

Kinetics and entropy production of force-induced oligomeric enzyme catalysis in a single trajectory: effect of multiple substrates

Here we have estimated the macroscopic entropy production for a mechanical force induced single oligomeric enzyme kinetics in a chemiostatic condition. This result is supported by the calculation of the single trajectory approach of forward and reverse path as a tool of stochastic thermodynamics. In the former case macroscopic entropy production is calculated from a master equation whereas in the later case single trajectory entropy production is obtained from the reaction kinetics itself. Through the approach of Kullback-Leibler distance in terms of average propensity for a particular reaction channel in the forward trajectory and the corresponding reverse reaction channel in the backward trajectory we explored the contribution of each and every sub-reactions or channels to the overall enzymatic processes and made the trajectory analysis of multiple kind of substrates. We have calculated the individual contribution of each sub-reaction in the overall medium entropy production which is exactly valid in the nonequilibrium steady state and it can give an insight about the mechanism of a complex chemical reaction, a step towards the nonequilibrium thermochemistry.

Keywords: Oligomeric enzyme kinetics, multiple substrates, Single trajectory, Nonequilibrium steady state

I. INTRODUCTION

In the last few decades there have been a growing interest to understand the irreversible macroscopic events^{1–37} in terms of the microscopic laws and tools of individual microscopic systems. Presently some theoretical methods in nonequilibrium systems are routinely tested in the process of describing the relationship^{3–9} between work and free energy involving the experiments of atomic force microscopy⁷ and optical tweezers^{8,9}. Stochastic dynamic approaches^{1,2,11–20} have developed the algorithm in which microscopic analogues of all relevant macroscopic quantities are precisely defined. Specially, nonequilibrium simulation of entropy production in single trajectory is gradually becoming a tool-kit of nonequilibrium methods and being applied to a variety of chemical^{1,2,11,12} and mechanical situations¹⁰. The common thread in all these developments is in the essence of the so called fluctuation theorems^{26–33} which are preparing the ground of generalizing thermodynamics to be applicable in far away from equilibrium state as well in the similar spirit of equilibrium thermodynamics.

Usually time dependent chemical processes are described by the chemical master equation^{22–25} to obtain the thermodynamic quantities like entropy production and heat dissipation rate which are generic properties of the systems far away from equilibrium^{1,12,13}. The thermodynamic quantities like entropy production are generally considered as an ensemble average property. However, very recently, Seifert has suggested that one can define entropy production along a single stochastic trajectory which can be negative as well, particularly at short time and for small systems. However, the average total entropy production remains always greater than or equal to zero according to the second law of thermodynamics. Trajectory analysis technique has been successfully applied to various systems¹¹, e.g, chemical reaction networks³⁴, driven colloidal particles³⁵, single two level systems³⁶ and also single biomolecular reactions³⁷. On the other hand, enzyme kinetics reaction at single molecular level has been extensively studied both theoretically and experimentally^{38–40}. Recent results of Gump et al. on the triggering of enzymatic activity through AFM at the single molecular level opens a new way to study the direct influence of force to manipulate biocatalytic reactions⁴¹. Recently we had theoretically studied the effect of external mechanical force on a single oligomeric enzyme kinetic reaction in non-equilibrium steady state(NESS) by proposing an approximate description of the system in terms of a master equation where the

substrate molecules randomly walk on the several sites of a single oligomeric enzyme. The strength of our master equation for the finite number of active sites of a single oligomeric enzyme is that it is possible to calculate the various statistical average properties of reaction kinetics. In the limit of a single active site the master equation gives the result of waiting time distribution of single molecule process and in the limit of many active sites its solution is a Poisson distribution in the steady state corresponding to the enzyme kinetics following the chemical master equation of small system. We had found that the net rate of the reaction averaged over several turn over cycles in chemiostatic condition is multiplied by the number of active sites and further enhanced by more than two orders of magnitude with the application of 10-100 pN force^{42,43}.

In continuation of our earlier work⁴², here we have studied the entropy production of the oligomeric enzyme kinetics with external mechanical force as a kinetic parameter. However, from the master equation approach we get the ensemble average entropy production over many active sites of an oligomeric enzyme. To find out the entropy production by a single oligomeric enzyme from the reaction kinetics in all active sites and to explore at the level of individual sub-reactions, we have performed the single trajectory analysis^{1,2,11,12} from the reaction kinetics itself with the help of the Gillespie stochastic simulation approach. Furthermore, by the analysis of the stochastic trajectory we have also shown⁴³ the validity of microscopic reversibility for this reaction system at NESS. Here we have asked the question about how the individual reaction channels or sub-reactions contribute to the overall entropy production. In this context we have generalized the single oligomeric enzyme reaction system for the case of one type of substrate to multiple types of substrates.

The paper is organized as follows. In sec. II, we have calculated the entropy production rate by the master equation approach for the oligomeric enzyme kinetics and then we have extended the master equation to describe the kinetics of the oligomeric enzyme when different types of substrate molecules can attach with the enzyme. The simulation procedure for the single trajectory stochastic thermodynamics is described in sec. III. The results are discussed in sec. IV for the entropy production including the individual reaction channels or sub-reactions contributing to the overall entropy production and also the validity of the detailed fluctuation theorem is shown. Finally, the paper is concluded in sec. V.

II. MASTER EQUATION APPROACH TO ENTROPY PRODUCTION IN AN OLIGOMERIC ENZYME KINETICS

In this section, we first give a brief description of an oligomeric enzyme kinetics at chemiostatic condition through the master equation approach⁴². Later in this section, we have calculated the time dependent entropy production rate from the master equation which gives the macroscopic result in the chemiostat.

A. Scheme of the reaction and the stochastic master equation

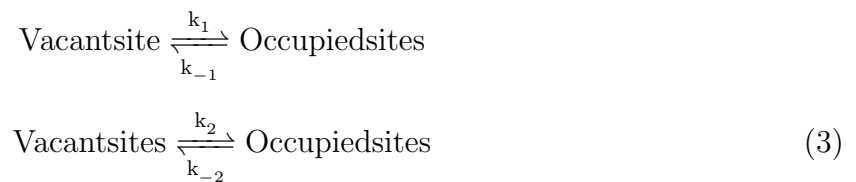
The familiar Michaelis-Menten enzyme kinetics reaction where an enzyme molecule has one active site is written as



