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Citation for published version:

Holehouse, J, Sukys, A & Grima, R 2020, 'Stochastic time-dependent enzyme kinetics: Closed-form solution and transient bimodality', *The Journal of Chemical Physics*, vol. 153, no. 16, 164113.
<https://doi.org/10.1063/5.0017573>

Digital Object Identifier (DOI):

[10.1063/5.0017573](https://doi.org/10.1063/5.0017573)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

The Journal of Chemical Physics

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Cite as: J. Chem. Phys. **153**, 164113 (2020); <https://doi.org/10.1063/5.0017573>

Submitted: 08 June 2020 . Accepted: 23 September 2020 . Published Online: 26 October 2020

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Submitted: 8 June 2020 • Accepted: 23 September 2020 •

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ABSTRACT

We derive an approximate closed-form solution to the chemical master equation describing the Michaelis–Menten reaction mechanism of enzyme action. In particular, assuming that the probability of a complex dissociating into an enzyme and substrate is significantly larger than the probability of a product formation event, we obtain expressions for the time-dependent marginal probability distributions of the number of substrate and enzyme molecules. For delta function initial conditions, we show that the substrate distribution is either unimodal at all times or else becomes bimodal at intermediate times. This transient bimodality, which has no deterministic counterpart, manifests when the initial number of substrate molecules is much larger than the total number of enzyme molecules and if the frequency of enzyme–substrate binding events is large enough. Furthermore, we show that our closed-form solution is different from the solution of the chemical master equation reduced by means of the widely used discrete stochastic Michaelis–Menten approximation, where the propensity for substrate decay has a hyperbolic dependence on the number of substrate molecules. The differences arise because the latter does not take into account enzyme number fluctuations, while our approach includes them. We confirm by means of a stochastic simulation of all the elementary reaction steps in the Michaelis–Menten mechanism that our closed-form solution is accurate over a larger region of parameter space than that obtained using the discrete stochastic Michaelis–Menten approximation.

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I. INTRODUCTION

The mechanistic basis of the simplest single-enzyme, single-substrate reaction consists of a reversible step between an enzyme and a substrate, yielding the enzyme–substrate complex, which subsequently forms the product. This reaction is commonly called the Michaelis–Menten (MM) reaction.^{1,2}

For over a century, the dynamics of this reaction have been extensively studied using deterministic rate equations. Because these equations do not admit an exact closed-form solution, various approximations have been devised to obtain insight into the underlying dynamics. Use of the quasi-equilibrium or quasi steady-state approximations leads to the famous Michaelis–Menten equation, an ordinary differential equation relating the rate of product formation and the substrate concentration (see Ref. 3 for a discussion of these approximations and their range of validity). This equation provides a simple means to extract the relevant kinetic parameters

(the Michaelis–Menten constant and the maximum velocity) from experimental data. The Michaelis–Menten equation has also been solved exactly, leading to explicit expressions for the time-evolution of the substrate (and product) concentration.⁴

The stochastic formulation of enzyme kinetics, while not studied as much as its deterministic counterpart, has received increasing attention since the 1960s when the chemical master equation (CME) for the MM reaction mechanism was first derived and studied by Bartholomay.⁵ The CME is a probabilistic discrete description of chemical reaction kinetics that is valid in well-mixed environments for point reacting particles.^{6,7} Its relevance lies in its ability to describe kinetics when the molecule numbers are low, conditions typical in intracellular environments, e.g., the median copy number per cell of most enzymes in *E. coli* is below a thousand.⁸ Research efforts concerning the MM mechanism in the area of stochastic chemical kinetics can be, broadly speaking, categorized into three types: (i) The search for a solution of the CME for the MM reaction

and its various extensions, i.e., obtaining a closed-form solution for the time-dependent or steady-state probability distribution of the molecule numbers of each species in the reaction system.^{9–11} (ii) The reduction of the CME and the construction of the stochastic equivalent of deterministic approximations (such as the fast equilibrium, quasi steady-state, and total quasi steady-state approximations) and understanding their regime of validity.^{12–25} (iii) The derivation of exact or approximate expressions for the mean of the stochastic rate of product formation and an investigation of the differences or similarities from the predictions of the deterministic Michaelis–Menten equation.^{26–32}

