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Enzyme efficiency: An open reaction system perspective

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A measure of enzyme efficiency is proposed for an open reaction network that, in suitable form, applies to closed systems as well. The idea originates from the description of classical enzyme kinetics in terms of cycles. We derive analytical expressions for the efficiency measure by treating the network not only deterministically but also stochastically. The latter accounts for any significant amount of noise that can be present in biological systems and hence reveals its impact on efficiency. Numerical verification of the results is also performed. It is found that the deterministic equation overestimates the efficiency, the more so for very small system sizes. Roles of various kinetics parameters and system sizes on the efficiency are thoroughly explored and compared with the standard definition k_2/K_M. Study of substrate fluctuation also indicates an interesting efficiency-accuracy balance. © *2015 AIP Publishing LLC.* [http://dx.doi.org/10.1063/1.4937792]

I. INTRODUCTION

Enzymes are natural catalysts of immense importance regulating numerous biological and physiological processes.[1,2] Their actions and mechanisms were thus explored in detail over the years, with studies ranging over various time scales as well as length scales.[3–8] In characterizing the enzyme kinetics, the Michaelis-Menten (MM) equation[1] stands as the benchmark for bulk *in vitro* experiments. It provides a ready estimation of key rate parameters. The data from modern single-molecule experiments are also compared and contrasted against the MM model, with the variables suitably modified.[7,9] However, recent theoretical studies indicate that in presence of noise, the MM equation can fail to accurately predict the rate of the reaction.[10,11] The quasi-steady-state approximation that lies at the core of the MM equation often breaks down in small systems with low copy number of reacting species[12] and also in open systems.[13] The latter observation is very important as it represents many naturally occurring systems associated with flow. In this work, therefore, we shall focus mainly on the enzyme kinetics in an open system framework.

The MM scheme for single enzyme-single substrate reaction is written as

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1'}{\rightleftharpoons}} \mathrm{C} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}. \tag{1}$$

The enzyme-substrate complex is denoted by C. A widely used measure of enzyme efficiency in converting the substrate into product is the k_2/K_M (or more generally k_{cat}/K_M) ratio.[1,2] This is because these parameters are the ones that are readily obtained from experiments, e.g., $K_M = (k_{-1} + k_2)/k_1'$, called the MM constant.

In a closed reaction volume (batch reactor), however, the system reaches equilibrium with complete conversion of substrate into product and full recovery of the enzyme. While this is true for any enzyme following MM scheme (1), the time required for this conversion seems to be a logical candidate in discussions on the "efficiency" of a specific enzyme. In this context, we may mention some recent works that address the enzyme kinetics problem from the standpoint of cycle-time.[14–16] The attention has thus been shifted from rate, though the original concept had been developed much earlier.[17] Assuming that the substrate is in large excess, the average duration of each cycle τ_c and the total average number of cycles required for the complete course of the reaction $\bar{n}_c$ have been shown to have the forms[16]

$$\tau_c = \frac{K_M + [\mathrm{S}]_0}{k_1' K_M [\mathrm{S}]_0}, \quad \bar{n}_c = \frac{(k_{-1} + k_2)[\mathrm{S}]_0}{k_2 [\mathrm{E}]_0}. \tag{2}$$

Here, $[\mathrm{S}]_0$ and $[\mathrm{E}]_0$ are the initial substrate and enzyme concentrations, respectively. Same results can also be derived by assuming chemiostatic conditions where the substrate number is kept fixed by some suitable mechanism.

One can immediately see that the product of the two quantities given in Eq. (2) provides an estimate of the reaction completion time in a closed system. Hence, we define the efficiency ζ as its inverse, i.e.,

$$\zeta = (\bar{n}_c \tau_c)^{-1} = \frac{k_2 [\mathrm{E}]_0}{K_M + [\mathrm{S}]_0}. \tag{3}$$

Along with the rate parameters, ζ explicitly contains the (initial) concentration terms. Eq. (3) is similar in structure to the turnover rate (per enzyme), with ζ having the dimension of rate. The only difference is that $[\mathrm{S}]_0$ also appears in the numerator of turnover rate. It follows desirably from Eq. (3) that for a fixed set of rate parameters and $[\mathrm{E}]_0$, higher $[\mathrm{S}]_0$ requires a longer time for full conversion into product, resulting in lower ζ. The proportionality of ζ to $[\mathrm{E}]_0$ can be similarly explained.

With this background, we now ask the following questions: (i) How can the enzyme efficiency be defined in a biological reaction system with flow that is consistent with Eq. (3)? (ii) What is the role of stochasticity or noise

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in such an open system? We shall address both the issues in what follows. In Sec. II, an open reaction network is analytically studied using both deterministic rate equation (DRE) and chemical master equation (CME) techniques. The steady-state (SS) results are compared to understand the effect of noise for different system sizes. We clarify that in our work, the SS refers to a situation out-of-equilibrium with non-zero stationary fluxes[18] and *time-independent populations of all the species*. This is unlike some other prevalent works where the product is kept out of the SS condition. This means, even in the SS, there is a non-zero product formation rate.[10,11] In Sec. III, we analyze the effects of various kinetic parameters and system sizes on the efficiency numerically. The paper is concluded in Sec. IV.

