Effect of Substrate Number Fluctuations in Stochastic Enzyme Kinetics

Divya Singh and Srabanti Chaudhury

Department of Chemistry, Indian Institute of Science Education and Research, Dr. Homi Bhabha Road, Pune 411008, Maharashtra, India

Supporting Information

ABSTRACT: Conventional studies on enzyme kinetics assume that the substrate concentration remains constant. However, for catalytic reactions taking place in intracellular compartments, the substrate molecules are fed in and out of the compartment and are catalyzed into product molecules within the compartment. In this work, we use a chemical master equation approach to study the stochastic kinetics of a single enzyme for different reaction pathways with one or more intermediate states. We obtain velocity expressions that deviate from the Michaelis–Menten expression. We study the coefficient of variation, which is a measure of the noise strength as a function of the mean substrate concentration for systems where there is influx or and outflux of substrate molecules. Our results show that the noise strength decreases with the increase in the substrate concentration and finally remains the same when the substrate is present in abundance.

I. INTRODUCTION

The Michaelis–Menten (MM) model for an enzymatic reaction is given by $E + S \rightleftharpoons ES \rightarrow E + P$, where substrate $S$ reversibly binds to free enzyme $E$ to form enzyme–substrate complex $ES$, which then dissociates to form the product. When the substrate is present in excess, the steady-state rate is given by

$$
\langle v \rangle = \frac{k_2[S][E]_T}{(k_{-1} + k_i) + [S]}
$$

(1)

where $k_{-1}$, $k_i$, and $k_2$ are the rate constants of formation of the ES complex, substrate release, and product formation, respectively. $[E]_T$ is the total enzyme concentration, $[E]_T = [E] + [ES]$ is the total enzyme concentration, and $[S]$ is the concentration of the substrate. Recent advances in single-molecule fluorescence microscopy enable one to study single turnovers of individual enzyme molecules in real time. In such single-molecule studies, it has been shown that the free enzyme, $E$, and the bound state, ES, undergo transitions between their different conformers on time scales longer than or comparable to the time scale of the product formation. The probability density function (pdf) of the turnover times between successive catalytic events is obtained from the experimental waiting times, and its first moment gives the average turnover rate. A series of previous theoretical studies have examined the effects of such conformational fluctuations of the enzyme on the reaction kinetics. In all of these studies, it is found that the average rate of the reaction catalyzed by a single enzyme follows the MM relation as obtained at the ensemble level. At the single-molecule level, using a chemical master equation (CME) approach, it has been shown that if the substrate is present in abundance and if the conformational detailed balance is satisfied, the dependence of the average rate of the reaction on the substrate concentration follows a MM form. This resemblance between the rate equations in the deterministic and stochastic limits is valid only under the assumption that the substrate is always in excess. However, in living cells, the assumption that the substrate is present in abundance may not always be valid. In reality, it is not possible to have an experimental setup where one can maintain a constant substrate concentration at the single-molecule level. Recently, Grima and Leier have used the chemical master equation technique to study a wide range of enzymatic reaction mechanisms and obtain the average rate of product formation without including the substrate abundance assumption. It has been shown that the reaction showing a dependence of the rate of product formation on the substrate concentration measured during the time when the reaction is going to complete is a logarithmically corrected MM type of equation. In this work, we study a more realistic situation; the catalytic activity of an enzyme is studied in an intracellular environment where substrate molecules are injected into the cellular compartment, they are catalyzed to a product, and the product molecules leave the compartment. We focus on the dependence of the catalytic turnover rate on the average substrate concentration using the chemical master equation approach for the probability of the system of being in various states. Because the substrate
fluctuations are taken into account, one must explicitly include the number of substrate molecules in the probability distribution required to describe the time evolution of the system. Our model is exactly solvable in the steady-state limit and demonstrates that in the case when substrate molecules move across the compartment membrane in one or both directions there is a deviation in the MM relation. To study the noise in the system, one can also calculate the higher moments of the pdf that cannot be obtained from deterministic kinetics. Thus, the distribution function can yield all of the statistical moments that provide a statistical measure of the fluctuations present in the system through a dimensionless quantity known as the coefficient of variation and defined as

$$\sigma = \sqrt{\langle n^2 \rangle - \langle n \rangle^2} / \langle n \rangle$$ \hspace{1cm} (2)$$

where $n$ is the number of substrate molecules present in the compartment at some definite time interval. Because the assumption of excess substrate concentration is not invoked into the system, the noise is proportional to the inverse of the square root of the mean substrate concentration. Grima has studied earlier the role of noise in multisubunit enzymes where one can obtain an expression for protein (substrate) fluctuations by considering an effective chemical master equation that was solved in the steady state using the linear noise assumption.\textsuperscript{13,14} The dependence of the noise strength was studied as a function of the protein concentration by measuring the coefficient of variation. The noise strength shows different scaling behaviors at low and high substrate concentrations depending on the number of binding sites of the multisubunit enzyme.

In this work, we consider two examples: an enzymatic reaction with one intermediate enzyme–substrate complex that corresponds to a MM mechanism and a reaction scheme with two intermediate enzyme–substrate complexes. In our earlier studies on enzymatic reaction with one or more intermediate complexes using a first passage time distribution formalism, we have shown that velocity expressions remain the same. However, in all of these studies, the substrate was considered being always present in excess.\textsuperscript{15,16} In this work, we consider a realistic situation where there is an influx and outflux of substrate molecules into and out of the reaction vessel. In Section II, we discuss these cases. In what follows in Section III, starting from the chemical master equation for the probability of the number of product molecules formed and the number of substrate molecules present in solution at a given time, we obtain an exact analytic solution of the CME in the steady state to study the effect of the substrate fluctuations on the velocity equation. From the higher moments of the probability distribution functions, we study the fluctuations in the number of substrate molecules by calculating the coefficient of variation.

