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Exact Product Formation Rates for Stochastic Enzyme Kinetics

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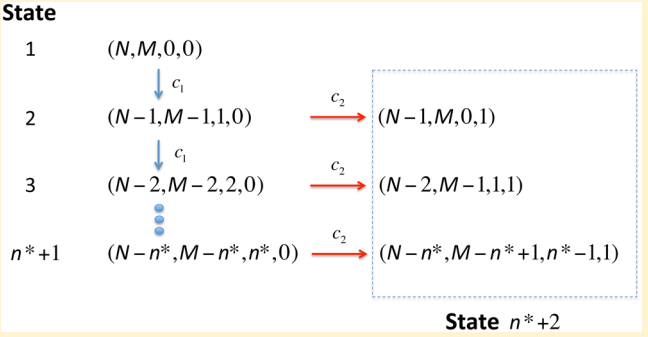
Supporting Information

ABSTRACT: The rate of product formation is an important measure of the speed of enzyme reactions. Classical studies of enzyme reactions have been conducted in dilute solutions and under conditions that justified the substrate abundance assumption. However, such assumption is well-known to break down in the context of cellular biochemistry. Instead, the concentration of available substrate can become rate limiting. Here we use the chemical master equation to obtain expressions for the instantaneous and time averaged rate of product formation without invoking the conventional substrate abundance assumption. The expressions are derived for a broad range of enzyme reaction mechanisms, including those that involve one or many enzyme molecules, require multiple substrates, and exhibit cooperativity and substrate inhibition. Novel results include: (i) the relationship between the average rate of product formation (calculated over the time it takes for the reaction to finish) and the substrate concentration, for a Michaelis−Menten (MM) reaction with one enzyme molecule, is approximately given by a logarithmically corrected MM form; (ii) intrinsic noise decreases the sharpness of cooperative switches but enhances the filtering response of substrate inhibition; (iii) the relationship between the initial average rate of product formation and the initial substrate concentration for a MM reaction with no reversible reaction and with any number of enzyme and substrate molecules is a sum of Michaelis−Menten equations.



INTRODUCTION

A main aim of enzymology is the inference of the molecular mechanisms underpinning enzyme catalysis and of the associated rate constants characterizing the reaction. For over a century the data utilized for such an endeavor has been time-course data gathered from ensemble experiments.[1] The classical approach to infer the kinetic constants from such experiments involves the proposal of a plausible mechanism and the algebraic manipulation of the (deterministic) chemical rate equations for this mechanism such that the kinetic parameters can be inferred from suitable linear plots of the experimental data. The most commonly proposed mechanism for single-substrate kinetics is the Michaelis−Menten (MM) reaction mechanism:

$$\mathrm{S} + \mathrm{E} \underset{k_1}{\overset{k_0}{\rightleftharpoons}} \mathrm{C} \xrightarrow{k_2} \mathrm{E} + \mathrm{P} \tag{1}$$

where S, E, C, and P denote substrate, free enzyme, complex and product species, respectively. The k's denote the associated rate constants. This mechanism was originated by Henri[2] but nowadays is commonly attributed to Michaelis and Menten.[3]

Writing the chemical rate equations and applying time scale separation via the quasi-steady-state assumption (QSSA) (by setting the time derivatives of the concentrations of E and C to zero) one obtains the rate of product formation:

$$\frac{d[\mathrm{P}]}{dt} = \frac{k_2 E_T [\mathrm{S}]}{K_M + [\mathrm{S}]} \tag{2}$$

where $[\mathrm{X}]$ denotes the concentration of species X, E_T is the total enzyme concentration and $K_M = (k_1 + k_2)/k_0$ is the MM constant. Eq 2 is the well-known MM form.[1] Strictly speaking, this equation is valid if the transients in the substrate concentration decay much slower than transients in the enzyme concentration; it is a valid approximation for short times provided the initial substrate concentration is much larger than the enzyme concentration (more general validity conditions are discussed here[4]). Hence $[\mathrm{S}]$ in the MM form is to be interpreted as the initial substrate concentration and $d[\mathrm{P}]/dt$ as the approximate rate of product formation for short times. In what follows, we shall conveniently refer to this as the initial rate of product formation with the understanding that we do not mean the rate at $t = 0$ but rather the rate over a short time interval). It is easy to see that eq 2 implies the kinetic parameters (K_M and k_2) can be obtained from the y-intercept and slope of a linear graph of the inverse rate of product formation versus the inverse initial substrate concentration. This so-called Lineweaver−Burk plot[5] constitutes the most

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popular means of obtaining the kinetic parameters from ensemble experimental data.

Recent years have seen the development of sophisticated experimental techniques capable of following reactions at the single molecule level using fluorescence correlation spectroscopy and related optical methods (see ref 6 for a review). In particular, Xie and collaborators have measured long time traces of enzymatic turnover events for a reaction catalyzed by a single enzyme molecule;[7] they also showed that kinetic parameters from this data can be obtained in the same way as from ensemble experiments, namely using a Lineweaver−Burk plot (see Figure 2b of ref 7). This is possible because it can be shown using a chemical master equation approach that if the number of substrate molecules is much larger than one and provided there is no conformational current,[8] the dependence of the inverse time between successive product formation events (the rate of the reaction) on the substrate concentration is precisely given by the MM form eq 2 with $E_T = 1/\Omega$, where Ω is the volume of the compartment.[9−11] Similarly it has been shown that when the substrate is much more abundant than enzyme then one can write a reduced chemical master equation in which the effective propensity function is of the MM type.[12]

The parallel between the analysis of single and ensemble experiments and of deterministic and stochastic analysis is remarkable given that the chemical rate equations from which eq 2 was initially derived are strictly speaking only valid in the limit of large numbers of molecules.[13] A common feature, however, of all these studies is (i) the assumption that the substrate is available much more abundantly than the enzyme and (ii) the enzyme reaction is described by the MM mechanism. While the assumption of excess substrate is usually valid in classical chemical kinetic studies that have been performed in dilute aqueous solutions, it is in general invalid in living cells where enzymatic systems often operate under excess enzyme conditions.[14−16] Even in some bioengineered systems, which usually run in very controlled conditions, excess substrate concentrations may not always be achieved.[17] It is therefore very important to study enzyme kinetics also under the nonexcess substrate condition. That said, a number of recent publications have reported stochastic rates of product formation in single enzyme kinetics and under the assumption of substrate abundance albeit for reaction schemes that differ from those studied here (e.g., refs 18,19).

In this article we seek to go beyond the restrictions mentioned above. For convenience we divide our results into two sections. In The Single Enzyme Case Section we consider the case of reactions catalyzed by a single enzyme molecule whereas in The Many Enzyme Case Section we consider the case of multiple enzyme molecules. In both cases, the number of substrate molecules can be any integer and hence our analysis covers all situations including those in which the substrate concentration is comparable or even smaller than the enzyme concentration. We take a mean first passage time approach to estimate the average rate of product formation (instantaneous or averaged over the time to make a certain number of product molecules) from the chemical master equation. The expressions are derived for a broad range of enzyme reaction mechanisms, including those that involve one or many enzyme molecules, which require multiple substrates and exhibit cooperativity and substrate inhibition. Two novel results which are of particular interest are (i) the relationship between the average rate of product formation (calculated over the time it takes for the reaction to finish) and the substrate concentration, for a Michaelis−Menten (MM) reaction with one enzyme molecule, is approximately given by a logarithmically corrected MM form. (ii) The relationship between the initial average rate of product formation and the initial substrate concentration for a MM reaction with no reversible reaction and with any number of enzyme and substrate molecules is a sum of Michaelis−Menten equations.