In chemiostatic condition where the concentration of substrate, S and product, P are maintained at constant values, the above reaction scheme can be reduced to



where $\lambda = k_1 + k_2$ and $\gamma = k_{-1} + k_{-2}$. The pseudo first order rate constants k_1 and k_2 are given by $k_1 = k_1'[S]$ and $k_2 = k_2'[P]$. In our case, the oligomeric enzyme consists of n_T number of homo type subunits which are linked through extra covalent bonding by using some cross linking reagents e.g., glutaraldehyde or dimethylsuberimidate⁴⁴ and each subunit has one active site. As each active site can form one ES complex, so the reaction scheme can be viewed in terms of the number of total active sites present at a particular time in the oligomeric enzyme. Here, the active sites which have already formed ES complex are referred to as occupied sites and those that do not form the ES complex at that moment are called vacant sites. Hence, the oligomeric enzyme catalyzed reaction scheme can be written as



consisting of four reaction channels or sub-reactions. Due to the random occurrence of various reaction channels, the number of occupied sites becomes a fluctuating quantity. If at time t , n number of occupied sites are present in the system, i.e, the system is in the n -th occupied state, then after a small time τ , the system goes to a new state, $(n + \nu_\mu)$ through any one of the four possible sub-reactions. Here ν_μ is designated as the stoichiometric coefficient of the μ -th reaction. Furthermore, among n_T number of total active sites, if n number of sites form ES complex at time t and $(n_T - n)$ number of sites remain vacant then the stochastic master equation can be written as

$$\frac{dP(n,t)}{dt} = \sum_{\mu=\pm 1}^{\pm 2} [w_\mu(n - \nu_\mu|n)P(n - \nu_\mu, t) - w_{-\mu}(n|n - \nu_\mu)P(n, t)] \quad (4)$$

where $\nu_\mu = -\nu_{-\mu} = 1$; and $P(n, t)$ is the probability of having n number of occupied state at time t . The transition probabilities are defined as follows,

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

and

$$w_\mu(n - \nu_\mu|n) = k_\mu(n - \nu_\mu), \mu < 0. \quad (5)$$

Then Eq.(4) can be rewritten as

$$\begin{aligned} \frac{dP(n,t)}{dt} = & (k_1 + k_2)(n_T - n + 1)P(n - 1, t) + (k_{-1} + k_{-2})(n + 1)P(n + 1, t) \\ & - (k_1 + k_2)(n_T - n)P(n, t) - (k_{-1} + k_{-2})nP(n, t). \end{aligned} \quad (6)$$

Solving the master equation using the standard approach of generating function method^{24,25,42} we get the probability distribution function as

$$\begin{aligned} P(n,t) = & \frac{n_T!}{n!(n_T - n)!} \left[\frac{1}{K_1 + K_2} \{K_2 + K_1 \exp[-(K_1 + K_2)t]\} \right]^{n_T - n} \\ & \times \left[\frac{K_1}{K_1 + K_2} \{1 - \exp[-(K_1 + K_2)t]\} \right]^n, \end{aligned} \quad (7)$$

where $K_1 = (k_1 + k_2)$ and $K_2 = (k_{-1} + k_{-2})$, assuming that initially all the active sites are unoccupied. With this initial condition the time dependent average number of occupied sites is given by

$$\langle n(t) \rangle = \frac{n_T K_1}{K_1 + K_2} [1 - \exp\{-(K_1 + K_2)t\}] \quad (8)$$

and the average number of vacant sites is expressed as

$$\langle n_T - n(t) \rangle = \frac{n_T}{(K_1 + K_2)} [K_2 + K_1 \exp\{-(K_1 + K_2)t\}]. \quad (9)$$

B. Calculation of the entropy production rate through the master equation

The Shannon entropy is defined as,

$$S_{\text{sys}}(t) = -k_B \sum_n P(n, t) \ln P(n, t). \quad (10)$$

In our case we set the Boltzmann constant, $k_B = 1$. Using the solution of the master equation $P(n, t)$ of the single oligomeric enzyme system, we get the entropy production rate of the system as^{12,13,45}

$$\frac{dS_{\text{sys}}(t)}{dt} = \frac{1}{2} \sum_{n,\mu} [w_\mu(n - \nu_\mu | n) P(n - \nu_\mu, t) - w_{-\mu}(n | n - \nu_\mu) P(n, t)] \ln \frac{P(n - \nu_\mu, t)}{P(n, t)} \quad (11)$$

Here $\dot{S}_{\text{sys}}$ is the entropy production rate (epr) due to the nonequilibrium processes (chemical reactions) occurring in the system and we have assumed ideal reservoirs (surroundings) with no inherent entropy production.

This entropy production rate can be splitted as¹³

$$\frac{dS_{\text{sys}}(t)}{dt} = \frac{dS_{\text{tot}}(t)}{dt} - \frac{dS_{\text{m}}(t)}{dt}. \quad (12)$$

Here the first term in the r.h.s. of Eq.(12) gives the total entropy production rate and the second term denotes the medium entropy production rate due to the entropy flux into surroundings. As the surroundings is treated as an ideal reservoir, its epr is simply $-\dot{S}_{\text{m}}$. For our master equation they are defined as

$$\frac{dS_{\text{tot}}(t)}{dt} = \frac{1}{2} \sum_{n,\mu} [w_\mu(n - \nu_\mu | n) P(n - \nu_\mu, t) - w_{-\mu}(n | n - \nu_\mu) P(n, t)] \ln \frac{w_\mu(n - \nu_\mu | n) P(n - \nu_\mu, t)}{w_{-\mu}(n | n - \nu_\mu) P(n, t)} \quad (13)$$

and

$$\frac{dS_{\text{m}}(t)}{dt} = \frac{1}{2} \sum_{n,\mu} [w_\mu(n - \nu_\mu | n) P(n - \nu_\mu, t) - w_{-\mu}(n | n - \nu_\mu) P(n, t)] \ln \frac{w_{-\mu}(n | n - \nu_\mu)}{w_\mu(n - \nu_\mu | n)}. \quad (14)$$

Using the expressions of the corresponding transition probabilities from Eq.(5), we get

$$\frac{dS_{\text{tot}}(t)}{dt} = \frac{1}{2} \sum_n [k_1(n_T - n + 1) P(n - 1, t) - k_{-1}n P(n, t) \ln \frac{k_1(n_T - n + 1) P(n - 1, t)}{k_{-1}n P(n, t)}]$$

$$\begin{aligned}
& +[k_{-1}(n+1)P(n+1, t) - k_1(n_T - n)P(n, t) \ln \frac{k_{-1}(n+1)P(n+1, t)}{k_1(n_T - n)P(n, t)}] \\
& +[k_2(n_T - n+1)P(n-1, t) - k_{-2}nP(n, t) \ln \frac{k_2(n_T - n+1)P(n-1, t)}{k_{-2}nP(n, t)}] \\
& +[k_{-2}(n+1)P(n+1, t) - k_2(n_T - n)P(n, t) \ln \frac{k_{-2}(n+1)P(n+1, t)}{k_2(n_T - n)P(n, t)}]. \quad (15)
\end{aligned}$$

After some algebra we finally get,

$$\frac{dS_{\text{tot}}(t)}{dt} = \langle n(t) \rangle \left[k_{-1} \ln \left(\frac{k_{-1}Y}{k_1X} \right) + k_{-2} \ln \left(\frac{k_{-2}Y}{k_2X} \right) \right] - \langle n_T - n(t) \rangle \left[k_1 \ln \left(\frac{k_{-1}Y}{k_1X} \right) + k_2 \ln \left(\frac{k_{-2}Y}{k_2X} \right) \right] \quad (16)$$

where $Y = \frac{K_1(1 - \exp(-(K_1+K_2)t))}{K_1+K_2}$ and $X = \frac{K_2+K_1 \exp(-(K_1+K_2)t)}{K_1+K_2}$ with $K_1 = (k_1 + k_2)$ and $K_2 = (k_{-1} + k_{-2})$.