II. MODEL

We consider four examples where a single enzyme catalyzes the formation of the product in an intercellular environment where substrate molecules are fed in at a constant rate $k_r$. In the first two cases, the enzymatic reaction involves a single intermediate complex, ES (Figure 1a,b). In the former, for simplicity, we assume that there is no export of substrate molecules out of the compartment. Such unidirectional transport mechanisms are common inside cells.\textsuperscript{17} In the latter case, we consider an additional backward reaction for the substrate outflux in the reaction scheme with an average rate equal to $k_0$, as shown in Figure 1b. Next, we consider two reaction schemes that involve two intermediate complexes, ES\textsubscript{1} and ES\textsubscript{2}, and there is only an influx of substrate molecules or substrate molecules go in and come out of the compartment. In this theoretical model, it is assumed that there is no backward transition from the product molecules. For all of these different models, we describe the system by $P[p, n, J, t]$, the probability that when the enzyme is in state $J$, $p$ molecules are processed and $n$ substrate molecules are present in the compartment at time $t$. The active site of the enzyme can be vacant ($J = 0$, free enzyme) or occupied, i.e., an enzyme–substrate complex is formed ($J = 1$) such that $P[p, n, J = 0, t] = P_0[p, n, t]$ and $P[p, n, J = 1, t] = P_1[p, n, t]$. This probability distribution satisfies a set of nonlinear master equations for which it is not possible to find an exact analytical solution. We use the steady-state approximation, which allows us to write these master equations as a set of reduced distributions that can be solved for the turnover rate, and also derive the moments of the distribution.\textsuperscript{18} We discuss this theoretical approach in our Results and Discussion section for all of the four reaction schemes given in Figure 1.

II.I. Single-Intermediate Catalytic Reaction. The chemical master equations for the probability distributions can be written in the following form\textsuperscript{18}.

**Table 1.** Conditions for the different reaction schemes.

| Reaction Scheme | Conditions |
|-----------------|------------|
| (a)             | $k_s$, $k_f$, $k_r$ |
| (b)             | $k_s$, $k_f$, $k_r$, $k_0$ |
| (c)             | $k_s$, $k_f$, $k_r$, $k_0$ |
| (d)             | $k_s$, $k_f$, $k_r$, $k_0$, $k_1$ |

Figure 1. Schematic representation of catalytic reaction schemes associated with (a) irreversible substrate flow with one intermediate, (c) irreversible substrate flow with one intermediate, (b) reversible substrate flow with one intermediate, and (d) reversible substrate flow with two intermediates.
Figure 2. Variation of reaction velocity \( V \) as a function of average number of substrate molecules \( \langle n \rangle \) for the scheme with one intermediate and irreversible substrate flow at (a) \( \epsilon_1 = 2, \epsilon_{-1} = 0.1 \) and (b) \( \epsilon_1 = 10, \epsilon_{-1} = 0.1 \). The solid line represents the velocity expression obtained from 6, and the dashed line represents the velocity in the MM limit (7).

\[
\frac{\partial P_0[p,n,t]}{\partial t} = -nkP_0[p,n,t] + k_{-1}P[p+1,n-1,t] + \epsilon_1 P_1[p,n,t] - k_{0}P_0[p,n,t] + k_{0}P[p,n-1,t]
\]

(3a)

\[
\frac{\partial P_1[p,n,t]}{\partial t} = (n+1)k_1P[p-1,n+1,t] - (k_{-1} + k_1)P_1[p,n,t] - k_{0}P[p,n,t] + k_{0}P[p,n-1,t]
\]

(3b)

where \( P_0[p,n,t] = P[p,n,f = 0,t] \) and \( P_1[p,n,t] = P[p,n,f = 1,t] \).

On rescaling \( \tau = kt \), \( \epsilon_{-1} = \frac{k_{-1}}{k_1} \), and \( \theta = \frac{k_0}{k_1} \), 3a and 3b can be written as

\[
\frac{\partial P_0[p,n,\tau]}{\partial \tau} = -nP_0[p,n,\tau] + c_{-1}P[p+1,n-1,\tau] + \epsilon_1 P_1[p,n,\tau] - k_0P_0[p,n,\tau] + k_0P[p,n-1,\tau]
\]

(4a)

\[
\frac{\partial P_1[p,n,\tau]}{\partial \tau} = (n+1)P[p-1,n+1,\tau] - (c_{-1})P_1[p,n,\tau] - k_0P[p,n,\tau] + k_0P[p,n-1,\tau]
\]

(4b)

The normalization condition \( \sum_{p,n} P_0[p,n,\tau] + P_1[p,n,\tau] = 1 \) is satisfied at all times.

The turnover rate is given by

\[
V = \frac{\partial (p)}{\partial \tau} = \sum_{p,n} p(P_0[p,n,\tau] + P_1[p,n,\tau])
\]

As given in Appendix A, we work with the reduced distributions, and the turnover rate is given by

\[
V = \frac{\epsilon_1}{2} \left( \langle n \rangle + z \right) \left[ 1 + \frac{4\langle n \rangle}{\left( \langle n \rangle + z \right)^2} - 1 \right]
\]

(6)

\begin{align*}
\text{where } z &= \epsilon_1 + \epsilon_{-1}.
\end{align*}

When \( \langle n \rangle + z \gg 2\sqrt{n} \), which is true at high substrate concentration or at large values of \( z \), 6 can be binomially expanded as

\[
V = \theta = \frac{\epsilon_1}{2} \langle n \rangle + z \left[ 1 + \left( \frac{1}{2} \right) \left( \frac{4\langle n \rangle}{\left( \langle n \rangle + z \right)^2} \right) - \left( \frac{1}{4} \right) \left( \frac{4\langle n \rangle}{(\langle n \rangle + z)^2} \right)^2 + \ldots - 1 \right]
\]

Neglecting the higher-order terms and after simplification, the above equation reduces to a MM-type relation given as

\[
V_{MM} = \theta = \frac{\epsilon_1 \langle n \rangle}{\langle n \rangle + z}
\]

(7)

Thus, at higher substrate concentrations and higher values of \( z \) (at a given value of \( \langle n \rangle \)), the velocity equation behaves as a MM-type equation. As shown in Figure 2a,b, we compare 6 with the MM relation (7) and different values of \( z \) by varying \( \epsilon_1 \). The difference between the two curves is more at lower values of \( \epsilon_1 \). Also, at a higher value of \( z \), the two curves come closer at higher substrate concentrations where the effect of fluctuations is less prominent. Thus, fluctuations in the substrate concentration are responsible for the strong deviations from the MM relation.