II. OPEN REACTION NETWORK

We consider an open network where the substrate is injected at a constant rate (of flow) γ_s and the product flows out of the system with a first-order rate constant k_p. The enzyme is present within the system from the onset of reaction with some initial population. The situation is shown schematically as[13]

$$\emptyset \xrightarrow{\gamma_s} \mathrm{S} + \mathrm{E} \underset{k_{-1}}{\overset{k_1'}{\rightleftharpoons}} \mathrm{C} \xrightarrow{k_2} \mathrm{E} + \mathrm{P} \xrightarrow{k_p} \emptyset. \tag{4}$$

Under suitable conditions, the reaction system reaches SS as described below. One may consider the scheme in (4) as a part of a larger (say, a metabolic) network.

A. Deterministic description

Using the DREs for network (4) (given in Appendix A), one obtains the SS concentrations as

$$\begin{aligned}[\mathrm{S}] &= \frac{\gamma_s K_\mathrm{M}}{k_2[\mathrm{E}]_0 - \gamma_s},\ [\mathrm{C}] = \frac{[\mathrm{E}]_0[\mathrm{S}]}{K_\mathrm{M} + [\mathrm{S}]},\\ [\mathrm{E}] &= [\mathrm{E}]_0 - [\mathrm{C}],\ [\mathrm{P}] = \frac{k_2[\mathrm{C}]}{k_p} = \frac{\gamma_s}{k_p}.\end{aligned} \tag{5}$$

Thus, to achieve the SS, one needs $k_2[\mathrm{E}]_0 > \gamma_s$.

Now, a general definition of efficiency must be formulated as a ratio of output to input. For the open system under study, the (deterministic) efficiency at any instant can be expressed as the ratio of product formation rate, or equivalently the outflow rate, and the substrate concentration inside the reaction volume as follows:

$$(\zeta)_\mathrm{det}(t) = \frac{k_p[\mathrm{P}](t)}{[\mathrm{S}](t)}. \tag{6}$$

The utility of such a definition is that, at SS, from Eq. (5), we get

$$(\zeta)_\mathrm{det} = \frac{k_2[\mathrm{E}]_0}{K_\mathrm{M} + [\mathrm{S}]}. \tag{7}$$

Interestingly, this is the same as Eq. (3), obtained from the cycle description of MM kinetics in closed system, except for the crucial fact that *here, the substrate concentration at SS is required rather than* $[\mathrm{S}]_0$. Putting the expression of [S] from Eq. (5) into Eq. (7), one gets

$$(\zeta)_\mathrm{det} = \frac{k_2[\mathrm{E}]_0 - \gamma_s}{K_\mathrm{M}}, \tag{8}$$

revealing explicitly the effect of γ_s, the inflow rate. For $\gamma_s = 0$, it follows from Eq. (8) that the efficiency (scaled by $[\mathrm{E}]_0$) reduces to the standard measure of k_2/K_M for closed systems.

B. Stochastic description

Here, we study the case of a single enzyme molecule. The CME with substrate inflow and product outflow is constructed separately for the two possible states of the single enzyme molecule as[10,11]

$$\begin{aligned}\partial_t \Pi_0(N,P,t) = &-k_1 N \Pi_0(N,P,t) + k_{-1}\Pi_1(N-1,P,t)\\ &+ k_2 \Pi_1(N,P-1,t) + k_s \Pi_0(N-1,P,t)\\ &- k_s \Pi_0(N,P,t) + k_p(P+1)\Pi_0(N,P+1,t)\\ &- k_p P \Pi_0(N,P,t)\end{aligned} \tag{9}$$

and

$$\begin{aligned}\partial_t \Pi_1(N,P,t) = &\,k_1(N+1)\Pi_0(N+1,P,t) - k_{-1}\Pi_1(N,P,t)\\ &- k_2 \Pi_1(N,P,t) + k_s \Pi_1(N-1,P,t)\\ &- k_s \Pi_1(N,P,t) + k_p(P+1)\Pi_1(N,P+1,t)\\ &- k_p P \Pi_1(N,P,t).\end{aligned} \tag{10}$$

In Eqs. (9) and (10), $\Pi_j(N,P,t)\,(j = 0,1)$ denotes the probability to realize the state of the system with N substrate molecules, P product molecules, and the enzyme in the free ($j = 0$) or in the bound ($j = 1$) state, at time t. The mesoscopic rate constants are related to the corresponding macroscopic ones by $k_1 = k_1'/\Omega$ and $k_s = \gamma_s\Omega$, Ω being the volume of the reaction compartment.

