Nonequilibrium thermodynamics and a fluctuation theorem for individual reaction steps in a chemical reaction network

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Abstract. We have introduced an approach to nonequilibrium thermodynamics of an open chemical reaction network in terms of the propensities of the individual elementary reactions and the corresponding reverse reactions. The method is a microscopic formulation of the dissipation function in terms of the relative entropy or Kullback-Leibler distance which is based on the analogy of phase space trajectory with the path of elementary reactions in a network of chemical process. We have introduced here a fluctuation theorem valid for each opposite pair of elementary reactions which is useful in determining the contribution of each sub-reaction on the nonequilibrium thermodynamics of overall reaction. The methodology is applied to an oligomeric enzyme kinetics at a chemiostatic condition that leads the reaction to a nonequilibrium steady state for which we have estimated how each step of the reaction is energy driven or entropy driven to contribute to the overall reaction.

1. Introduction

Approach of chemical kinetics of bulk reaction systems needs serious modification [1, 2, 3] to apply for small a system containing a few number of molecules to accommodate the significant fluctuation of the species [1, 2, 3, 4, 5, 6, 7, 8, 9]. Stochastic approach becomes the theoretical tool for the description of the inherent statistical nature of the fluctuating dynamics of reaction system [1, 2, 10, 11, 12], as well as chemical master equation [4, 5, 6]. In single molecule kinetics [13, 14], the concentration terms are replaced by the corresponding probabilities and number of molecules of a reacting species is a fluctuating quantity [1, 2, 11, 12, 15, 16, 17].

In terms of master equation the stochastic thermodynamic description of such systems are given starting from a definition of the system entropy in terms of state probabilities [10, 18, 19, 20, 21]. It has been established that closed systems tend to equilibrium whereas open systems can reach a nonequilibrium steady state(NESS) characterized by positive total entropy production rate (EPR) [10, 12]. In chemical reaction systems, NESS can be attained in presence of chemiostatic condition where concentrations of some species are kept constant throughout the reaction [10]. Such situation can arise in a living cell where, e.g., an enzyme catalysis occurs with substrate and product concentrations inside the cell maintained at a constant value through some cellular mechanism that continuously supplies the substrate to and withdraws the product from the system [22]. Recently, the master equation approach has been used...
formulate the nonequilibrium thermodynamics describing generally the first and second laws in terms of various entropy production rates [23, 24, 25] and energetics of reactions occurring far from equilibrium [10, 11, 12, 22].

Here our aim is to determine the role of individual elementary reactions in governing the nonequilibrium thermodynamics of open chemical reaction. We have formulated a theory of nonequilibrium thermodynamics of a chemical reaction using Kullback-Leibler (KL) distance in terms of propensity [4, 5] of each elementary reaction. We are interested to know the work done and various energy functions of the nonequilibrium chemical system, like heat dissipation and work done as well as state functions like internal energy, free energy and system entropy. We have studied how the kinetics and energetics are coupled in this nonequilibrium system, specially at NESS and how the fluctuation relations are connected with it. For this purpose the total epr is formulated using the propensities of an elementary reaction and its reverse reaction assuming that microscopic reversibility holds, similar to the relative entropy or Kullback-Leibler (KL) distance formalism of the dissipation function in terms of forward and (time-)reversed phase space trajectories [26, 27, 28, 29]. We have applied the theory on a single oligomeric enzyme catalysis reaction reaching NESS [11, 12, 22, 15, 30] and studied using real parameters of a single Escherichia coli β- galactosidase enzyme under chemiostatic condition.

The paper is organized as follows. In Sec. II, we have given the scheme of an open nonequilibrium reaction network described by a chemical master equation. Expressions of different entropy production rates corresponding to the irreversible processes are given from this master equation. In Sec. III, a method of calculation of total entropy production rate is devised in terms of the individual reactions based on propensity distance. The theory is applied for the kinetics of a single oligomeric enzyme catalysis in Sec. IV. We have discussed on propensity approach to total EPR of individual sub-reaction in Section V. A Fluctuation Theorem is introduced in Section VI for the total EPR of elementary reactions. A thorough analysis of the contributions of elementary reactions are performed for the single molecule kinetics of a Escherichia coli β- galactosidase enzyme in Sec. VII. The paper is concluded Sec. VIII.

2. Master equation for nonequilibrium reaction

We consider a nonequilibrium steady state reaction network

$$\sum_{i=1}^{a} \nu_{<\mu} i A_i + \sum_{j=1}^{b} \nu_{<\mu} j Z_j \frac{k_{p}}{k_{\mu}} \sum_{i=1}^{a} \nu_{>\mu} i A_i + \sum_{j=1}^{b} \nu_{>\mu} j Z_j .$$

(1)

Here $Z_j$ are the intermediate species and $A_i$ are species whose concentrations are kept constant by connecting the reaction system to reservoirs, known as chemiostatic condition. The system is assumed to be at thermal equilibrium with the reservoir kept at some temperature, $T$. $\mu$ is the elementary reaction index with $\mu = \pm 1, \pm 2, \ldots, \pm k$. So every transition in the population state space due to the reaction $\mu$ has a counterpart in the reverse transition by the reaction $-\mu$. The stoichiometric coefficient, $\nu_\mu^j$, of the species $Z_j$ involved in the $\mu$-th reaction is defined as

$$\nu_\mu^j = \nu_{>\mu}^j - \nu_{<\mu}^j, \quad (j = 1, \ldots, b),$$

(2)

where $\nu_{<\mu}^j$ and $\nu_{>\mu}^j$ are the corresponding coefficient of the $j$-th species for reactant and product, respectively for the $\mu$ reaction. The number of molecules of different intermediate species are collectively denoted by the vector $Z = \{Z_j\}, (j = 1, \ldots, b)$ and the corresponding vector of the stoichiometric coefficients associated with the $\mu$-th reaction is denoted by $\nu_\mu$. The chemical master equation governing the stochastic reaction event is given by [10, 19, 20, 21]

$$\frac{dP(Z,t)}{dt} = \sum_{\mu = \pm 1}^{\pm k} \left[ w_\mu (Z - \nu_\mu | Z) P(Z - \nu_\mu, t) - w_{-\mu} (Z | Z - \nu_\mu) P(Z, t) \right].$$