THE SINGLE ENZYME CASE

Single Substrate Enzyme Reaction. Consider the MM reaction mechanism (eq 1) with one enzyme molecule confined in a volume. The stochastic dynamics of this system has been studied without the assumption of substrate abundance previously by Aranyi and Toth,[20] where they derived the exact solution of the probability distribution for all times, computed the time course of the mean substrate and enzyme concentrations, and compared with those obtained by numerical integration of the chemical rate equations. Here we analyze the stochastic dynamics from a different perspective, namely by studying the rate of product formation, which, as we shall see, cannot be obtained from the probability distribution but rather from the first passage time distribution.

We start by assuming that the enzymatic processes can be modeled by a Markov process. This is a common assumption[13] whose validity rests primarily on that of the well-mixing condition. Let T_m be the time it takes for the enzyme reaction to produce m product molecules. It then follows that

$$T_m = \sum_{i=1}^{m} t_{i-1,i} \tag{3}$$

where $t_{i-1,i}$ is the time for the number of product molecules to change from $i-1$ to i given that there are already $i-1$ product molecules. T_m is a stochastic quantity and we are interested in obtaining its mean which is given by

$$\tau_m = \langle T_m \rangle = \sum_{i=1}^{m} \langle t_{i-1,i} \rangle \tag{4}$$

where the angled brackets denote the statistical average.

Next we derive the probability distribution of $t_{i-1,i}$ from which we can obtain the averages needed to explicitly evaluate eq 4. Consider the case where initially we have N substrate molecules and the enzyme is in its unbound (E) state. As the reaction proceeds, the number of substrate molecules decreases and the number of product molecules correspondingly increases. After each product molecule is formed, the enzyme returns to its unbound state and is ready for the next round of catalysis.

Now consider the point in time at which the counter of the number of product molecules turns to $i-1$. As a new product molecule has just been formed, the enzyme is back in its unbound state. Also the corresponding number of substrate molecules is $N - i + 1$. In Table 1, we show the progression from this state to the state in which the successive product molecule is formed, i.e., the state in which we have i product

Table 1. Progression of the MM Mechanism

state	S mols	E mols	C mols	P mols
0	$N - i + 1$	1	0	$i - 1$
1	$N - i$	0	1	$i - 1$
2	$N - i$	1	0	i

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molecules. Note that "mols" is an acronym standing for molecules.

Now we want to calculate the distribution of $t_{i-1,\,i}$ i.e., the time to move from state 0 to state 2, given that there are already $i-1$ molecules of product. Let P_i be the probability that the system is in state i at time t. Given that we are assuming Markovian dynamics, we have to solve the following time evolution equations (the master equations):

$$\partial_t P_0(t) = -(N+1-i)\frac{k_0}{\Omega}P_0(t) + k_1 P_1(t) \tag{5}$$

$$\partial_t P_1(t) = (N+1-i)\frac{k_0}{\Omega}P_0(t) - (k_1+k_2)P_1(t) \tag{6}$$

with the initial condition $P_0(0) = 1$. The volume of the compartment in which the reaction is confined is denoted by Ω. The probability that $t_{i-1,\,i} = t$, which we shall denote as $\pi_i(t)$, is then given by the probability of entering the state 2 in the time interval $(t, t+dt)$ which is equal to the catalytic rate constant multiplied by the probability of being in the previous state, i.e., $k_2 P_1(t)$. Solving eqs 5 and 6 simultaneously we obtain

$$\begin{aligned}\tilde{\pi}_i(s) &= k_2\tilde{P}_1(s)\\ &= k_2\frac{k_0'(i-N-1)}{k_0'(i-N-1)(k_2+s) - s(k_1+k_2+s)}\end{aligned} \tag{7}$$

where $k_0' = k_0/\Omega$ and the tilde refers to the Laplace Transform which is defined as $\tilde{P}_i(s) = \int_0^\infty P_i(t)\, e^{-st}\, dt$ (the use of this transform simplifies the calculations). Hence it follows that the mean of $t_{i-1,\,i} = t$ is given by

$$\langle t_{i-1,i}\rangle = -\frac{d}{ds}\tilde{\pi}_i(0) = \frac{1}{k_2}\left(1 + \frac{K_M'}{h_i}\right) \tag{8}$$

where $K_M' = K_M\,\Omega$ and $h_i = 1-i+N$. Note that this implies that $\langle t_{i-2,\,i-1}\rangle < \langle t_{i-1,\,i}\rangle$, i.e., the mean first passage time to change from $i-2$ to $i-1$ product molecules is less than the mean first passage time to change from $i-1$ to i product molecules. The catalytic process thus becomes slower with time; this is because as the reaction progresses, there is a monotonic decrease in the number of substrate molecules which implies slower effective association rates between enzyme and substrate.

Substituting eq 8 in eq 4 we obtain an expression for the mean total time to make m product molecules:

$$\tau_m = \frac{m}{k_2}\left(1 + \frac{K_M'}{z_m}\right) \tag{9}$$

where $z_m = m/\sum_{j=1+N-m}^{N} j^{-1}$.

We next discuss in some detail the implications of this equation and its relationship to special cases which are already reported in the literature. The inverse of the quantity τ_m/m, which appears on the left-hand side of eq 9, can be interpreted as the average rate of change of the product numbers, calculated over the average time period in which m turnovers are observed. We shall refer to this as $r_m = m/\tau_m$. Hence it follows that

$$r_m = \frac{k_2 z_m}{z_m + K_M'} \tag{10}$$

This result is particularly interesting because it has the form of the conventional MM form (see eq 2) except that (i) our result holds for all times not just short times and (ii) the substrate concentration is now replaced by z_m. The intuition for this is that z_m is the harmonic mean of the set $\{N, N-1, ..., N-m+1\}$, so it is a measure of the mean substrate concentration over the time period in which m turnovers are observed. (iii) We did not invoke any time scale separation arguments. (iv) Our analysis takes into account the intrinsic stochasticity of reaction kinetics. There are two special cases of eq 10 which are worth pointing out:

$$m = 1,\ r_1 = \frac{k_2 N}{N + K_M'} \tag{11}$$

$$m = N,\ r_N \simeq \frac{k_2(N/\log N)}{(N/\log N) + K_M'} \tag{12}$$

The first result, eq 11, is the so-called single molecule MM form first reported by Xie and collaborators.[11] This result holds only when the reaction rate is measured shortly after the reaction starts. More precisely, this is the rate after one product molecule is formed. This is indeed consistent with the derivation in[11] which implicitly assumed that the substrate concentration is constant (pseudo-first-order kinetics) and which of course is a good approximation only for short times. Note that r_1/Ω is the initial rate of change of product concentration and this equals exactly the QSSA result, eq 2, with $E_T = 1/\Omega$ and $[S] = N/\Omega$; hence eq 11 is referred to as a single-molecule MM form.

The second result, eq 12, is a logarithmically corrected MM type of equation. Here we use the approximation $z_N \simeq (N/\log N)$, which is obtained by replacing the sum by an integral in the definition of z_m with $m = N$. This approximation is good for large enough N; the relative error between $N/\log N$ and z_N is about 10% for $N = 100$. This curious, novel dependence manifests when the rate of reaction is measured over the time it takes for the reaction to finish, i.e, for all the substrate to be converted into product. The inverse of the average rate from start to finish provides an estimate of the time for the reaction to finish, which is a physically relevant and measurable quantity. This would for example be particularly useful when estimating time scales for intracellular reactions characterized by very low enzyme concentrations.