At the nonequilibrium steady state we have $d\dot{S}_{\text{sys}} = 0$. As here the forward cycle flux becomes equal to the backward cycle flux⁴⁶, from Eq.(11) we get

$$w_1(n-1|n)P(n-1|n) - w_{-1}(n|n-1)P(n|n-1) = w_{-2}(n|n-1)P(n|n-1) - w_2(n-1|n)P(n-1|n) = 0.$$

Then the total entropy production rate at the steady state can be written as

$$\begin{aligned}
\frac{dS_{\text{tot}}}{dt} &= \sum_n [w_1(n-1|n)P(n-1|n) - w_{-1}(n|n-1)P(n|n-1)] \\
&\times \ln \left(\frac{w_1(n-1|n)P(n-1|n) \times w_{-2}(n|n-1)P(n|n-1)}{w_{-1}(n|n-1)P(n|n-1) \times w_2(n-1|n)P(n-1|n)} \right). \quad (17)
\end{aligned}$$

Now putting the values of $\langle n \rangle$ and $\langle (n_T - n) \rangle$ at steady state in Eq.(16), we obtain

$$\frac{dS_{\text{tot}}}{dt} = n_T \ln \left(\frac{k_{-1}k_2}{k_1k_{-2}} \right) \left[\left(\frac{k_2k_{-1} - k_1k_{-2}}{k_1 + k_{-1} + k_2 + k_{-2}} \right) \right]. \quad (18)$$

C. Multiple types of substrates

An enzyme mainly catalyses the transformation of its substrate into a product. Some enzymes show absolute specificity, catalyzing the transformation of only one specific substrate to yield a unique product. Other enzymes carry out a particular reaction but act on a class of compounds. For example, hexokinase (ATP : hexose-6-phosphotransferase) will carry out the ATP-dependent phosphorylation of a number of hexoses at the 6-position, including glucose⁴⁷. In this section, we have considered the possibility of attachment of different types of substrates with an oligomeric enzyme and calculated the entropy production rate for this

system. If M number of different substrates, $S_1, S_2, \dots, S_M$, can bind independently with the enzyme then the master equation can be written as

$$\frac{dP(n, t)}{dt} = \sum_{j=1}^M \sum_{\mu=\pm 1}^{\pm 2} [w_{\mu j}(n - \nu_{\mu j}|n)P(n - \nu_{\mu j}, t) - w_{-\mu j}(n|n - \nu_{\mu j})P(n, t)] \quad (19)$$

and the average number of occupied and unoccupied sites can be written as

$$\langle n(t) \rangle = \frac{n_T K'_1}{K'_1 + K'_2} [1 - \exp\{-(K'_1 + K'_2)t\}], \quad (20)$$

$$\langle n_T - n(t) \rangle = \frac{n_T}{(K'_1 + K'_2)} [K'_2 + K'_1 \exp\{-(K'_1 + K'_2)t\}] \quad (21)$$

where K'_1 and K'_2 can be expressed as, $K'_1 = \sum_{j=1}^M (k_{1j} + k_{2j})$ and $K'_2 = \sum_{j=1}^M (k_{-1j} + k_{-2j})$.

The total entropy production rate can be written as

$$\frac{dS_{\text{tot}}(t)}{dt} = \frac{1}{2} \sum_{j, n, \mu} [w_{\mu j}(n - \nu_{\mu j}|n)P(n - \nu_{\mu j}, t) - w_{-\mu j}(n|n - \nu_{\mu j})P(n, t)] \ln \frac{w_{\mu j}(n - \nu_{\mu j}|n)P(n - \nu_{\mu j}, t)}{w_{-\mu j}(n|n - \nu_{\mu j})P(n, t)}. \quad (22)$$

Putting the transition probabilities in the above equation one obtains

$$\begin{aligned} \frac{dS_{\text{tot}}(t)}{dt} = \sum_j \langle n(t) \rangle & \left[\left\{ k_{-1j} \ln \left(\frac{k_{-1j} Y'}{k_{1j} X'} \right) \right\} + \left\{ k_{-2j} \ln \left(\frac{k_{-2j} Y'}{k_{2j} X'} \right) \right\} \right] \\ & - \langle n_T - n \rangle \left[\left\{ k_{1j} \ln \left(\frac{k_{-1j} Y'}{k_{1j} X'} \right) \right\} + \left\{ k_{2j} \ln \left(\frac{k_{-2j} Y'}{k_{2j} X'} \right) \right\} \right] \end{aligned} \quad (23)$$

where $Y' = \frac{K'_1(1 - \exp(-(K'_1 + K'_2)t))}{K'_1 + K'_2}$ and $X' = \frac{K'_2 + K'_1 \exp(-(K'_1 + K'_2)t)}{K'_1 + K'_2}$ with $K'_1 = \sum_j (k_{1j} + k_{2j})$ and $K'_2 = \sum_j (k_{-1j} + k_{-2j})$.

III. SINGLE TRAJECTORY APPROACH

In the previous section, we have calculated the ensemble average entropy production from the master equation approach. In this section, we have calculated the entropy production for a single stochastic trajectory from the stoichiometric of the reaction kinetics.

According to Seifert, the entropy production along a single stochastic trajectory can be defined as¹¹

$$s(t) = -\ln p(n, t) \quad (24)$$

where $p(n, t)$ is the solution of the stochastic master equation for a given initial condition, $p(n_0, t_0)$, taken along the specific trajectory $n(t)$. Note that, the single trajectory entropy is denoted by s whereas the average entropy production (whether being an ensemble average obtained from the master equation or averaged over many trajectories generated in the simulation) is denoted by S . Now the time dependent total entropy production, Δs_{tot} can be split into a system part, Δs_{sys} and a medium contribution, Δs_{m} . Hence the change of total entropy along a trajectory can be written as^{11,48,49}

$$\Delta s_{\text{tot}} = \Delta s_{\text{m}} + \Delta s_{\text{sys}} \quad (25)$$

where,

$$\Delta s_{\text{sys}} = \ln \frac{p(n_0, t_0)}{p(n, t)} \quad (26)$$

and

$$\Delta s_{\text{m}} = \sum_j \ln \frac{w(n_{j-1}; \nu_{\mu}^j)}{w(n_j; -\nu_{\mu}^j)}. \quad (27)$$

Here $w(n_{j-1}; \nu_{\mu}^j)$ denotes the transition probability from the state (n_{j-1}) to the n_j state through a reaction channel μ with the stoichiometric coefficient ν_{μ}^j . At the microscopic level, the number of occupied sites of the oligomeric enzyme becomes a fluctuating quantity due to the random occurrence of the reaction events within the random time interval. This develops the concept of different trajectories and the trajectory dependent total entropy production^{48,49} calculated using the Gillespie stochastic simulation approach^{50,51}. A stochastic trajectory, $n(t)$ starts at n_0 and jumping at times t_j from n_{j-1} to n_j ending up at n_1 with $t = t_1$,

$$n(t) \equiv (n_0, t_0) \xrightarrow{\nu_{\mu}^1} (n_1, t_1) \xrightarrow{\nu_{\mu}^2} \dots \rightarrow (n_{j-1}, t_{j-1}) \xrightarrow{\nu_{\mu}^j} (n_j, t_j) \rightarrow \dots \rightarrow (n_{l-1}, t_{l-1}) \xrightarrow{\nu_{\mu}^1} (n_l, t_l). \quad (28)$$

Here $n_j = n_{j-1} + \nu_{\mu}^j$ and $t_j = t_{j-1} + \tau_j$ where τ_j is the time interval between two successive jumps.