The variance of the substrate fluctuations is defined as

\[
\langle n^2 \rangle = \mu_2^{(0)} + \mu_2^{(1)}
\]

(8)

As shown in Appendix A, the moments \( \mu_2^{(0)} \) and \( \mu_2^{(1)} \) can be calculated and \( \langle n^2 \rangle \) is given by

\[
\langle n^2 \rangle = \frac{\partial^2 (2z + \epsilon_1) + \theta(c_1z^2 - \langle n \rangle c_1) - \langle n \rangle c_1^2 (1 + z)}{\epsilon_1(\theta - \epsilon_1)}
\]

(9)

and \( \theta \) is defined in 6.

9 is then used to calculate the coefficient of variation, \( \sigma \), using 2.

For the catalytic reaction scheme described in Figure 1b with an additional backward reaction at an average rate equal to \( k_{0} \), the master equations are given by

\[
\frac{\partial P_0[p,n,t]}{\partial t} = -nkP_0[p,n,t] + k_{-1}P[p+1,n-1,t] + k_1P[p,n,t] + k_{0}P_0[p,n,t] + k_{0}P[p,n+1,t] - (k_{0} + k_{0})P_0[p,n,t]
\]

(10a)

\[
\frac{\partial P_1[p,n,t]}{\partial t} = (n+1)k_1P[p-1,n+1,t] - (k_{-1} + k_1)P[p,n,t] - (k_{0} + k_{0})P[p,n,t] + k_{0}P[p,n+1,t]
\]

(10b)

and satisfy the normalization condition \( \sum_{p,n} (P_0[p,n,\tau] + P_1[p,n,\tau]) \). As shown in Appendix B, the turnover rate is given by

\[
V = \theta = \frac{\epsilon_1}{2} \langle n \rangle + z \left[ 1 + \left( \frac{1}{2} \right) \left( \frac{4\langle n \rangle}{\left( \langle n \rangle + z \right)^2} \right) - \left( \frac{1}{4} \right) \left( \frac{4\langle n \rangle}{(\langle n \rangle + z)^2} \right)^2 + \ldots - 1 \right]
\]
Figure 3. (a) Variation of reaction velocity $V$ as a function of average number of substrate molecules $\langle n \rangle$ for the scheme with one intermediate and reversible substrate flow (influx and outflux). The solid line represents the velocity expression (11) at $\epsilon_1 = 2$, $\epsilon_{-1} = 0.1$, and $\eta = 1$. The black circles represent the velocity expression obtained when the limit $\eta \to 0$ is applied to 11, and the dashed line represents the velocity expression obtained from 6. A comparison of the reaction velocity expression obtained from 11 (solid line) with that obtained from the MM equation (13, dashed line) at (b) $\epsilon_1 = 2$, $\epsilon_{-1} = 0.1$, $\eta = 1$ and (c) $\epsilon_1 = 20$, $\epsilon_{-1} = 0.1$, $\eta = 1$.

$$V = \theta$$

$$= \frac{\epsilon_1}{2} (\langle n \rangle + z - \frac{2n}{\epsilon_1}) \left[ 1 + \frac{4\langle \eta \rangle + \eta(z - 1 - \eta)}{\epsilon_1 (\langle n \rangle + z - \frac{2n}{\epsilon_1})} - 1 \right]$$

(11)

Thus, the functional form of the turnover rate equation changes when we include the reverse outflow reaction. For the velocity function to exist over the entire range of substrate concentration, $\eta \ll \frac{\epsilon_1 (\langle n \rangle + z)}{2}$ at given value of $z$ and at a given substrate concentration $\langle n \rangle$. In Figure 3a, we plot the velocity as a function of average substrate concentration. We show that in the limit when $\eta = 0$, 11 reduces to 6 (the dotted line and the black circles coincide with each other).

In the limit when $\langle n \rangle + z = \frac{2n}{\epsilon_1} \gg 2 \left( \frac{\langle \eta \rangle + \langle n \rangle (z - 1 - \eta)}{\epsilon_1} \right)$, which is true at high substrate concentration or at large values of $z$ and small values of $\eta$ (at a given substrate concentration), 19 can be binomially expanded, and neglecting the higher-order terms and simplifying, we get

$$V = \frac{\langle n \rangle (\epsilon_1 + \eta) + \eta(z - 1) - \eta^2}{\langle n \rangle + z - \frac{2n}{\epsilon_1}}$$

(12)

Furthermore, in the limit when $z \gg \eta$, the above equation reduces the velocity expression in the MM form

$$V_{MM} = \frac{\langle n \rangle (\epsilon_1 + \eta)}{\langle n \rangle + z - \frac{2n}{\epsilon_1}}$$

(13)

If we put $\eta = 0$, 14 reduces to 9. In Figure 4, we compare the coefficients of variation for the enzyme-catalyzed reactions with and without substrate molecules. As the concentration of the substrate increases, the noise strength decreases.