(3)
Here $P(Z,t)$ is the probability of having population vector $Z$ of the intermediate species at time $t$ and $w_{\mu}(Z - \nu_{\mu}|Z)$ is the transition probability for the jump $Z - \nu_{\mu} \rightarrow Z$ in the population state space via reaction $\mu$. The rate of entropy production from the above master equation (Eq.3), from system’s Shannon entropy [10, 16, 18, 19, 20, 21]

$$S_{sys}(t) = -\sum_{Z} P(Z,t) \ln P(Z,t),$$  \hspace{1cm} (4)

and using Eq.(3), we get the system entropy production rate (EPR) as

$$\dot{S}_{sys}(t) = \frac{1}{2} \sum_{Z,\mu} [w_{\mu}(Z - \nu_{\mu}|Z)P(Z - \nu_{\mu},t) - w_{-\mu}(Z|Z - \nu_{\mu})P(Z,t)] \times \ln \frac{P(Z - \nu_{\mu},t)}{P(Z,t)}. \hspace{1cm} (5)$$

Then the system EPR can be splitted as $\dot{S}_{sys}(t) = \dot{S}_{tot}(t) - \dot{S}_{m}(t)$, where the first term in the r.h.s. gives the total EPR and the second term denotes the medium EPR due to the entropy flux into the surroundings given by

$$\dot{S}_{tot}(t) = \frac{1}{2} \sum_{Z,\mu} [w_{\mu}(Z - \nu_{\mu}|Z)P(Z - \nu_{\mu},t) - w_{-\mu}(Z|Z - \nu_{\mu})P(Z,t)] \times \ln \frac{w_{\mu}(Z - \nu_{\mu}|Z)P(Z - \nu_{\mu},t)}{w_{-\mu}(Z|Z - \nu_{\mu})P(Z,t)}. \hspace{1cm} (6)$$

and

$$\dot{S}_{m}(t) = \frac{1}{2} \sum_{Z,\mu} [w_{\mu}(Z - \nu_{\mu}|Z)P(Z - \nu_{\mu},t) - w_{-\mu}(Z|Z - \nu_{\mu})P(Z,t)] \times \ln \frac{w_{\mu}(Z - \nu_{\mu}|Z)}{w_{-\mu}(Z|Z - \nu_{\mu})}. \hspace{1cm} (7)$$

It is evident from Eq.(6) that $\dot{S}_{tot}(t) \geq 0$. The medium EPR is identified as the temperature-scaled heat dissipation rate that can be positive or negative. Recently, Esposito et al. [24, 25] showed that the total EPR, $\dot{S}_{tot}(t)$ can be separated into adiabatic and nonadiabatic parts, $\dot{S}_{a}(t)$ and $\dot{S}_{na}(t)$, respectively which for this reaction network case can be given by

$$\dot{S}_{a}(t) = \frac{1}{2} \sum_{Z,\mu} [w_{\mu}(Z - \nu_{\mu}|Z)P(Z - \nu_{\mu},t) - w_{-\mu}(Z|Z - \nu_{\mu})P(Z,t)] \times \ln \frac{w_{\mu}(Z - \nu_{\mu}|Z)P^{st}(Z - \nu_{\mu})}{w_{-\mu}(Z|Z - \nu_{\mu})P^{st}(Z)}. \hspace{1cm} (8)$$

and

$$\dot{S}_{na}(t) = \frac{1}{2} \sum_{Z,\mu} [w_{\mu}(Z - \nu_{\mu}|Z)P(Z - \nu_{\mu},t) - w_{-\mu}(Z|Z - \nu_{\mu})P(Z,t)] \times \ln \frac{P(Z - \nu_{\mu},t)P^{st}(Z)}{P^{st}(Z - \nu_{\mu})P(Z,t)}. \hspace{1cm} (9)$$

where $P^{st}(Z)$ is the steady state probability of having the population vector $Z$. Unlike the system and medium contributions, the adiabatic and nonadiabatic parts characterize the rates
of internal energy ($U$) change, free energy ($F$) change and first law can be given in terms of rates of heat dissipation and irreversible work done [23]. It is also shown that the nonadiabatic EPR is negative of the temperature-scaled free energy dissipation rate, $\dot{S}_{na}(t) = -\dot{F}(t)/T$ and the adiabatic EPR, $\dot{S}_a(t)$ is the rate of irreversible work done (temperature-scaled) on the system. The rate of internal energy change behaves as $\dot{U}(t)/T = \dot{S}_a(t) - \dot{S}_{na}(t)$ like first law.

3. Propensity distance for individual reactions

We consider a transition along a stochastic trajectory in the population state space of the chemical reaction network as $(Z,t) \rightarrow (Z + \nu_{\mu}, t + \Delta t)$. This can be described as a transition from the state $(Z)$ to $(Z + \nu_{\mu})$ at time $t$ and then considering a time interval $\Delta t$ such that no further transition takes place in this interval. The system entropy production [31, 32, 33] for the transition due to the elementary reaction $\mu$ over the time interval $\Delta t$ can be written as

$$\Delta s^{(\mu)}_{sys} = -\ln P(Z + \nu_{\mu}, t + \Delta t) - (-\ln P(Z, t)) = \ln \frac{P(Z, t)}{P(Z + \nu_{\mu}, t + \Delta t)}. \quad (10)$$