The rate of the reaction v, quantified in terms of the rate of (mean) product formation, is given by

$$\begin{aligned}v = \frac{d\langle P\rangle}{dt} &= \frac{d}{dt}\sum_{N,P} P(\Pi_0(N,P,t) + \Pi_1(N,P,t))\\ &= k_2 \sum_{N,P} \Pi_1(N,P,t) - k_p\langle P\rangle.\end{aligned} \tag{11}$$

At SS, we set $v = 0$. Here, we depart from the previous works.[10,11] Following Eq. (6), the (stochastic) efficiency at SS should be naturally expressed as

$$(\zeta)_\mathrm{sto} = \frac{k_p\langle P\rangle}{\langle N\rangle}. \tag{12}$$

So, the mean substrate and product populations are needed to evaluate ζ. To this end, we choose the approach of Stefanini *et al.*[10] to define

$$Q_j(N,t) \equiv \sum_P \Pi_j(N,P,t), \quad \eta_q^{(j)} \equiv \sum_N N^q Q_j(N,t). \tag{13}$$

Using Eqs. (9) and (10) and the first expression of Eq. (13), one can hence write down the evolution equations of Q_0 and Q_1. Then, setting those equations to zero, one obtains the

following two sets of algebraic equations at SS:

$$(k_1 N + k_s)Q_0(N) = k_{-1}Q_1(N-1) + k_2 Q_1(N) + k_s Q_0(N-1), \tag{14}$$

$$k_1(N+1)Q_0(N+1) + k_s Q_1(N-1) = (k_{-1} + k_2 + k_s)Q_1(N). \tag{15}$$

Summing over "N," we get from Eqs.(14) and (15),

$$\eta_1^{(0)} = K_M' \eta_0^{(1)}, \tag{16}$$

where $K_M' = \frac{k_{-1}+k_2}{k_1} = K_M\Omega$. Multiplying Eq. (14) by "N" and then summing over "N" give

$$\eta_2^{(0)} = K_M' \eta_1^{(1)} + \frac{k_{-1}}{k_1}\eta_0^{(1)} + \frac{k_s}{k_1}\eta_0^{(0)}. \tag{17}$$

A similar procedure yields from Eq. (15),

$$\eta_2^{(0)} = K_M' \eta_1^{(1)} + \eta_1^{(0)} - \frac{k_s}{k_1}\eta_0^{(1)}. \tag{18}$$

At SS, it follows from Eqs. (11), (13), (17), and (18) that

$$k_2 \eta_0^{(1)} = k_s = k_p \langle P \rangle. \tag{19}$$

Eq. (19) actually gives the flux-balance at SS and confirms the validity of the analytical treatment.

Now, multiplying Eqs. (14) and (15) by "N^2," summing over "N," and then eliminating $\eta_3^{(0)}$, we get (for details, see Appendix B)

$$2k_1\eta_2^{(0)} = k_s + 2k_s\langle N \rangle + k_1\eta_1^{(0)} + k_{-1}\eta_0^{(1)} + 2k_{-1}\eta_1^{(1)}, \tag{20}$$

where $\langle N \rangle = \eta_1^{(0)} + \eta_1^{(1)}$ and $\eta_0^{(0)} + \eta_0^{(1)} = 1$ give the normalization. Eliminating $\eta_2^{(0)}$ between Eqs. (17) and (20), one gets

$$\eta_0^{(1)} = \frac{k_s + 2\langle N \rangle (k_2 - k_s)}{k_2 + 2(k_s + k_2 K_M')}. \tag{21}$$

Next, eliminating $\eta_0^{(1)}$ between Eqs. (19) and (21), we finally have

$$\langle N \rangle = \frac{k_s(k_s + k_2 K_M')}{k_2(k_2 - k_s)}. \tag{22}$$

Eq. (22) can also be expressed as

$$\frac{\langle N \rangle}{\Omega} = \frac{\gamma_s(\gamma_s + k_2 K_M)}{k_2(k_2\Omega^{-1} - \gamma_s)}. \tag{23}$$

For $k_2 K_M \gg \gamma_s$, Eq. (23) reduces to the deterministic form (see Eq. (5))

$$(\langle N \rangle/\Omega)_{det} \equiv [S] = \frac{\gamma_s K_M}{(k_2[E]_0 - \gamma_s)}, \tag{24}$$

where, for the single enzyme molecule, $[E]_0 = \Omega^{-1}$. Then, from Eqs. (23) and (24), we can determine the deviation due to noise as

$$\frac{(\langle N \rangle/\Omega)}{[S]} \equiv \Delta = 1 + \frac{\gamma_s}{k_2 K_M} = 1 + \frac{k_s}{k_2 K_M'}. \tag{25}$$

According to Eq. (23), SS is attained for $k_2\Omega^{-1} > \gamma_s$ (see Eq. (5)). Let us choose $k_2 = \alpha\gamma_s\Omega\,(\alpha > 1)$. Then, Eq. (25) becomes

$$\Delta = 1 + \frac{1}{\alpha K_M \Omega}. \tag{26}$$

So, for fixed rate parameters (and fixed α), the deviation due to noise increases for smaller reaction volume and vanishes in the $\Omega \to \infty$ limit, as expected. For a given Ω, on the other hand, the lower be the value of K_M and/or α, higher will be the deviation.

It is easy to see from Eqs. (12), (19), and (22) that

$$\frac{(\zeta)_{det}}{(\zeta)_{sto}} = \Delta \tag{27}$$

with

$$(\zeta)_{sto} = \frac{k_2(k_2 - k_s)}{k_s + k_2 K_M'}, \quad (\zeta)_{det} = \frac{k_2 - k_s}{K_M'} = \frac{k_2[E]_0 - \gamma_s}{K_M}. \tag{28}$$

For a particular enzyme-substrate pair with a given Ω and k_s, it is therefore evident from Eq. (28) that

$$k_2/K_M' > (\zeta)_{det} > (\zeta)_{sto}. \tag{29}$$

Thus, Eq. (25) and Eq. (27) show that *the deterministic equations underestimate the mean substrate population and overestimate the efficiency*. The mean product population remains the same in either the presence or the absence of noise, i.e., $\langle P \rangle/\Omega = \gamma_s/k_p = [P]$.