II.II. Two-Intermediate Catalytic Reaction. We consider an enzymatic reaction where two intermediate enzyme-substrate complexes are formed before the product is released out of the compartment, as shown in Figure 1c. Instead of two values of $J$, one needs to consider the number of substrate molecules present and number of product molecules formed in the ES$_2$ state $(J = 2)$ such that $P[p, n, J = 2, \tau] = P_{2}[p, n, \tau]$. With a unidirectional flow of substrate molecules into the compartment, the master equations are given by

$$\frac{\partial P_{2}}{\partial \tau} = -nkP_0[p, n, \tau] + k_{-1}P[p + 1, n - 1, \tau]$$

$$+ k_{2}P_{2}[p, n, \tau] - k_{-2}P_{0}[p, n, \tau] + k_{o}P[p, n - 1, \tau]$$

(15a)
The turnover rate is given by

\[
V = \frac{\partial(p)}{\partial t} = \frac{\partial}{\partial t} \sum_{n,p} [P_0(p, n, \tau) + P_1(p, n, \tau) + P_2(p, n, t)]
\]

(16)

As shown in Supporting Information S1, the reaction velocity is given as

\[
V = \theta = \frac{\langle B^* \rangle + \langle n \rangle}{2A^*} - \left[ 1 + \frac{4A^*e_1e_3\langle n \rangle}{(e_2 + \Omega_2)(B^* + \langle n \rangle)^2} \right]^{-1}
\]

(17)

where

\[
A^* = \frac{(e_2 + \Omega_2)^2 - e_2e_3}{e_3(e_2 + \Omega_2)}, \quad B^* = \frac{e_2e_3\Omega_2 + e_1\Omega_3}{e_3(e_2 + \Omega_2)}
\]

\[
\Omega_1 = e_{-1} + e_2, \quad \Omega_2 = e_{-2} + e_3
\]

The functional form of the turnover rate relation with only the substrate input does not change by including an additional intermediate state in the catalysis reaction (6).

In the limit, when \(B^* + \langle n \rangle \gg 2\sqrt{\frac{A^*e_1e_3\langle n \rangle}{e_2 + \Omega_2}}\), carrying out the binomial expansion, we obtain the velocity expression in the MM form

\[
V_{\text{MM}} = \theta = \frac{e_1e_3\langle n \rangle}{(B^* + \langle n \rangle)(e_2 + \Omega_2)}
\]

(18)

This condition is satisfied either at a very high substrate concentration or at high values of \(e_2\) and \(e_1\) for a given value of \(\langle n \rangle\). This is depicted in Figure 5a,b, where the MM form can be obtained when the velocity equation, 17, is considered in these limits.

From the different moments obtained in S1, the coefficient of variation can be obtained by 2, where \(\langle n^2 \rangle\) is given by

\[
\langle n^2 \rangle = a_4 + a_3\langle n \rangle
\]

(19)

where

\[
a_i = \frac{2}{(\Omega_2 + e_3)} + (H + P - C_1c_1 + D(\Omega_2 + e_3))\theta^2 + (1 + \frac{\Omega_1}{e_2})\theta_1 + (1 + \frac{\Omega_1}{e_2}) - E_{c_1}c_1.
\]

\[
a_i = \theta(\Omega_2 + e_3) - e_1e_3, \quad a_i = \frac{a_1}{a_3} \text{ and } a_3 = \frac{a_2}{a_3}
\]

Constants \(I, H, C, D, E, L, \) and \(J\) are defined in S1.

Next, we consider the same enzymatic reaction as described in Figure 1d with reversible substrate influx and outflux. The master equations are given by

\[
\frac{\partial P_0(p, n, t)}{\partial t} = -nkP_0(p, n, t) + k_2P_2[p, n - 1, t] + k_0P_0[p, n, t] - \langle k_0 + k_0' \rangle P_0[p, n, t]
\]

(20a)

\[
\frac{\partial P_1(p, n, t)}{\partial t} = (n + 1)k_0P_0[p, n, t] + k_0P_0[p, n - 1, t] + k_2P_2[p, n, t] - \langle k_0 + k_0' \rangle P_0[p, n, t] - \langle k_0 + k_0' \rangle P_1[p, n, t]
\]

(20b)

\[
\frac{\partial P_2(p, n, t)}{\partial t} = k_2P_2[p, n, t] + k_0P_0[p, n - 1, t] + k_0P_0[p, n, t] - \langle k_0 + k_0' \rangle P_1[p, n, t]
\]

(20c)

The normalization condition \(\sum_{p,n} [P_0(p, n, \tau) + P_1(p, n, \tau) + P_2(p, n, \tau)] = 1\) is satisfied at all times, where \(\tau = kt\).

The turnover rate is given by
In the limit when \( \eta \to 0 \), \(22\) reduces to \(17\). This is also shown graphically in Figure 6a.

In the limit when \( (B^* - 2\eta + \langle n \rangle) \gg 2 \)

\[
A = \left[ A^* \left( \frac{C_e \epsilon_3}{\epsilon_2 + \Omega_2} + \eta \right) \langle n \rangle + \eta \langle B^* - 1 \rangle - \eta^2 A^* \right]^{-1}
\]

\[
V = \theta = \left( \frac{C_e \epsilon_3}{\epsilon_2 + \Omega_2} + \eta \right) \langle n \rangle + \eta \langle B^* - 1 \rangle - \eta^2 A^* \frac{A}{B^* - 2\eta + \langle n \rangle}
\]

(23)

In the limit when \( \epsilon_2 \) and \( \epsilon_3 \) are much higher than \( \eta \), \(23\) reduces to a MM form of velocity as shown in Figure 6b,c

\[
V_{\text{MM}} = \theta = \left( \frac{C_e \epsilon_3}{\epsilon_2 + \Omega_2} + \eta \right) \langle n \rangle \frac{A^* \langle n \rangle}{B^* - 2\eta + \langle n \rangle}
\]

(24)

As shown in S1, the other moments of the distribution are also calculated and the second moment is given by

\[
\langle n^2 \rangle = a_9 + a_{10} \langle n \rangle
\]

(25)

Figure 6. (a) Variation of reaction velocity \( V \) as a function of average number of substrate molecules \( \langle n \rangle \) for the scheme with two intermediates and reversible substrate flow (influx and outflux). The solid line represents the velocity expression \(22\) at \( \epsilon_2 = 3 \), \( \epsilon_{-1} = \epsilon_{-2} = 0.1 \), \( \epsilon_3 = 10 \), and \( \eta = 1 \). The black circles represent the velocity expression obtained when the limit \( \eta \to 0 \) is applied to \(22\), and the dashed line represents the velocity expression obtained from \(17\). A comparison of the reaction velocity expression obtained from \(22\) (solid line) with that from the MM equation \(24\) (dashed line) at (b) \( \epsilon_2 = 3 \), \( \epsilon_{-1} = \epsilon_{-2} = 0.1 \), \( \epsilon_3 = 10 \), \( \eta = 1 \) and (c) \( \epsilon_2 = 30 \), \( \epsilon_{-1} = \epsilon_{-2} = 0.1 \), \( \epsilon_3 = 10 \), \( \eta = 1 \).

