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Propensity approach to nonequilibrium thermodynamics of a chemical reaction network: Controlling single E-coli β-galactosidase enzyme catalysis through the elementary reaction steps[a)]

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In this work, we develop an approach to nonequilibrium thermodynamics of an open chemical reaction network in terms of the elementary reaction propensities. The method is akin to the microscopic formulation of the dissipation function in terms of the Kullback-Leibler distance of phase space trajectories in Hamiltonian system. The formalism is applied to a single oligomeric enzyme kinetics at chemiostatic condition that leads the reaction system to a nonequilibrium steady state, characterized by a positive total entropy production rate. Analytical expressions are derived, relating the individual reaction contributions towards the total entropy production rate with experimentally measurable reaction velocity. Taking a real case of *Escherichia coli* β-galactosidase enzyme obeying Michaelis-Menten kinetics, we thoroughly analyze the temporal as well as the steady state behavior of various thermodynamic quantities for each elementary reaction. This gives a useful insight in the relative magnitudes of various energy terms and the dissipated heat to sustain a steady state of the reaction system operating far-from-equilibrium. It is also observed that, the reaction is entropy-driven at low substrate concentration and becomes energy-driven as the substrate concentration rises. © *2013 AIP Publishing LLC*. [http://dx.doi.org/10.1063/1.4844195]

I. INTRODUCTION

Chemical kinetics of small reaction systems requires a stochastic approach to accommodate the significant fluctuation in species concentration.[1–12] The theoretical tools are well-established in the literature in the context of chemical master equation.[4–6] Recent experiments on single molecules, e.g., temporal fluctuation of catalytic rate of a single enzyme,[13,14] keep the interest growing in this field. In recent years, this theoretical approach has been enormously used to describe mesoscopic systems, e.g., single bio-molecular processes, in which the time evolution is considered as a random-walk in the conformational state space.[11,12,15–17] The master equation approach also provides the stochastic thermodynamic description of such systems, starting from a definition of the system entropy in terms of state probabilities.[10,18–29] The total entropy production rate (EPR) due to the chemical reactions can be calculated which is always non-negative and becomes zero at equilibrium.[10] It has been established that, closed systems tend to such equilibrium whereas, open systems can reach a nonequilibrium steady state (NESS),[30–36] characterized by a positive total EPR.[12] In chemical reaction systems, such NESS can be attained in presence of chemiostatic condition, where concentrations of some species are kept fixed throughout the reaction.[10] This is the simplest assumption that can model an open chemical system, e.g., a living cell in homeostatis.[22]

Recently, Kawai *et al.*[23] gave a phase space picture of dissipation in terms of the relative entropy or Kullback-Leibler (KL) distance[33,34] of probabilities of realizing forward and (time-) reversed phase space trajectories. This formulation goes to the core of the realization of the development of macroscopic irreversibility from the microscopic reversibility and the arrow of time.[24,25] In the same spirit, here our main aim is to formulate an expression of total EPR in a chemical reaction network, using suitable trajectory probabilities in population state space in terms of the propensities of each elementary reaction as introduced by Gillespie.[4,5] The evolution of the reaction system involves jump from one population state to another along trajectories in forward and backward directions. Now, the characteristic feature of such a system is that, one or more, *but not all*, of the elementary reactions can be responsible for a particular state change. This automatically leads one to construct the theory on the basis of individual elementary reactions. Determination of the role of each reaction event in governing the nonequilibrium thermodynamics of an open chemical reaction network should give new insights in the temporal energetics, complementing the kinetic knowledge. In this regard, we thoroughly analyze both the first and second laws for nonequilibrium system, developed from a master equation description of the system.[26–29] The theory has been applied on a single oligomeric enzyme catalysis reaction reaching NESS.[11,12,15,22]

The paper is organized as follows. In Sec. II, we discuss on the formulation of total EPR using the elementary reaction probability densities, denoted here as propensities, of an

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open nonequilibrium reaction network. The theory is applied on a single oligomeric enzyme kinetics in Sec. III, giving analytical relations between the EPR and experimental observables, like net reaction velocity. Section IV is devoted to a thorough analysis of the contributions of elementary reactions in various state functions, like internal energy, free energy, and also path functions, like heat dissipation and irreversible work. In this context, we take a real example in the form of *Escherichia coli* β-galactosidase enzyme. The paper is concluded in Sec. V.

II. EPR IN AN OPEN REACTION SYSTEM: ELEMENTARY REACTION PROPENSITIES

Following Gaspard[10] we consider a reaction network

$$\sum_{i=1}^{a} \nu^i_{<\mu} A_i + \sum_{j=1}^{b} \nu^j_{<\mu} Z_j \underset{k_{-\mu}}{\overset{k_\mu}{\rightleftharpoons}} \sum_{i=1}^{a} \nu^i_{>\mu} A_i + \sum_{j=1}^{b} \nu^j_{>\mu} Z_j. \quad (1)$$
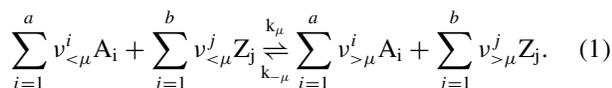
Here Z_j are the intermediate species and A_i are species whose concentrations are kept constant by connecting the reaction system to reservoirs. This is known as the chemiostatic condition, leading the system to a NESS. The system is assumed to be at thermal equilibrium with the reservoir, kept at temperature T. An elementary reaction is denoted by the index μ with $\mu = \pm1, \pm2, \ldots, \pm k$. The condition of microscopic reversibility is the fundamental requirement for the construction of the nonequilibrium thermodynamic theory, as it needs to consider the (time-)reversed process. Thus, every transition in the population state space due to the reaction μ has a counterpart in the reverse transition through the reaction $-\mu$. Also, none of the rate constants of the elementary reactions can be exactly zero. The stoichiometric coefficient, ν^j_μ of the species Z_j involved in the μth reaction is defined as

$$\nu^j_\mu = \nu^j_{>\mu} - \nu^j_{<\mu}, \quad (j = 1, \ldots, b). \quad (2)$$

We denote the population vector of the intermediate species by $\mathbf{Z} (= \{Z_j\}, j = 1, \ldots, b)$ and the stoichiometric coefficient vector of the μth reaction is given by $\boldsymbol{\nu_\mu}$. The chemical master equation governing the stochastic reaction events is given by[10, 19–21]

$$\frac{dP(\mathbf{Z}, t)}{dt} = \sum_{\mu=\pm1}^{\pm k} [w_\mu(\mathbf{Z} - \boldsymbol{\nu_\mu}|\mathbf{Z})P(\mathbf{Z} - \boldsymbol{\nu_\mu}, t)$$
$$- w_{-\mu}(\mathbf{Z}|\mathbf{Z} - \boldsymbol{\nu_\mu})P(\mathbf{Z}, t)]. \quad (3)$$

Here $P(\mathbf{Z}, t)$ is the probability of having population vector $\mathbf{Z}$ of the intermediate species at time t and $w_\mu(\mathbf{Z}|\mathbf{Z} + \boldsymbol{\nu_\mu})$ is the transition probability for the jump $\mathbf{Z} \to \mathbf{Z} + \boldsymbol{\nu_\mu}$ along a stochastic trajectory via reaction μ.