The medium entropy production or entropy flow associated with this transition along the trajectory is defined as [31, 32, 33, 25]

$$\Delta s^{(\mu)}_{m} = \ln \frac{w_{\mu}(Z|Z + \nu_{\mu})}{w_{-\mu}(Z + \nu_{\mu}|Z)}. \quad (11)$$

Thus we write the total entropy production as

$$\Delta s^{(\mu)}_{tot} = \Delta s^{(\mu)}_{sys} + \Delta s^{(\mu)}_{m} = \ln \frac{P_{\mu}(Z, t|Z + \nu_{\mu})}{P_{-\mu}(Z + \nu_{\mu}, t + \Delta t|Z)}. \quad (12)$$

Here we introduce the propensity, $P_{\mu}(Z, t|Z + \nu_{\mu}) = w_{\mu}(Z|Z + \nu_{\mu})P(Z, t)$ which is the reaction probability density discussed by Gillespie [4, 5, 6]. Then $P_{\mu}(Z, t|Z + \nu_{\mu})dt$ is the probability that the system at time $t$ will go from state $Z$ to state $Z + \nu_{\mu}$ due to the occurrence of an elementary reaction $\mu$ in the next infinitesimal time interval $dt$.

Now all the above expressions of entropy production are for a specific transition as indicated by the lower case ‘s’. Thus we need to average over all the transitions due to the $\mu$-th reaction considering all possible values of the population vector $Z$ to get the overall entropy production due to this elementary reaction. This averaging is done with the probability, $P_{\mu}(Z, t|Z + \nu_{\mu})\Delta t$. Here $\Delta t$ is considered to be small enough so that over this interval $P_{\mu}$ remains constant. Then

$$\Delta S^{(\mu)}_{tot} = \langle \Delta s^{(\mu)}_{tot} \rangle = \sum_{Z} (P_{\mu}(Z, t|Z + \nu_{\mu})\Delta t) \ln \frac{P_{\mu}(Z, t|Z + \nu_{\mu})}{P_{-\mu}(Z + \nu_{\mu}, t + \Delta t|Z)}. \quad (13)$$

Thus the expression of the total entropy production rate becomes

$$\lim_{\Delta t \rightarrow 0} \frac{\Delta S^{(\mu)}_{tot}}{\Delta t} = \dot{S}^{(\mu)} = \sum_{Z} P_{\mu}(Z, t|Z + \nu_{\mu}) \ln \frac{P_{\mu}(Z, t|Z + \nu_{\mu})}{P_{-\mu}(Z + \nu_{\mu}, t|Z)}. \quad (14)$$

The structure of Eq.(14) is similar to the relative entropy or the Kullback-Leibler (KL) distance of two probability distributions [26, 27, 28, 34, 35]. Hence a KL distance type relation given in terms of the forward and reverse propensities, $P_{\mu}$ and $P_{-\mu}$ gives rise to the total entropy production rate due to the $\mu$-th reaction in the forward direction which is similar to the description of the total entropy production given by the KL distance of forward and backward trajectories in phase space [26, 28].
4. Single oligomeric enzyme catalysis

To apply this formulation we consider an example of a prototype chemical reaction network as a single oligomeric enzyme kinetics where the reaction of an active site is written as [15, 17]

\[ \text{E} + \text{S} \xrightleftharpoons[k_{-1}']{k_1} \text{ES} \xrightleftharpoons[k_{-2}']{k_2} \text{E} + \text{P}. \]  

Under chemiostatic condition [10, 12, 22] with concentrations of substrate, S and product, P maintained constant, the above reaction scheme can be reduced to

\[ \text{E} \xrightleftharpoons[K_1]{K_2} \text{ES}, \]  

where \( K_1 = k_1 + k_2 \) and \( K_2 = k_{-1} + k_{-2} \). The pseudo first order rate constants \( k_1 \) and \( k_2 \) are given by \( k_1 = k_1[S] \) and \( k_2 = k_2[P] \). We consider the enzyme to be consisting of \( n_T \) number of identical non-interacting subunits where each subunit has one active site. During the progress of the reaction, the active sites which have already formed the ES-complex are referred to as occupied sites and those lying vacant at that moment are called the vacant sites. The reaction system can thus be viewed in terms of the number of occupied sites present at a particular time. Due to the random occurrence of the four elementary reactions, the number of occupied sites of a single oligomeric enzyme becomes a fluctuating quantity. The state of the system is denoted by the random variable, \( n \) where \( n \) is the number of occupied sites present at time \( t \). After a small time \( \tau \), the system can move to a new state, \( (n + \nu_\mu) \) through any one of the four elementary reactions. Here \( \nu_\mu \) is the stoichiometric coefficient of the \( \mu \)-th reaction with rate constant \( k_\mu \).

Then the stochastic master equation can be written as [15, 16, 17]

\[ \frac{dP(n,t)}{dt} = \sum_{\mu=\pm1}^{\pm2} [w_\mu(n - \nu_\mu|n)P(n - \nu_\mu,t) - w_{-\mu}(n|n - \nu_\mu)P(n,t)]. \]  

Here \( P(n,t) \) is the probability of having \( n \) number of occupied states at time \( t \) with \( \nu_\mu = 1 \) for \( \mu > 0 \) and \( \nu_\mu = -1 \) for \( \mu < 0 \). The transition probabilities are defined as follows,

\[ w_\mu(n - \nu_\mu|n) = k_\mu(n_T - (n - \nu_\mu)), \mu > 0 \]

and

\[ w_\mu(n - \nu_\mu|n) = k_\mu(n - \nu_\mu), \mu < 0. \]  

Then Eq.(17) can be rewritten as

\[ \frac{dP(n,t)}{dt} = K_1(n_T - n + 1)P(n - 1,t) + K_2(n + 1)P(n + 1,t) - K_1(n_T - n)P(n,t) - K_2nP(n,t). \]  

Solving the master equation with the standard approach of generating function method [36, 37] we get the probability distribution function and average number of occupied site [15, 16] assuming that initially all the active sites are vacant.
5. Propensity approach to Total EPR of elementary sub-reaction

Here we shall introduce the approach to calculate the total EPR of individual sub-reactions in terms of propensities. We shall describe the master equation in terms of propensity equation of each sub-reaction. By this procedure one can obtain the property of the irreversible chemical flow system using the microscopic reversibility in the chemical reaction steps.