During the jump from the (n_{j-1}) state to the n_j state, any one of the four sub-reactions will occur and the time interval τ_j between the two jumps is a random variable following the exponential distribution

$$p(\tau_j) = a \exp(-a\tau_j) \quad (29)$$

with $a = \sum_{\mu=\pm 1}^{\pm 2} w(n_j - 1; \nu_{\mu}^j)$.

Now a time reversed trajectory can be defined as,

$$n^R(t) \equiv (n_1, t_1) \xrightarrow{-\nu_\mu^1} (n_{1-1}, t_{1-1}) \xrightarrow{-\nu_\mu^{1-1}} \dots \rightarrow (n_j, t_j) \xrightarrow{-\nu_\mu^j} (n_{j-1}, t_{j-1}) \dots \rightarrow (n_1, t_1) \xrightarrow{-\nu_\mu^1} (n_0, t_0). \quad (30)$$

This time reversed trajectory is generated due to the occurrence of a reaction channel whose state changing vector $-\nu_\mu^j$ is exactly opposite to the state changing vector ν_μ^j of the forward reaction channel. The ratio of probabilities of the forward trajectory path $p(n(t)|n(t_0))$ and the backward trajectory path $p(n^R(t)|n_1)$ of the reaction system is given by the quantity $e^{\Delta S_{\text{tot}}}$ ^{11,21}, obtained by applying the above stochastic simulation approach^{50,51}. Hence this gives a microscopic reversibility of the entropy production of the chemically reacting system in terms of the single trajectories of evolution.

A. A different estimate of medium entropy production by the trajectory approach

Here we have provided an approximate formula of calculating the medium entropy production to explore the contribution of each and every sub-reactions or channels to the overall enzymatic process. More specifically, we have formulated the medium entropy production or entropy flow to the surroundings, ΔS_m as an average over the contributions of all the individual sub-reactions for a single trajectory of length n_{step} of the evolving reaction system.

The four reaction channels of the overall enzyme kinetics in an active site are (1)(E + S) $\xrightarrow{k_1}$ (ES), (2)(ES) $\xrightarrow{k_{-1}}$ (E + S), (3)(ES) $\xrightarrow{k_{-2}}$ (E + P) and (4)(E + P) $\xrightarrow{k_3}$ (ES). The medium entropy production in the overall trajectory consisting of n number of sub-reactions is given by

$$\Delta S_m(t) = n_{\text{step}} \sum_i \left(p_i \ln \frac{a_i}{a_i^R} \right), \quad i = \pm 1, \pm 2. \quad (31)$$

Here $p_i = \frac{n_i}{n_{\text{step}}}$, gives the probability of reaction- i over a trajectory where n_i being the number of times the i -th reaction occurs over this n_{step} trajectory. a_i is the average propensity for the i -th reaction in the forward trajectory and a_i^R is the corresponding quantity for the reverse reaction channel in the backward trajectory.

We define the average individual reaction propensities as, $a_{1,2} = k_{1,2} \left(\frac{e_i + e_f}{2} \right)$ and $a_{-1,-2} = k_{-1,-2} \left(\frac{x_i + x_f}{2} \right)$. Here e_i and e_f are the number of vacant sites at the start and at the end of the trajectory, respectively and x_i and x_f are the corresponding values for the occupied sites. As this formulation is in terms of the average quantities defined above, it is exactly valid at

the NESS. The average individual i -th reaction contribution to Δs_m for a single trajectory is

$$\Delta s_m^i = n_{\text{step}}(p_i \ln \frac{a_i}{a_i^R}). \quad (32)$$

This heuristic formula of medium entropy production can be rationalized in terms of the basic structure of the expression for the relative entropy or the Kullback-Leibler distance between two probability distributions $P(n)$ and $\tilde{P}(n)$ of a set of random variables n given by²¹

$$D_{\text{KL}}(P||\tilde{P}) = \sum_n P(n) \ln \frac{P(n)}{\tilde{P}(n)}. \quad (33)$$

If n represent the trajectories followed by the system corresponding to a set of variables and $P(n)$ and $\tilde{P}(n)$ denote normalized probability densities of these trajectories traversed in the forward and reverse directions, respectively then the above expression gives the total entropy production over the ensemble of trajectories. For a single n -th trajectory, the total entropy production is given by $\Delta s_{\text{tot}} = \ln \frac{P(n)}{\tilde{P}(n)}$. In the light of these formulae, Eq.(31) can be understood as the average per step medium entropy production for a single trajectory of the chemical reaction system with the average being performed over all the reaction channels comprising the trajectory. It is straightforward to see that this average individual reaction contribution to the entropy production can also be written for ΔS_{tot} in a single trajectory as $\Delta s_{\text{tot}} = \Delta s_m + \Delta s_{\text{sys}}$.

IV. RESULT AND DISCUSSION

In the oligomeric enzyme reaction system, we have calculated the total entropy production rate (epr), $\frac{dS_{\text{tot}}}{dt}$ from the master equation approach (see Eq.(16)). Here we have given its relation with the net rate of the reaction. Then we have checked the validity of the detailed fluctuation theorem by employing the results of entropy production using single trajectory simulation and its consequence thereon. Here we have taken the rate parameters as $k_1 = 15$, $k_{-1} = 7$, $k_{-2} = 2$ and $k_2 = 1$. The total number of subunits is considered as $n_T = 20$.

The macroscopic total entropy production rate(epr) are studied in figure 1, as a function of time. It is observed that the epr decreases sharply with time until it reaches the non-equilibrium steady state (NESS). The epr is higher at the NESS for higher force as the dissociation rate of the enzyme-substrate complex, ES to the product is increased with force.