Let us now consider an evolution of the system's distribution given as $P(\mathbf{Z}, t) \to P(\mathbf{Z} + \boldsymbol{\nu_\mu}, t + \Delta t)$. This can be described as a transition from the state-$\mathbf{Z}$ to the state-$(\mathbf{Z} + \boldsymbol{\nu_\mu})$ at time t and then considering a time interval Δt such that no further transition takes place in this interval. According to the definition of stochastic system entropy given by Seifert,[30–32] the system entropy production for this transition due to the

elementary reaction μ can be written as

$$\Delta s^{(\mu)}_{sys} = -\ln P(\mathbf{Z} + \boldsymbol{\nu_\mu}, t + \Delta t) - (-\ln P(\mathbf{Z}, t))$$
$$= \ln \frac{P(\mathbf{Z}, t)}{P(\mathbf{Z} + \boldsymbol{\nu_\mu}, t + \Delta t)}. \quad (4)$$

The medium entropy production or entropy flow associated with this transition is defined as[28, 30–32]

$$\Delta s^{(\mu)}_m = \ln \frac{w_\mu(\mathbf{Z}|\mathbf{Z} + \boldsymbol{\nu_\mu})}{w_{-\mu}(\mathbf{Z} + \boldsymbol{\nu_\mu}|\mathbf{Z})}. \quad (5)$$

Then, the corresponding total entropy production becomes

$$\Delta s^{(\mu)}_{tot} = \Delta s^{(\mu)}_{sys} + \Delta s^{(\mu)}_m = \ln \frac{R_\mu(\mathbf{Z}, t|\mathbf{Z} + \boldsymbol{\nu_\mu})}{R_{-\mu}(\mathbf{Z} + \boldsymbol{\nu_\mu}, t + \Delta t|\mathbf{Z})}. \quad (6)$$

Here we introduce the propensity, $R_\mu(\mathbf{Z}, t|\mathbf{Z} + \boldsymbol{\nu_\mu}) = w_\mu(\mathbf{Z}|\mathbf{Z} + \boldsymbol{\nu_\mu})P(\mathbf{Z}, t)$, in the same spirit of the reaction probability density given by Gillespie.[4–6] Thus, $R_\mu(\mathbf{Z}, t|\mathbf{Z} + \boldsymbol{\nu_\mu})dt$ is the probability that the system at time t will go from state-$\mathbf{Z}$ to state-$(\mathbf{Z} + \boldsymbol{\nu_\mu})$ due to the occurrence of an elementary reaction μ 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." To get the overall entropy production due to an elementary reaction, we need to average over all the transitions due to the μth reaction considering all possible values of the population vector $\mathbf{Z}$. This averaging is done with the probability, $R_\mu(\mathbf{Z}, t|\mathbf{Z} + \boldsymbol{\nu_\mu})\Delta t$. Here, Δt is considered to be small enough so that R_μ remains constant over this interval. Then we have

$$\Delta S^{(\mu)}_{tot} = \langle \Delta s^{(\mu)}_{tot} \rangle = \sum_{\mathbf{Z}} (R_\mu(\mathbf{Z}, t|\mathbf{Z} + \boldsymbol{\nu_\mu})\Delta t)\ln$$
$$\times \frac{R_\mu(\mathbf{Z}, t|\mathbf{Z} + \boldsymbol{\nu_\mu})}{R_{-\mu}(\mathbf{Z} + \boldsymbol{\nu_\mu}, t + \Delta t|\mathbf{Z})}. \quad (7)$$

The expression of the total EPR follows from Eq. (7)

$$\lim_{\Delta t \to 0} \frac{\Delta S^{(\mu)}_{tot}}{\Delta t} = \dot{S}^{(\mu)}_{tot}$$
$$= \sum_{\mathbf{Z}} R_\mu(\mathbf{Z}, t|\mathbf{Z} + \boldsymbol{\nu_\mu})\ln \frac{R_\mu(\mathbf{Z}, t|\mathbf{Z} + \boldsymbol{\nu_\mu})}{R_{-\mu}(\mathbf{Z} + \boldsymbol{\nu_\mu}, t|\mathbf{Z})}. \quad (8)$$

The structure of Eq. (8) is similar to the relative entropy or the Kullback-Leibler (KL) distance of two probability distributions but *not identical to it*. In our case, the KL probability distance function, $D(\rho||\tilde{\rho}) = \langle \ln \frac{\rho}{\tilde{\rho}} \rangle$, also called relative entropy of ρ versus $\tilde{\rho}$, gets replaced by the propensity distance function for the μth reaction, $\langle \ln \frac{R_\mu(\mathbf{Z},t|\mathbf{Z}+\boldsymbol{\nu_\mu})}{R_{-\mu}(\mathbf{Z}+\boldsymbol{\nu_\mu},t|\mathbf{Z})} \rangle$ where the average is over the forward process, $R_\mu(\mathbf{Z}, t|\mathbf{Z} + \boldsymbol{\nu_\mu})$. Thus, a KL distance-type relation in terms of the forward and reverse propensities, R_μ and $R_{-\mu}$, respectively, gives rise to the total EPR due to the μth reaction in the forward direction and $-\mu$th reaction in the backward direction. However, as R_μ and $R_{-\mu}$ are not normalized, so $\dot{S}^{(\mu)}_{tot}$ can be negative also. The splitting of $\dot{S}^{(\mu)}_{tot}$ in Eq. (8) into system and medium contributions is straightforward.

Recently, Esposito and Van den Broeck[27,28] introduced another separation of the total EPR, $\dot{S}_{tot}(t)$ into adiabatic and nonadiabatic parts, $\dot{S}_a(t)$ and $\dot{S}_{na}(t)$, respectively. In our scheme of reaction network, they become

$$\dot{S}_a^{(\mu)}(t) = \sum_{\mathbf{Z}} R_\mu(\mathbf{Z}, t|\mathbf{Z} + \boldsymbol{\nu_\mu}) \ln \frac{R_\mu^{ss}(\mathbf{Z}, t|\mathbf{Z} + \boldsymbol{\nu_\mu})}{R_{-\mu}^{ss}(\mathbf{Z} + \boldsymbol{\nu_\mu}, t|\mathbf{Z})}, \quad (9)$$
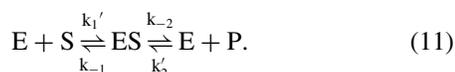
and

$$\dot{S}_{na}^{(\mu)}(t) = \sum_{\mathbf{Z}} R_\mu(\mathbf{Z}, t|\mathbf{Z} + \boldsymbol{\nu_\mu}) \ln \frac{P(\mathbf{Z}, t) P^{ss}(\mathbf{Z} + \boldsymbol{\nu_\mu})}{P^{ss}(\mathbf{Z}) P(\mathbf{Z} + \boldsymbol{\nu_\mu}, t)}. \quad (10)$$