Now we give a description of the total entropy production rate, where the elementary reactions $\mu = \pm 1$ and $\mu = \pm 2$ form the reaction-'counter'reaction pairs on the basis of ES-complex formation and dissociation. The transition between two states of the system, say state $m$ and state $l$, are governed by Eq.(17). As $\nu_\mu = \pm 1$, so $l = m \pm 1$. In terms of state variable, $n$ we can write $m, l = (n - 1), n$ and $(n + 1)$, for example, for a forward transition, $l > m$ and for a backward transition, $l < m$. Now a specific reaction is not responsible for all the possible transitions between the system states. For example, the system can go from the state $(n - 1)$ to state $n$ by the elementary reaction $\mu = 1$ or $\mu = 2$ but not by $\mu = -1$ or $\mu = -2$. More clearly, for $\mu > 0$, 'l' must be greater than 'm' with $m = (n - 1), n$. Similarly for $\mu < 0$, 'l' is less than 'm' with $m = n, (n + 1)$. If one considers transition from state $m$ to state $l$ at time $t$ via the reaction $\mu$ with the propensity $P_\mu(m, t|l)$ (having the dimension of rate), then the reverse transition occurs through the reaction channel $-\mu$ with the corresponding propensity being $P_{-\mu}(l, t|m)$.

The master equation Eq.(17) can be cast into the form of propensity equation as

$$P_\mu(m, t|l) = w_\mu(m|l)P(m, t)$$

where we call $P_\mu(m, t|l)dt$ is the probability at time $t$ that the system will go from state $m$ to state $l$ in the time interval $dt$ through the $\mu$-th reaction channel. Then one can define the total entropy production rate at time $t$ due to the reaction $\mu$ as

$$\dot{S}_{tot}^{(\mu)}(t) = \sum_{m,l} P_\mu(m, t|l) \ln \left( \frac{P_\mu(m, t|l)}{P_{-\mu}(l, t|m)} \right) \delta_{l,m+1}, \mu > 0$$

$$= \sum_{m,l} P_\mu(m, t|l) \ln \left( \frac{P_\mu(m, t|l)}{P_{-\mu}(l, t|m)} \right) \delta_{l,m-1}, \mu < 0. \quad (21)$$

We now come to specific cases. For the reaction $\mu = 1$, using Eq.(18) along with its solution in Eq.(21), we get

$$\dot{S}_{tot}^{(1)}(t) = \sum_m P_1(m, t|m + 1) \ln \left( \frac{P_1(m, t|m + 1)}{P_{-1}(m + 1, t|m)} \right)$$

$$= \frac{1}{2} \left[ \sum_{n-1} P_1(n - 1, t|n) \ln \left( \frac{P_1(n - 1, t|n)}{P_{-1}(n, t|n - 1)} \right) + \sum_n P_1(n, t|n + 1) \ln \left( \frac{P_1(n, t|n + 1)}{P_{-1}(n + 1, t|n)} \right) \right]$$

$$= (k_1 n_T Y) \ln \left( \frac{k_1 n_T Y}{k_{-1} n_T X} \right). \quad (22)$$

The entropy production rate for other reactions can be calculated similarly. They are given in a compact form as

$$\dot{S}_{tot}^{(\mu)}(t) = (k_\mu n_T Y) \ln \left( \frac{k_\mu n_T Y}{k_{-\mu} n_T X} \right), \mu > 0$$

$$= (k_\mu n_T X) \ln \left( \frac{k_\mu n_T X}{k_{-\mu} n_T Y} \right), \mu < 0, \quad (23)$$
and we can obviously write the overall total EPR as
\[
\dot{S}_{\text{tot}}(t) = \sum_{\mu = \pm 1}^{\pm 2} \dot{S}_{\text{tot}}^{(\mu)}(t).
\] (24)

One can easily verify that this is the same expression one will get using the master equation description starting from the definition of Shannon entropy, as given in Eq.(6). However, Eq.(6) implies that contribution of reaction \( \mu \) towards \( \dot{S}_{\text{tot}}(t) \) is the same as that of reaction \(-\mu\). Now according to Eq.(21), the individual reaction contributions to the total EPR are distinct from each other following the propensity distance formalism. Using this approach, it is also straightforward to calculate the reaction contributions to the various other entropy production rates. This approach has subtle physical connection with the microscopic description of the second law of thermodynamics and the macroscopic law of irreversibility of arrow of time [26, 27, 28].

Now from Eq.(21), we can easily calculate the medium EPR, \( \dot{S}_{m}^{(\mu)}(t) \) and system EPR, \( \dot{S}_{\text{sys}}^{(\mu)}(t) \) for individual reaction and they can be expressed as
\[
\dot{S}_{m}^{(\mu)}(t) = \sum_{m,l} P_{\mu}(m, t|l) \ln \left( \frac{w_{\mu}(m, t|l)}{w_{-\mu}(l, t|m)} \right) \delta_{l,m+1}, \mu > 0
\]
\[
= \sum_{m,l} P_{\mu}(m, t|l) \ln \left( \frac{w_{\mu}(m, t|l)}{w_{-\mu}(l, t|m)} \right) \delta_{l,m-1}, \mu < 0.
\] (25)
and
\[
\dot{S}_{\text{sys}}^{(\mu)}(t) = \sum_{m,l} P_{\mu}(m, t|l) \ln \left( \frac{P(m, t)}{P(l, t)} \right) \delta_{l,m+1}, \mu > 0
\]
\[
= \sum_{m,l} P_{\mu}(m, t|l) \ln \left( \frac{P(m, t)}{P(l, t)} \right) \delta_{l,m-1}, \mu < 0.
\] (26)