III. NUMERICAL RESULTS AND DISCUSSION

In this section, we will numerically analyze how the system size and various rate parameters affect the disparity (Δ) between deterministic and stochastic results. The fluctuation of substrate population is quantified in terms of the coefficient of variation, $CV = (\langle N^2 \rangle - \langle N \rangle^2)^{1/2}/\langle N \rangle$. The derivation of the second moment of "N" is given in Appendix C. In all the cases, mesoscopic rate constants are used with the "mole" unit converted into numbers. The enzyme kinetics parameters are taken over appropriate ranges, following a recent study on enzyme efficiency.[19]

A. The role of inflow rate and system volume

Equation (22) suggests that $\langle N \rangle$ will rise sharply with increase in k_s. Thus, efficiency will decrease with k_s. This is shown in Fig. 1 for both stochastic and deterministic cases, along with the standard measure k_2/K_M' (in mesoscopic form). They approach each other as $k_s \to 0$, as already mentioned. Other parameters are kept fixed at $k_1' = 10^8$ $M^{-1}s^{-1}$, $k_{-1} = 1\ s^{-1}$, $k_2 = 10\ s^{-1}$ that give $K_M = 0.11\ \mu M$. We have also checked that for $K_M \approx 100\ \mu M$, the median value obtained from a large data set of enzyme parameters,[19] the effect of noise is significant only for $\Omega \leq 10^{-18}$ L. This is in line with earlier findings.[8]

The deviations Δ are plotted in Fig. 2(a) as a function of k_s for three different system volumes Ω with $K_M = 0.11\ \mu M$. For a given Ω and rate constants, Δ increases linearly with k_s as expected from Eq. (25) and rises with reduction of Ω. The corresponding CVs of substrate population are shown in Fig. 2(b). The CVs are higher for lower Ω and at a given Ω value falls with k_s as $\langle N \rangle$ rises. It is interesting to note that for a fixed system size, a higher Δ does not necessarily mean a higher CV. Actually, the opposite is true for the cases shown in Fig. 2. We also perform simulations, using the standard

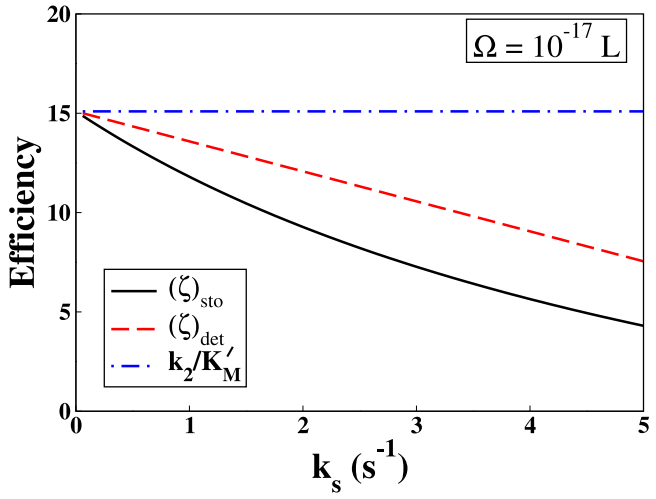

FIG. 1. Variation of efficiency as a function of k_s for stochastic and deterministic cases with $\Omega = 10^{-17}$ L and $K_M = 0.11$ μM. The standard measure (in mesoscopic form) k_2/K'_M is compared with these estimates.

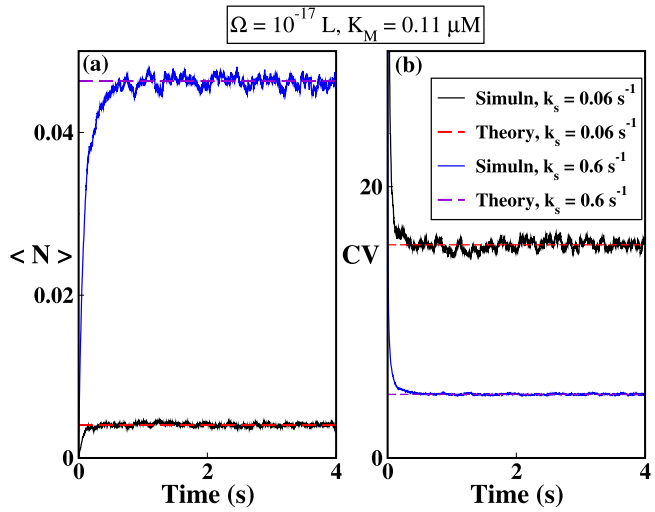

FIG. 3. Variation of (a) $\langle N \rangle$ and (b) the CV as a function of time for $\Omega = 10^{-17}$ L and $K_M = 0.11$ μM. The results obtained from simulation are seen to nicely approach the corresponding steady-state analytical results.

stochastic simulation algorithm (SSA),[20,21] to test the sanity of our analytical results. The initial condition is chosen as follows: $N = 0$, $N_E = 1$, $N_C = 0$, and $P = 0$, where N_E and N_C denote the number of enzyme and complex, respectively. The number of runs is set to 50 000 and code is written in FORTRAN. Some representative cases are depicted in Fig. 3. The results obtained from simulation are found to approach the corresponding steady-state analytical results nicely.