Here $R_\mu^{ss}(\mathbf{Z}, t|\mathbf{Z} + \boldsymbol{\nu_\mu}) = w_\mu(\mathbf{Z}|\mathbf{Z} + \boldsymbol{\nu_\mu}) P^{ss}(\mathbf{Z})$ with $P^{ss}(\mathbf{Z})$ being the steady state probability of having the population vector $\mathbf{Z}$. Here and throughout the paper, the "ss" superscript denotes the quantities at NESS. Unlike the system and medium contributions, the adiabatic and nonadiabatic parts are always individually positive. Recently, Ge and Qian[26] discussed the nonequilibrium thermodynamics of a system governed by a master equation, characterizing the rates of internal energy change, $\dot{U}$ and free energy change, $\dot{F}$. They also constructed the first law in terms of the rate of heat dissipation, related to the medium EPR, $\dot{S}_m$ and the rate of irreversible work done, given by the adiabatic EPR, $\dot{S}_a(t)$. The nonadiabatic EPR is found to be negative of the temperature-scaled free energy dissipation rate, $\dot{S}_{na}(t) = -\dot{F}(t)/T$. The rate of internal energy change, given as $\dot{U}(t)/T = \dot{S}_a(t) - \dot{S}_m(t)$, then behaves like first law.

III. SINGLE OLIGOMERIC ENZYME CATALYSIS

To get some physical understanding, here we take the simplest case of a single oligomeric enzyme kinetics with a single intermediate. A generalized version is given in Appendix A. The reaction of an active site of the enzyme is written following a reversible Michaelis-Menten (MM) scheme

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1'}{\rightleftharpoons}} \mathrm{ES} \underset{k_2'}{\overset{k_{-2}}{\rightleftharpoons}} \mathrm{E} + \mathrm{P}. \quad (11)$$

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

$$\mathrm{E} \underset{K_2}{\overset{K_1}{\rightleftharpoons}} \mathrm{ES}, \quad (12)$$

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 consist of n_T number of identical non-interacting subunits where each subunit has one active site. During 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. With the total number of sites being conserved, the reaction system can be described in terms of a single random variable, the number of occupied sites, n, at a particular time t. The corresponding master equation is written as[15,16]

$$\frac{dP(n,t)}{dt} = \sum_{\mu=\pm 1}^{\pm 2} [w_\mu(n - \nu_\mu|n) P(n - \nu_\mu, t)$$
$$- w_{-\mu}(n|n - \nu_\mu) P(n, t)], \quad (13)$$

where $P(n, t)$ being the probability of having n number of occupied states at time t. The stoichiometric coefficients, $\nu_\mu = 1$ for $\mu > 0$ and $\nu_\mu = -1$ for $\mu < 0$. The rate constant of the elementary reaction, μ is denoted by k_μ. The transition probabilities are defined as follows:

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

and

$$w_\mu(n - \nu_\mu|n) = k_\mu(n - \nu_\mu), \ \mu < 0. \quad (14)$$

Then Eq. (13) can be rewritten as

$$\frac{dP(n,t)}{dt} = K_1(n_T - n + 1) P(n - 1, t) + K_2(n + 1) P(n + 1, t)$$
$$- K_1(n_T - n) P(n, t) - K_2 n P(n, t). \quad (15)$$

Solution of the master equation, obtained using the generating function method,[35,36] is given as[15,16]

$$P(n, t) = \frac{n_T!}{n!(n_T - n)!} X^n Y^{n_T - n}, \quad (16)$$

where $X = \frac{K_1(1 - \exp(-(K_1 + K_2)t))}{K_1 + K_2}$, $Y = \frac{K_2 + K_1 \exp(-(K_1 + K_2)t)}{K_1 + K_2}$, assuming that initially all the active sites are vacant.

Now we give a description of the total EPR, $\dot{S}_{tot}$ for the enzyme kinetics scheme (11), based on the KL distance type formalism, described in Sec. II. 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, is governed by Eq. (13). 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 $(n + 1)$. In a forward transition, $\mu > 0$ and $l > m$ whereas, for a backward transition, $\mu < 0$ and $l < m$. Then one can define the total EPR at time t due to the reaction μ in terms of the forward and backward reaction propensities as

$$\dot{S}_{tot}^{(\mu)}(t) = \sum_{m,l} R_\mu(m, t|l) \ln \left(\frac{R_\mu(m, t|l)}{R_{-\mu}(l, t|m)} \right) \delta_{l,m+1}, \ \mu > 0$$
$$= \sum_{m,l} R_\mu(m, t|l) \ln \left(\frac{R_\mu(m, t|l)}{R_{-\mu}(l, t|m)} \right) \delta_{l,m-1}, \ \mu < 0. \quad (17)$$

Using Eqs. (14) and (16) in Eq. (17), we get the desired EPR in a concise form as

$$\dot{S}_{tot}^{(\mu)}(t) = (k_\mu n_T Y) \ln \left(\frac{k_\mu n_T Y}{k_{-\mu} n_T X} \right), \ \mu > 0$$
$$= (k_\mu n_T X) \ln \left(\frac{k_\mu n_T X}{k_{-\mu} n_T Y} \right), \ \mu < 0. \quad (18)$$

The total EPR of the whole reaction system is obviously

$$\dot{S}_{tot}(t) = \sum_{\mu=\pm 1}^{\pm 2} \dot{S}_{tot}^{(\mu)}(t). \quad (19)$$

We clarify here that, using the usual master equation description starting from the definition of Shannon entropy, one will get the same expression as given in Eq. (19). However, as also mentioned in Sec. I, the construction of various EPRs in terms of the forward and backward reaction propensities has

deeper physical meaning, giving a microscopic description of the second law of thermodynamics. Also, use of the standard formulation can give rise to a technical problem in the determination of the individual reaction contributions towards total EPR. No such confusion arises in the case of Eq. (8). This is discussed in Appendix B. It has been proved that, both the adiabatic and the nonadiabatic entropy productions (but neither system nor medium entropy production) can be written as a relative entropy or KL distance of suitably defined trajectory probabilities.[28] We show in Appendix C that, following our formulation, the nonadiabatic EPR can be written in such a form.

A. EPR of the elementary reactions at NESS

In this subsection, we focus on the total EPR due to the elementary reactions of the enzyme kinetics scheme (11) and their relations, if any, with experimental observables. We mention that the system EPR and the nonadiabatic EPR are zero at NESS both being state functions. So, one gets $\dot{S}_{tot}^{ss} = \dot{S}_m^{ss} = \dot{S}_a^{ss}$.

1. Michaelis-Menten analogues for total EPR

The kinetics scheme in Eq. (11) tends to the traditional MM one for $k_2 \to 0$. The net velocity of the MM enzyme catalysis at NESS can be defined as

$$v_{net}^{ss} = k_{-2}\langle n \rangle^{ss} = n_T k_{-2} X^{ss} = \frac{n_T k_{-2}[S]}{K_M + [S]}. \quad (20)$$

This is the famous MM equation of enzyme catalysis, shown here for the single oligomeric enzyme case, where $K_M = \frac{k_{-1}+k_{-2}}{k_1'} = \frac{K_2}{k_1'}$ is the usual MM constant.