We test our theory by computing the mean time to make m product molecules numerically via the matrix exponential method (see Appendix A). Throughout the rest of this article we refer to this method as the numerics. In particular, since the main result eq 10 has the form of a MM form, we test it by obtaining r_m and z_m from the numerics for several pairs of (m, N) and then plotting a Lineweaver–Burk plot, i.e., a graph of $1/r_m$ vs $1/z_m$. As shown in Figure 1 the plot is linear and agrees perfectly with that given by the modified MM form eq 10 (where z_m replaces the substrate concentration) derived from stochastic theory. This result is particularly striking when one considers that for the parameters used, the deterministic MM form given by eq 2 is not expected to be a good approximation since the condition $K_M/E_T \gg 1$ is not met.[4]

Two Substrate Enzyme Reactions. Next we consider a sequential ordered reaction[1] in which two substrates S_1 and S_2 interact successively and in a specific order with enzyme leading to the production of two products P_1 and P_2:

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J. Phys. Chem. B 2017, 121, 13−23

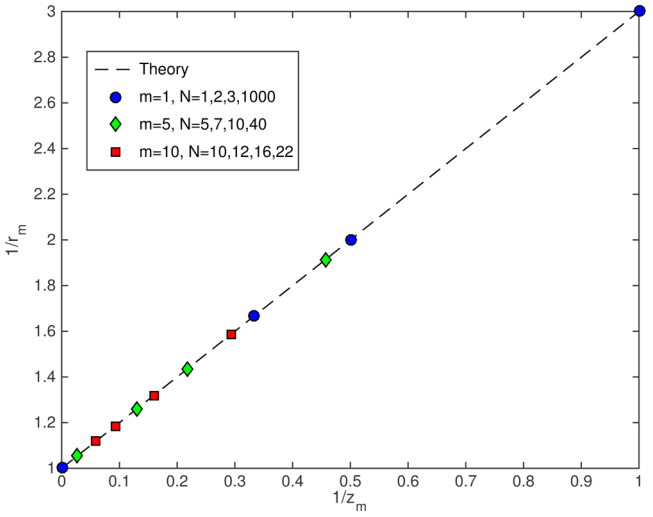

Figure 1. Single substrate stochastic dynamics. Here, we plot the mean time to make m product molecules ($1/r_m$) as a function of the parameter $1/z_m$ which depends on m and N (the total number of substrate molecules) for the reaction system (eq1). The theory is given by eq 10 and the numerics by points. The parameters are $\Omega = 1$, $k_0 = k_1 = k_2 = 1$.

$$S_1 + E \underset{k_1}{\overset{k_0}{\rightleftharpoons}} C_1,$$

$$S_2 + C_1 \underset{k_3}{\overset{k_2}{\rightleftharpoons}} C_2,$$

$$C_2 \xrightarrow{k_4} C^* + P_1,$$

$$C^* \xrightarrow{k_5} E + P_2 \tag{13}$$

This can also be seen as a chemical and gate since two inputs (two substrates) are needed to have an output (the product).

Writing the deterministic rate equations and applying time scale separation via the QSSA assumption (setting the time derivatives of the concentrations of E, C_1, C_2, C* to zero) one obtains the rates of product formation:

$$\frac{d[P_1]}{dt} = \frac{d[P_2]}{dt} = \frac{\alpha_0[S_1][S_2]}{\alpha_1 + \alpha_2[S_1] + \alpha_3[S_1][S_2] + \alpha_4[S_2]} \tag{14}$$

where $\alpha_0 = E_T k_0 k_2 k_4 k_5$, $\alpha_1 = k_1 k_5 (k_3+k_4)$, $\alpha_2 = k_0 k_5 (k_3+k_4)$, $\alpha_3 = k_0 k_2 (k_4+k_5)$, $\alpha_4 = k_2 k_4 k_5$, and E_T is the total enzyme concentration. As for the MM mechanism, in the above equation the substrate concentrations are the initial ones and the rates of product formation are for short times.

Next we consider the stochastic analysis of the dynamics for the case where the initial number of the two substrates is the same and equal to N while the number of enzyme molecules equals one (the derivation which proceeds can be done for an arbitrary number of the two types of substrates but here we choose equal initial numbers to simplify the calculation). The analysis proceeds analogously to that for the earlier case, i.e., we use the master equation approach to calculate the quantity $\langle t_{i-1,\,i}\rangle$. In Table 2, we show the progression from the state with $i-1$ product molecules of P_1 and P_2 to the state in which the successive product molecule is formed, i.e., the state in which we have i product molecules of P_1 and P_2.

The master equations for the probability of being in each state are given by

$$\partial_t P_0(t) = -k_0 \frac{N-i+1}{\Omega} P_0(t) + k_1 P_1(t) \tag{15}$$

$$\partial_t P_1(t) = k_0 \frac{N-i+1}{\Omega} P_0(t) - k_1 P_1(t) - k_2 \frac{N-i+1}{\Omega} P_1(t) + k_3 P_2(t) \tag{16}$$

$$\partial_t P_2(t) = k_2 \frac{N-i+1}{\Omega} P_1(t) - (k_3 + k_4) P_2(t) \tag{17}$$

$$\partial_t P_3(t) = k_4 P_2(t) - k_5 P_3(t) \tag{18}$$

with initial condition $P_0(0) = 1$. Note that this initial condition is consistent with the fact that $\langle t_{i-1,\,i}\rangle$ is defined conditional on there being $i-1$ molecules initially (see previous section).

The probability $\pi_i(t)$ that $t_{i-1,\,i} = t$ is then given by the probability of entering the state 4 in the time interval $(t, t + dt)$ which is equal to the catalytic rate constant multiplied by the probability of being in the previous state, i.e., $k_5 P_3(t)$. The above time-dependent equations can be solved using the Laplace transform. Finally we can obtain an expression for the mean time for the system to jump from state 0 to state 4:

$$\langle t_{i-1,i}\rangle = -\frac{d}{ds}\tilde{\pi}_i(0) = \frac{\alpha_3}{\Omega\alpha_0} + \frac{\alpha_2 + \alpha_4}{\alpha_0(1-i+N)} + \frac{\alpha_1\Omega}{\alpha_0(1-i+N)^2} \tag{19}$$

Substituting the above expression in eq 4 we obtain τ_m, the average time to make m product molecules (of P_1 and P_2). Thus, the rate of change of the product numbers calculated over the average time period in which m turnovers are observed is given by

$$r_m = \frac{m}{\tau_m} = \frac{\alpha_0\Omega z_m w_m}{\Omega w_m(\alpha_2 + \alpha_4) + \alpha_1\Omega^2 z_m + \alpha_3 w_m z_m} \tag{20}$$

where z_m is as defined earlier and $w_m = m/\sum_{j=1+N-m}^{N} j^{-2}$. This is the two substrate reaction analog of the one substrate reaction result given by eq 10.

Again there are two special cases of particular interest:

Table 2. Progression of the Two Substrate Reaction

state	S_1 mols	S_2 mols	E mols	C_1 mols	C_2 mols	$i-1$* mols	P_1 mols	P_2 mols
0	$N-i+1$	$N-i+1$	1	0	0	0	$i-1$	$i-1$
1	$N-i$	$N-i+1$	0	1	0	0	$i-1$	$i-1$
2	$N-i$	$N-i$	0	0	1	0	$i-1$	$i-1$
3	$N-i$	$N-i$	0	0	0	1	i	$i-1$
4	$N-i$	$N-i$	1	0	0	0	i	i

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J. Phys. Chem. B 2017, 121, 13−23

$$r_1 = \frac{\alpha_0 \Omega (N/\Omega)^2}{\alpha_1 + (\alpha_2 + \alpha_4)(N/\Omega) + \alpha_3 (N/\Omega)^2} \quad (21)$$

$$r_N \simeq \frac{\alpha_0 \Omega (N/\log N)}{((\pi^2/6)\alpha_1 \Omega^2/\log N) + \Omega(\alpha_2 + \alpha_4) + \alpha_3 (N/\log N)} \quad (22)$$

In the last line, we used again that $z_N \simeq (N/\log N)$ which follows by replacing the sum by an integral in the definition of z_m, whereas $w_N \simeq 6N/\pi^2$ which follows from the approximation that $\sum_{j=1}^{N} j^{-2} \simeq \pi^2/6$ for large N.