The medium EPR, \( \dot{S}_{m}^{(\mu)}(t) \) is related with the heat dissipation rate, \( h_{d} \) as \( h_{d} = T \dot{S}_{m}^{(\mu)}(t) \), where \( T \) is the equilibrium temperature of the surrounding. Now following Esposito et al. [24, 25], the total EPR, \( \dot{S}_{\text{tot}}^{(\mu)}(t) \) can be separated into adiabatic and nonadiabatic EPRs, \( \dot{S}_{a}^{(\mu)}(t) \) and \( \dot{S}_{na}^{(\mu)}(t) \), respectively and they can be expressed as
\[
\dot{S}_{a}^{(\mu)}(t) = \sum_{m,l} P_{\mu}(m|l) \ln \left( \frac{P_{\mu}^{(\text{NESS})}(m|l)}{P_{-\mu}^{(\text{NESS})}(|l|m)} \right) \delta_{l,m+1}, \mu > 0,
\]
\[
= \sum_{m,l} P_{\mu}(m|l) \ln \left( \frac{P_{\mu}^{(\text{NESS})}(m|l)}{P_{-\mu}^{(\text{NESS})}(|l|m)} \right) \delta_{l,m-1}, \mu < 0
\] (27)
and
\[
\dot{S}_{na}^{(\mu)}(t) = \sum_{m,l} P_{\mu}(m|l) \ln \left( \frac{P(m)P(l)^{(\text{NESS})}}{P(l)P(m)^{(\text{NESS})}} \right) \delta_{l,m+1}, \mu > 0,
\]
\[
= \sum_{m,l} P_{\mu}(m|l) \ln \left( \frac{P(m)P(l)^{(\text{NESS})}}{P(l)P(m)^{(\text{NESS})}} \right) \delta_{l,m-1}, \mu < 0.
\] (28)
6. Fluctuation Theorem for the total EPR of elementary reactions

Here we focus entirely on the entropy production rates at NESS of elementary reactions. As the system EPR and the nonadiabatic EPR are zero at NESS both being state functions, so we have \( \dot{S}_{\text{tot}}^{\text{NESS}} = \dot{S}_m^{\text{NESS}} = \dot{S}_a^{\text{NESS}} \). We estimate here the individual reaction contributions to the total EPR and the fluctuation relations in the total entropy production and its possible implications.

The total entropy production during a time interval from \( t \) to \( t + \Delta t \) when the system remains at NESS is simply given by

\[
\Delta S_{\text{tot}}^{(\text{NESS})} = \int_t^{t+\Delta t} \dot{S}_{\text{tot}}^{(\text{NESS})} \, dt = \sum_{\mu} \dot{S}_{\text{tot}}^{\mu(\text{NESS})} \Delta t = \sum_{\mu} \Delta S_{\text{tot}}^{\mu(\text{NESS})}. \tag{29}
\]

\( \Delta S_{\text{tot}}^{\mu(\text{NESS})} \) is the total entropy production due to the elementary reaction \( \mu \) at NESS defined as

\[
\Delta S_{\text{tot}}^{\mu(\text{NESS})} = \sum_{m,l} (P_\mu^{(\text{NESS})})(m|l) \Delta t \ln \left( \frac{P_\mu^{(\text{NESS})}(m|l)}{P_{-\mu}^{(\text{NESS})}(l|m)} \right) \delta_{l,m+1}, \mu > 0 \\
= \sum_{m,l} (P_\mu^{(\text{NESS})})(m|l) \Delta t \ln \left( \frac{P_\mu^{(\text{NESS})}(m|l)}{P_{-\mu}^{(\text{NESS})}(l|m)} \right) \delta_{l,m-1}, \mu < 0. \tag{30}
\]

Now for a particular transition, say from state \( m \) to state \( l \) \((l = m \pm 1)\) via the elementary reaction \( \mu \) at NESS, the entropy production per step (written with lowercase s) can be written following Eq.(12) as

\[
\Delta s_{\text{tot}}^{\mu(\text{NESS})}(\Delta t) = \ln \left( \frac{P_\mu^{(\text{NESS})}(m|l) \Delta t}{P_{-\mu}^{(\text{NESS})}(l|m) \Delta t} \right), \tag{31}
\]

where \( \Delta s_{\text{tot}}^{\mu(\text{NESS})} = \langle \Delta s_{\text{tot}}^{\mu(\text{NESS})} \rangle \), the average being taken with respect to the time-integrated reaction propensity, \( P_\mu^{(\text{NESS})}(m|l) \Delta t \). One can check that the average over all possible transitions for the reaction channel \( \mu \) in terms of the reaction probability, \( P_\mu^{(\text{NESS})} \Delta t \) gives back \( \langle \Delta s_{\text{tot}}^{\mu(\text{NESS})} \rangle \). The fluctuation theorem for a particular transition \( m \rightarrow l \) via the reaction channel \( \mu \) with the entropy production, \( \Delta s_{\text{tot}}^{\mu(\text{NESS})}(\Delta t) \) for some arbitrary time, \( \Delta t \) at NESS follows

\[
P_\mu^{(\text{NESS})}(m|l) = e^{\Delta s_{\text{tot}}^{\mu(\text{NESS})}(\Delta t)}. \tag{32}
\]

As an example, we consider a transition from state \( m \) to state \((m + 1)\) via the elementary reaction \( \mu = 1 \). From Eq.(18), at NESS we get

\[
\Delta s_{\text{tot}}^{1(\text{NESS})} = \ln \left( \frac{k_1K_2}{k \cdot K_1} \right). \tag{33}
\]

