad \phi^-(c) = \hat{c}^- = (c_1^-, c_2^-, \ldots, c_M^-) \]

and

\[ \phi^0(c) = \hat{c} = (c_1, c_2, \ldots, c_M) \]

holds:

1. \( c_i = c_i^+ - c_i^- \), \( c_i^+ \geq 0, c_i^- \geq 0 \), where \( i = 1, 2, \ldots, M \).

2. \( \sum_{i=1}^{M} (c_i^+ + c_i^-) = s \).

Lemma 3.5. From lemma (3.2) and definition (3.3), for cycle \( c = [y_1, y_2, \ldots, y_s] \),

\[
q_{y_1y_2}q_{y_2y_3} \cdots q_{y_sy_1} = \mathcal{V} \left[ N_A \cdot \left( \prod_{i=1}^{N_1} (\alpha_i)^{\Xi_i^{y_1}} \right) \left( \prod_{i=1}^{N_2} (\beta_i^{y_1} + 1)^{\Xi_i^{y_2}} \right) \cdots \left( \prod_{i=1}^{N_3} (\gamma_i^{y_1} + 1)^{\Xi_i^{y_3}} \right) \cdots \left( \prod_{i=1}^{N_s} (\delta_i^{y_1} + 1)^{\Xi_i^{y_2}} \right) \right]
\]

(3.5)

**Proof.** From definition (3.3), denote the corresponding reaction cycle of \( c = [y_1, y_2, \ldots, y_s] \) as \( \hat{c} = [c_1, c_2, \ldots, c_M] \), forward reaction cycle as \( \hat{c}^+ = [c_1^+, c_2^+, \ldots, c_M^+] \), back reaction cycle as \( \hat{c}^- = [c_1^-, c_2^-, \ldots, c_M^-] \). There are \( c_i^+(i = 1, 2, \ldots, M) \) states in the cycle on which the \( i \)-th forward reaction occurs and \( c_i^-(i = 1, 2, \ldots, M) \) states on which the \( i \)-th backward reaction occurs. Rearrange the cycle \( c \) as an ordered sequence \( \hat{c} \),

\[
\hat{c} = (y_1^{i_1}, y_2^{i_2}, \ldots, y_s^{i_s}, y_1^{M}, y_2^{M}, \ldots, y_s^{M})
\]

(3.4)

where \( y_\ell^s, \ell = 1, 2, \ldots, M \) are the \( i \)-th state along the cycle \( c \) that on which occurs \( \ell \)-th forward and backward reaction that convert one state to another. Denote by \( \hat{c}^+ \) the concentrations vector of \( c \) corresponding to \( \hat{c} \),

\[
\hat{c}^+ = (\hat{c}_1^+, \hat{c}_2^+, \ldots, \hat{c}_M^+)
\]

(3.5)

where all

\[ \hat{c}_i^+ = y_i^{i+} \cdots y_i^{i-} / (MN_A) \]

are \( N_2 \)-dimensional vectors. Furthermore, denote

\[ \hat{c}_i^+, j = 1, 2, \ldots, N_2 \]
the $j$-th element of the vector $\beta_{i,c+1}^{+}$. Thus,

\[
q_{1}s_{2}s_{3} \cdots q_{s} \cdot w_{1} = \left( r_{1}(a, \beta_{i,1}^{+}; V) r_{1}(a, \beta_{i,2}^{+}; V) \cdots r_{+1}(a, \beta_{i,c+1}^{+}; V) \right) \left( r_{+2}(\beta_{2,1}^{+}; V) r_{+2}(\beta_{2,2}^{+}; V) \cdots r_{+2}(\beta_{2,c+2}^{+}; V) \right) \cdots
\]

\[
+ (r_{+M}(a, \beta_{i,1}^{+}; V) r_{+M}(a, \beta_{i,2}^{+}; V) \cdots r_{+M}(a, \beta_{i,M+1}^{+}; V))
\]