Note that r_1/Ω is the initial rate of change of product concentration; this agrees with the deterministic result eq 14 with $[S_1] = [S_2] = N/\Omega$ (this agreement parallels that earlier shown for the single substrate MM reaction). Note also that in the limit of small volumes and large enough N, r_N has the same form as that obtained for the single substrate MM reaction mechanism, namely the form of a MM form with variable $(N/\log N)$.

Hence, a conclusion of our theory is that the stochastic reaction dynamics, as described by the rate r_m (the number of product molecules divided by the mean time to produce them), allows one to easily distinguish the single and two substrate reactions at short times (compare eq 11 and eq 21); at longer times it becomes progressively more difficult to tell the two reaction dynamics apart as they both tend (in the limit of long times, i.e., $m \simeq N$, and when N is large) to a logarithmically corrected MM type of equation (compare eq 12 and eq 22).

We finally test our theory using numerics. In particular, the main result, eq 20, implies a straight line graph if we plot $1/r_m$ versus the quantity $(\alpha_2+\alpha_4)/(\alpha_0 z_m) + \alpha_1 \Omega/(\alpha_0 w_m) + \alpha_3/(\alpha_0 \Omega)$. This is verified in Figure 2 for several pairs of (m, N) obtained from numerics.

Cooperativity and Substrate Inhibition. We now consider two phenomena which are well studied using the deterministic approach. These are cooperativity and substrate inhibition.[21]

A set of reactions which is capable of displaying both phenomena is

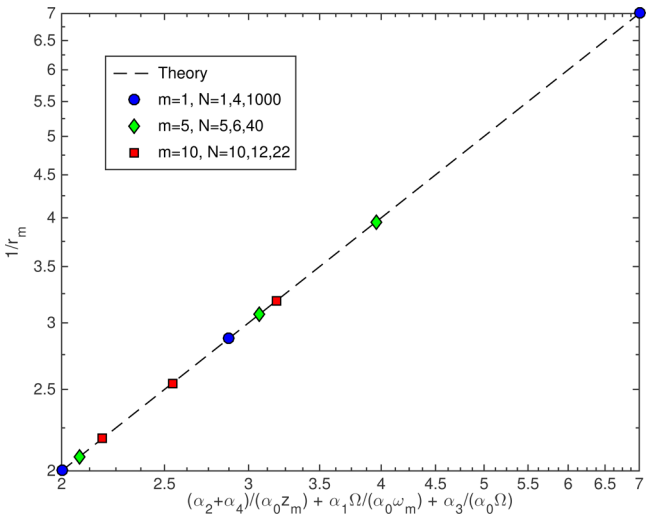

Figure 2. Two substrate stochastic dynamics. We here plot the mean time to make m product molecules $(1/r_m)$ as a function of the parameter $(\alpha_2+\alpha_4)/(\alpha_0 z_m) + \alpha_1 \Omega/(\alpha_0 w_m) + \alpha_3/(\alpha_0 \Omega)$ for the reaction system (eq 13). The theory is given by eq 20 and the numerics by points. The parameters are $\Omega = 1$, $k_0 = k_1 = k_2 = k_3 = k_4 = k_5 = 1$.

$$S + E \underset{k_1}{\overset{k_0}{\rightleftharpoons}} C \xrightarrow{k_2} E + P \quad (23)$$

$$S + C \underset{k_4}{\overset{k_3}{\rightleftharpoons}} C^* \xrightarrow{k_5} C + P \quad (24)$$

Writing the deterministic rate equations and applying time scale separation via the QSSA (setting the time derivatives of the concentrations of E, C, C* to zero) one obtains the rate of product formation:

$$\frac{d[P]}{dt} = \frac{\frac{E_T[S]}{K_M^1}\left(\frac{k_5[S])}{K_M^2} + k_2\right)}{1 + \frac{[S]}{K_M^1} + \frac{[S]^2}{K_M}} \quad (25)$$

where $K_M^1 = (k_1+k_2)/k_0$, $K_M^2 = (k_4+k_5)/k_3$, $K_M = K_M^1 K_M^2$, and E_T is the total enzyme concentration.

Next we consider the stochastic analysis of the enzyme dynamics for the case where the initial number of substrate molecules is equal to N. The analysis proceeds analogously as for the earlier cases, though for this system we shall only calculate the time to form the first product molecule as the analysis gets considerably complicated for more product molecules.

In Table 3, we show the progression from the initial state with 0 product molecules of P to the state in which we have 1 product molecules of P.

Table 3. Progression of the Cooperative or Substrate Inhibited Reaction

state	S mols	E mols	C mols	C* mols	P mols
0	N	1	0	0	0
1	$N-1$	0	1	0	0
2	$N-1$	1	0	0	1
3	$N-2$	0	0	1	0
4	$N-2$	0	1	0	1

The master equations for the probability of being in each state are given by

$$\partial_t P_0(t) = -k_0 \frac{N}{\Omega} P_0(t) + k_1 P_1(t) \quad (26)$$

$$\partial_t P_1(t) = k_0 \frac{N}{\Omega} P_0(t) - (k_1 + k_2) P_1(t) - k_3 \frac{N-1}{\Omega} P_1(t) + k_4 P_3(t) \quad (27)$$

$$\partial_t P_3(t) = k_3 \frac{N-1}{\Omega} P_1(t) - (k_4 + k_5) P_3(t) \quad (28)$$

with initial condition $P_0(0) = 1$. The probability $\pi_0(t)$ that a product molecule appears at time t is then equal to the sum of the probability of entering the state 2 and the probability of entering the state 4 in the time interval $(t, t + dt)$, i.e., $\pi_0(t) = k_2 P_1(t) + k_5 P_3(t)$. The above set of coupled equations can be solved using the Laplace transform and the inverse mean time to produce a product molecule is then given by

$$r_1 = \frac{1}{\langle t_{0,1} \rangle} = -\frac{1}{\frac{d}{ds}\tilde{\pi}_0(0)} = \frac{\frac{k_5}{K_M}\left(\frac{N}{\Omega}\right)^2 + \left(\frac{k_2}{K_M^1} - \frac{k_5}{K_M \Omega}\right)\left(\frac{N}{\Omega}\right)}{1 - \frac{k_5}{k_0 K_M \Omega} + \frac{1}{k_0}\left(\frac{k_5}{K_M} + \frac{k_0}{K_M^1} - \frac{k_0}{K_M \Omega}\right)\left(\frac{N}{\Omega}\right) + \frac{1}{K_M}\left(\frac{N}{\Omega}\right)^2} \quad (29)$$