The entropy production per step due to reaction \( \mu = 1 \) is thus the same for all the reaction steps at NESS and this is also true for all the other elementary reactions. The above expression can be splitted as

\[
\Delta s_{\text{tot}}^{1(\text{NESS})} = \ln \left( \frac{k_1(nT - m)}{k \cdot (m + 1)} \right) + \ln \left( \frac{K_2(m + 1)}{K_1(nT - m)} \right). \tag{34}
\]

The first term in the right hand side of Eq.(34) contains the logarithm of the ratio of the propensities of the reactions \( \mu = 1 \) and \( \mu = -1 \) for a transition between states \( m \) and \((m + 1)\). The second term gives the logarithm of the ratio of the propensities for the overall dissociation.
(K_2 = k_{-1} + k_{-2}) and formation (K_1 = k_1 + k_2) reactions and it is easy to see that this term is common in all the Δs^μ_{tot}(NESS) expressions except for a sign inversion. Hence the per step entropy production due to an elementary reaction μ at NESS can be separated into local and global contributions.

The deeper significance of the above fluctuation theorem can be shown to be reflected in the relation between the net velocity and total EPR of elementary reactions at NESS. It will be very important to have a relationship between the experimentally measurable v^{NESS}_net and the entropy production rates of the elementary reactions.

By setting k'_{2} ≈ 0, the enzyme kinetics in Eq.(15) tends to the traditional Michaelis-Menten scheme. In this limit, k_2 ≈ 0 with finite values of [P] and K_1 ≈ k_1. The net velocity of the Michaelis-Menten enzyme catalysis at NESS can be defined as v^{NESS}_net = k_{-2}(n)^{NESS}. Using the expression of ⟨n⟩ at NESS, we have

\[ v^{NESS}_\text{net} = nT k_{-2} \frac{K_1}{K_1 + K_2} = \frac{nT k_{-2} [S]}{K_M + [S]} \]  

(35)

This is the celebrated Michaelis-Menten equation of enzyme catalysis which is shown here for the single oligomeric enzyme case with nT number of active sites with K_M = \frac{k_{-1} + k_{-2}}{k_1'} = \frac{k_2'}{k_1'} is the Michaelis-Menten constant. Now at NESS, from Eq.(22) we get

\[ \dot{S}^{1(NESS)}_{\text{tot}} = (k_1 nT Y^{NESS}) \ln \left( \frac{k_1 nT Y^{NESS}}{k_{-1} nT X^{NESS}} \right) \]

\[ = \frac{nT K_2 \ln f[S]}{K_M + [S]}, \quad f = \frac{K_2}{k_1}, k_2' \approx 0. \]  

(36)

Similar treatment for the other reactions gives

\[ \dot{S}^{1(NESS)}_{\text{tot}} = -\frac{nT k_{-1} \ln f[S]}{K_M + [S]}, \]  

(37)

\[ \dot{S}^{2(NESS)}_{\text{tot}} = \frac{nT k_{-2} K_M \ln f'[P]}{K_M + [S]}, \]  

(38)

and

\[ \dot{S}^{2(NESS)}_{\text{tot}} = -\frac{nT k_{-2} \ln f'[S]}{K_M + [S]}, \]  

(39)

where f' = \frac{k_{-2} K_M P}{k_1' K_2 [S]}. From the above equations it is clear that the total EPR for the reactions \( \mu = \pm 1 \) show hyperbolic dependence on substrate concentration at NESS whereas for the reactions \( \mu = \pm 2 \), the dependence is more complicated as the factor f' is a function of [S] and [P]. The Eqs.(36)-(39) can be designated as the ‘Michaelis-Menten analogue’ for the total entropy production rates due to the elementary reactions.

Again from Eq.(36) and Eq.(37), we have

\[ \dot{S}^{1(NESS)}_{\text{tot}} + \dot{S}^{1(NESS)}_{\text{tot}} = v^{NESS}_\text{net} \ln f \]  

(40)

and from Eq.(38) and Eq.(39)

\[ \dot{S}^{2(NESS)}_{\text{tot}} + \dot{S}^{2(NESS)}_{\text{tot}} \approx \dot{S}^{2(NESS)}_{\text{tot}} = -v^{NESS}_\text{net} \ln f'. \]  

(41)
Figure 1. Total EPR, $\dot{S}_{tot}$ and adiabatic EPR, $\dot{S}_a$ are plotted as a function of time at substrate concentration $[S] = 100 \mu M$. The rate constants are: $k_1 = 5 \times 10^7 M^{-1} s^{-1}$, $k_{-1} = 18,300 s^{-1}$, $k_{-2} = 730 s^{-1}$, $k_2 = 1 \times 10^{-5} M^{-1} s^{-1}$.

As a consequence, we obtain

$$\dot{S}^{(NESS)}_{tot} = v^{(NESS)}_{net} \ln \gamma,$$

where $\gamma = \frac{k_1 k_{-2}}{k_{-1} k_2}$. Thus the total entropy production rate often signifies as an arrow of time which is directly measured from the deviation from equilibrium where for $\gamma = 1$, $\dot{S}^{(NESS)}_{tot} = 0$ corresponds to equilibrium.

The method is based on the analogy of a trajectory in phase space of a Hamiltonian system with the path of elementary reactions in a network of chemical process which in general can be an open system. The analogy is very qualitative in nature as it serves here as a motivation for study. It is also interesting to understand more quantitative meaning of this analogy which may provide some new methodology as well as some insight into problems of nonequilibrium thermodynamics of chemical reaction where kinetics and time dependent energetics of the process are highly coupled. Here we have applied the nonequilibrium thermodynamic relations including the fluctuation theorem for a model of oligomeric enzyme kinetics as it is an useful class of reactions and equally applicable to other reactions. Another important point is that the condition of microscopic reversibility is the fundamental requirement for the theory to hold as it needs to consider the time-reversed process. So any rate constant of the elementary reactions can be small but can not be taken exactly zero which may appear as limitation in some cases.