Now at NESS, from Eq. (18) with $\mu = 1$, we get

$$\dot{S}_{tot}^{1(ss)} = (k_1 n_T Y^{ss})\ln\left(\frac{k_1 n_T Y^{ss}}{k_{-1} n_T X^{ss}}\right)$$

$$= \frac{n_T K_2 \ln f[S]}{K_M + [S]}, \quad f = \frac{K_2}{k_{-1}}, \ k_2' \approx 0. \quad (21)$$

Similar treatment for other reactions gives

$$\dot{S}_{tot}^{-1(ss)} = -\frac{n_T k_{-1}\ln f[S]}{K_M + [S]}; \ \dot{S}_{tot}^{2(ss)} = \frac{n_T k_2' K_M \ln f'[P]}{K_M + [S]};$$

$$\dot{S}_{tot}^{-2(ss)} = -\frac{n_T k_{-2}\ln f'[S]}{K_M + [S]}, \quad (22)$$

where $f' = \frac{k_2' K_2 [P]}{k_1' k_{-2}[S]}$. It is clear from the above equations that the total EPR for the reactions $\mu = \pm 1$ show hyperbolic dependence on $[S]$ at NESS. For the reactions $\mu = \pm 2$, this dependence is not clear, although the structure of the equations are similar. Thus, Eqs. (21) and (22) can be designated as the "MM analogues" for the total EPR due to the elementary reactions.

2. Relation between the net velocity and total EPR

It will be very important to have a relationship between the experimentally measurable v_{net}^{ss} and the total EPR. Using

Eqs. (21) and (22) and neglecting $\dot{S}_{tot}^{2(ss)}$, we have

$$\dot{S}_{tot}^{(ss)} = \dot{S}_{tot}^{1(ss)} + \dot{S}_{tot}^{-1(ss)} + \dot{S}_{tot}^{-2(ss)} = v_{net}^{ss}\ln\gamma, \quad (23)$$

the desired relationship, with $\gamma = \frac{k_1 k_{-2}}{k_{-1}k_2}$. At the condition of detailed balance, $\gamma = 1$ one obtains, $\dot{S}_{tot}^{(ss)} = 0$, corresponding to equilibrium.

IV. RESULT AND DISCUSSION

In this section, we have numerically studied the individual reaction contributions to various thermodynamic quantities. To this end, a single *Escherichia coli β*-galactosidase enzyme is considered, which is tetrameric[39–41] and follows the MM kinetics.[13, 14] We have taken the rate constant data from the single molecule experiment of English *et al.*[13] They are as follows: $k_1' = 5 \times 10^7$ M^{-1} s^{-1}, $k_{-1} = 18\,300$ s^{-1}, and $k_{-2} = 730$ s^{-1}, In this regard, we want to clarify that here we have used the value of k_{-2} without the dynamic disorder, taken in their study.[13] We set the rate constant $k_2 = 1 \times 10^{-5}$ s^{-1}, as the calculation of the nonequilibrium thermodynamic quantities does not permit any rate constant to be exactly zero. However, the rate constant is small enough to ensure that the effect of this reaction step on the overall kinetic and thermodynamic properties is negligible, as will be shown below. The substrate concentration is set at $[S] = 100$ μM.

We have plotted the time evolution of the elementary reaction contributions to the total EPR for $\mu = \pm 1, \pm 2$ in Figs. 1(a)–1(d). The plots reveal four main features: (i) $\dot{S}_{tot}^{(1)}(t)$, $\dot{S}_{tot}^{(-2)}(t)$ remain positive over the time course whereas, $\dot{S}_{tot}^{(-1)}(t)$ remains negative throughout. (ii) Initially, the major contribution to $\dot{S}_{tot}(t)$ comes from $\dot{S}_{tot}^{(1)}(t)$. This is because contributions of elementary reactions $\mu = -1, -2$ to total EPR starts from zero. (iii) At NESS, $\dot{S}_{tot}^{(1)}$ and $\dot{S}_{tot}^{(-1)}$ almost cancel out each other and hence the total EPR is governed by $\dot{S}_{tot}^{(-2)}$. (iv) At intermediate times, $\dot{S}_{tot}^{(1)}(t)$ and $\dot{S}_{tot}^{(-2)}(t)$ continuously decreases and increases, respectively, whereas $\dot{S}_{tot}^{(-1)}(t)$ passes through a minimum. This gives rise to the minimum in $\dot{S}_{tot}(t)$. $\dot{S}_{tot}^{(2)}(t)$ remains insignificant relative to others. In Appendix E, we give the condition for $\dot{S}_{tot}^{(\mu)}(t)$ to change its sign during time evolution.
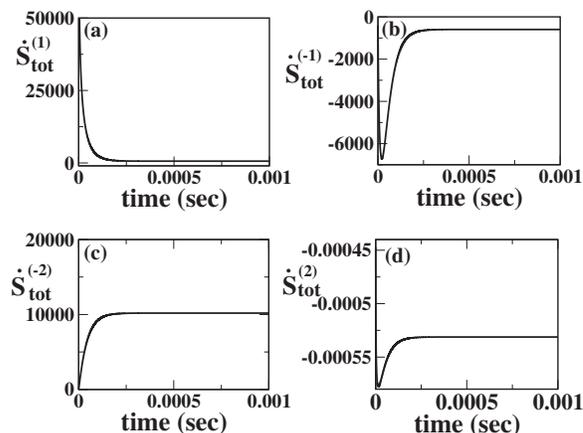

FIG. 1. (a)–(d) Contributions of the individual reactions to the total EPR, $\dot{S}_{tot}^{(\mu)}$ as a function of time, t. The rate constants are $k_1' = 5 \times 10^7$ M^{-1}s^{-1}, $k_{-1} = 18\,300$ s^{-1}, $k_{-2} = 730$ s^{-1}, $k_2 = 1 \times 10^{-5}$ s^{-1}. The substrate concentration is set at $[S] = 100$ μM.
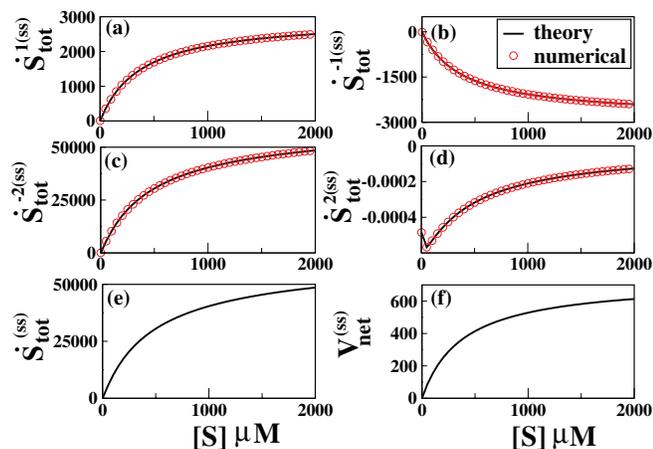
FIG. 2. (a)–(f) The individual reaction contributions in total EPR, $\dot{S}_{tot}^{(\mu)}$ and net velocity of reaction are plotted as a function of substrate concentration, $[S]$ at NESS. The rate constants are: $k_1' = 5 \times 10^7$ M^{-1} s^{-1}, $k_{-1} = 18\,300$ s^{-1}, $k_{-2} = 730$ s^{-1}, $k_2 = 1 \times 10^{-5}$ s^{-1}.
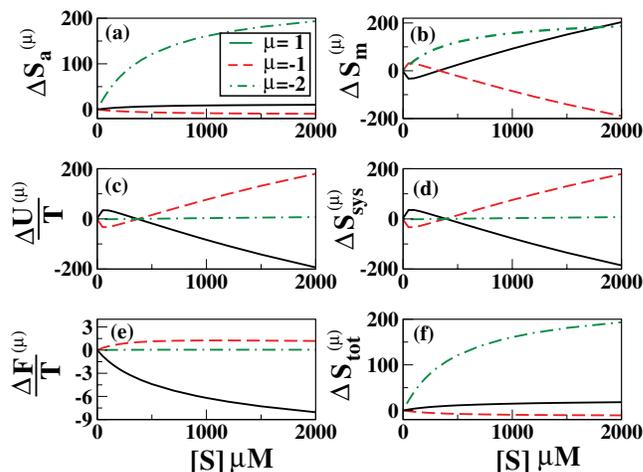