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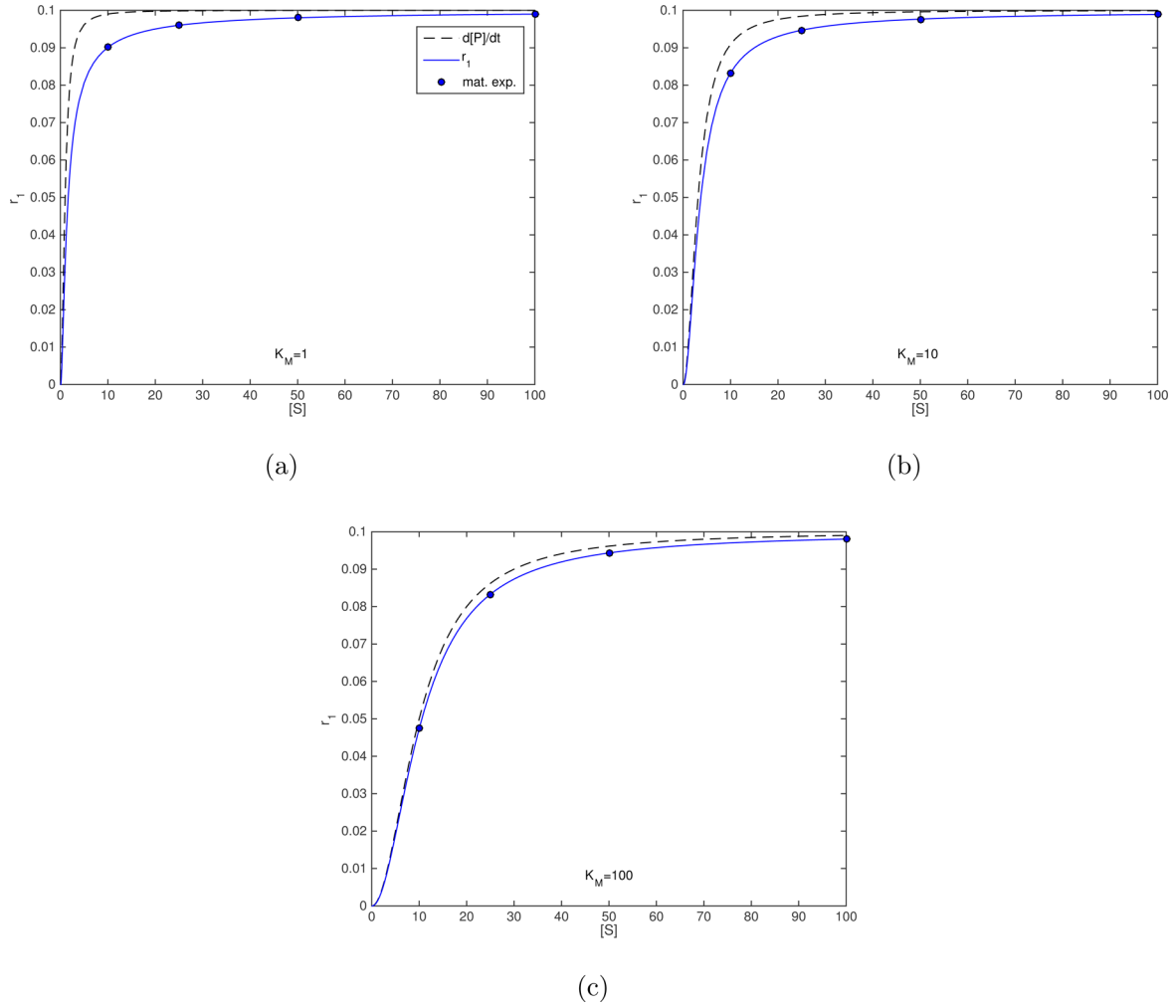

Figure 3. Stochastic dynamics of positive cooperativity. We plot the initial rate of change of the product concentration (r_1/Ω according to the stochastic approach, and $d[\mathrm{P}]/dt$ according to the deterministic approach) as a function of the substrate concentrations [S]. The theory is given by eq 31 for the stochastic approach (solid line) and by eq 30 for the deterministic approach (dashed lines) while the numerics (for the stochastic approach) is given by points. The parameters are $k_0 = k_5 = 1$, $\Omega = 10$, $k_1 = 1 \times 10^7$, $k_2 = k_4 = 1$, and (a) $k_3 = 2 \times 10^7$, $K_M = 1$, (b) $k_3 = 2 \times 10^6$, $K_M = 10$, and (c) $k_3 = 2 \times 10^5$, $K_M = 100$.

Note that the initial rate of change of the product concentration according to the stochastic approach is given by r_1/Ω. This expression is different to the one given by the deterministic QSSA approach eq 25 with $E_T = 1/\Omega$ and $[\mathrm{S}] = N/\Omega$. This is particularly interesting because for the previous cases of single and two substrate enzyme kinetics, the stochastic and deterministic approaches agreed.

Cooperativity. Positive cooperativity is obtained[21] by applying the limits $K_M^1 \to \infty$ and $K_M^2 \to 0$ at constant K_M. This implies that the affinity of the enzyme for the substrate increases, after a substrate molecule binds the free enzyme. In other words, the first enzyme reaction is slow to occur but, once it does, it is very quickly succeeded by the second enzyme reaction. In this cooperative limit, the deterministic expression given by eq 25 reduces to

$$\frac{d[\mathrm{P}]}{dt} = \frac{\frac{k_5 E_T [\mathrm{S}]^2}{K_M}}{1 + \frac{[\mathrm{S}]^2}{K_M}} \tag{30}$$

which is a Hill function with Hill coefficient 2.

Similarly, by taking the same limit, the stochastic expression given by eq 29 simplifies to

$$r_1 = \frac{\frac{k_5}{K_M}\left(\frac{N}{\Omega}\right)^2 - \frac{k_5}{K_M \Omega}\left(\frac{N}{\Omega}\right)}{1 - \frac{k_5}{k_0 K_M \Omega} + \frac{1}{k_0 K_M}\left(k_5 - \frac{k_0}{\Omega}\right)\left(\frac{N}{\Omega}\right) + \frac{1}{K_M}\left(\frac{N}{\Omega}\right)^2} \tag{31}$$

Again, r_1/Ω evaluated using the above expression does not agree with that given by the deterministic QSSA approach, eq 30 with $E_T = 1/\Omega$ and $[\mathrm{S}] = N/\Omega$, except of course in the limits of small and large substrate concentration. However, note that both stochastic and deterministic approaches qualitatively agree in the sense that in the cooperative limit both approaches predict the initial rate of change of the product concentration increases monotonically with substrate concentration.

In Figure 3 we use numerics to verify the differences between the stochastic and deterministic results in the cooperative limit. We also show that stochasticity causes the average rate of product formation to always be less than that predicted by the deterministic approach, i.e., intrinsic noise decreases the sharpness of the enzyme switch. These differences between the two approaches go to zero as K_M increases.

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J. Phys. Chem. B 2017, 121, 13−23

Substrate Inhibition. Substrate inhibition is obtained[21] by setting $k_5 = 0$. This implies that C* is an inactive state of the enzyme which results from high substrate concentrations. Thus, here we expect that in both low and high substrate concentrations the rate of product formation is very small while it reaches a maximum at intermediate concentrations. This is in clear contrast to the cooperative case where the rate of product formation increases monotonically with substrate concentration. Thus, while cooperativity leads to switch-like behavior, substrate inhibition leads to a selective filter.[21]

Applying $k_5 = 0$ to the deterministic approach eq 25 and to the stochastic approach eq 29 leads to

$$\frac{d[\mathrm{P}]}{dt} = \frac{\frac{k_2 E_T[\mathrm{S}]}{K_M^1}}{1 + \frac{[\mathrm{S}]}{K_M^1} + \frac{[\mathrm{S}]^2}{K_M}} \tag{32}$$

$$r_1 = \frac{\frac{k_2}{K_M^1}\frac{N}{\Omega}}{1 + \left(\frac{1}{K_M^1} - \frac{1}{K_M \Omega}\right)\left(\frac{N}{\Omega}\right) + \frac{1}{K_M}\left(\frac{N}{\Omega}\right)^2} \tag{33}$$

respectively. Note that the initial rate of change of the product concentration according to the stochastic approach, r_1/Ω, is always larger than the deterministic prediction $d[\mathrm{P}]/dt$ (with $E_T = 1/\Omega$ and $[\mathrm{S}] = N/\Omega$.). These differences are pronounced for small values of K_M.

However, in both the deterministic and stochastic approaches, the maximum rate of change of the product concentration occurs at a substrate concentration of $\sqrt{K_M}$ (follows from finding the maxima of eqs 32 and 33). Therefore, noise does not alter the critical substrate concentration at which the filter works but instead it enhances its response. This is confirmed by comparison to the numerics in Figure 4.