The majority of the literature has focused on (ii) and (iii). There are very few studies that focus on (i), principally, because the CME is notoriously difficult to solve analytically.²³ In this paper, we are interested in deriving new solutions of the CME for enzyme kinetic systems, and hence, next, we briefly review the known solutions (see also Ref. 33 for a lengthier discussion). Arányi and Tóth⁹ were the first to exactly solve the CME introduced by Bartholomay for the special case in which there is only one enzyme molecule with several substrate molecules in a closed compartment; in particular, they obtained an exact expression for the joint distribution of the number of substrate and enzyme molecules as a function of time (since the original paper is rather difficult to find, in Appendix A, we have reproduced the derivation in a concise manner). Another exact solution is reported in Ref. 10 by Schnoerr *et al.* who derived the exact steady-state solution for the CME describing the MM reaction system with one enzyme molecule and augmented with a substrate production reaction step (to model, for example, the production of substrate via translation). To our knowledge, there are no known exact solutions for the time-dependent probability distribution of the CME of the MM reaction system with multiple enzyme molecules. However, an approximate closed-form solution was derived by Dóka and Lente,¹¹ using a so-called stochastic equivalent of the quasi steady-state approximation. Namely, they made an ansatz that the joint distribution of the number of substrate and enzyme molecules takes the form of a product of a time-dependent function and a constant value that characterizes the state occupied by the system. Using this assumption and a number of heuristic arguments, the authors reduced the problem to a one-variable master equation, which they then solved iteratively. However, one could argue that the derivation outlined in Ref. 11 lacks a certain degree of rigor, and the analysis of the accuracy of the solution over time and parameter space is rather limited, which raises questions about the validity of the approximation.

In this paper, our aim is to (a) derive an expression for the approximate time-dependent solution of the CME of the MM reaction system with multiple enzyme molecules under quasi-equilibrium conditions using an approach that is more rigorous and systematic than in previously published works, (b) compare and contrast this solution with the solution of an often used reduced CME for the MM reaction in the literature, and (c) use the closed-form solution to identify interesting dynamical phenomena. We verify our approximate analytic results against the benchmark stochastic simulation algorithm (SSA).⁶ Our paper is divided as follows. In Sec. II, we briefly review the main results known for deterministic enzyme kinetics, focusing, in particular, on the quasi-equilibrium approximation (QEA). In Secs. III A and III B, we introduce our method by first applying it to the MM reaction with a single

enzyme molecule and subsequently to the case of multiple enzyme molecules. The method consists of three steps: (1) using a time scale separation method called averaging³⁴ to define groups of rapidly equilibrating states, which then allows the derivation of a master equation describing the Markovian dynamics of these groups on the slower time scale; (2) solving the resultant time-dependent, single variable master equation for the group dynamics using the method developed in Ref. 35, which has the advantage of bypassing the calculation of the eigenvectors of the transition matrix and, hence, considerably simplifies the analytical computations; (3) using the time-dependent solution describing the group dynamics to construct the marginal time-dependent distributions for both the numbers of substrate and enzyme molecules. We use the closed-form solution to find the regions of parameter space where transient bimodality of the distribution of substrate molecules occur. In Sec. IV, we show that our solution is accurate over a wider region of parameter space than the solution of a commonly used reduced master equation with a propensity that has the same hyperbolic dependence on the number of substrate molecules as the deterministic Michaelis–Menten equation (an approach popularized by Rao and Arkin¹³). In Sec. V, we show that the same three-step method used in Secs. III A and III B can be used to derive time-dependent distributions for multi-substrate enzyme reactions. We finish by discussing our results in Sec. VI.