\[
- (r_{-1}(a, \beta_{i,1}^{-}; V) r_{-1}(a, \beta_{i,2}^{-}; V) \cdots r_{-1}(a, \beta_{i,c+1}^{-}; V)) \left( r_{-2}(\beta_{2,1}^{-}; V) r_{-2}(\beta_{2,2}^{-}; V) \cdots r_{-2}(\beta_{2,c+2}^{-}; V) \right) \cdots
\]

\[
+ (r_{-M}(a, \beta_{i,1}^{-}; V) r_{-M}(a, \beta_{i,2}^{-}; V) \cdots r_{-M}(a, \beta_{i,M+1}^{-}; V))
\]

\[
= \left( k_{1}^{+} \sum_{i=1}^{N_{1}}(a_{i})^{+} \sum_{j=1}^{N_{2}}(\beta_{i,j}^{+})^{+} \right) \left( k_{2}^{+} \sum_{i=1}^{N_{1}}(a_{i})^{+} \sum_{j=1}^{N_{2}}(\beta_{i,j}^{+})^{+} \right) \cdots
\]

\[
+ \left( k_{M}^{+} \sum_{i=1}^{N_{1}}(a_{i})^{+} \sum_{j=1}^{N_{2}}(\beta_{i,j}^{+})^{+} \right) \left( k_{M}^{-} \sum_{i=1}^{N_{1}}(a_{i})^{-} \sum_{j=1}^{N_{2}}(\beta_{i,j}^{-})^{-} \right) + O(V^{-1})
\]

\[
= V^{N_{A}} \cdot \left( k_{1}^{+} \sum_{i=1}^{N_{1}}(a_{i})^{+} \sum_{j=1}^{N_{2}}(\beta_{i,j}^{+})^{+} \right) \left( k_{2}^{+} \sum_{i=1}^{N_{1}}(a_{i})^{+} \sum_{j=1}^{N_{2}}(\beta_{i,j}^{+})^{+} \right) \cdots
\]

\[
+ \left( k_{M}^{+} \sum_{i=1}^{N_{1}}(a_{i})^{+} \sum_{j=1}^{N_{2}}(\beta_{i,j}^{+})^{+} \right) \left( k_{M}^{-} \sum_{i=1}^{N_{1}}(a_{i})^{-} \sum_{j=1}^{N_{2}}(\beta_{i,j}^{-})^{-} \right) + O(V^{-1})
\]

(3.6)
**Theorem 3.6.** The following limit exists:

\[
J_x = \lim_{V \to \infty} \frac{\omega(V)}{V}
\]

\[
= \sum_{s=1}^{N} \sum_{c \in [y_1, y_2, \ldots, y_s]} (-1)^{s-1} N_A^s \left( \prod_{i=1}^{N} (\alpha_i)^{\Xi^{-1}+Y} \right) \prod_{j=1}^{N} (\beta_j)^{\Xi^{-1}+Y} \right)
\]

\[
\left( k_{+M}^{\ell} \prod_{j=1}^{N} (\beta_j)^{\Xi^{-1}+Y} \right) \cdots \left( k_{+M}^{M} \prod_{j=1}^{N} (\beta_j)^{\Xi^{-1}+Y} \right)
\]

\[
\cdot \left( Q \left( \{y_1, y_2, \ldots, y_s\} \right) \right) \left( \prod_{j \in S} \left( \sum_{e=1}^{N-1} \sum_{e=1}^{N} (\Xi^{-1}+Y) - 1 \right) \left( y_{e+1} + y_e, y_s \right) \right)
\]