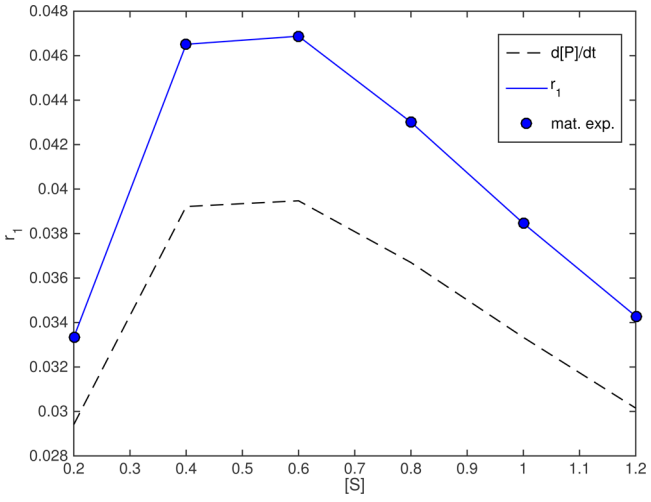

Figure 4. Stochastic dynamics of substrate inhibition. We here plot the initial rate of change of the product concentration (r_1/Ω according to the stochastic approach, and $d[P]/dt$ according to the deterministic approach) as a function of the substrate concentrations [S]. The theory is given by eq 33 for the stochastic approach (solid line) and by eq 32 for the deterministic approach (dashed lines) while the numerics (for the stochastic approach) is given by points. The parameters are $\Omega = 5$, $k_0 = 2$, $k_1 = k_2 = 1$, $k_3 = 4$, $k_4 = 1$, and $k_5 = 0$, implying $K^1_M = 1$ and $K_M = 0.25$. The substrate concentration $[\mathrm{S}] = N/\Omega$ where $N = 1, 2, 3, 4, 5, 6$.

THE MANY ENZYME CASE

In this section we present an exact result for the case of many enzymes. Due to the fact that there are many enzymes, the paths connecting the initial and final states are numerous and very complicated. Hence, unlike the single enzyme case, in practice it is typically not possible to obtain compact meaningful expressions for the mean first passage times.

The exception to this, as we now show, is the van-Slyke–Cullen mechanism.[22] This is a special case of the MM reaction mechanism (eq 1), obtained by considering the bimolecular reaction between substrate and enzyme to be irreversible:

$$\mathrm{S} + \mathrm{E} \overset{k_0}{\rightarrow} \mathrm{C} \overset{k_2}{\rightarrow} \mathrm{E} + \mathrm{P} \tag{34}$$

In what follows we shall define $c_1 = k_0/\Omega$ and $c_2 = k_2$, both of which have units of inverse time. The approach will be to first find a general expression for the first passage time distribution (FPT) and then to use this to calculate the mean first passage time to produce the first product molecule.

Let the number of substrate and enzyme molecules at time $t = 0$ be N and M, respectively. We also assume that there is no complex or product molecules initially. To calculate the FPT we have to consider all intermediate states of the system which connect the initial state with the states in which a single product molecule has been formed. These relevant states are schematically shown in Figure 5.

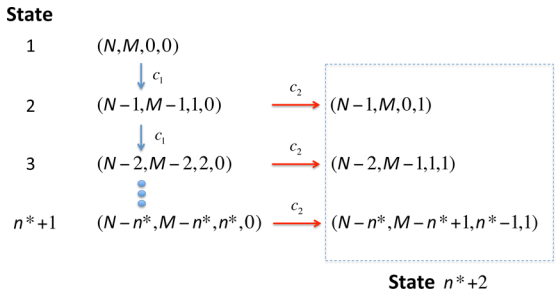

Figure 5. Schematic showing the relevant state space for the calculation of the FPT of the van-Slyke–Cullen mechanism (eq 34). There are $n^* + 2$ states where the initial state is labeled state 1 and the final state $n^* + 2$ is a lumped state consisting of all the absorbing states of the system, i.e., those with one product molecule. The state of the system is represented as (a, b, c, d) where a is the number of substrate molecule, b is the number of enzyme molecules, c is the number of complex molecules, and d is the number of product molecules. The blue and red arrows show transitions between states due to the bimolecular reaction $\mathrm{E} + \mathrm{S} \rightarrow \mathrm{C}$ and due to the unimolecular reaction $\mathrm{C} \rightarrow \mathrm{E} + \mathrm{P}$, respectively.

Defining $n^* = \min(N, M)$, one finds that there are $n^* + 2$ of these states, of which the first is the initial state of the system and the last is the absorbing state, a lumped state consisting of all those states with a single product molecule. The master equation describing the stochastic dynamics of the system as it moves from the initial state to the absorbing state is given by

$$\partial_{t'} \mathrm{P}(t') = A\mathrm{P}(t') \tag{35}$$

where t' is a dimensionless variable defined as $t' = c_2\, t$ (t is time), $\mathrm{P}(t') = (\mathrm{P}_1(t'), \mathrm{P}_2(t'), \ldots, \mathrm{P}_{n^*+2}(t'))$ is a vector of probabilities of the system to be in its allowed states, $\mathrm{P}_i(t')$ is the probability of the system being in state i at time t' (refer to Figure 1 for state classification) and A is a matrix with nonzero entries

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J. Phys. Chem. B 2017, 121, 13–23

$$A_{i,i} = -(K\Omega)^{-1}(N - i + 1)(M - i + 1) - (i - 1), \quad i = 1, ..., n - 1 \tag{36}$$

$$A_{i+1,i} = (K\Omega)^{-1}(N - i + 1)(M - i + 1), \quad i = 1, ..., n - 2 \tag{37}$$

$$A_{n,i} = (i - 1), \quad i = 1, ..., n - 1, \tag{38}$$

and all other entries of the matrix are zero. Here we have defined $n = n^* + 2$ and $K = k_2/k_0$ for convenience; note that the latter is the MM constant of the van-Slyke−Cullen reaction mechanism. The initial condition is specified by requiring $P_1(0) = 1$ and $P_i(0) = 0$, $\forall i \neq 1$. It then follows that the FPT is given by

$$f(t') = \partial_t P_n(t') = \sum_{i=2}^{n-1} (i - 1) P_i(t') \tag{39}$$

Applying the Laplace transform to the master eq 35, we obtain

$$s\tilde{P}_1(s) - 1 = A_{1,1}\tilde{P}_1(s) \tag{40}$$

$$s\tilde{P}_i(s) = A_{i,i-1}\tilde{P}_{i-1}(s) + A_{i,i}\tilde{P}_i(s), \quad i = 2, ..., n - 1 \tag{41}$$

This set of recurrence relations can be solved exactly, yielding

$$\tilde{P}_i(s) = \frac{1}{s - A_{11}} \prod_{j=2}^{i} \frac{A_{j,j-1}}{s - A_{j,j}} \tag{42}$$

It then follows from eq 39 that the Laplace transform of the FPT is given by

$$\tilde{f}(s) = \frac{1}{s - A_{11}} \sum_{i=2}^{n-1} (i - 1) \prod_{j=2}^{i} \frac{A_{j,j-1}}{s - A_{j,j}} \tag{43}$$

Applying the inverse Laplace transform yields the FPT distribution, described by a sum of convolutions of exponentials.[23,24] However, as we shall now see, it is not necessary to perform the inverse transform to obtain the mean first passage time.