II. DETERMINISTIC ENZYME KINETICS

Before progressing to stochastic enzyme kinetics, we first briefly outline some of the main results known for deterministic enzyme kinetics. We consider the following chemical reaction system:

where S denotes the substrate species, E denotes the enzyme species, C denotes the enzyme–substrate complex, and P denotes the product. This system can be thought of as a reduction of the more biologically realistic set of reactions,

where the unbinding of the product from the enzyme is very fast. For simplicity, we assume the initial condition for this system is that all enzymes are unbound to the substrate. There are two conservation laws for this system: $[E] + [C] = [E]_0$ and $[S] + [C] + [P] = [S]_0$, where $[i]$ denotes the concentration of species i and $[i]_0$ denotes the initial concentration of species i . Assuming well-mixed conditions and the law of mass action, the deterministic dynamics of the reaction system in Eq. (1) are described by a set of coupled ordinary differential equations (commonly called the rate equations) describing the time-evolution of the substrate and complex concentrations,

$$\begin{aligned} \frac{d[S(t)]}{dt} &= -k_0[S(t)]([E]_0 - [C(t)]) + k_1[C(t)], \\ \frac{d[C(t)]}{dt} &= -(k_1 + k_2)[C(t)] + k_0[S(t)]([E]_0 - [C(t)]). \end{aligned} \quad (3)$$

Note that the time-dependent concentrations of E and P can be straightforwardly obtained from the time-dependent solutions of C and S by means of the conservation laws previously stated. Although seemingly simple, the rate equations given by Eq. (3) are not easy to solve analytically for the time-dependent analytic solution, and as such, one is limited to finding approximate solutions. Two of the most common approximations used in the literature are the (i) *quasi steady-state assumption* (QSSA) and (ii) the *quasi-equilibrium approximation* (QEA), also called the rapid equilibrium approximation or the reverse quasi steady-state assumption. The QSSA, derived by Briggs and Haldane,³⁶ assumes that after a short transient, the concentration of the complex (and enzyme) is in a quasi steady-state (with regard to the substrate and product); thus, under the QSSA, it is assumed that $d[C(t)]/dt \approx 0$. See Ref. 37 for a detailed discussion of this approximation and for its range of validity. On the other hand, the QEA assumes that substrate binding and dissociation occur much more rapidly than product formation such that the substrate, enzyme, and complex are approximately in equilibrium. Thus, under the QEA, it is assumed that $d[S(t)]/dt \approx 0$; this approximation, popularized by Michaelis and Menten,¹ is commonly used in the analysis of various biochemical models.³⁸

Enforcing either the QSSA or QEA leads to the following effective rate equation describing the time-evolution of the substrate concentration:

$$\frac{d[S(t)]}{dt} = \frac{-V_{\max}[S(t)]}{k + [S(t)]}, \quad (4)$$

where $V_{\max} = k_2[E]_0$, $k = (k_1 + k_2)/k_0$ if the QSSA is used, $k = k_1/k_0$ if the QEA is used, and where the conservation law $[S] + [P] = [S]_0$ holds. Note that a necessary limitation of Eq. (4) is that we have assumed that $[S] + [C] \approx [S]$, which is true in the limit $[S]_0/[E]_0 \gg 1$. Equation (4) has been solved perturbatively in a number of studies, all of which also assessed the validity of the QSSA.^{37,39} An exact solution was reported in Ref. 4, which is given by

$$\langle n(t) \rangle_a = \Omega[S(t)] = \Omega k W\left(\frac{[S]_0}{k} \exp\left(\frac{-V_{\max}t + [S]_0}{k}\right)\right), \quad (5)$$

where $\langle n(t) \rangle_a$ gives the (deterministic) number of bound and unbound substrate molecules obtained in the limit $[S]_0/[E]_0 \gg 1$ at time t , Ω is the volume of the system, and $W(\cdot)$ is the principal branch of the Lambert W function (also known as the Omega function). Note that within van Kampen's *system size expansion*⁴⁰ for monostable systems, the rate equations are obtained as the macroscopic limit of the stochastic description of a well-mixed chemical system; within this formalism, the concentration of a species i multiplied by the volume is the same as the mean number of molecules of species i . Hence, in our case, $\langle n(t) \rangle_a$ can also be interpreted as the mean number of substrate molecules in the macroscopic limit. In the rest of this article, we study the stochastic equivalent of the QEA, and thus, we shall use $k = k_1/k_0$.