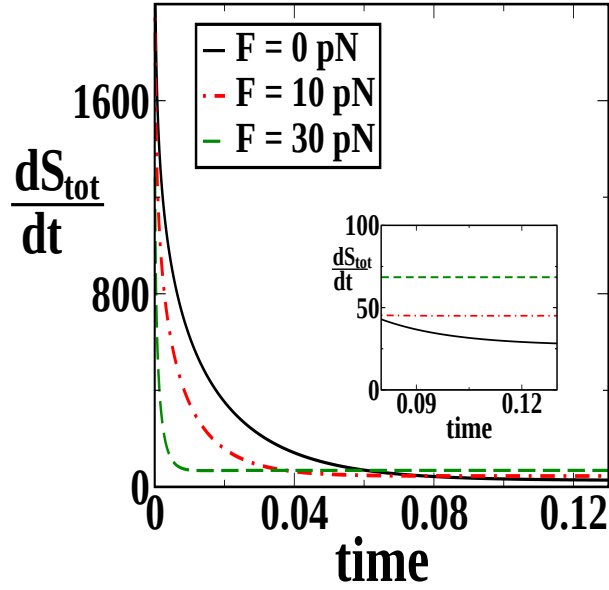


FIG. 1. Plots of entropy production rate versus time for different force parameters with rate parameters $k_1 = 15$, $k_{-1} = 7$, $k_{-2} = 2$, $k_2 = 1$ and $n_T = 20$

Initially, the dissociation rate of the ES complex in forming the product is less important as the ES complex is low in number and the rate constant of the product formation is low as well. Hence the overall reaction is mostly guided by the ES formation kinetics and its dissociation, giving back the substrate (and not forming the product). For higher force values, the importance of the product formation reaction increases corresponding to a decrease in epr at a faster rate. The value of epr at the NESS rises with increased force but it goes to saturation quickly at high forces as is evident from the inset in the figure. From the nature of the curves of epr at different forces, it is also clear that the epr values at very short times (before attaining the steady state value) are actually lower for higher forces. Hence the total entropy production rate shows opposite behaviors for short and long time intervals as a function of the force parameter.

In our earlier work⁴², we have calculated the net velocity of the reaction to show the dependence of the kinetic parameters namely, the external mechanical force and other chemical rate constants. The net velocity of the reaction system is defined as $v_{\text{net}} = k_{-2}\langle n \rangle - k_2\langle n_T - n \rangle$. Here we have shown how the trend in the net velocity is related to the rate of the total en-

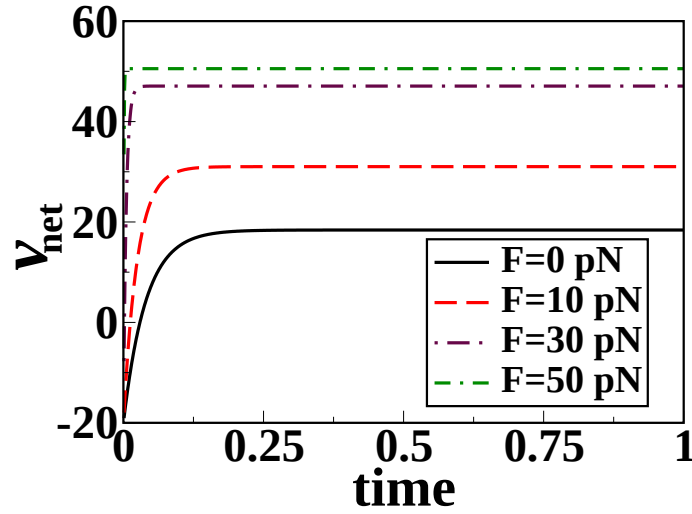


FIG. 2. Plot of the net velocity of the reaction versus time for different values of force with rate parameters, same as in figure 1

tropy production. The net velocity of the reaction increases with force and reaches a steady value as the ES-complex dissociation is enhanced by the force parameter kinetically. At lower force value and in small time, net velocity becomes negative which means no product formation has occurred but a consumption of product is taking place which is a typical feature of chemiostatic condition where the reactants and products are maintained at a constant concentration externally. However, after a certain time, net velocity becomes positive. With increase in force, net velocity becomes positive more quickly which is shown in figure 2. It is interesting to note that the net velocity of the reaction, v_{net} and the rate of the total entropy production, $\frac{dS_{\text{tot}}}{dt}$ both saturates at a quicker rate with increase in the value of force.

Next, we have determined the total, system and medium entropy production using the Eqs.(25-27) from the averaging over trajectories obtained from the simulation (over 2×10^5 trajectories). We have also theoretically determined ΔS_{tot} by integration of the epr formula (Eq.(16)) over a specified time interval. In the figure 3(a), we have plotted ΔS_{tot} as a function of time obtained from both the ways discussed above for two different force parameters. The stochastic simulation results and the master equation results tally very well. ΔS_{tot} increases with time, the rise being linear after it reaches the steady state (NESS). The steady state comes earlier for higher force as shown in figure 3(a). The lower ΔS_{tot} at short times and higher ΔS_{tot} at longer times for $F=30$ pN compared to $F=0$ pN is clear from the figure and is

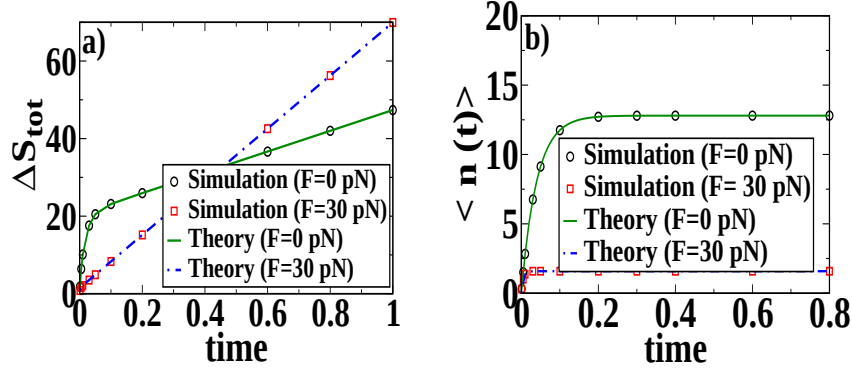


FIG. 3. (a) ΔS_{tot} is calculated from master equation (theory). Simulation results is obtained by averaging over 2×10^5 of single trajectories for different force values, $F=0$ and $F=30$ pN with the same rate parameters as in figure 1. (b) average number of occupied sites $\langle n \rangle$, is also calculated by using the same parameters for different force values.

consistent with the idea obtained from the epr versus time curve. The average occupied site population, $\langle n(t) \rangle$, obtained at two different forces is plotted in figure 3(b) $\langle n(t) \rangle$, decreases significantly with the application of force as the force increases the dissociation rate of the ES complex. This is a more detailed check of the validity of the master equation proposed approximately in the earlier publication⁴².