FIG. 3. (a)–(f) The individual reaction contributions in the various entropy productions, free energy change and the internal energy change are plotted as a function of $[S]$. The rate constants are: $k_1' = 5 \times 10^7$ M^{-1} s^{-1}, $k_{-1} = 18\,300$ s^{-1}, $k_{-2} = 730$ s^{-1}, $k_2 = 1 \times 10^{-5}$ s^{-1}.

We have calculated $\dot{S}_{tot}^{\mu(ss)}$ as a function of substrate concentration, $[S]$ from Eqs. (21) and (22). We have also determined the quantities directly from the master equation, Eq. (15). Results obtained from the two approaches tally very well as depicted in Figs. 2(a)–2(d). In Figs. 2(a)–2(c), it is observed that the variation of $\dot{S}_{tot}^{1(ss)}$, $\dot{S}_{tot}^{-1(ss)}$, and $\dot{S}_{tot}^{-2(ss)}$ as a function of $[S]$ are hyperbolic in nature. Also, $\dot{S}_{tot}^{-1(ss)}$ remains negative throughout, its magnitude almost same as that of $\dot{S}_{tot}^{1(ss)}$. The value of $\dot{S}_{tot}^{2(ss)}$ remains negligible throughout, as shown in Fig. 2(d). Therefore, $\dot{S}_{tot}^{(ss)}$ is mainly governed by reaction $\mu = -2$. In Figs. 2(e) and 2(f), we have plotted the total EPR, $\dot{S}_{tot}^{(ss)}$ and the net velocity, v_{net}^{ss}, both exhibiting hyperbolic dependence on $[S]$ at NESS.

Next, we focus on various entropy productions, determined by integrating the corresponding rates over a time interval starting from $t = 0$ to a fixed final time, when the system resides in NESS. We have plotted the individual reaction contributions as a function of $[S]$ at NESS shown in Figs. 3(a)–3(f). In the figure, we have not shown the contribution of reaction $\mu = 2$ due to its smallness. First of all, we investigate how the irreversible work done in sustaining the NESS is utilized by the reactions. We remind the reader that, the adiabatic entropy production, ΔS_a is the measure of the (temperature-scaled) irreversible work done. $\Delta S_a^{(\mu)}$ is plotted as a function of $[S]$ at NESS, depicted in Fig. 3(a). In figure, it is evident that, $\Delta S_a^{(1)}$ remains positive whereas, $\Delta S_a^{(-1)}$ remains negative and they are close in magnitude. So, the irreversible work done on the system is mostly consumed by the reaction $\mu = -2$, more so at higher $[S]$. Now according to first law, some amount of the irreversible work done on the system changes its internal energy and rest of it dissipates as heat to the surroundings. We have plotted the measure of heat dissipation, $\Delta S_m^{(\mu)}$ and the temperature-scaled internal energy change of the system, $\Delta U^{(\mu)}/T$ as a function of $[S]$ in Figs. 3(b) and 3(c), respectively. In Fig. 3(b), it is observed that $\Delta S_m^{(1)}$ is negative at small values of $[S]$, indicating heat absorption by the system from surroundings. As $[S]$ is increased however, $\Delta S_m^{(1)}$ becomes positive and increases linearly, indicating growing heat dissipation to surroundings. For reaction

$\mu = -1$, the trend is almost exactly opposite and cancels out the contribution of the reaction $\mu = 1$. Hence, again the reaction $\mu = -2$ governs the total heat dissipation, as $\Delta S_m^{(-2)}$ remains positive and rises with $[S]$ to saturation.

Interestingly, the trend of variation of $\Delta S_m^{(-2)}$ is quantitatively similar to $\Delta S_a^{(-2)}$, as one can see from Figs. 3(a) and 3(b). 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. This is indeed the case, as shown in Fig. 3(c). The variation of the corresponding contributions of reactions $\mu = \pm 1$ towards the internal energy change, $\Delta U^{(\pm 1)}/T$ show opposite trends as a function of $[S]$ compared to the variation of $\Delta S_m^{(\pm 1)}$(see Fig. 3(b)). This is expected as heat absorption by the system due to an elementary reaction leads to an increase in internal energy 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. 3(d). The difference between $\Delta U^{(\mu)}/T$ and $\Delta S_{sys}^{(\mu)}$ gives the temperature-scaled free energy change, $\Delta F^{(\mu)}/T$(which is also the nonadiabatic entropy production, $\Delta S_{na}^{(\mu)}$), shown in Fig. 3(e). 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 $\Delta S_{sys}^{(\mu)}$. We see from Fig. 3(e), 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 $\mu = 1$. Finally, we have plotted $\Delta S_{tot}^{(\mu)}$ as a function of $[S]$ in Fig. 3(f). As the magnitude and trend of variations of $\Delta S_m^{(\mu)}$ and $\Delta S_{sys}^{(\mu)}$ are opposite for the reactions $\mu = 1$ and $\mu = -1$, so the value of $\Delta S_{tot}^{(\pm 1)}$ remains small compared to the reaction $\mu = -2$ over the range of $[S]$.