III. STOCHASTIC QEA ANALYSIS

A. Single enzyme

For simplicity, we first illustrate the method by solving the enzyme system described in Eq. (1) for the case of one enzyme molecule with initially N substrate molecules. Since there are no birth-death processes coupled to any species, the conservation equations $n_E + n_C = 1$ and $n + n_C + n_P = N$ hold, where n denotes the number of substrate molecules and all other n_i denote the number of species i .

We label the microstate of the reaction network in Eq. (1) as (n, n_E) , which fully specifies the state of the system due to the conservation laws stated previously. The possible transitions between all the discrete microstates of this system are illustrated in Fig. 1(i): the system starts from the state $(N, 1)$ and eventually ends up in the state $(0, 1)$. Our goal now will be to find the marginal probability distribution $P(n; t)$, i.e., the probability of observing n substrate molecules at a time t .

FIG. 1. Illustration of the enzymatic system described by a single enzyme and N initial substrate molecules. (i) Markovian dynamics of the enzyme kinetic system described by a single enzyme. The initial condition for the system is $(N, 1)$, and as $t \rightarrow \infty$, the microstate of the system is guaranteed to be that of the absorbing state $(0, 1)$, with no remaining substrate and one free enzyme. (ii) Markovian dynamics in the reduced model, where processes occurring in a group are assumed to be much faster than the interactions between the groups themselves. The label “group m ” denotes the set of microstates that exist when m product molecules have been formed, given that there are no product molecules initially; hence, it is easily seen that there are $N + 1$ groups in total with labels $m = \{0, 1, 2, \dots, N - 1, N\}$.

Assuming Markovian dynamics,²³ it follows that the time-evolution of $P(n, n_E; t)$ (the probability of observing n substrate molecules and n_E enzyme molecules at a time t) is given by the CME,

$$\begin{aligned} \frac{\partial P(n, n_E; t)}{\partial t} = & k_0(n+1)(n_E+1)P(n+1, n_E+1; t) \\ & + (2-n_E)(k_1P(n-1, n_E-1; t) \\ & + k_2P(n, n_E-1; t)) \\ & - (k_0n n_E + (1-n_E)(k_1+k_2))P(n, n_E; t). \end{aligned} \quad (6)$$

Note that this CME is valid only for a single enzyme system, i.e., $n_E \in \{0, 1\}$. Furthermore, note that the bimolecular propensity is inversely proportional to the volume Ω , but for simplicity, we set $\Omega = 1$ (a convention throughout this article). The standard approach involves introducing the time-dependent marginal generating functions $G_{n_E}(z; t) = \sum_n z^n P(n, n_E; t)$ and attempting to solve the generating function partial differential equations, e.g., using eigenfunction methods.⁷ However, this standard method quickly leads one to mathematical difficulty. An analytic solution only presents itself in a non-cumbersome form where one assumes that the initial state contains a single substrate molecule.⁹ In Appendix A, we summarize the single enzyme solution provided by Ref. 9, and its complexity even in the single substrate molecule case motivates the analysis we present below.

We take a different approach. We first simplify the problem through the use of averaging.^{34,41,42} Specifically, the procedure lumps together microstates equilibrating on a fast timescale in groups, which then allows one to write a master equation describing the dynamics of the groups on the slow timescale. We shall assume that the slow timescale is that associated with product formation, i.e., k_2 is sufficiently small (we will be more precise what this really means later), and hence, the averaging procedure is in the same spirit as the QEA discussed in Sec. II.

Since k_2 is small, it follows that we can group all microstates that are in rapid equilibrium with each other (due to the fast processes of binding and unbinding of substrate from the enzyme), as shown in Fig. 1(ii); group m is then the set of microstates of the system accessible when m product molecules have been produced. We define $p_m^g(t)$ as the probability to be in group m at a time t and $p_{i,m}^{qe}$ as the probability of having i free enzymes for the *reduced* system given by considering only reactions among microstates in group m . Once these probabilities are found, we can construct $P(n; t)$, based on the fact that there are two microstates that contain n substrate molecules: $(n, 0)$ and $(n, 1)$ associated with groups $N - (n + 1)$ and $N - n$, respectively. This means that under the stochastic QEA,

$$P(n; t) = p_{N-n}^g(t)p_{1,N-n}^{qe} + p_{N-(n+1)}^g(t)p_{0,N-(n+1)}^{qe}. \quad (7)$$