Figure 7. Plot of the coefficient of variation, \( \sigma \), of fluctuations in substrate concentration versus the mean substrate concentration, \( \langle n \rangle \), for the catalytic reaction scheme with two intermediate steps in the absence of substrate outflow (solid line) obtained from \(19\) and in the presence of substrate outflow (dashed line) obtained from \(25\). Parameter values are \( \epsilon_2 = 3 \), \( \epsilon_{-1} = \epsilon_{-2} = 0.1 \), \( \epsilon_3 = 10 \), and \( \eta = 1 \).
III. CONCLUSIONS

In this work, we study the role of noise in enzymatic reactions that take place in intracellular environments. The stochastic description of the system is obtained by solving the chemical master equation under the steady-state approximation. Our results show that irrespective of the number of intermediates present in the reaction scheme, substrate fluctuations can lead to deviation from the classical MM equation. This is in contrast to previously reported enzyme reactions where the MM-type relation is followed even at the single-molecule level under the substrate abundance assumption. The velocity expressions have a different functional form when there is a unidirectional or bidirectional transport of substrate molecules into the intracellular compartment. Thus, the effect of the substrate fluctuations on the catalytic turnover rate also depends on the manner in which substrate molecules are injected into the compartment. For unidirectional as well as bidirectional transport, the velocity equation cannot distinguish between single- and multiple-intermediate states. At high substrate concentration, all of these velocity equations reduce to the classical MM-type equation. We also study the fluctuations in the substrate concentration by measuring the coefficient of variation, \( \sigma \). For a catalytic reaction with one intermediate or more intermediate states, \( \sigma \) decreases with the increase in the mean substrate number and reaches a constant value at high substrate concentrations. Also, the fluctuations are larger in the presence of both substrate influx and outflux at low substrate concentrations as compared to those in reactions where there is only unidirectional flow of the substrate into the compartment (Figures 5 and 7). Thus, noise plays an important role in cellular compartments in which there are often a small number of substrate molecules involved in the catalytic reaction. As shown in Figure S1, we have compared the noise for the models with one- and two-intermediate states. For both the models, the noise level decreases as we move from low to moderate substrate concentrations. At high substrate number, \( \sigma \) remains the same with an increase in the mean substrate concentration. At high substrate concentration, when most of the enzyme is in the substrate-bound state, the presence of slow fluctuations between the two enzyme–substrate conformers leads to higher noise levels in the two-state model. Our theoretical results go beyond those of single-molecule enzymatic experiments where it is assumed that the substrate is always present in abundance.

\[ \sigma \]

\[ \mu \]

\[ A \]

\[ B \]

\[ C \]

\[ D \]

\[ E \]

\[ F \]

\[ G \]

\[ H \]

\[ I \]

\[ J \]

\[ K \]

\[ L \]

\[ M \]

\[ N \]

\[ O \]

\[ P \]

\[ Q \]

\[ R \]

\[ S \]

\[ T \]

\[ U \]

\[ V \]

\[ W \]

\[ X \]

\[ Y \]

\[ Z \]

\[ \sum_{n} n^2 Q_n(n, \tau) = \mu_{(0)}^{(0)} \]

\[ \sum_{n} n^2 Q_1(n, \tau) = \mu_{(1)}^{(1)} \]

\[ \text{Thus, the velocity expression in A.2 reduces to} \]

\[ V = \mu_{(0)}^{(0)} - \epsilon_{-1} \mu_{(1)}^{(1)} \]

\[ \text{In the steady-state approximation,} \]

\[ \frac{\partial Q_{n}[n, \tau]}{\partial \tau} = 0 \]

\[ \frac{\partial Q_{n}[n, \tau]}{\partial \tau} = 0 \]

\[ (n + \theta)Q_{n}[n, \tau] = \epsilon_{-1} Q_{n}[n - 1, \tau] + \epsilon_{1} Q_{n}[n, \tau] \]

\[ + \theta Q_{n}[n - 1, \tau] \]

\[ (\epsilon_{1} + \epsilon_{-1} + \theta)Q_{n}[n, \tau] \]

\[ = (n + 1)Q_{n}[n + 1, \tau] + \theta Q_{n}[n - 1, \tau] \]

\[ \text{Summing A.5a over} \ n, \ \text{we get} \]

\[ \mu_{1}^{(0)} = (\epsilon_{1} + \epsilon_{-1}) \mu_{(1)}^{(1)} \]

\[ \text{Summing A.5a over} \ n \text{and weighting it by} \ n, \ \text{we get} \]

\[ \mu_{2}^{(0)} = (\epsilon_{1} + \epsilon_{-1}) \mu_{(1)}^{(1)} + \epsilon_{-1} \mu_{(0)}^{(1)} + \theta \mu_{0}^{(0)} \]

\[ \text{Summing A.5a over} \ n \text{and weighting it by} \ n^2, \ \text{we get} \]

\[ \mu_{3}^{(0)} = (\epsilon_{1} + \epsilon_{-1}) \mu_{2}^{(1)} + \epsilon_{-1} \mu_{0}^{(1)} + 2\epsilon_{-1} \mu_{0}^{(1)} + \theta \mu_{0}^{(0)} + 2 \theta \mu_{1}^{(0)} \]

\[ \text{Summing A.5a over} \ n \text{and weighting it by} \ n, \ \text{we get} \]

\[ \mu_{4}^{(0)} = (\epsilon_{1} + \epsilon_{-1}) \mu_{2}^{(1)} + \epsilon_{-1} \mu_{0}^{(1)} + 3\epsilon_{-1} \mu_{2}^{(1)} + 3 \epsilon_{-1} \mu_{1}^{(1)} + \theta \mu_{0}^{(0)} + 3 \theta \mu_{1}^{(0)} + 3 \theta \mu_{2}^{(0)} \]

\[ \text{Similarly, summing A.5b over} \ n, \ \text{we get} \]