The mean first passage time is the first moment of the FPT and hence given by

$$\langle t' \rangle = \int_0^\infty t' f(t') dt' = -\frac{d}{ds}\tilde{f}(s)|_{s=0} \tag{44}$$

It follows by substituting eq 43 in eq 44 that the general expression for the mean first passage time is given by

$$\langle t' \rangle = -\sum_{i=2}^{n-1} (i - 1)\left(\prod_{j=2}^{i} A_{j,j-1}\right)\frac{d}{ds} r_i(0) \tag{45}$$

where $r_j(s) = \prod_{w=1}^{j} \gamma_w(s)$ and $\gamma_w(s) = 1/(s - A_{w,w})$. What remains is to calculate $d/ds\, r_i(0)$ which is reported next. From the definition of $r_j(s)$ we can obtain the recurrence relation

$$\frac{d}{ds} r_i(0) = -\frac{r_{i-1}(0)}{A_{i,i}^2} - \frac{1}{A_{i,i}} \frac{d}{ds} r_{i-1}(0), \quad i \geq 2 \tag{46}$$

where $d/ds\, r_1(0) = -A_{1,1}^{-2}$. This can be solved exactly yielding

$$\frac{d}{ds} r_i(0) = \frac{(-1)^i}{\prod_{r=1}^{i} A_{r,r}} \sum_{j=1}^{i} A_{j,j}^{-1} \tag{47}$$

Hence the final expression for the mean first passage time is given by substituting the latter in eq 45 leading to

$$\langle t' \rangle = -\sum_{i=2}^{n-1} (-1)^i (i - 1) \frac{\prod_{r=1}^{i-1} A_{r+1,r}}{\prod_{r=1}^{i} A_{r,r}} \sum_{j=1}^{i} A_{j,j}^{-1} \tag{48}$$

Note that the $f = M + 2$ since A does not change either when swapping the values for N and M: the terms $A_{r,r}$ and $A_{r+1,r}$ are identical for $(N, M) = (x, y)$ and $(N, M) = (y, x)$. Obviously, this no longer holds for n−th arrival times with $n \leq 2$, when swapping N and M for $N \neq M$ changes the state space and hence the transition matrix A.

Due to its complex dependence on the elements of the matrix A, the equation for the mean first passage time is difficult to interpret, at first glance, for general number of enzyme and substrate molecules. To gain insight, we proceed by considering some specific cases. In particular, we consider the catalysis of N substrate molecules by one, two, and three enzymes. Substituting $M = 1, 2, 3$ in eq 48, and recalling $t' = c_2\, t$ (where is t is time) one obtains the following results:

$$\frac{1}{\langle t \rangle} = \frac{c_2 N}{K' + N}, \quad M = 1 \tag{49}$$

$$\frac{1}{\langle t \rangle} = \frac{2c_2 N((K' - 1) + N)}{K'(K' - 1) + (3K' - 1)N + N^2}, \quad M = 2 \tag{50}$$

$$\frac{1}{\langle t \rangle} = \frac{3c_2 N(2(2 - 3K' + K'^2) + (-6 + 5K')N + 2N^2)}{2K'(2 - 3K' + K'^2) + (4 - 18K' + 11K'^2)N + (-6 + 11K')N^2 + 2N^3}, \quad M = 3, \tag{51}$$

where $K' = K\Omega$ is the nondimensional MM constant.

A special case of this result which is of particular interest is $K' = 1$. Substituting this value in eqs 49−51 one finds the simple result:

$$\frac{1}{\langle t \rangle} = \frac{M c_2 N}{N + M} \tag{52}$$

We have verified this law to be true for arbitrary values of M (using Mathematica) though a general proof remains elusive. Hence for $K' = 1$, the inverse of the mean first passage time for arbitrary number of enzyme molecules M is given by a MM form. We confirm this result using numerics in Figure 6.

More generally note that the eqs 49−51 are all of the form

$$\frac{1}{\langle t \rangle} = \frac{\sum_{i=1}^{M} a_i N^i}{\sum_{i=0}^{M} b_i N^i} \tag{53}$$

where a_i and b_i are functions of c_2 and K. Note that N is any integer (greater than, equal to, or less than M). We have explicitly verified (using Mathematica) that this form holds for at least $M \leq 50$ which strongly suggests that this form is general for all M. Interestingly, one finds that eq 53 can always be written as a sum of MM terms

$$\frac{1}{\langle t \rangle} = \sum_{i=1}^{M} \frac{d_i N}{e_i + N} \tag{54}$$

where d_i and e_i are complicated functions of a_i and b_i. This result can be seen as a generalization of the results presented in ref 11, there it was shown that the inverse of the mean first passage time for a reaction catalyzed by a single enzyme molecule is a MM form, whereas here we show that generally, for an arbitrary number of enzyme molecules M, the inverse of

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J. Phys. Chem. B 2017, 121, 13−23

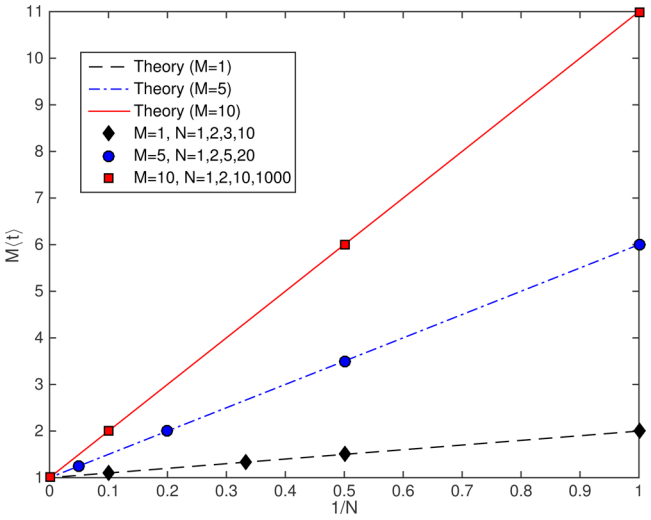

Figure 6. Stochastic dynamics of the van-Slyke−Cullen mechanism with M enzyme molecules. We plot the mean time to make the first product molecules $\langle t \rangle$ (multiplied by M) as a function of the initial number of substrate molecules N for the reaction system (eq 34). The theory is given by eq 52 and the numerics by points. The parameters are $\Omega = 1$, $c_1 = c_2 = 1$ such that $K' = 1$.

the mean first passage time is a sum of M Michaelis−Menten equations.

The same approach can be used to obtain expressions for the mean first passage time for the van-Slyke−Cullen mechanism with two substrate reactions and with cooperative reactions. We note that in these cases, due to the complexity involved, the evaluation of the expressions might be as tedious as the actual numerics and hence little intuition can likely be derived from the theory—the results are reported in the Supporting Information for completeness sake. Lastly, a comparison of our results (eqs 49−51) with those obtained under the substrate abundance assumption is provided in Appendix B.

CONCLUSION

In this article we have used the chemical master equation to derive closed-form expressions for the instantaneous average rate of product formation as well as for the rate of product formation averaged over the time to make a specified number of product molecules, without invoking the substrate abundance assumption. Our results go beyond the bulk of the existing stochastic enzyme kinetic results, which either directly or implicitly invoke the substrate abundance assumption,[9−12,25,26] sometimes in the form of the QSSA.