In the case of the single enzyme system studied in this section, the quasi-equilibrium probabilities are trivial (since there are only two microstates in each group) and are given by

$$p_{1,N-n}^{qe} = \frac{k_1}{k_1 + k_0n} \quad \text{and} \quad p_{0,N-(n+1)}^{qe} = \frac{k_0(n+1)}{k_1 + k_0(n+1)}. \quad (8)$$

All that remains is the task of finding $p_m^g(t)$. To do this, we first write the master equation for the transitions between groups. Rescaling time as $t' = k_2t$ and making use of the previous definition, $k = k_1/k_0$, the master equation for the groups is

$$\partial_{t'} p_m^g(t') = a_m p_{m-1}^g(t') - a_{m+1} p_m^g(t'), \quad (9)$$

where

$$a_m = \frac{N - (m - 1)}{k + N - (m - 1)}, \quad 1 \leq m \leq N + 1 \quad (10)$$

and $a_{i \leq 0} = 0$. Note that a_m is the probability of the jump from group $m - 1$ to group m in a unit interval of rescaled time. From Fig. 1(ii), the probability of the jump from group $m - 1$ to group m in a unit interval of normal time is equal to k_2 multiplied by the probability of being in the microstate $(N - m, 0)$, which under the rapid equilibrium assumption is $k_0(N - m + 1)/(k_1 + k_0(N - (m - 1)))$. Due to time rescaling, the factor of k_2 disappears and, hence, follows Eq. (10).

Since there are $N + 1$ groups in total, Eq. (9) corresponds to a system of $N + 1$ ODEs, which can be concisely written as the matrix equation,

$$\partial_{t'} \underline{p}^g(t') = \mathcal{Q} \cdot \underline{p}^g(t'), \quad (11)$$

where $\underline{p}^g(t')$ is a $N + 1$ element column vector defined by $\underline{p}^g(t') = (p_0^g(t'), p_1^g(t'), \dots, p_N^g(t'))$ and $\mathcal{Q}$ is a $(N + 1) \times (N + 1)$ lower-bidiagonal square matrix defined by

$$\mathcal{Q} = \begin{pmatrix} -a_1 & & & & \\ a_1 & -a_2 & & & \\ & a_2 & -a_3 & & \\ & & \ddots & \ddots & \\ & & & a_N & -a_{N+1} \end{pmatrix}. \quad (12)$$

As we will describe below, we solve the set of ODEs given by Eq. (11) using the method described in Ref. 35, which provides an *exact* time-dependent solution for any one-variable one-step master equation with a finite number of microstates *as long as one can find the eigenvalues of the transition rate matrix exactly*. In our case, the eigenvalues of $\mathcal{Q}$ are trivial since $\mathcal{Q}$ is lower-bidiagonal, and they are given by the diagonal elements. Hence, the eigenvalues of $\mathcal{Q}$ are given by $\lambda_i = -a_i$, $1 \leq i \leq N + 1$. Note that $\lambda_{N+1} = 0$ and is the largest eigenvalue, with all $\lambda_{1 \leq i \leq N} < 0$.

We now proceed to use these eigenvalues to find the time-dependent solution to Eq. (11). The solution to this set of ODEs is formally given by

$$\underline{p}^g(t') = \exp(\mathcal{Q}t') \cdot \underline{p}^g(0), \quad (13)$$

where $\exp(\mathcal{Q}t')$ is defined as a matrix exponential. For a general master equation, this matrix exponential is typically difficult to deal with; however, in our case, $\mathcal{Q}$ is lower-bidiagonal, and hence, we

can proceed via the method of Ref. 35. We first consider Cauchy's integral formula for matrices, explicitly given by⁴³

$$\underline{f}(\mathcal{Q}) = \frac{1}{2\pi i} \oint_C (zI - \mathcal{Q})^{-1} \cdot \underline{f}(z) dz, \quad (14)$$

where C is a closed contour in the complex plane that encloses all the eigenvalues of $\mathcal{Q}$ and I is the identity matrix. Taking $\underline{f}(z) = e^{zt'} \underline{p}^g(0)$, we then arrive at

$$\underline{p}^g(t') = \frac{1}{2\pi i} \oint_C (zI - \mathcal{Q})^{-1} \cdot \underline{p}^g(0) e^{zt'} dz. \quad (15)$$