**Remark 3.7.** When \( V \to \infty \), it follows from Lemma 3.2 that

\[
r_{+\ell} = V \left( k_{+\ell} N_A \prod_{j=1}^{N} (\beta_j)^{\Xi^{-1}+Y} + O(V^{-1}) \right), \quad \ell = 2, 3, \ldots, M
\]

\[
r_{-\ell} = V \left( k_{-\ell} N_A \prod_{j=1}^{N} (\beta_j)^{\Xi^{-1}+Y} + O(V^{-1}) \right), \quad \ell = 1, 2, \ldots, M - 1
\]

or

\[
r_{+1} = V \left( k_{+1} N_A \prod_{i=1}^{N} (\alpha_i)^{\Xi^{-1}+Y} \prod_{j=1}^{N} (\beta_j)^{\Xi^{-1}+Y} + O(V^{-1}) \right)
\]

\[
r_{-M} = V \left( k_{-M} N_A \prod_{i=1}^{N} (\alpha_i)^{\Xi^{-1}+Y} \prod_{j=1}^{N} (\beta_j)^{\Xi^{-1}+Y} + O(V^{-1}) \right).
\]

Denote

\[
a_{+\ell} = k_{+\ell} N_A \prod_{j=1}^{N} (\beta_j)^{\Xi^{-1}+Y}, \quad \ell = 2, 3, \ldots, M
\]

\[
a_{-\ell} = k_{-\ell} N_A \prod_{j=1}^{N} (\beta_j)^{\Xi^{-1}+Y}, \quad \ell = 1, 2, \ldots, M - 1
\]

or

\[
a_{+1} = k_{+1} N_A \prod_{i=1}^{N} (\alpha_i)^{\Xi^{-1}+Y} \prod_{j=1}^{N} (\beta_j)^{\Xi^{-1}+Y}
\]

\[
a_{-M} = k_{-M} N_A \prod_{i=1}^{N} (\alpha_i)^{\Xi^{-1}+Y} \prod_{j=1}^{N} (\beta_j)^{\Xi^{-1}+Y}.
\]
Then
\[ r_{+\ell} = V(a_{+\ell} + O(V^{-1})) , \quad \ell = 2, 3, \ldots , M \]
\[ r_{-\ell} = V(a_{-\ell} + O(V^{-1})) , \quad \ell = 1, 2, \ldots , M - 1 \]
or
\[ r_{+1} = V(a_{+1} + O(V^{-1})) \]
\[ r_{-M} = V(a_{-M} + O(V^{-1})) . \] (3.10)

Replace all \( r_{\pm \ell} \) in matrix \( Q \) with \( a_{\pm \ell} \), there appear a new matrix \( \tilde{Q} \). Obviously, \( \tilde{Q} \) is computable in our model.

Then, it’s easy to verify that
\[ \lim_{V \to \infty} \frac{1}{V} Q(\{y_1, y_2, \cdots , y_s\}^c) = \tilde{Q}(\{y_1, y_2, \cdots , y_s\}^c) \],
for any \( \{y_1, y_2, \cdots , y_s\} \in [S] \).

**Proof.** From equation 2.10, we have
\[ \omega_c = (-1)^{s-1} q_{y_1, y_2, y_3, \ldots , y_s, y_1} \left( \frac{1}{\sum_{j \in S} |Q(\{j\}^c)|} |Q(\{y_1, y_2, \cdots , y_s\}^c)| \right) \]

Then,
\[ \frac{|Q(\{y_1, y_2, \cdots , y_s\}^c)|}{\sum_{j \in S} |Q(\{j\}^c)|} = \frac{V^{-s} \frac{1}{V} Q(\{y_1, y_2, \cdots , y_s\}^c)}{V^{-1} \sum_{j \in S} \tilde{Q}(\{j\}^c)} = \frac{|Q(\{y_1, y_2, \cdots , y_s\}^c)|}{\sum_{j \in S} |Q(\{j\}^c)|} \]