\[ \mu_{2}^{(0)} = (\epsilon_{1} + \epsilon_{-1}) \mu_{1}^{(1)} + \mu_{1}^{(1)} - \theta \mu_{0}^{(1)} \]

\[ \text{Summing A.5b over} \ n \text{and weighting it by} \ n, \ \text{we get} \]

\[ \mu_{2}^{(0)} = (\epsilon_{1} + \epsilon_{-1}) \mu_{1}^{(1)} + \mu_{1}^{(1)} - \theta \mu_{0}^{(1)} \]

\[ \text{Summing A.5b over} \ n \text{and weighting it by} \ n^2, \ \text{we get} \]

\[ \mu_{2}^{(0)} = (\epsilon_{1} + \epsilon_{-1}) \mu_{1}^{(1)} + \mu_{1}^{(1)} - \theta \mu_{0}^{(1)} \]
\[ \mu_3^{(0)} = (\epsilon_1 + \epsilon_-)\mu_2^{(1)} - \mu_1^{(0)} + 2\mu_2^{(0)} - \theta \mu_0^{(1)} - 2\theta \mu_1^{(1)} \]  
(A.7c)

Summing A.5b over \( n \) and weighting it by \( n^3 \), we get
\[ \mu_3^{(0)} = (\epsilon_1 + \epsilon_-)\mu_3^{(1)} + \mu_1^{(0)} + 3\mu_2^{(0)} - 3\mu_2^{(0)} - \theta \mu_0^{(1)} - 3\theta \mu_1^{(1)} \]
(A.7d)

Equating A.6b and A.7b and using the normalization condition \( \mu_0^{(0)} + \mu_0^{(1)} = 1 \), we get
\[ V = \theta = \mu_1^{(0)} - \epsilon_- \mu_0^{(1)} \]  
(A.8)

Equating A.6c and A.7c we get
\[ \mu_1^{(0)} = \left( \frac{\theta + \epsilon_-}{2} \right) \mu_0^{(1)} + (\theta + \epsilon_-)\mu_1^{(1)} + \frac{\theta}{2} \mu_0^{(0)} + \left( \theta + \frac{1}{2} \right) \mu_1^{(0)} \]  
(A.9)

Equating A.6d and A.7d, we get
\[ \begin{aligned} 
\mu_2^{(0)} &= \left( \frac{\theta + \epsilon_-}{3} \right) \mu_0^{(0)} + (\theta + \epsilon_-)\mu_1^{(1)} + \frac{\theta}{2} \mu_2^{(0)} + \left( \theta + \frac{1}{3} \right) \mu_1^{(1)} \\
+ (\theta + 1)\mu_2^{(0)} + \frac{\theta}{3} \mu_0^{(0)} + \left( \theta + 1 \right) \mu_1^{(0)} 
\end{aligned} \]  
(A.10)

From A.9 and A.5b, we get the desired equation to be solved for \( \theta \)
\[ \begin{aligned} 
\left( \frac{\theta + \epsilon_-}{2} \right) \mu_0^{(1)} + (\theta - \epsilon_1)\mu_1^{(1)} - \frac{\theta}{2} \mu_0^{(0)} + \left( \theta + \frac{1}{2} \right) \mu_1^{(0)} &= 0 \\
\end{aligned} \]  
(A.11)

For obtaining an equation in terms of variable \( \theta \) that represents the reaction velocity, we need the moments of respective states present in A.11 in terms of reaction rate constants.

From the normalization condition and definition of \( \langle n \rangle \), we have
\[ \mu_0^{(0)} + \mu_0^{(1)} = 1 \text{ and } \mu_1^{(0)} + \mu_1^{(1)} = \langle n \rangle \]

Putting A.6a in A.8, we get
\[ \mu_0^{(1)} = \frac{\theta}{\epsilon_1} \]  
(A.12)

Putting A.12 in A.6a, we obtain
\[ \mu_1^{(0)} = \theta + \frac{\epsilon_-}{\epsilon_1} \theta \]  
(A.13)

From the definition of \( \langle n \rangle \), we get
\[ \mu_1^{(1)} = \langle n \rangle - \theta - \frac{\epsilon_-}{\epsilon_1} \theta \]  
(A.14)

From normalization, we get
\[ \mu_0^{(0)} = 1 - \frac{\theta}{\epsilon_1} \]  
(A.15)

Substituting A.12–A.15 into A.11 and rearranging, we obtain a quadratic equation in terms of variable \( \theta \)
\[ \begin{aligned} 
\frac{\theta^2}{\epsilon_1} + \theta (\langle n \rangle + z) - \langle n \rangle \epsilon_1 &= 0 \\
\end{aligned} \]  
(A.16)

Solution of A.16 gives 6 given in the text.

**Second Moment**

By definition
\[ \langle n^2 \rangle = \mu_2^{(0)} + \mu_2^{(1)} \]  
(A.17)

Substituting A.12, A.14, and A.15 in A.6b, we get
\[ \mu_2^{(0)} = -\frac{\theta^2}{\epsilon_1} + \theta \left[ \frac{z(1 - z)}{\epsilon_1} \right] + z(n) \]  
(A.18)

where \( z = \epsilon_1 + \epsilon_- \)

Equating A.10 and A.17c, we obtain
\[ \mu_2^{(1)} = \frac{\theta^3 + \theta^2 z (1 + z) + \theta (\epsilon_1 z - \epsilon_1 (1 + z) n) - \epsilon_1^2 n}{\epsilon_1 (\theta - \epsilon_1)} \]  
(A.19)

Substituting A.18 and A.19 into A.17 gives 14 given in the text.