We have plotted the values of ΔS_{tot} , ΔS_{m} and ΔS_{sys} in figure 4 obtained from the simulation at different forces as a function of time. Normally the medium entropy production grows linearly with time at NESS but the system entropy production remains a constant; this picture is clarified in terms of the variation of the force parameter. The variation of ΔS_{m} is shown in figure 4(a). ΔS_{m} increases with time, following the same trend as that of ΔS_{tot} (shown in figure 4(c)). It also shows similar behavior as a function of the force parameter

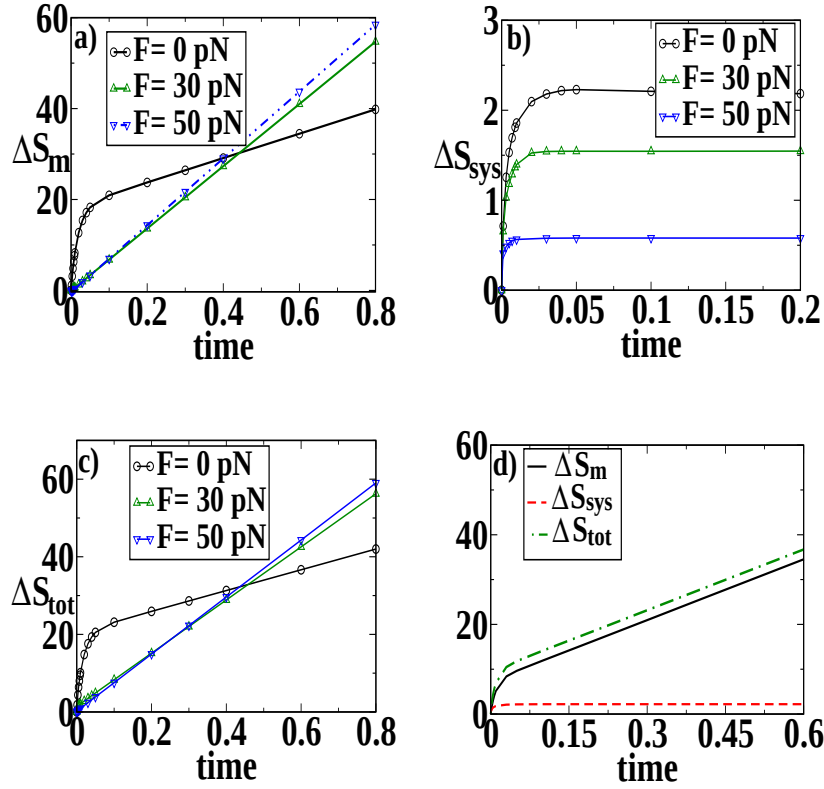


FIG. 4. Plot of (a) ΔS_m versus time, (b) ΔS_{sys} versus time and (c) ΔS_{tot} versus time for force, $F=0$ pN, $F=30$ pN and $F=50$ pN. The rate parameters are same as in figure 1. (d) ΔS_m , ΔS_{sys} and ΔS_{tot} are plotted against time for $F=10$ pN with the same rate parameters.

regarding the values at short and long time intervals. The value of ΔS_m is lower for higher force value at early time which is shown in the figure 4(a). In figure 4(b) it is observed that after a small time the ΔS_{sys} reaches a steady value and with increase in force value it reaches the steady value earlier and the magnitude of the ΔS_{sys} decreases with increase in force. The value of ΔS_{tot} , is the summation of the ΔS_{sys} and the ΔS_m . However, the magnitude of the ΔS_{sys} is small compared to the value of the ΔS_m . Hence the nature of the curve of ΔS_{tot} versus time follows the ΔS_m versus time plot which is confirmed from the figure 4(d). Therefore, it can be said that at $t \rightarrow \infty$, $\Delta S_m \rightarrow \Delta S_{tot}$ which is very useful when ΔS_{sys} is not known as any rate equation is not available although the detailed steps of the reaction may be known.

From figure 5, we first give a comparison of reaction probabilities of the four sub-reactions.

This analysis will clarify the mechanism of the overall reaction and show how the individual rates of the sub-reactions compete with each other in the time evolution on an average over a bunch of trajectories. We see that the probabilities (average over 2×10^5 trajectories) of second and third reactions increase with time and that for first and fourth reactions decrease with time with all of them reaching a point of saturation indicating the NESS. Now with increasing force, the dissociation of the ES complex is favored by the second and third reactions. So their probabilities increase with force and the probabilities of first and fourth reactions decrease to balance the effect. In our system, the probabilities of first and second reactions are higher than that for third and fourth reactions (except at very short time). In addition to that, the probability of the first reaction is always greater than that of the second but the difference between these two probabilities reduces with increasing force. The probabilities of third and fourth reactions show a different behaviour at short times. At force, $F=0$, the reaction probability of third remains lower than that of the fourth reaction for longer times. With higher forces, the probability of the third reaction becomes greater than that of the fourth reaction almost instantly. Also the probability of the third reaction is always greater than that of the fourth reaction at NESS. Opposite to the case of reaction pair one and two, here the difference between the two reaction probabilities, third and fourth, enhance with increasing force except at very short time where the behaviour can be different.

In figure 6, we have studied the average contribution of individual sub-reactions (1-4) in a single trajectory to the overall medium entropy production, ΔS_m as a function of time. The ΔS_m is calculated according to Eq. (31) for a single trajectory and then ensemble average is taken. We have found a reasonable tally between this result and the exact simulation result with the result obtained from Eq. (32) mainly differing by a constant from the exact simulation. We will show this result later in this section. As the entropy production in the stochastic events of sub-reactions can be both positive and negative, so it is highly interesting to find out how an individual reaction contributes to the negative and positive entropy production in a single trajectory. The average contribution of each sub-reaction to the overall medium entropy should also depend on the propensities of the individual reaction channels. In figure 6, it is observed that the contribution of reaction one to ΔS_m , i.e, $|\Delta S_m^1|$ increases with time almost linearly (except some transient behaviour at lower forces). For high force values like $F=50$ pN, it's sign and contribution becomes negative due to the very

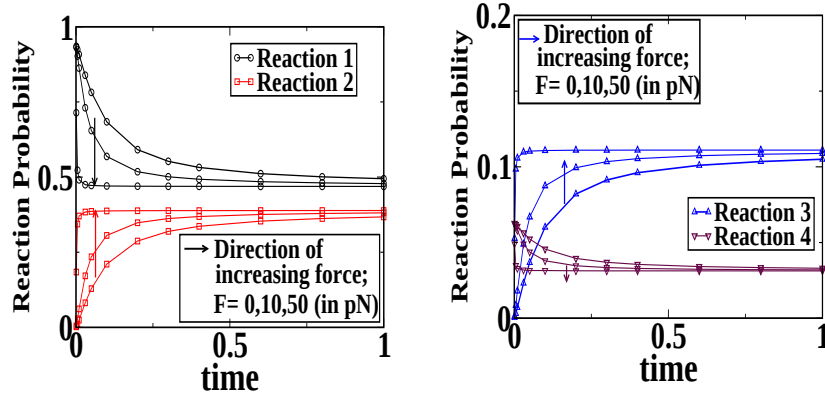


FIG. 5. Reaction probabilities are plotted against time for reactions (1-4) for different force values. The rate parameters of the reactions are same as in figure 1.