B. The role of rate constants k_1, k_2, k_{-1}

It follows from Eqs. (22) and (25) that $\langle N \rangle$ falls off in a hyperbolic manner and Δ rises linearly with increase in k_1, respectively. So, the CV is expected to rise to a saturation with k_1. These are shown to be the cases in Fig. 4 for $k_{-1} = 100$ s^{-1}, $k_2 = 10$ s^{-1}, $\Omega = 10^{-17}$ L, and $k_s = 6$ s^{-1}. The efficiency estimates, on the contrary, need a closer look. Equation (28) predicts a linear rise of $(\zeta)_{\text{det}}$ as a function of k_1, whereas $(\zeta)_{\text{sto}}$ is expected to grow to a saturation. These analytical findings tally with the plots shown in Fig. 4(b).

Next, the effect of k_2 on various properties is depicted in Fig. 5 with $k'_1 = 10^9$ $M^{-1}s^{-1}$, $k_{-1} = 100$ s^{-1}, $\Omega = 10^{-17}$ L, and $k_s = 6$ s^{-1}. An increase in k_2 results in higher K'_M and lower Δ (see Eq. (25)). For the given set of parameters, $\langle N \rangle$ falls, and hence, efficiency and CV rise with k_2, similar to the trend observed in the case of k_1 variation. However, all the efficiency estimates tend to merge at high k_2, which is easy to follow from Eq. (28).

Finally, Eqs. (22) and (24) show that $\langle N \rangle$ grows linearly in both stochastic and deterministic cases and Δ decreases in a hyperbolic fashion with k_{-1} (see Eq. (28)). Also, the CV and all the efficiency measures diminish as k_{-1} increases (not shown in figures).

It can be clearly seen from the figures and discussions so far that (i) $(\zeta)_{\text{sto}}$ shows a qualitatively similar trend of variation as that of CV, and (ii) a higher $(\zeta)_{\text{sto}}$ value is associated with a higher CV. Thus, a more efficient enzyme causes a greater

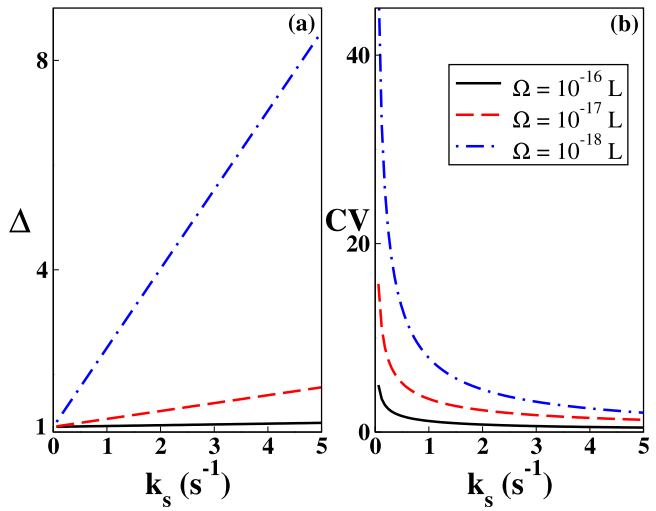

FIG. 2. (a) The deviation Δ and (b) the CV as a function of k_s for three different system volumes with $K_M = 0.11$ μM.

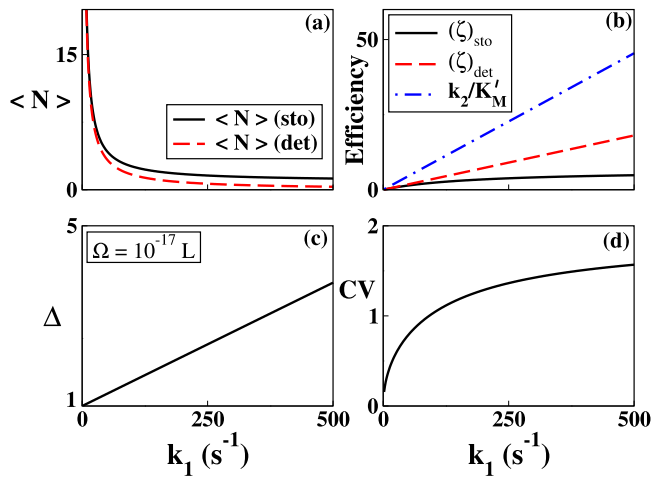

FIG. 4. Variation of (a) $\langle N \rangle$ and (b) different efficiency estimates along with (c) Δ and (d) CV, all as functions of k_1 with $\Omega = 10^{-17}$ L.