From lemma 3.5,
\[ q_{y_1, y_2, y_3, \ldots , y_s, y_1} \]
\[ = V^s \left[ N_1^s \cdot \left( k_{i,j}^{-1} \prod_{i=1}^{N_1} (\alpha_i)^\Xi_{i+1} \right)^{1+} \prod_{i=1}^{N_2} (\beta_{i,j})^{\Xi_{i+1}} \right] \left( k_{i,j}^{-1} \prod_{i=1}^{N_1} (\alpha_i)^\Xi_{i+1} \right)^{1-} \prod_{i=1}^{N_2} (\beta_{i,j})^{\Xi_{i+1}} \right] \]
\[ \ldots \left( k_{i,j}^{-1} \prod_{i=1}^{N_1} (\alpha_i)^\Xi_{i+1} \right)^{1+} \prod_{i=1}^{N_2} (\beta_{i,j})^{\Xi_{i+1}} \right] \left( k_{i,j}^{-1} \prod_{i=1}^{N_1} (\alpha_i)^\Xi_{i+1} \right)^{1-} \prod_{i=1}^{N_2} (\beta_{i,j})^{\Xi_{i+1}} \right] \]
\[ \ldots \left( k_{i,j}^{-1} \prod_{i=1}^{N_1} (\alpha_i)^\Xi_{i+1} \right)^{1+} \prod_{i=1}^{N_2} (\beta_{i,j})^{\Xi_{i+1}} \right] \left( k_{i,j}^{-1} \prod_{i=1}^{N_1} (\alpha_i)^\Xi_{i+1} \right)^{1-} \prod_{i=1}^{N_2} (\beta_{i,j})^{\Xi_{i+1}} \right] + O(V^{-1}) \] (3.11)
Thus,

\[
\lim_{V \to \infty} \frac{\omega_c}{V} = (-1)^{s-1} \lim_{V \to \infty} \frac{1}{V} \left| \mathcal{Q}(\{y_1, y_2, \ldots, y_s\}) \right| \cdot V^s
\]

\[
\cdot N_A \cdot \left( \prod_{i=1}^{N_1} (\alpha_i)^{\Xi_{i+1}} \right)^{c_1} \left( \prod_{j=1}^{N_2} (\beta_{i,j})^{\Xi_{i+1}} \right) \left( \prod_{k=1}^{N_3} (\beta_{i,j})^{\Xi_{i+1}} \right) \ldots
\]

\[
= (-1)^{s-1} N_A \cdot \left( \prod_{i=1}^{N_1} (\alpha_i)^{\Xi_{i+1}} \right)^{c_1} \left( \prod_{j=1}^{N_2} (\beta_{i,j})^{\Xi_{i+1}} \right) \left( \prod_{k=1}^{N_3} (\beta_{i,j})^{\Xi_{i+1}} \right) \ldots
\]

\[
\frac{\hat{Q}(\{y_1, y_2, \ldots, y_s\})}{\sum_{j \in S} \left| \hat{Q}(\{j\}) \right|}
\]

Therefore,

\[
\mathcal{J}_c = \lim_{V \to \infty} \frac{1}{V} \sum_{s=1}^{N} \sum_{c \in \{y_1, y_2, \ldots, y_s\}} \left( w_c \cdot \prod_{l=1}^{M} \left\{ \sum_{h=1}^{s} (1_{(y_{h+1} = y_h + 2y_i^l})^{1} (1_{(y_{h+1} = y_h - 2y_i^l)})^{c_l}) \right\} \right)
\]

\[
= \sum_{s=1}^{N} \sum_{c \in \{y_1, y_2, \ldots, y_s\}} \left( \lim_{V \to \infty} \frac{w_c}{V} \prod_{l=1}^{M} \left\{ \sum_{h=1}^{s} (1_{(y_{h+1} = y_h + 2y_i^l})^{1} (1_{(y_{h+1} = y_h - 2y_i^l)})^{c_l}) \right\} \right)
\]

\[
= \sum_{s=1}^{N} \sum_{c \in \{y_1, y_2, \ldots, y_s\}} (-1)^{s-1} N_A \cdot \left( \prod_{i=1}^{N_1} (\alpha_i)^{\Xi_{i+1}} \right)^{c_1} \left( \prod_{j=1}^{N_2} (\beta_{i,j})^{\Xi_{i+1}} \right) \left( \prod_{k=1}^{N_3} (\beta_{i,j})^{\Xi_{i+1}} \right) \ldots
\]