large rate constant K_2 . The behaviour of the counter reaction channel two is opposite as expected. Its contribution, $|\Delta S_m^2|$, shows the same linear increase with time and becomes positive at high force values. The opposite trends of these two reactions regarding the ΔS_m contribution is also seen for the reaction-pair three and four. But in the case of this reaction-pair, the difference between the individual reaction contributions, ΔS_m^3 and ΔS_m^4 , increases more rapidly with force compared to the reaction-pair of one and two. That ultimately leads to higher ΔS_m of the overall reaction system for higher forces at NESS. So one can say that the steep rise of ΔS_m^3 actually gives the major net positive contribution to the ΔS_m at higher forces. Now for low to moderate force values, at short times, the probability of reaction one is sufficiently higher compared to other reaction probabilities. So the nature of the overall ΔS_m curve is governed by the nature of ΔS_m^1 which shows lower (positive) values for higher forces (from $F=0$ pN to $F=30$ pN) at short times. If we closely look at the ΔS_m curves at different forces (fig. 4a), we can see that the time at which the ΔS_m curve for a higher force crosses that of the next lower force becomes progressively smaller with the increasing

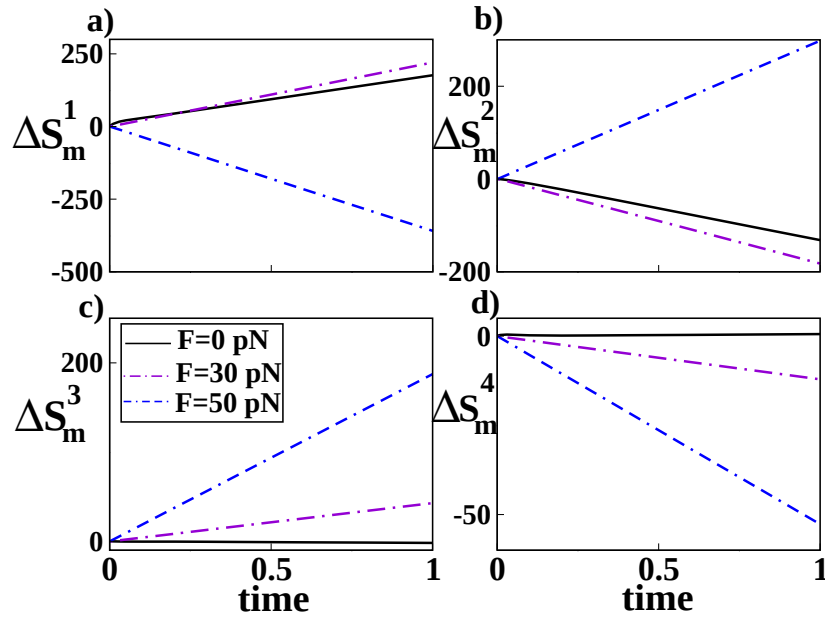


FIG. 6. Contribution of reactions (1-4) to ΔS_m as a function of time are plotted for different force values. The rate parameters of the reactions are taken same as in fig.1.

magnitude of the force. That shows the increasing reaction probability effect for reactions two and three for higher forces. Particularly, at $F=50$ pN, both ΔS_m^2 and ΔS_m^3 give large positive contributions.

A. Numerical demonstration for two types of substrates

Here we have considered that two different types of substrates namely, S_1 and S_2 can independently attach with the single oligomeric enzyme with different rate parameters. Therefore, two sets of reactions with different rate parameters are considered for two different substrates as already discussed in Sec.IIc. Reactions (1-4), $(1)E + S_1 \xrightarrow{k_{11}} ES_1$, $(2)ES_1 \xrightarrow{k_{-11}} E + S_1$, $(3)E + P_1 \xrightarrow{k_{21}} ES_1$ and $(4)ES_1 \xrightarrow{k_{-21}} E + P_1$, are considered for substrate S_1 and (5-8), $(5)E + S_2 \xrightarrow{k_{12}} ES_2$, $(6)ES_2 \xrightarrow{k_{-12}} E + S_2$, $(7)E + P_2 \xrightarrow{k_{22}} ES_2$ and $(8)ES_2 \xrightarrow{k_{-22}} E + P_2$, are for substrate, S_2 . From figure 7(a) and (b), it is observed that if the rate parameters of the reactions of substrate S_2 is half of that of substrate S_1 , then the reaction probabilities of the reactions involving substrate S_2 becomes half of the reaction probabilities of the reactions of substrates S_1 . If the rate parameters of the reactions of S_2 is double than that of S_1 , the reaction probabilities of the reactions of S_2 become double also, which is shown in figure

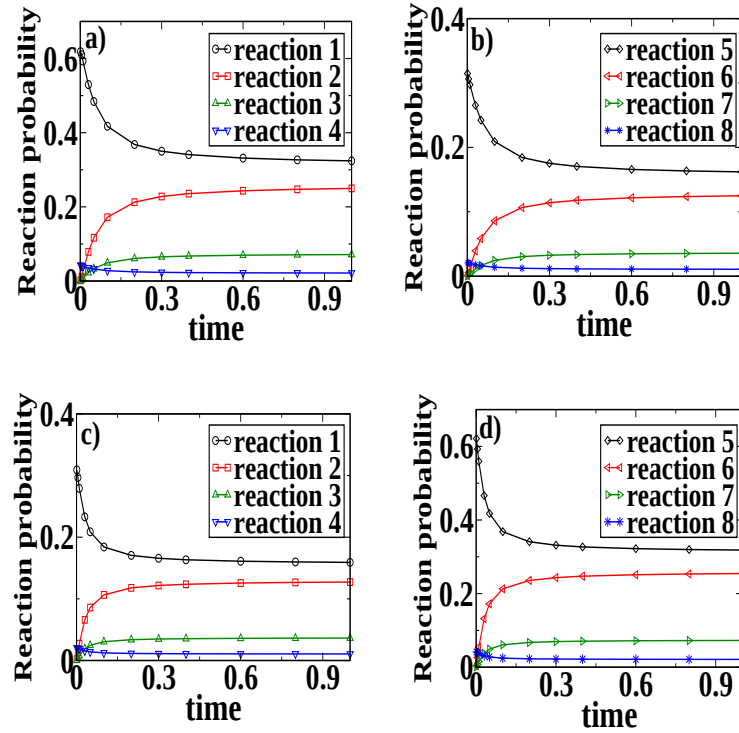


FIG. 7. Reaction probability versus time is plotted for the mixture of two substrates. In figure (a) and (b), the rate parameters of the reactions (5-8) is half of the rate parameters of reaction (1-4). The rate parameters are; $k_{11} = 15$, $k_{-11} = 7$, $k_{-21} = 2$, $k_{21} = 1$, $k_{12} = 7.5$, $k_{-12} = 3.5$, $k_{-22} = 1$ and $k_{22} = 0.5$. In figure (c) and (d), the rate parameters of the reactions (5-8) is double of the rate parameters of reaction (1-4). The rate parameters are; $k_{11} = 15$, $k_{-11} = 7$, $k_{-21} = 2$, $k_{21} = 1$, $k_{12} = 30$, $k_{-12} = 14$, $k_{-22} = 4$ and $k_{22} = 2$. The force parameter is set as zero.

7(c) and (d). All the results are calculated for zero force value.