7. Result and Discussion

Here we have studied the individual elementary reaction contributions to various thermodynamic quantities at chemostatic condition for a single Escherichia coli $\beta$- galactosidase enzyme at NESS as well as their time variation. The enzyme is tetrameric with four independent catalytic sites [40, 41, 42] and follows the Michaelis-Menten kinetics [13, 14]. To calculate the thermodynamic quantities for individual reactions of this system, we have taken the rate constants, $k_1 = 5 \times 10^7 M^{-1} s^{-1}$, $k_{-1} = 18,300 s^{-1}$ and $k_{-2} = 730 s^{-1}$, from the single molecule experiment [13]. We set the vanishly small rate constant $k_2 = 1 \times 10^{-5} M^{-1} s^{-1}$ as the calculation of the nonequilibrium thermodynamic quantities as the formalism does not permit any rate constant to be exactly zero. Here $k_1$ and $k_2$ are pseudo first order rate constants depending linearly on the substrate and product concentrations, respectively.

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*Figure 1.* Total EPR, $\dot{S}_{tot}$ and adiabatic EPR, $\dot{S}_a$ are plotted as a function of time at substrate concentration $[S] = 100 \mu M$. The rate constants are: $k_1 = 5 \times 10^7 M^{-1} s^{-1}$, $k_{-1} = 18,300 s^{-1}$, $k_{-2} = 730 s^{-1}$, $k_2 = 1 \times 10^{-5} M^{-1} s^{-1}$.

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At intermediate times, it was observed that the variation of $\dot{S}$ and numerical results tally very well as depicted in Fig.3(a)-(d). In Fig.3(a), (b) and (c), $\dot{S}$ and $\dot{\mu}$ are almost same as that of $\dot{S}$ whereas $\dot{\mu}$ compared to others and plays no part in governing the thermodynamics. (iii) At NESS, $\dot{\mu}$ is observed to be zero only when some species is absent, e.g., when no ES-complex is formed. (iv) At NESS, $\dot{\mu}$ starts from zero. $\dot{\mu}$ is throughout negligible compared to others and plays no part in governing the thermodynamics. (iii) At NESS, $\dot{\mu}$ and $\dot{\mu}^{-1}$ almost cancel each other and hence the total EPR is governed by $\dot{\mu}^{-2}$. (iv) At intermediate times, $\dot{\mu}^{1}$ and $\dot{\mu}^{-2}$ continuously decreases and increases, respectively, whereas $\dot{\mu}^{(-1)}$ passes through a minimum.

To investigate the individual reaction contributions in total EPR at NESS, we have calculated $\dot{S}^{\mu(NESS)}$ as a function of substrate concentration, $[S]$ from Eqs.(36)-(39). We have also determined the quantities by numerically solving the master equation (Eq.19). The theoretical and numerical results tally very well as depicted in Fig.3(a)-(d). In Fig.3(a), (b) and (c), it is observed that the variation of $\dot{S}^{1(NESS)}$, $\dot{S}^{-1(NESS)}$ and $\dot{S}^{2(NESS)}$ as a function of substrate concentration are hyperbolic in nature with $\dot{S}^{-1(NESS)}$ being negative throughout, its magnitude almost same as that of $\dot{S}^{1(NESS)}$, $\dot{S}^{2(NESS)}$ also remains negative throughout and at higher substrate concentration, it approaches zero as shown in Fig.3(d). However, its value is negligible. Therefore, as $\dot{S}^{1(NESS)}$ and $\dot{S}^{-1(NESS)}$ almost cancel out each other, $\dot{S}^{\mu(NESS)}$ is governed by

**Figure 2.** Total EPR, $\dot{S}_{tot}$ and adiabatic EPR, $\dot{S}_{a}$ are plotted as a function of time at substrate concentration $[S] = 100 \mu M$. The rate constants are: $k_1 = 5 \times 10^7 M^{-1}s^{-1}$, $k_{-1} = 18,300s^{-1}$, $k_{-2} = 730s^{-1}$, $k_2 = 1 \times 10^{-5} M^{-1}s^{-1}$.

In Fig.1(a) and (b), we have plotted the total EPR, $\dot{S}_{tot}(t)$ and the adiabatic EPR, $\dot{S}_{a}(t)$, respectively, as a function of time at substrate concentration $[S] = 100 \mu M$. From Fig.1(a), it is observed that $\dot{S}_{tot}(t)$ shows a sharp decay with time, passes through a minimum and finally attains a constant non-zero value as the system reaches NESS. From Fig.1(b), we see that $\dot{S}_{a}(t)$ increases with time and also saturates at NESS characterizing the irreversible work that has to be done on the system to sustain the NESS by continuous injection of substrate and ejection of product maintaining the chemiostatic condition. It follows from Eq.(8), that if the system obeys detailed balance, $\dot{S}_{tot}(t)$ remains positive for all time; it becomes zero only when some species is absent, e.g., in our case at $t = 0$ when no ES-complex is formed.

Next we have plotted the time evolution of total EPR due to the elementary reactions, $\mu = \pm 1, \pm 2$ in Fig.2(a)-(d). The plots reveal four main features: (i) $\dot{S}^{(1)}_{tot}(t)$, $\dot{S}^{-2}_{tot}(t)$ remain positive over the time course whereas $\dot{S}^{(-1)}_{tot}(t)$, $\dot{S}^{(2)}_{tot}(t)$ remain negative throughout. (ii) Initially, the major contribution to $\dot{S}_{tot}(t)$ (see Fig.1) comes from $\dot{S}^{(1)}_{tot}(t)$. This is because contributions of elementary reactions $\mu = -1, -2$ to total EPR starts from zero. $\dot{S}^{(2)}_{tot}(t)$ is throughout negligible compared to others and plays no part in governing the thermodynamics. (iii) At NESS, $\dot{S}^{(1)}_{tot}$ and $\dot{S}^{(-1)}_{tot}$ almost cancel each out each other and hence the total EPR is governed by $\dot{S}^{(-2)}_{tot}$. (iv) At intermediate times, $\dot{S}^{(1)}_{tot}(t)$ and $\dot{S}^{-2}_{tot}(t)$ continuously decreases and increases, respectively, whereas $\dot{S}^{(-1)}_{tot}(t)$ passes through a minimum.
the entropy production rate of reaction $\mu = -2$ only.