A typical initial condition is $\underline{p}_m^g(0) = \delta_{m,0}$, meaning that we always start in group 0, which contains the microstates $(N, 1)$ and $(N - 1, 0)$, as is shown in Fig. 1(i). Note that $\delta_{i,j}$ is the Kronecker delta. Using this initial condition, Eq. (15) becomes

$$p_m^g(t') = \frac{1}{2\pi i} \oint_C (zI - \mathcal{Q})_{m+1,1}^{-1} e^{zt'} dz. \quad (16)$$

We show at the end of this section how to extend the time-dependent solution for a general initial distribution. Since it is bidiagonal, the inverse of $zI - \mathcal{Q}$ can easily be found via Cramer's rule,⁴⁴

$$(zI - \mathcal{Q})_{ij}^{-1} = \begin{cases} 0, & i < j, \\ \frac{1}{a_i + z}, & i = j, \\ \frac{1}{a_j + z} \prod_{k=j+1}^i \frac{a_{k-1}}{a_k + z}, & i > j. \end{cases} \quad (17)$$

Substituting this into Eq. (16), then, gives us

$$p_m^g(t') = \begin{cases} 0, & m < 0, \\ \frac{1}{2\pi i} \oint_C \frac{e^{zt'}}{z - \lambda_1} dz, & m = 0, \\ \frac{1}{2\pi i} \{(-1)^m \prod_{k=1}^m \lambda_k\} \times \left\{ \oint_C \frac{e^{zt'}}{\prod_{k=1}^{m+1} (z - \lambda_k)} dz \right\}, & m > 0, \end{cases} \quad (18)$$

where we have utilized the relation $\lambda_i = -a_i$. These integrals can then be evaluated using Cauchy's residue theorem,⁴⁵ explicitly stated as

$$\oint_C f(z) dz = 2\pi i \sum_k \text{Res}(f(z), z_k), \quad (19)$$

where the values $z = z_k$ are poles of $f(z)$ within C and the residues are $\text{Res}(f(z), z_k) = \lim_{z \rightarrow z_k} (z - z_k) f(z)$ for the simple poles in Eq. (18). Note that the poles of the complex integrals in Eq. (18) are the eigenvalues of $\mathcal{Q}$. Therefore, from Eq. (18), we finally get an expression for $p_m^g(t')$ as

$$p_m^g(t') = \begin{cases} 0, & m < 0, \\ e^{\lambda_1 t'}, & m = 0, \\ \{(-1)^m \prod_{k=1}^m \lambda_k\} \times \left\{ \sum_{k=1}^{m+1} \frac{e^{\lambda_k t'}}{\prod_{j=1, j \neq k}^{m+1} (\lambda_k - \lambda_j)} \right\}, & m > 0. \end{cases} \quad (20)$$

Hence, the time-dependent probability distribution $P(n; t)$ is given by Eq. (7) together with Eqs. (8) and (20). The extension to a more general initial distribution is then relatively simple. Consider some initial distribution $\underline{p}^g(0) = \underline{q}$, where $\underline{q}$ is an $N + 1$ element vector; the time-dependent group probability $p_{m|q}^g(t')$ is then given by the weighted sum,

$$p_{m|q}^g(t') = \sum_{j=0}^N p_{m|q_j}(t') q_j. \quad (21)$$

This initial condition could be useful to model variation in the initial number of substrate molecules due to uncertainty introduced by an experimental error or else due to the intrinsic noise in the reaction mechanism generating the substrate. Note that if $q_m = \delta_{m,0}$, one clearly recovers the analysis shown above. For the rest of this paper, we only consider the initial condition $\underline{p}_m^g(0) = \delta_{m,0}$, specifically where all enzymes are initially unbound to the substrate and where there are initially zero product molecules, but note that the analysis that follows can be easily extended for more general initial distributions.