After the detailed analyses regarding the role of the elementary reactions in governing the nonequilibrium thermodynamics of the enzyme catalysis, next we explore the overall changes. For this purpose, we have determined the changes in the state functions, ΔS_{sys}, $\Delta F/T$, $\Delta U/T$ shown in Fig. 4(a) as well as the changes in the path functions ΔS_{tot}, ΔS_m, and ΔS_a plotted in Fig. 4(b) as a function of $[S]$. In Fig. 4(a), we
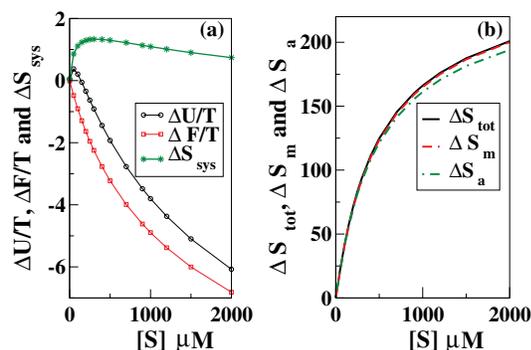
FIG. 4. Variations of (a) ΔS_{sys}, $\Delta U/T$, $\Delta F/T$ and (b) ΔS_{tot}, ΔS_m, ΔS_a are plotted as a function of $[S]$. The rate constants are $k_1' = 5 \times 10^7$ M^{-1} s^{-1}, $k_{-1} = 18\,300$ s^{-1}, $k_{-2} = 730$ s^{-1}, $k_2 = 1 \times 10^{-5}$ s^{-1}.

find out that $\Delta F/T$ becomes more and more negative with $[S]$, as expected. On the other hand, ΔS_{sys} remains positive and passes through a maximum. Now, from the same plot, we observe that at low $[S]$, $\Delta U/T$ is positive. So in this region, the reaction is entropy-driven. As $[S]$ increases, $\Delta U/T$ decreases steadily, changes sign and runs parallel to $\Delta F/T$. Therefore, at higher $[S]$, the reaction gradually becomes energy-driven. In Fig. 4(b), one can see that, all the path functions are almost the same at low $[S]$ and begin to differ as $[S]$ increases. Their magnitudes are much greater than the corresponding state function quantities, again indicating the fact that the irreversible work done on the system dissipates almost entirely as heat.

V. CONCLUSION

We have theoretically formulated the total EPR in an open chemical reaction system in terms of a KL-type distance between forward and backward elementary reaction propensities, equivalent to the reaction probability density, introduced by Gillespie.[4,5] The formalism is inspired by a recent study on the dissipation function in phase space.[23] A general nonequilibrium thermodynamic scenario is discussed in presence of chemiostatic condition that allows the system to sustain a NESS. The theory has been applied to the stochastic kinetics of a single oligomeric enzyme obeying reversible MM kinetics. Analytical expressions of the contributions of each elementary reaction to the total EPR are derived, providing important insights in the connection of these theoretical quantities to experimental observables, like the net velocity of the reaction at NESS.

As a real case study, we have taken the single molecule kinetics of the tetrameric *Escherichia coli β*-galactosidase enzyme under chemiostatic condition. The variation of total EPR for individual reactions is analyzed with time as well as with substrate concentration at NESS. In both situations, we find that the contributions of the enzyme-substrate binding reaction to form the ES-complex and its reverse reaction, i.e., $\mu = \pm 1$ balance out each other and the overall dissipation is mostly governed by the product-formation reaction, $\mu = -2$. We have proceeded further in determining the various entropy productions due to these reactions over the time course of the enzyme reaction from start to reaching NESS. The adiabatic entropy production, representing the irreversible work done in driving the system to NESS and then to sustain it, is shown to
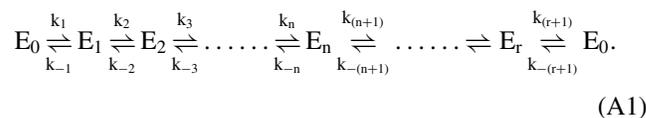
be almost entirely consumed by the product formation step. This, in turn, mostly dissipates as heat. So, the internal energy change and entropy change of the system come out to be much lower in magnitude. Again the contributions of reactions $\mu = \pm 1$ cancel out each other over the range of substrate concentration studied. Interestingly, the free energy change of the overall process is governed by the enzyme-substrate binding reaction, $\mu = 1$. Exploring the energy and entropy contributions to the overall free energy change, we have found that the reaction is entropy-driven at low substrate concentration and becomes energy-driven as the substrate concentration rises. Therefore, a detailed understanding of the mechanism of energy expenditure of the open reaction system is achieved under conditions arbitrarily away from thermodynamic equilibrium. In the light of the intimate relation between detailed balance and total EPR, we have shown a similar connection between circular balance and nonadiabatic EPR, that can also be written in the propensity distance formalism. Furthermore, we point out that with $k_2' \approx 0$, the MM kinetics is applicable to several other real cases, e.g., acid-base equilibrium occurring ahead of a first-order or pseudo first-order step and unimolecular reactions in the gas phase described by Lindemann mechanism.[37,38] However, to simulate the processes inside a small volume, fluctuations in all the concentrations should be taken into account more accurately. Recent experimental and theoretical studies also indicate that the mechanism of substrate input has a significant effect in governing the overall stochastic kinetics and thermodynamics.[42–45] Specifically, investigations on stochastic substrate generation in bursts reveal novel phenomena, like concentration inversion,[46] and provide physical insights into the mechanism of single cell rhythms.[47] Usually inside cells substrate cannot be constant and is produced via translation; what is really constant is the gene concentration and translation gives rise to production of substrate and other proteins in bursts. These works unequivocally confirm the need to go beyond the chemiostatic condition and the present formalism can be extended to more generic ways of introducing substrates through a bursty way to understand the role of stochasticity in biological systems.

APPENDIX A: TOTAL EPR IN A GENERAL CYCLIC REACTION SCHEME

For the sake of completeness, we consider a cyclic reaction scheme as given below

$$E_0 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E_1 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E_2 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \ldots\ldots \underset{k_{-n}}{\overset{k_n}{\rightleftharpoons}} E_n \underset{k_{-(n+1)}}{\overset{k_{(n+1)}}{\rightleftharpoons}} \ldots\ldots \rightleftharpoons E_r \underset{k_{-(r+1)}}{\overset{k_{(r+1)}}{\rightleftharpoons}} E_0.$$

$$\text{(A1)}$$

Here number of intermediate species are taken to be r. The reaction index is $\mu = \pm 1, \pm 2, \ldots, \pm(r + 1)$ with each elementary reaction having a reverse counterpart. The rate constant, k_μ of reaction μ can either be simple first order or can be pseudo first order. In the latter case, the reaction system contains species other than $E_n(n = 0, 1, \ldots, r)$ (not shown in the above scheme) which react with the species E_n and/or are generated due to the reactions but

their concentrations are assumed fixed under chemiostatic condition. The number of molecules of different intermediate species, E_n ($n = 1, 2, \ldots, r$) are collectively denoted by the vector $\mathbf{Z} = \{Z_n\}$ and the corresponding vector of the stoichiometric coefficients associated with the μth reaction is denoted by $\boldsymbol{v_\mu}$ with $v_\mu^n = \pm 1$. In this case the total EPR due to the μth reaction can be explicitly written as