\[
\cdot \left( \prod_{i=1}^{N_1} (\alpha_i)^{\Xi_{i+1}} \right)^{c_1} \left( \prod_{j=1}^{N_2} (\beta_{i,j})^{\Xi_{i+1}} \right) \left( \prod_{k=1}^{N_3} (\beta_{i,j})^{\Xi_{i+1}} \right) \ldots
\]

\[
\frac{\hat{Q}(\{y_1, y_2, \ldots, y_s\})}{\sum_{j \in S} \left| \hat{Q}(\{j\}) \right|}
\]

(3.12)
\[
\frac{Q((y_1, y_2, \ldots, y_s)\,|\, \sum_{j \in S} Q_j(\{j\}))}{\prod_{l=1}^M \Pi \left\{ \sum_{h=1}^{y_h+1} y_h + \Xi Y_{l} - 1 (y_h+1 = y_h - \Xi Y_{l}) \right\}}.
\]

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References

[1] Jia C, Liu X F, Qian M P, Jiang D Q and Zhang Y P 2010. Kinetic behavior of the general modifier mechanism of botts and morales with non-equilibrium binding. Journal of Theoretical Biology, 296(7):13-20.
[2] Jia C, Qian M, and Jiang D Q 2014. Overshoot in biological systems modelled by Markov chains: a non-equilibrium dynamic phenomenon. Iet Systems Biology, 8(4):138-145.
[3] Ge H, and Qian H 2017. Mathematical formalism of nonequilibrium thermodynamics for nonlinear chemical reaction systems with general rate law, J. Stat. Phys. 166, 190
[4] Jiang D Q, Qian M, and Qian M P 2004. Mathematical theory of nonequilibrium steady states : on the frontier of probability and dynamical systems Springer Berlin Heidelberg
[5] Qian H and Qian M 2012. Stochastic theory of nonequilibrium steady states. part ii: applications in chemical biophysics. Physics Reports: A Review Section of Physics Letters (Section C), 510(3), 87-118
[6] GreenLee and Marty 2009. Chemical biophysics: quantitative analysis of cellular systems. SIAM Review, 51(2), 444-445
[7] Jia C, Li Y, and Qian M P 2013. A General Analysis of Single IP3 Receptors Modulated by Cytosolic Ca \(^{2+}\) and IP3. The Third International Symposium on Optimization and Systems Biology, (2013).
[8] Gupta P B, Fillmore C M, Jiang G Z, Shapira S D, Tao K, Kuperwasser C and Lander E S 2011. Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells. Cell
[9] Peng Y, Qian H, Beard D A, and Ge H 2020. Universal relation between thermodynamic driving force and one-way fluxes in a nonequilibrium chemical reaction with complex mechanism.
[10] Rao R, and Esposito M 2016. Nonequilibrium thermodynamics of chemical reaction networks: wisdom from stochastic thermodynamics. Physical Review X, 6(4).
[11] Mcquarrie D A 1967/ Stochastic approach to chemical kinetics. Journal of Applied Probability, 4(3), 413-478.
[12] Qian C, Qian M, and Qian M P 1981. Markov chain as a model of Hill’s theory on circulation, Sci. Sin. 24, 1431.
[13] Jiang D Q, Qian M, and Qian M P 2004. Circulation Distribution, Entropy Production and Irreversibility of Finite Markov Chains with Continuous Parameter (Springer, Berlin), pp. 45-66.
[14] Jiang D Q, Qian M, and Qian M P 2004. Circulation Distribution, Entropy Production and Irreversibility of Denumerable Markov Chains (Springer, Berlin)
[15] Polettini M and Esposito M 2014. Irreversible thermodynamics of open chemical networks. I. Emergent cycles and broken conservation laws, J. Chem.Phys. B 141, 024117 .
[16] Ethier S N and Kurtz T G 1986. Markov Processes: Characterization and Convergence. Biometrics, p.454.
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