So far we have discussed about the time-dependence of the total EPR as well as its variation with substrate concentration at NESS, analyzing in detail the role of each elementary reaction. Now we determine various entropy productions by integrating the corresponding rates over the time interval starting from $t = 0$ to a fixed final time when the system resides in NESS. We have plotted the individual reaction contributions in these entropy productions as well as the free energy and internal energy changes as a function of $[S]$ at NESS shown in Figs.4(a)-(d). In the figures we have not shown the contribution of reaction $\mu = 2$ due to its negligible value. Keeping in mind the special role of adiabatic entropy production, $\Delta S_a$ as a measure of the (temperature-scaled) irreversible work done on the system that drives the system to NESS and then sustains it.

To investigate how the irreversible work is utilized by the reactions, we have plotted $\Delta S_{\text{tot}}^{(\mu)}$ as it is equal to $\Delta S_{a}^{(\mu)}$ at NESS which is depicted in Fig.4(a). In the figure it is observed that $\Delta S_{\text{tot}}^{(1)}$ has positive contribution whereas $\Delta S_{\text{tot}}^{(-1)}$ has negative contribution towards $\Delta S_{\text{tot}}$ and they are close in magnitude. Thus the irreversible work done on the system due to reaction $\mu = 1$ is almost compensated by the work performed by reaction $\mu = -1$. It becomes evident from Fig.4(a) that the irreversible work done on the system is mostly consumed by the reaction $\mu = -2$, more so at higher substrate concentration. Now first law suggests that some amount of the irreversible work done on the system changes the internal energy of the system and rest of it dissipates as heat to the surroundings. To find out the contribution of individual reactions in the change of free energy and internal energy of the system as a function of $[S]$ in Fig.4(b) and (c), respectively.

The difference between $\Delta U^{(\mu)}/T$ and $\Delta S_{\text{sys}}^{(\mu)}$ gives the temperature-scaled free energy change, $\Delta F^{(\mu)}/T$ (which is also the nonadiabatic entropy production, $\Delta S_{\text{na}}^{(\mu)}$) due to each elementary reaction as shown in Fig.4(b). The magnitude of $\Delta F^{(\mu)}/T$ is small compared to the other quantities due to the quantitative similarity of $\Delta U^{(\mu)}/T$ and $\Delta S_{\text{sys}}^{(\mu)}$. In Fig.4(b), we see that the contribution of reactions $\mu = -1, -2$ are positive as well as small and the major, negative contribution in the total free energy change comes from the reaction $\mu = 1$. This indicates that the irreversible work consumed by the reaction $\mu = -2$ almost entirely dissipates as heat and therefore, the contribution of this reaction to internal energy change, $\Delta U^{(-2)}/T$ is little as
Figure 4. The individual reaction contributions in the system and total entropy productions along with the internal energy change are plotted as a function of $[S]$. The rate constants are: $k_1 = 5 \times 10^7 M^{-1}s^{-1}$, $k_{-1} = 18,300 s^{-1}$, $k_{-2} = 730 s^{-1}$, $k_2 = 1 \times 10^{-5} M^{-1}s^{-1}$ shown in Fig.4(c). The variation of the corresponding contributions of reactions $\mu = \pm 1$ towards the internal energy change, $\Delta U^{(\pm 1)}/T$ show the opposite trend as a function of $[S]$ compared to the variation of $\Delta S_{tot}^{(\pm 1)}$ (see Fig.4(c)). This is expected as heat absorption by the system for an elementary reaction leads to an increase in internal energy due to that reaction whereas heat released by the system results in fall of its internal energy. Same is true for the reaction contributions in the system entropy change, $\Delta S_{sys}^{(\mu)}$ plotted in Fig.4(d).

8. Conclusion
We have formulated the total entropy production rate due to a complex chemical reaction in the spirit of Gillespie’s [4, 5] propensity-concept for each elementary reaction. The formalism is based on the relative entropy or Kullback-Leibler distance of probability distributions for a chemical reaction associated with an elementary reaction and its microscopic reversible ‘counter’ reaction similar to the approach of forward and time-reversed trajectories in phase space. A general chemical reaction network is discussed in presence of chemiostatic condition that leads the open reaction system to a nonequilibrium steady state. To understand how the overall work done on the reaction system is used up in changing the internal energy of the reactants and heat dissipation, we have thoroughly analyzed path functions like heat dissipation and work done as well as state functions like internal energy, free energy and system entropy for each elementary steps. As a real case study, we have taken a single molecule kinetics of the tetrameric Escherichia coli $\beta$-galactosidase enzyme under chemiostatic condition. The variation of total entropy production rate for individual reactions are analyzed with time as well as with substrate concentration at steady state.

To understand the role of each elementary sub-reactions in governing the overall kinetics and thermodynamics, we have shown that it is the fluctuation relations which maintains an intrinsic interdependence of each step. As an implication of fluctuation theorem arises for each sub-reaction at nonequilibrium steady state, we have established relations for total entropy production rate due to each elementary reaction analogous to the Michaelis-Menten equation of enzymatic reaction rate. Total entropy production due to each reaction step is determined and the ratio of the total entropy production rate due to a reaction and its reverse reaction is shown to be constrained by this quantity at steady state. One can also estimate how each step of the
reaction is energy driven or entropy driven to contribute to the overall reaction. Therefore, one can control the overall reaction more effectively by the choice of the proper parameters of the individual steps.

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