Our main results can be summarized as follows: (i) For the MM reaction mechanism with one enzyme molecule, we find that the average rate of product formation, calculated over the average time to produce m product molecules, follows a MM form in which the initial substrate molecule number N is replaced by another variable $z(N, m)$. For the case $m = 1$ (the average rate of product formation for the first turnover) this reduces to the single-molecule MM form reported by Xie and collaborators;[11] for the case $m = N$ this reduces to a novel logarithmically corrected MM form when N is large. We find that these corrections are present even when the reaction dynamics are further complicated, for example by increasing the number of substrates. (ii) While the expression for the average rate of product formation for noncooperative reactions (one or multiple substrate reactions with one enzyme molecule) obtained from the stochastic model agrees with the deterministic result obtained using the QSSA, this is not the case for cooperative enzyme reactions. (iii) For a positively cooperative enzyme reaction with one enzyme molecule, intrinsic noise leads to a less sharp switch from low to high rates of product formation (as the substrate concentration is varied) than that predicted by the deterministic Hill equation. (iv) For a single enzyme reaction capable of exhibiting substrate inhibition, noise enhances the rate of product formation at the critical substrate concentration relative to the deterministic prediction using the QSSA. (v) For a MM reaction mechanism with an arbitrary number M of enzyme molecules and with no reversible reaction, the rate of initial product formation is generally a sum of M Michaelis−Menten equations. This can be seen as a generalization of the result that for a single enzyme molecule MM reaction mechanism, the rate of initial product formation is a single MM form.[11]

OBTAINING m-TH PASSAGE TIMES VIA MATRIX EXPONENTIALS OR CTMC-BASED APPROACHES

Let $p_i(t) = p(S_i, t|S_1,0)$ be the probability for a reaction system to be in state S_i at time t given it is in state S_1 at time $t = 0$. We denote with $p(t) = [p_1(t), p_2(t), ..., p_n(t), p_{n+1}(t)]^T$ the probability vector of $n+1$ states, namely the n states that the system can be prior to reaching the absorbing state S_{n+1}. This absorbing state is a lumped state consisting of all the states with m product molecules, following directly from a state with $m-1$ product molecules.

To obtain $p(S_{n+1}, t|S_1,0)$ we solve $\frac{d}{dt}p(t) = Ap(t)$, where A denotes the matrix describing the transitions between the $n+1$ states. The solution of this differential equation is the matrix exponential mapping the initial probability to the probability at time t: $p(t) = e^{At}\, p(0)$, where $p(0) = [1\ 0\cdots\ 0]^T$. The last entry of this solution corresponds then to the cumulative distribution function (CDF) of the mean first passage time distribution to produce m product molecules at time t.

We use Roger Sidje's software Expokit[27] for numerical calculation of the matrix exponentials. The CDF is calculated for a discrete number of equidistant time points, while we ensure that the CDF takes value 1 for a large enough time point T and that reducing the distance between time points does not significantly change the outcome of the numerical differentiation, which yields the pdf $f(t)$. The mean first passage time is then calculated as $\int_0^T t\, f(t)$ which is numerically approximated as a Riemann sum with very small step size.

Alternatively, one can use known results about finite state continuous time Markov chains (CTMC) to obtain mean first passage times. In the CTMC context, first passage times are often referred to as hitting times, which follow a so-called phase type distribution. Such distributions have known closed forms for their moments while their PDFs and CDFs are usually described using matrix exponentials. The mean hitting time distribution for a $m+1$ state CTMC with one absorbing state can be written in the form

$$-v_0 G^{-1}\mathbf{1}$$

where v_0 is the probability row vector describing the initial probability of the process to start in each of the m non-absorbing states, $\mathbf{1}$ is a $m\times 1$ vector with all elements being 1, and G is a $m \times m$ matrix known as the infinitesimal generator of the Markov chain and closely related to the transition matrix A described above. We obtain G from A by removing in A the

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J. Phys. Chem. B 2017, 121, 13−23

column and the row that refer to the absorbing state and by taking the transpose of the resulting matrix. For instance, eq 8 is the expected hitting time of a three state ($m = 2$) CTMC, where state 2 is the absorbing state (cf. Table 1). Assuming that we start in state 0, i.e. $v_0 = (1,0)$ we can write down the mean hitting time (of state 2) as

$$\langle t_{i-1,i}\rangle = -(1,\,0)\begin{bmatrix} -h_i{*}k_0' & h_i{*}k_0' \\ k_1 & -k_1 - k_2 \end{bmatrix}^{(-1)} (1,\,1)^T \tag{55}$$

We can use the same approach to obtain all other expressions of mFPTs presented in this work.

COMPARISON WITH PSEUDO-FIRST ORDER KINETICS FOR SUBSTRATE AND ENZYME INTERACTION

As mentioned in the Introduction Section, it is common in the literature to invoke the substrate abundance assumption. Here

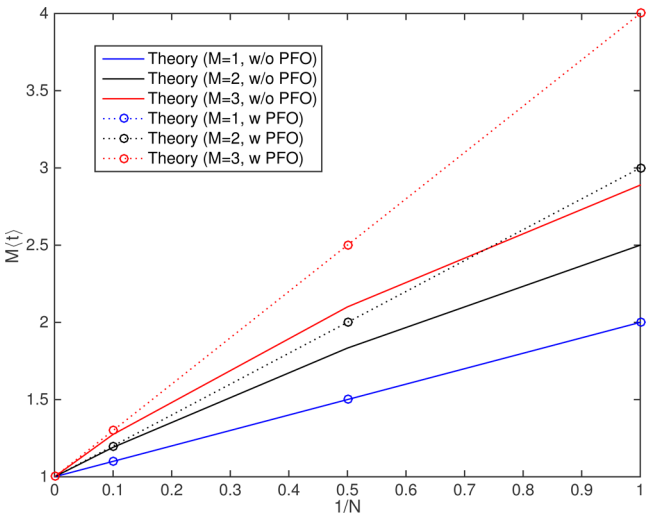

Figure 7. Stochastic dynamics of the van-Slyke–Cullen mechanism with pseudo-first order (PFO) reaction and $M = 1, 2, 3$ enzyme molecules. We plot the mean time to make the first product molecules $\langle t\rangle$ (multiplied by M) as a function of the initial number of substrate molecules N for the reaction system (eq 56). We compare the theory given by eqs 60–62 (dotted lines) with the theory for the reaction system (eq 34) (solid lines), given by eqs 49–51). The parameters are $\Omega = 1$, $c_1 = c_2 = 1$ such that $K' = 1$.

we briefly investigate the differences between our expressions eqs 49–51 and the ones obtained under the latter assumption. If the substrate is much more abundant than enzyme then eq 34 reduces to

$$\mathrm{E} \xrightarrow{k_0 N/\Omega} \mathrm{C} \xrightarrow{k_2} \mathrm{E} + \mathrm{P} \tag{56}$$

where we replace the bimolecular reaction between enzyme and substrate by a pseudo-first order reaction with an effective rate constant. In this case the matrix entries for A in eq 35 change to

$$A_{i,i} = -(K\Omega)^{-1}N(M - i + 1) - (i - 1),\ i = 1, \ldots, n - 1 \tag{57}$$

$$A_{i+1,i} = (K\Omega)^{-1}N(M - i + 1),\ i = 1, \ldots, n - 2 \tag{58}$$

$$A_{n,i} = (i - 1),\ i = 1, \ldots, n - 1 \tag{59}$$

This leads to the following inverse mean first passage time for the production of a product molecule:

$$\frac{1}{\langle t\rangle} = \frac{c_2 N}{K' + N},\ M = 1 \tag{60}$$

$$\frac{1}{\langle t\rangle} = \frac{2c_2 N(K' + N)}{(K' + N)^2 + K'N},\ M = 2 \tag{61}$$

$$\frac{1}{\langle t\rangle} = \frac{3c_2 N(2K' + N)(K' + 2N))}{(K' + N)(2K'^2 + 9K'N + 2N^2)},\ M = 3 \tag{62}$$

where $K' = K\Omega$ is the non-dimensional MM constant. Comparison of the above equations with eqs 49–51 shows that the substrate abundance assumption leads to an average rate of product formation which is always more than the true value (except for the case of one enzyme molecule $M = 1$ in which case the assumption leads to the correct value). This fact is illustrated in Figure 7. In particular one finds that eqs 49–51 become equal to eqs 60–62 in the limit of $K' \gg 1$, i.e., the error in the predictions of the substrate abundance assumption increases with decreasing volumes Ω (at constant K).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.6b08891.

van-Slyke–Cullen mechanism with two substrate reactions (multiple enzyme molecule case) and van-Slyke–Cullen with cooperative reactions (multiple enzyme molecule case) (PDF)

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Notes

The authors declare no competing financial interest.

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J. Phys. Chem. B 2017, 121, 13−23