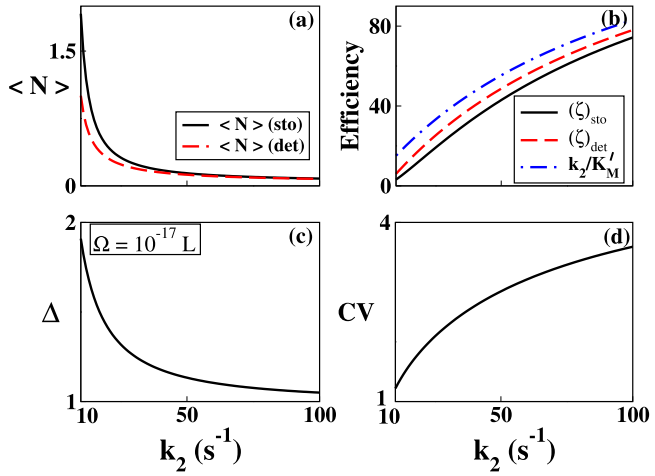

FIG. 5. Variation of (a) $\langle N \rangle$ and (b) different efficiency estimates along with (c) Δ and (d) CV, all as functions of k_2 with $\Omega = 10^{-17}$ L.

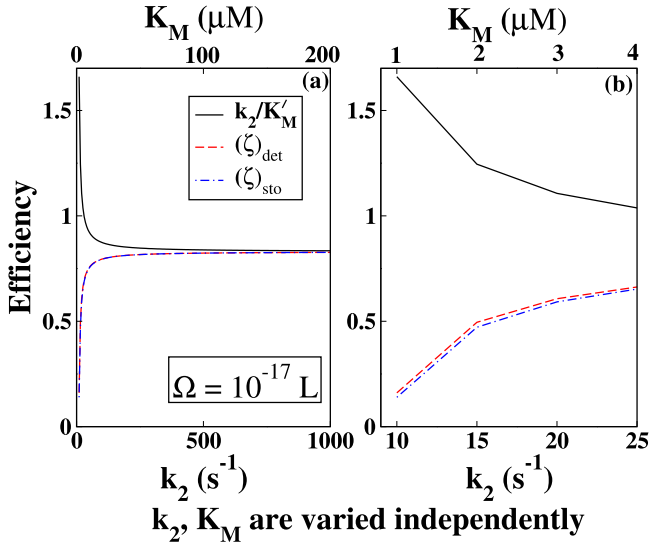

FIG. 6. Evolution of different efficiency measures when both k_2 and K_M are varied independently such that lowering of k_2 and K_M results in higher k_2/K_M. We set $\Omega = 10^{-17}$ L and $k_s = 6\ s^{-1}$.

amount of fluctuation. Also, due to the overestimation by deterministic equations, an enzyme that is considered highly efficient via some *in vitro* experiment may turn out to be only moderately efficient under *in vivo* environments.

In the context of the above discussion on relative magnitudes of the various efficiency estimates, let us now probe the matter more deeply. An important question to ask now is as follows: does a higher k_2/K_M' necessarily mean a higher $(\zeta)_{det}$ or $(\zeta)_{sto}$? While comparing two arbitrary cases with different k_2/K_M' with, say, the corresponding $(\zeta)_{det}$, one must keep both Ω and k_s ($< k_2$) fixed. Then, α, or equivalently k_2, and/or K_M can be varied independently. It is easy to show from Eq. (28) that if a higher k_2/K_M results from the combinations of (a) higher k_2, higher K_M or (b) higher k_2, lower K_M, then the system with higher k_2/K_M will have higher $(\zeta)_{det}$ as well as higher $(\zeta)_{sto}$, but always maintaining the inequality $(\zeta)_{sto} < (\zeta)_{det}$.

However, if the higher k_2/K_M is generated from both a lower k_2 and lower K_M, then it may so happen that the higher k_2/K_M case is associated with a lower $(\zeta)_{det}$ (and $(\zeta)_{sto}$). It is particularly so if k_2 itself is low and k_s ($<k_2$) is necessarily fixed at a still lower value. We show such a case here in Fig. 6. The curves of different efficiency measures in Fig. 6 are generated by varying k_2 and K_M *independently* and not just as a function of k_2 only. The idea is to represent various arbitrary enzyme-substrate systems. Both k_2 and K_M are changed independently which should also become clear from the fact that a decrease in k_2 only results in the lowering of k_2/K_M. By zooming in the low k_2 zone, as shown in Fig. 6(b), one finds that Eq. (29) remains valid. So, enzyme-substrate pairs with a low k_2 and still lower K_M, placed in a high k_2/K_M region,[19] will be displaced at the low range of $(\zeta)_{det}$. This may cause a difference between the shapes of the distributions of $(\zeta)_{det}$ and k_2/K_M. However, there are several limitations to compare our results with those existing in the literature. Some of these are as follows: (i) most of the available data are from standard *in vitro* laboratory experiments. These kinetic parameters may differ considerably when the same reaction is studied *in vivo* only where the effect of Ω is significant. (ii) To experimentally test the role of Ω on the efficiency, data from small-volume flow reactors are needed. Nonetheless, we believe that with more experimental data becoming available in future for small flow reactor systems, such comparisons will become more exciting.