In the beginning of this derivation, we stated that the main assumption is that k_2 is sufficiently small. This statement can be made more precise as follows. From Fig. 1(ii), it is clear that the exit from group m can only occur when the enzyme is bound to the substrate, i.e., from state $(N - m - 1, 0)$. Now, given that we are in this state, it follows that only two reactions can occur: either a reaction that causes a group change, i.e., $(N - m - 1, 0) \rightarrow (N - m - 1, 1)$, which occurs with rate k_2 , or a reaction that leads to no group change, i.e., $(N - m - 1, 0) \rightarrow (N - m, 1)$, which occurs with rate k_1 . Hence, the probability of leaving the group is $k_2/(k_1 + k_2)$, from which it follows that the microstates in each group will achieve quasi-equilibrium if $k_2 \ll k_1$. Therefore, this is the condition under which our method provides a good approximation to the distribution of substrate molecules at all times.

We test the distributions predicted by Eq. (7) against the SSA in Figs. 2(A-i)–2(A-iii) and Figs. 2(B-i)–2(B-iii). In Figs. 2(A-i)–2(A-iii) we show that the solution is accurate for small $N = 8$, over a time range from $t' = 1$ near the initial condition to $t' = 12$ close to the absorbing state, where the validity criterion $k_1 \gg k_2$ holds. In Figs. 2(B-i)–2(B-iii) we observe that our solution agrees similarly well with the SSA for larger values of N . For a more general comparison of the exact solution to SSA through time, we can compute the mean and standard deviation from Eq. (7),

$$\langle n(t') \rangle = \sum_{n=0}^N n P(n; t'), \quad (22)$$

$$\sigma(t') = \sqrt{\left(\sum_{n=0}^N n^2 P(n; t') \right) - \langle n(t') \rangle^2}. \quad (23)$$

The stochastic mean number of substrate $\langle n \rangle$ can then be compared to the deterministic mean number $\langle n \rangle_d$ obtained from the rate

FIG. 2. Comparison of the analytic time-dependent probability distribution of substrate molecules for the enzyme reaction in (1) with one enzyme molecule, i.e., $M = 1$, and N initial substrate molecules to the distribution obtained from the SSA.⁶ Note that the analytic solution is given by Eq. (7) together with Eqs. (8) and (20). In all cases, we enforce $k_1/k_2 \gg 1$ such that the quasi-equilibrium assumption behind the QEA is justified. We show the time-evolution of the distribution for substrate numbers, from near the initial condition to near the absorbing state, in two cases: (A-i)–(A-iii) are for $N = 8$, $k_0 = k_1 = 10^3$, $k_2 = 1$, meaning that $k = k_1/k_0 = 1$. (B-i)–(B-iii) are for $N = 50$, and all rate parameters as in the previous case. Note that the analytical solution (green lines) matches the SSA (black dots) for all times, for both a small and large initial number of substrate molecules. In (A-iv) and (B-iv), we show the corresponding plots of the time-evolution of the mean $\langle n \rangle$ and of the standard deviation σ of the distributions of substrate molecules, as predicted by our theory; these are compared with the mean calculated from the SSA and the mean $\langle n \rangle_d$ obtained from the numerical solution of the deterministic rate equations given by Eq. (3). Note that the deterministic mean is a better approximation to the stochastic mean for larger N . As shown in (B-iii), and mildly in (A-ii), the distribution can be bimodal at intermediate times. Each SSA probability distribution is constructed from 10^5 individual reaction trajectories.

equations. That is, we numerically solve Eq. (3) for $[S(t)]$ with $k_2 = 1$, noting that $\langle n \rangle_d = [S(t)]$ as we have previously set $\Omega = 1$.