$$
\begin{aligned}
\dot{S}_{tot}^{(\mu)}(t) &= \sum_{\mathbf{Z},\mathbf{Z}'} R_\mu(Z_1, \ldots, Z_r, t | Z_1', \ldots, Z_r') \ln\left(\frac{R_\mu(Z_1, \ldots, Z_r, t | Z_1', \ldots, Z_r')}{R_{-\mu}(Z_1', \ldots, Z_r', t | Z_1, \ldots, Z_r)} \right) \delta_{Z_1', Z_1 \pm 1}, \ \mu = \pm 1 \\
&= \sum_{\mathbf{Z},\mathbf{Z}'} R_\mu(Z_1, \ldots, Z_r, t | Z_1', \ldots, Z_r') \ln\left(\frac{R_\mu(Z_1, \ldots, Z_r, t | Z_1', \ldots, Z_r')}{R_{-\mu}(Z_1', \ldots, Z_r', t | Z_1, \ldots, Z_r)} \right) \delta_{Z_r', Z_r \mp 1}, \ \mu = \pm(r + 1) \\
&= \sum_{\mathbf{Z},\mathbf{Z}'} R_\mu(Z_1, \ldots, Z_r, t | Z_1', \ldots, Z_r') \ln\left(\frac{R_\mu(Z_1, \ldots, Z_r, t | Z_1', \ldots, Z_r')}{R_{-\mu}(Z_1', \ldots, Z_r', t | Z_1, \ldots, Z_r)} \right) \delta_{Z_{n-1}', Z_{n-1} \mp 1} \delta_{Z_n', Z_n \pm 1},
\end{aligned}
$$

$$\mu = \pm n (n = 2, 3, \ldots, r). \tag{A2}$$

APPENDIX B: TOTAL EPR FROM SHANNON ENTROPY

Starting from the definition of system's Shannon entropy[10, 16, 18–21]

$$S_{sys}(t) = - \sum_{\mathbf{Z}} P(\mathbf{Z}, t) \ln P(\mathbf{Z}, t), \tag{B1}$$

and using Eq. (3), we get the total EPR as

$$
\begin{aligned}
\dot{S}_{tot}(t) &= \frac{1}{2} \sum_{\mathbf{Z},\mu} [w_\mu(\mathbf{Z} - \boldsymbol{v_\mu} | \mathbf{Z}) P(\mathbf{Z} - \boldsymbol{v_\mu}, t) \\
&\quad - w_{-\mu}(\mathbf{Z} | \mathbf{Z} - \boldsymbol{v_\mu}) P(\mathbf{Z}, t)] \\
&\quad \times \ln \frac{w_\mu(\mathbf{Z} - \boldsymbol{v_\mu} | \mathbf{Z}) P(\mathbf{Z} - \boldsymbol{v_\mu}, t)}{w_{-\mu}(\mathbf{Z} | \mathbf{Z} - \boldsymbol{v_\mu}) P(\mathbf{Z}, t)}.
\end{aligned} \tag{B2}
$$

Now, Eq. (B2) implies that contribution of reaction μ towards $\dot{S}_{tot}(t)$ is the same as that of reaction $-\mu$. This is not physically meaningful. Actually, this problem is encountered not only in the case of total EPR but also for all the other EPRs. However, the individual reaction contributions to the total EPR are distinct from each other following the propensity distance formalism, as given in Eq. (17).

APPENDIX C: CIRCULAR BALANCE AND FREE ENERGY DISSIPATION RATE IN SINGLE ENZYME CATALYSIS

Splitting of total EPR into adiabatic and nonadiabatic contributions is particularly relevant when the system does not satisfy detailed balance. This is so in our case, with the enzyme kinetics in Eq. (11) under chemiostatic condition.

Now the question arises: can the formalism of constructing the total EPR from forward and backward reaction propensities also be used to express the adiabatic and nonadiabatic parts as KL distances? Here we show that, at least the nonadiabatic EPR, which is the negative of the temperature-scaled free energy dissipation rate, can be cast in such a form. For the steady state solution of the master equation, Eq. (13), one can use two kinds of balance conditions: the detailed balance and the circular balance. The former gives the equilibrium state whereas the latter gives the NESS. The detailed balance condition is given as $w_\mu(m|l) P(m, t) = w_{-\mu}(l|m) P(l, t)$, i.e., $R_\mu(m, t|l) = R_{-\mu}(l, t|m)$. As the total EPR becomes zero at equilibrium, so the expression of the total EPR must be built from these two reaction propensities in a relative entropy kind of structure as is already shown in Sec. III. This gives the clue to construct the appropriate reaction propensities for the description of the nonadiabatic EPR. As, by definition (see Eq. (10)), the nonadiabatic EPR becomes zero at the stationary state (whether equilibrium or NESS), the balance condition that is valid for NESS (and obviously for equilibrium) must be linked to this EPR. This leads us to the circular balance condition which is given in terms of the overall formation and dissociation reaction propensities of the ES-complex as

$$R_f(n, t|n + 1) = R_d(n + 1, t|n), \tag{C1}$$

where $R_f(n, t|n + 1) = K_1(n_T - n) P(n, t)$ and $R_d(n + 1, t|n) = K_2(n + 1) P(n + 1, t)$ with K_1, K_2 already defined in Sec. III. Then the contribution of the formation reaction to the

nonadiabatic EPR, $\dot{S}_{na}$ is written as

$$
\begin{aligned}
\dot{S}_{na}^{(f)}(t) = \frac{1}{2}\Bigg[&\sum_{n-1} R_f(n-1,t|n)\ln\left(\frac{R_f(n-1,t|n)}{R_d(n,t|n-1)}\right) \\
&+ \sum_n R_f(n,t|n+1)\ln\left(\frac{R_f(n,t|n+1)}{R_d(n+1,t|n)}\right)\Bigg] \\
= &(K_1 n_T Y)\ln\left(\frac{K_1 n_T Y}{K_2 n_T X}\right).
\end{aligned}
\tag{C2}
$$

Here X and Y are the same as those defined in Sec. III. Similarly, we get the contribution of the dissociation reaction as

$$
\dot{S}_{na}^{(d)}(t) = (K_2 n_T X)\ln\left(\frac{K_2 n_T X}{K_1 n_T Y}\right),
\tag{C3}
$$

with

$$
\dot{S}_{na}(t) = \dot{S}_{na}^{(f)}(t) + \dot{S}_{na}^{(d)}(t).
\tag{C4}
$$

So, the nonadiabatic EPR or the free energy dissipation rate can be written in the same relative entropy kind of formalism in terms of the overall formation (forward) and dissociation (backward) reactions. Thus, *for total EPR, the detailed balance gives the appropriate reaction propensities whereas, for the nonadiabatic EPR, the circular balance provides the suitable choices.*

APPENDIX D: INEQUALITIES IN $\dot{S}_{tot}^{\mu(ss)}$ IN THE MM KINETICS

For the MM kinetics with $k_2' \approx 0$, we have the following inequalities:

$$
\frac{\dot{S}_{tot}^{1(ss)}}{\dot{S}_{tot}^{-1(ss)}} = -\frac{K_2}{k_{-1}} = -\left(1 + \frac{k_{-2}}{k_{-1}}\right) < -1,
\tag{D1}
$$

and

$$
\frac{\dot{S}_{tot}^{-2(ss)}}{\dot{S}_{tot}^{2(ss)}} = -\frac{k_{-2}K_1}{k_2 K_2} = -\frac{k_{-2}[S]}{k_2 K_M} \ll -1.
\tag{D2}
$$