Similarly from figure 8(a) and (b), it is observed that when the rate parameters of the reactions of substrate S_2 are half of those for substrate S_1 , then the contribution of the reactions of S_2 in ΔS_m becomes half of the contribution of the reactions of substrate S_1 . Again the contribution becomes double when the rate parameters of the reactions of S_2 is double of the reactions of S_1 which is shown in figure 8(c) and (d). Therefore, it can be said that the probability of occurrence of a reaction and more importantly, its contribution to the medium entropy production, ΔS_m is proportional to the rate parameter of the reaction. So the extensivity of the entropy production with respect to the detailed sub-reactions and rate

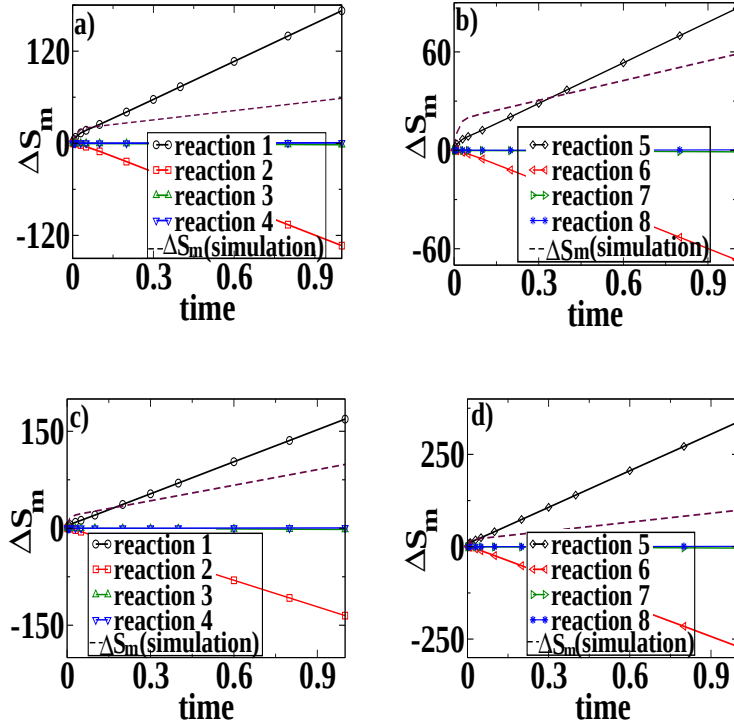


FIG. 8. Contribution of the reactions to ΔS_m against time is plotted in figure (a) to (d). In first two plots, (a) and (b), rate constants of reaction (5-8) are half of reactions (1-4). The rate parameters are $k_{11} = 15$, $k_{-11} = 7$, $k_{-21} = 2$, $k_{21} = 1$, $k_{12} = 7.5$, $k_{-12} = 3.5$, $k_{-22} = 1$ and $k_{22} = 0.5$. In next two plots, (c) and (d), rate constants of reaction (5-8) are double of reactions (1-4). The rate parameters are $k_{11} = 15$, $k_{-11} = 7$, $k_{-21} = 2$, $k_{21} = 1$, $k_{12} = 30$, $k_{-12} = 14$, $k_{-22} = 4$ and $k_{22} = 2$. The force parameter is set as zero.

constants are established here which is necessary to implement thermochemistry at NESS.

We have constructed a heuristic formula of medium entropy production as an average over the contributions of the sub-reactions to calculate the ΔS_m . The result from the formula shows a good correspondence with the simulation result differing mainly by a constant value at the steady state which is shown in figure 9(a) and (b). Figure 9(a) is plotted by considering only one type of substrate molecule whereas mixture of two substrates are considered to obtain figure 9(b). From the expression in the formula (Eq.31), $\Delta S_m = n_{\text{step}} \sum_i p_i \ln \frac{a_i}{a_i^R}$, for a trajectory of length n_{step} , it is evident that the difference between the actual simulation result and that obtained from this formula is a measure of how far the system is from the

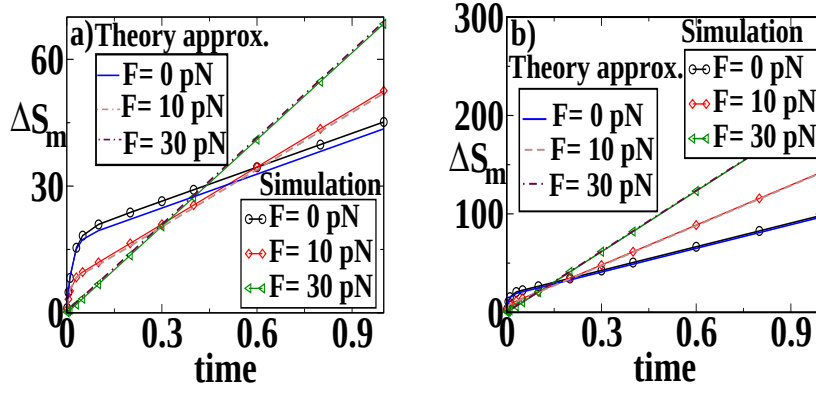


FIG. 9. (a) Plot of ΔS_m versus time for different force values by considering only one type of substrate molecule. ΔS_m values are obtained from simulation and our predicted formula. The rate parameters are $k_1 = 15$, $k_{-1} = 7$, $k_{-2} = 2$ and $k_2 = 1$. In figure (b), ΔS_m versus time is plotted by considering the mixture of two substrate molecules. The rate parameters are $k_{11} = 15$, $k_{-11} = 7$, $k_{-21} = 2$, $k_{21} = 1$, $k_{12} = 30$, $k_{-12} = 14$, $k_{-22} = 4$ and $k_{22} = 2$.

NESS at the initial condition. That is why for higher force parameters, where the steady state is obtained more and more quickly, the discrepancy between the two results gradually vanishes. At $F = 30$ pN, the two results are almost the same throughout the time span studied. The formula is exact at the NESS. Hence this gives a microscopic description of the medium entropy production (flow) at the level of individual reactions for a single trajectory from the knowledge of the reaction probabilities and the initial and final populations of the species concerned over the trajectory.

V. CONCLUSION

In our previous work we had constructed the stochastic master equation to describe the kinetics of an oligomeric enzyme with many active sites where an externally controllable mechanical force could affect the rate constant of the enzyme-substrate complex and we had calculated the net rate of the reaction at NESS. Using the same master equation, as a first step, we have shown the relation between the macroscopic total entropy production rate with the net rate of the reaction for this system.

We have calculated the entropy production along a single stochastic trajectory by using the Gillespie stochastic simulation approach to study the individual contribution of each reaction channel or sub-reaction to the medium entropy production by the formalism of forward and reverse trajectory approach. Here we have constructed a heuristic formula for individual reaction contribution to the medium entropy. The formula gives a microscopic description of the medium entropy production at the level of individual sub-reactions from the knowledge of the individual sub-reaction probabilities and the initial and final populations of the species concerned over the trajectory. We have generalized the single oligomeric enzyme reaction system for one specific substrate to multiple substrates case. From the consequence of the fluctuation theorem about the individual contribution of each reaction channel to the overall medium entropy production, one can get an insight about the mechanism of a multi-step reaction, a step towards the nonequilibrium thermochemistry.

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