C. The case of multiple enzyme molecules

So far in this paper, we have considered a single enzyme molecule to study the behavior of the efficiency estimate given in Eq. (12). In a realistic situation, e.g., inside a cell, more than a single enzyme molecule is present. Some recent works focus on such open reaction systems with many enzyme molecules by employing various theoretical tools and approximations as well as numerical simulation.[8,11,22] In this section, we treat scenarios starting with multiple enzyme molecules exactly by using the SSA. As already derived for a single enzyme molecule in Eq. (23), $\langle N \rangle/\Omega$ contains the initial enzyme concentration in the form of $1/\Omega$. So, by logical extrapolation of Eq. (22), for a starting enzyme population of N_{E0}, we propose the mean substrate population at SS as

$$\langle N \rangle = \frac{k_s(k_s + k_2 K_M')}{k_2(k_2 N_{E0} - k_s)}. \tag{30}$$

It is easy to follow that in the same limit of $k_2 K_M \gg \gamma_s$ (see Eq. (23)), Eq. (30) reduces to the deterministic form with $[E]_0 = N_{E0}\,\Omega^{-1}$. Now, for multiple enzyme molecules, it may be useful to scale the efficiency by N_{E0}. Then, one obtains from Eqs. (12) and (30)

$$\frac{(\zeta)_{sto}}{N_{E0}} = \frac{k_2(k_2 - k_s N_{E0}^{-1})}{(k_s + k_2 K_M')}. \tag{31}$$

According to Eq. (31), the scaled (stochastic) efficiency increases with the number of starting enzyme molecules and ultimately saturates at the value $k_2^2/(k_s + k_2 K_M')$ for very large N_{E0}. Also, for $k_2 \gg k_s$, the efficiency per enzyme *becomes independent of* N_{E0} *even for finite* N_{E0}.

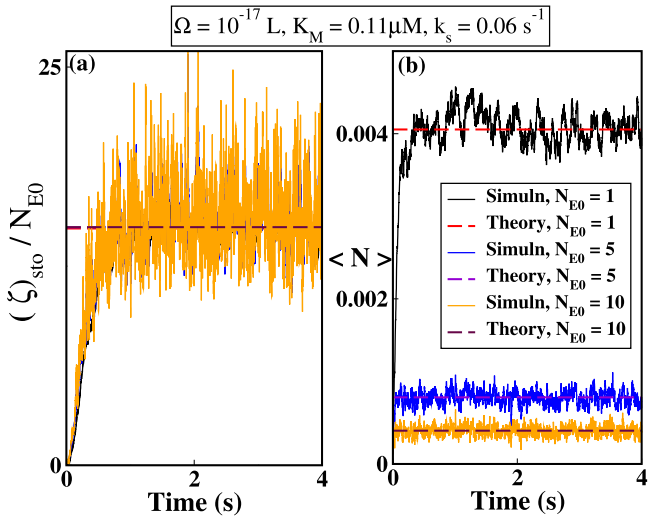

FIG. 7. Variation of (a) $(\zeta)_{\rm sto}/N_{\rm E0}$ and (b) $\langle N\rangle$ as a function of time for $\Omega = 10^{-17}$ L, $K_{\rm M} = 0.11$ μM, and $k_s = 0.06$ s^{-1}. The results obtained from SSA are found to approach the corresponding steady-state analytical results, obtained from Eqs. (30) and (31), nicely.

We show the numerical results for the scaled efficiency and mean substrate population in Figs. 7(a) and 7(b), respectively, for $N_{\rm E0} = 5$ and $N_{\rm E0} = 10$, along with $N_{\rm E0} = 1$. Other parameters are $\Omega = 10^{-17}$ L, $K_{\rm M} = 0.11$ μ M, $k_2 = 10$ s^{-1}, and $k_s = 0.06$ s^{-1}. The scaled efficiency values are almost identical for different $N_{\rm E0}$, as already discussed above for the given choice of parameters. Comparison of the numerical data with those obtained from Eq. (30) reveals that the latter can be useful to represent the mean SS substrate population in the multi-enzyme case. The relatively large spread of $(\zeta)_{\rm sto}$ for $N_{\rm E0} = 10$ arises out of the very low $\langle N\rangle$. We mention that Eq. (30) predicts the deviation Δ to be independent of the initial enzyme population. This feature can be used to test the validity of Eq. (30).

IV. CONCLUSION

In summary, we have proposed here a new measure of enzyme efficiency that can be applied to closed as well as open reaction systems. In closed systems, this efficiency is related to the completion time of the reaction, defined in terms of the cycle description of the kinetics with substrate in excess. It is shown that a similar expression has emerged in the case of a system with flow at SS. Importantly, the definition of efficiency, Eq. (12) (or Eq. (6)), is applicable to any flow system with arbitrarily complex reaction network where a single species is injected from outside and another single species comes out of the reaction medium.

It is known that biological systems are not only open but also subjected to significant noise. Therefore, we have felt it mandatory to analyze the efficiency and related properties in both deterministic and stochastic scenarios. Significant differences are observed between the ensuing results that establish the strong role of noise in such systems. The reliability of our analytical results is verified with numerical data generated from stochastic simulations. The effects of inflow rate, the system volume, and various rate constants are thoroughly studied in this context, including the case of multiple enzyme molecules. Our efficiency estimate is also compared with the age-old measure $k_2/K_{\rm M}$ and is found to merge with the latter under suitable limiting conditions. We have further shown that higher efficiency is linked with greater fluctuation, indicating a trade-off between efficiency and accuracy.