APPENDIX E: SIGN INVERSION OF $\dot{S}_{tot}^{(\mu)}(t)$

Let us consider the equality $k_\mu Y = k_{-\mu} X$ in Eq. (18) to determine the time, t_μ^* when the curve of $\dot{S}_{tot}^{(\mu)}(t)$ crosses the time axis, if at all. It is given by

$$
t_\mu^* = \frac{1}{K_1 + K_2}\ln\left(\frac{K_1(k_\mu + k_{-\mu})}{k_{-\mu}K_1 - k_\mu K_2}\right).
\tag{E1}
$$

Hence $\dot{S}_{tot}^{(\mu)}(t)$ can change sign with time if $k_{-\mu}K_1 > k_\mu K_2$. With our parameters, this condition gets satisfied only for the reaction, $\mu = 2$. Hence, the total EPR due to the reactions $\mu = \pm 1, -2$ remain positive or negative throughout the time evolution up to the NESS, as shown in Fig. 1. For reaction

$\mu = 2$, t_2^* comes out to be $\sim 10^{-12}$ s which is beyond our numerical precision (time-steps of 10^{-7} s). Hence, the sign change is not detected in Fig. 1(d).

[1] D. A. McQuarrie, J. Chem. Phys. **38**, 433 (1963).
[2] D. A. McQuarrie, C. J. Jachimowski, and M. E. Russell, J. Chem. Phys. **40**, 2914 (1964).
[3] D. A. McQuarrie, J. Appl. Probab. **4**, 413 (1967).
[4] D. T. Gillespie, J. Comput. Phys. **22**, 403 (1976).
[5] D. T. Gillespie, J. Phys. Chem. **81**, 2340 (1977).
[6] D. T. Gillespie, Physica A **188**, 404 (1992).
[7] M. Delbruck, J. Chem. Phys. **8**, 120 (1940).
[8] K. Ishida, Bull. Chem. Soc. Jpn. **33**, 1030 (1960).
[9] A. Bartholomay, Bull. Math. Biophys. **20**, 175 (1958).
[10] P. Gaspard, J. Chem. Phys. **120**, 8898 (2004).
[11] H. Qian and E. L. Elson, Biophys. Chem. **101–102**, 565 (2002).
[12] H. Qian, Annu. Rev. Phys. Chem. **58**, 113 (2007).
[13] H. P. Lu, L. Xun, and X. S. Xie, Science **282**, 1877 (1998).
[14] B. P. English, W. Min, A. M. van Oijen, K. T. Lee, G. Luo, H. Sun, B. J. Cherayil, S. C. Kou, and X. S. Xie, Nat. Chem. Biol. **2**, 87 (2006).
[15] B. Das and G. Gangopadhyay, J. Chem. Phys. **132**, 135102 (2010).
[16] B. Das, K. Banerjee, and G. Gangopadhyay, J. Math. Chem. **51**, 588 (2013).
[17] K. Banerjee, B. Das, and G. Gangopadhyay, J. Chem. Phys. **136**, 154502 (2012).
[18] L. Jiu-li, C. Van den Broeck, and G. Nicolis, Z. Phys. B **56**, 165 (1984).
[19] G. Nicolis and I. Prigogine, *Self-Organization in Nonequilibrium Systems* (Willey, New York, 1977).
[20] G. Nicolis and I. Prigogine, Proc. Natl. Acad. Sci. U.S.A. **68**, 2102 (1971).
[21] G. Nicolis, J. Stat. Phys. **6**, 195 (1972).
[22] W. Min, L. Jiang, J. Yu, S. C. Kou, H. Qian, and X. S. Xie, Nano Lett. **5**, 2373 (2005).
[23] R. Kawai, J. M. R. Parrondo, and C. Van den Broeck, Phys. Rev. Lett. **98**, 080602 (2007).
[24] E. H. Feng and G. E. Crooks, Phys. Rev. Lett. **101**, 090602 (2008).
[25] J. M. R. Parrondo, C. Van den Broeck, and R. Kawai, New. J. Phys. **11**, 073008 (2009).
[26] H. Ge and H. Qian, Phys. Rev. E **81**, 051133 (2010).
[27] M. Esposito and C. Van den Broeck, Phys. Rev. E **82**, 011143 (2010).
[28] M. Esposito and C. Van den Broeck, Phys. Rev. Lett. **104**, 090601 (2010).
[29] T. J. Xiao, Z. Hou, and H. Xin, J. Chem. Phys. **129**, 114506 (2008).
[30] U. Seifert, Phys. Rev. Lett. **95**, 040602 (2005).
[31] T. Schmiedl and U. Seifert, J. Chem. Phys. **126**, 044101 (2007).
[32] T. Schmiedl, T. Speck, and U. Seifert, J. Stat. Phys. **128**, 77 (2007).
[33] S. Kullback and R. A. Leibler, Ann. Math. Stat. **22**, 79 (1951).
[34] G. E. Crooks and D. A. Sivak, J. Stat. Mech.: Theor. Exp. **2011**, P06003.
[35] C. W. Gardiner, *Handbook of Stochastic Methods for Physics, Chemistry and the Natural Sciences*, 2nd ed. (Springer, New York, 1985).
[36] N. G. Vankampen, *Stochastic Processes in Physics and Chemistry* (Elsevier, Amsterdam, The Netherlands, 1992).
[37] J. I. Steinfeld, J. S. Francisco, and W. L. Hase, *Chemical Kinetics and Dynamics*, 2nd ed. (Prentice-Hall, Upper Saddle River, NJ, 1999).
[38] M. Rae and M. N. Berberan-Santos, J. Chem. Ed. **81**, 436 (2004).
[39] R. H. Jacobson, X. J. Zhang, R. F. DuBose, and B. W. Matthews, Nature (London) **369**, 761 (1994).
[40] J. P. Richard, R. E. Huber, C. Heo, T. L. Amyes, and S. Lin, Biochemistry **35**, 12387 (1996).
[41] S. L. Marchesi, Jr., E. Steers, and S. Shifrin, Biochim. Biophys. Acta **181**, 20 (1969).
[42] M. O. Stéfanini, A. J. McKane, and T. J. Newman, Nonlinearity **18**, 1575 (2005).
[43] E. M. Ozbudak, M. Thattai, I. Kurtser, A. D. Grossman, and A. van Oudenaarden, Nat. Genet. **31**, 69 (2002).
[44] J. Yu, J. Xiao, X. Ren, K. Lao, and X. S. Xie, Science **311**, 1600 (2006).
[45] H. Ge and H. Qian, Phys. Rev. E **87**, 062125 (2013).
[46] R. Ramaswamy, N. González-Segredo, I. F. Sbalzarini, and R. Grima, Nat. Commun. **3**, 779 (2012).
[47] D. L. K. Toner and R. Grima, Sci. Rep. **3**, 2438 (2013).