**APPENDIX B**

Calculation of Reaction Velocity and Second Moment with One Intermediate and Reversible Substrate Influx

**First Moment and Velocity.** With
\[ c_{-1} = \frac{k_{-1}}{k}, \quad \epsilon_1 = \frac{k_1}{k}, \quad \eta = \frac{k_0}{k} \]

and \( \theta = \frac{k}{\epsilon} \) the master equations in the reduced state are given by
\[
\frac{\partial Q_a[n, \tau]}{\partial \tau} = -nQ_a[n, \tau] + \epsilon_{-1}Q_a[n - 1, \tau] + \epsilon_1 Q_a[n, \tau] + \eta Q_a[n + 1, \tau] + (\theta + \eta)Q_a[n, \tau] \\
+ (\epsilon_1 + \epsilon_{-1})Q_a[n, \tau] + \eta Q_a[n + 1, \tau] + (\theta + \eta)Q_a[n, \tau] 
\]

(B.1a)

In the steady state, the turnover rate in \( S \) is given by
\[
V = \mu_1^{(0)} - \epsilon_- \mu_0^{(1)} 
\]

(B.2)

In the steady-state approximation, \( \frac{\partial Q_a[n, \tau]}{\partial \tau} = 0 \) and \( \frac{\partial Q_a[n, \tau]}{\partial n} = 0 \), which give the following set of equations
\[
\begin{aligned} 
(n + \theta + \eta)Q_a[n, \tau] &= \epsilon_{-1}Q_a[n - 1, \tau] + \epsilon_1 Q_a[n, \tau] + \theta Q_a[n - 1, \tau] + \eta Q_a[n + 1, \tau] \\
+ (\epsilon_1 + \epsilon_{-1} + \theta + \eta)Q_a[n, \tau] &= (n + 1)Q_a[n + 1, \tau] + \theta Q_a[n - 1, \tau] + \eta Q_a[n + 1, \tau] 
\end{aligned} \]  
(B.3a)

(B.3b)

Summing B.3a over \( n \) and weighting it by \( n, n^2, \) and \( n^3 \)
\[ \begin{aligned} 
\mu_1^{(0)} &= (\epsilon_1 + \epsilon_{-1})\mu_0^{(1)} \\
\mu_2^{(0)} &= (\epsilon_1 + \epsilon_{-1})\mu_1^{(1)} + \epsilon_{-1}\mu_0^{(1)} + (\theta - \eta)\mu_0^{(0)} \\
\mu_3^{(0)} &= (\epsilon_1 + \epsilon_{-1})\mu_2^{(1)} + \epsilon_{-1}\mu_1^{(1)} + 2\epsilon_{-1} \\
\mu_1^{(1)} &= (\theta + \eta)\mu_0^{(0)} + 2(\theta - \eta)\mu_1^{(0)} 
\end{aligned} \]  
(B.4a)

(B.4b)

(B.4c)
\[ \mu_i^{(0)} = (e_i + e_{-i})\mu_0^{(1)} + e_{-i}\mu_0^{(1)} + 3e_{-i}\mu_2^{(1)} + 3e_{+i}\mu_1^{(1)} + (\theta - \eta)\mu_0^{(0)} + 3(\theta - \eta)\mu_2^{(0)} + 3(\theta + \eta)\mu_1^{(0)} \] (B.4d)

Summing B.3b over \( n \) and weighting it by \( n, n^2, \) and \( n^3 \)

\[ \mu_1^{(0)} = (e_1 + e_{-1})\mu_0^{(1)} \] (B.5a)

\[ \mu_2^{(0)} = (e_1 + e_{-1})\mu_1^{(1)} + \mu_1^{(0)} - (\theta - \eta)\mu_0^{(1)} \] (B.5b)

\[ \mu_3^{(0)} = (e_1 + e_{-1})\mu_2^{(1)} - \mu_1^{(0)} + 2\mu_2^{(0)} - (\theta + \eta)\mu_0^{(0)} - 2(\theta - \eta)\mu_1^{(1)} \] (B.5c)

\[ \mu_4^{(0)} = (e_1 + e_{-1})\mu_3^{(1)} + \mu_1^{(0)} + 3\mu_3^{(0)} - 3\mu_2^{(0)} - (\theta - \eta)\mu_0^{(0)} - 3(\theta - \eta)\mu_2^{(0)} - 3(\theta + \eta)\mu_1^{(0)} \] (B.5d)

Equating B.4b and B.5b and using the normalization condition, we get

\[ V = \theta = \mu_1^{(0)} - e_{-i}\mu_0^{(1)} + \eta \] (B.6)

Equating B.4c and B.5c, we get

\[ \mu_2^{(0)} = \frac{(\theta + \eta + e_{-1})}{2}\mu_0^{(1)} + (\theta - \eta + e_{-1})\mu_1^{(1)} + \frac{(\theta + \eta)}{2}\mu_0^{(0)} + (\theta - \eta + \frac{1}{2})\mu_1^{(0)} \] (B.7)

Equating B.4d and B.5d, we get

\[ \mu_3^{(0)} = \frac{(\theta - \eta + e_{-1})}{3}\mu_0^{(1)} + (\theta - \eta + e_{-1})\mu_2^{(1)} + (\theta + \eta + e_{-1})\mu_1^{(1)} + (\theta - \eta + 1)\mu_2^{(0)} + (\theta - \eta) \]

\[ \mu_0^{(0)} + (\theta - \eta) - \frac{1}{3}\mu_1^{(0)} \] (B.8)

From B.7 and B.4b, we get the desired equation to be solved for \( \theta \)

\[ \mu_i^{(1)} = \theta^2(1 + z) - 3\eta + 2\eta \] (B.15)

Substituting B.15 and B.16 into \( \langle n^2 \rangle \), we get 22 given in the text.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b00611.

Reaction velocity and second moment calculation for the two-intermediate scheme with irreversible substrate transport and reaction velocity for the scheme with two intermediates and reversible substrate influx and outflux (PDF)

\[ \frac{(\theta + \eta)}{2} + (\theta - \eta)(\eta) - \frac{e_{-1}}{2}\mu_0^{(1)} - e_{+1}\mu_1^{(1)} - (\theta - \eta)\mu_0^{(0)} + \frac{1}{2}\mu_1^{(0)} = 0 \] (B.9)

To obtain an equation in terms of variable \( \theta \) that represents the reaction velocity, we need to calculate the moments in B.9 in terms of reaction rate constants by using the above set of relations.