Finally, our approach has also established that both the $k_2/K_{\rm M}$ ratio and the deterministic results overestimate the efficiency compared to its stochastic assessment. Therefore, the present endeavor provides a clue to the understanding of why some enzyme showing high efficacy in bulk laboratory experiments can become considerably less efficient inside a cell or a sub-cellular compartment. Experiments in open *in vitro* and *in vivo* systems, e.g., in small-volume flow reactors, with controlled inflow and outflow rates of reacting species can be performed to test the validity of these results. However, maintaining a (non-equilibrium) steady state in a small reaction-volume flow reactor, e.g., a cell, can be quite a non-trivial task. We sincerely hope that such experiments may unveil more fascinating features in future.

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APPENDIX A: DREs FOR OPEN REACTION NETWORK (4)

$$d_t[\mathrm{S}] = \gamma_s - k_1'[\mathrm{E}][\mathrm{S}] + k_{-1}[\mathrm{C}], \tag{A1}$$

$$d_t[\mathrm{E}] = -d_t[\mathrm{C}] = -k_1'[\mathrm{E}][\mathrm{S}] + (k_{-1} + k_2)[\mathrm{C}], \tag{A2}$$

$$d_t[\mathrm{P}] = k_2[\mathrm{C}] - k_p[\mathrm{P}]. \tag{A3}$$

The SS concentrations are determined by setting the l.h.s. of all the rate equations equal to zero.

APPENDIX B: DERIVATION OF EQ. (20)

Multiplying Eq. (14) by "N^2" and then summing over "N" give

$$k_1\eta_3^{(0)} = (k_{-1} + k_2)\eta_2^{(1)} + 2k_{-1}\eta_1^{(1)} + k_{-1}\eta_0^{(1)} + 2k_s\eta_1^{(0)} + k_s\eta_0^{(0)}. \tag{B1}$$

Similar treatment with Eq. (15) yields

$$k_1\eta_3^{(0)} = (k_{-1} + k_2)\eta_2^{(1)} + 2k_1\eta_2^{(0)} - k_1\eta_1^{(0)} - 2k_s\eta_1^{(1)} - k_s\eta_0^{(1)}. \tag{B2}$$

Equating the r.h.s. of Eqs. (B1) and (B2), we get the desired expression given in Eq. (20).

APPENDIX C: DETERMINATION OF $\langle N^2 \rangle$

Multiplying Eq. (14) by "N^3" and then summing over "N" give

$$k_1\eta_4^{(0)} = k_{-1}(\eta_3^{(1)} + 3\eta_2^{(1)} + 3\eta_1^{(1)} + \eta_0^{(1)}) + k_s(3\eta_2^{(0)} + 3\eta_1^{(0)} + \eta_0^{(0)}) + k_2\eta_3^{(1)}. \quad \text{(C1)}$$

Similar treatment with Eq. (15) yields

$$k_1\eta_4^{(0)} = k_1(3\eta_3^{(0)} - 3\eta_2^{(0)} + \eta_1^{(0)}) + (k_{-1} + k_2)\eta_3^{(1)} - k_s(3\eta_2^{(1)} + 3\eta_1^{(1)} + \eta_0^{(1)}). \quad \text{(C2)}$$

From Eqs. (C1) and (C2), one obtains

$$k_1\eta_3^{(0)} = k_s\left(\langle N^2\rangle + \langle N\rangle + \frac{1}{3}\right) + k_{-1}\left(\eta_2^{(1)} + \eta_1^{(1)} + \frac{\eta_0^{(1)}}{3}\right) + \eta_2^{(0)} - \frac{\eta_1^{(0)}}{3}, \quad \text{(C3)}$$

where we have used $\langle N^2\rangle = \eta_2^{(0)} + \eta_2^{(1)}$, $\langle N\rangle = \eta_1^{(0)} + \eta_1^{(1)}$, and $\eta_0^{(0)} + \eta_0^{(1)} = 1$. Using Eqs. (B1) and (C3), we finally get

$$(k_2 - k_s)\langle N^2\rangle = k_s\left(\langle N\rangle + \frac{1}{3}\right) + (k_1 + k_2)\eta_2^{(0)} - \left(2k_s + \frac{k_1}{3}\right)\eta_1^{(0)} - k_{-1}\eta_1^{(1)} - \frac{2k_{-1}}{3}\eta_0^{(1)} - k_s\eta_0^{(0)}. \quad \text{(C4)}$$

All the quantities in the r.h.s. of Eq. (C4) are already determined at SS. For example, $\eta_0^{(1)} = k_s/k_2$ (from Eq. (19)), $\eta_1^{(0)} = K'_M\eta_0^{(1)}$ (from Eq. (16)), $\eta_1^{(1)} = \langle N\rangle - \eta_1^{(0)}$, where $\langle N\rangle$ is given in Eq. (22). Then, using Eq. (17) and $\eta_0^{(0)} = 1 - \eta_0^{(1)}$, $\eta_2^{(0)}$ can also be evaluated. Thus, we can determine $\langle N^2\rangle$ and subsequently the CV.

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