As shown in Appendix A, using the above equations, the normalization condition, and the definition of \( \langle n \rangle \), we get

\[ \mu_0^{(1)} = \frac{(\theta - \eta)}{e_1} \] (B.10)

\[ \mu_1^{(0)} = (\theta - \eta)\left( \frac{e_1 + e_{-1}}{e_1} \right) \] (B.11)

\[ \mu_1^{(1)} = (\theta - \eta)\left( \frac{e_1 + e_{-1}}{e_1} \right) \] (B.12)

\[ \mu_0^{(0)} = 1 - (\theta - \eta) \] (B.13)

Substituting B.10–B.13 into B.9 and after some rearrangement, we get a quadratic equation in terms of variable \( \theta \)

\[ \frac{\theta^2}{e_1} + \frac{\theta}{e_1} + \langle n \rangle - \frac{2\eta}{e_1} - \left[ e_1\langle n \rangle + \eta(\langle z \rangle + \langle n \rangle - 1) - \frac{\eta^2}{e_1} \right] = 0 \] (B.14)

where \( z = e_1 + e_{-1} \) whose solution is given by 19.

**Second Moment.** By definition, \( \langle n^2 \rangle = \mu_1^{(0)} + \mu_1^{(1)} \). As shown in Appendix A, for the irreversible substrate flux reaction scheme, \( \mu_2^{(1)} \) and \( \mu_2^{(1)} \) are calculated and are given by

\[ \mu_2^{(1)} = -\frac{\theta^2}{e_1} + \theta \left( \frac{\eta(\langle z \rangle - 1) - \frac{\eta^2}{e_1} - \langle n \rangle}{\langle z \rangle} \right) \] (B.15)

where \( z = e_1 + e_{-1} \).

\[ \langle n^2 \rangle = \frac{\theta^2}{3} + 2\eta \left( 1 + \frac{\eta(\langle z \rangle - 1)}{\langle z \rangle} \right) - \frac{\eta^2}{3} \] (B.16)

**AUTHOR INFORMATION**

Corresponding Author

*E-mail: srabanti@iiserpune.ac.in. Tel: 912025908140.

ORCID

Srabanti Chaudhury: 0000-0001-6718-8886

Notes

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

The authors acknowledge support from IISER Pune. S.C. acknowledges DST-SERB grant GAP/DST/CHE-15-184 for funding.

5582
REFERENCES

(1) Michaelis, L.; Menten, M. L. Die Kinetik Der Invertinwirkung. Biochem. Z. 1913, 49, 333.

(2) English, B. P.; Min, W.; van Oijen, A. M.; Lee, K. T.; Luo, G.; Sun, H.; Cherayil, B. J.; Kou, S. C.; Xie, X. S. Ever-Fluctuating Single Enzyme Molecules: Michaelis Menten Equation Revisited. Nat. Chem. Biol. 2006, 2, 87–92.

(3) Lu, H. P.; Xun, L.; Xie, X. S. Single-Molecule Enzymatic Dynamics. Science 1998, 282, 1877–1882.

(4) Min, W.; English, B. P.; Luo, G.; Cherayil, B. J.; Kou, S. C.; Xie, X. S. Fluctuating Enzymes: Lessons from Single-Molecule Studies. Acc. Chem. Res. 2005, 38, 923.

(5) Min, W.; Gopich, I. V.; English, B. P.; Kou, S. C.; Xie, X. S.; Szabo, A. When Does the Michaelis-Menten Equation Hold for Fluctuating Enzymes? J. Phys. Chem. B 2006, 110, 20093–20097.

(6) Kou, S. C.; Cherayil, B. J.; Min, W.; English, B. P.; Xie, X. S. Single-Molecule Michaelis-Menten Equations. J. Phys. Chem. B 2005, 109, 19068–19081.

(7) Singh, D.; Chaudhury, S. Statistical Properties of Fluctuating Enzymes with Dynamic Cooperativity Using a First Passage Time Distribution Formalism. J. Chem. Phys. 2017, 146, No. 145103.

(8) Kolomeisky, A. B. Michaelis-Menten Relations for Complex Enzymatic Networks. J. Chem. Phys. 2011, 134, No. 155101.

(9) Cao, J. MichaelisMenten Equation and Detailed Balance in Enzymatic Networks. J. Phys. Chem. B 2011, 115, 5493–5498.

(10) Gardiner, C. W., Handbook of Stochastic Methods: For Physics, Chemistry, and the Natural Sciences; Springer: New York, 1996.

(11) Holwerda, E. K.; Lynd, L. R. Testing Alternative Kinetic Models for Utilization of Crystalline Cellulose (Avicel) by Batch Cultures of Clostridium thermocellum. Biotechnol. Bioeng. 2013, 110, 2389–2394.

(12) Grima, R.; Leier, A. Exact Product Formation Rates for Stochastic Enzyme Kinetics. J. Phys. Chem. B 2017, 121, 13–23.

(13) Grima, R. Anomalous Fluctuation Scaling Laws in Stochastic Enzyme Kinetics: Increase of Noise Strength with the Mean Concentration. Phys. Rev. E 2014, 89, No. 012710.

(14) Kampen, N. G. V. Stochastic Processes in Physics and Chemistry, 4th ed.; Elsevier: North-Holland, 2007.

(15) Chaudhury, S.; Cao, J.; Sinitsyn, N. A. Universality of Poisson Indicator and Fano Factor of Transport Event Statistics in Ion Channels and Enzyme Kinetics. J. Phys. Chem. B 2013, 117, 503–509.

(16) Chaudhury, S. Poisson Indicator and Fano Factor for Probing Dynamic Disorder in Single-Molecule Enzyme Inhibition Kinetics. J. Phys. Chem. B 2014, 118, 10405–10412.

(17) Alberts, B.; Watson, J.; Bray, D.; Lewis, J. Molecular Biology of the Cell, 4th ed.; Garland Science: New York, 2008.

(18) Stéfanini, M. O.; mckeane, A. K.; Newman, T. J. Single Enzyme Pathways and Substrate Fluctuations. Nonlinearity 2005, 18, 1575–1595.