In Figs. 2(A-iv) and 2(B-iv), we plot the evolution of the stochastic and deterministic mean substrate numbers in time and compare them to the SSA for parameters sets $N = 8$, $k = 1$ and $N = 50$, $k = 1$, respectively. We also show the standard deviation about the mean, i.e., $\langle n \rangle \pm \sigma$, where we have dropped the time-dependence for brevity, given in the blue envelope. Clearly, $\langle n \rangle$ from Eq. (22) matches the mean predicted by the SSA for most times, aside from the initial condition at $t' = 0$, where a step-like drop is observed in the mean predicted by the SSA to the value predicted by the quasi-equilibrium analysis. This step-like drop to the quasi-equilibrium value of the mean is known as the *initial transient* and is seen in more detail in Appendix B. The explanation of the initial transient follows by considering the system after quasi-equilibrium has been reached between the two microstates in group 0, $(N, 1)$ and

$(N - 1, 0)$, after a time $t'_c \approx 1/\min\{k_0N, k_1\}$, which is small under the rapid equilibrium assumption. Because of the discreteness of the substrate molecules, $\langle n \rangle$ after a time $t'_c \ll 1$ becomes an average over $n = N$ and $n = N - 1$ weighted by the quasi steady-state probabilities $p_{1,0}^{qe}$ and $p_{0,0}^{qe}$, respectively, hence the step-like drop in the mean predicted by the SSA at $t' = t'_c$. The method of averaging in the stochastic QEA assumes the immediate occurrence of the equilibrium in group 0 at $t' = 0$, hence the dispatch of $\langle n(t' = 0) \rangle$ from the exact initial condition. This also explains why the standard deviation predicted by the stochastic QEA [notably in Fig. 2(A-iv)] appears to be non-zero at $t' = 0$: since the system is modeled to be in quasi-equilibrium at initiation, equilibrium fluctuations are present even at $t' = 0$. However, so long as the SSA parameters are chosen such that $k_1/k_2 \gg 1$, the stochastic QEA provides a very good approximation even for small times so long as $t' > t'_c$. Additionally, we compared $\langle n \rangle$ to the deterministic

mean number of the free substrate, $\langle n \rangle_d$, and predicted the numerical solution of Eq. (3). Overall, the deterministic solution is found to be in good agreement with the mean predicted by the SSA and the stochastic QEA; however, there does exist a small disagreement where the mean number of substrate molecules is small [seen more explicitly in Fig. 2(B-iv)]. This disagreement occurs since molecular discreteness is very important where $\langle n \rangle$ is very small, and properly accounting for it leads to differing dynamics for $\langle n \rangle$ in this region, whereas the behavior of $\langle n \rangle_d$ does not change compared to $\langle n \rangle_d \gtrsim 1$ since the deterministic analysis considers the molecule number to be continuous. As we shall see later, increasing the number of enzyme molecules removes this discrepancy between the stochastic and deterministic means, highlighting that the discrepancy seen here is because we do not consider enzyme molecules to be discrete in the deterministic analysis.

From Fig. 2(B-iii), we observe that the distribution of substrate molecule numbers can be bimodal at intermediate times (there are two peaks at $n = 0$ and $n = 6$ at $t' = 45$). This bimodality, though less conspicuous, can, in fact, also be observed in Fig. 2(A-ii) with peaks at $n = 0$ and $n = 2$. From Figs. 2(A-iv) and 2(B-iv), we can see that in both cases, the bimodality occurs at a time t' when $\langle n \rangle - \sigma \approx 0$, i.e., when the fluctuations are large enough to cause frequent transitions to the absorbing state. This type of dynamical phase transition (which we shall refer to as transient bimodality), from a unimodal distribution to a bimodal one and then back to a unimodal one, as time progresses, has also been recently observed in genetic feedback loops⁴¹ and is known in non-biological systems.^{46,47} We will discuss this phenomenon more extensively in Sec. III B 2.

B. Multiple enzymes

We now extend the solution to enzyme system (1) to the case where initially there are N free substrate molecules and M free

enzyme molecules with the constraint of substrate abundance, i.e., $N \geq M$. Note that the solution to the system with $M \geq N$ follows as a special case of the $N \geq M$ system, discussed at the end of this section.

We proceed in solving this system as we did in the single enzyme case: assuming that k_2 is sufficiently small, we group the microstates governed by the fast processes together to form $N + 1$ groups between which the transitions are significantly slower than those between the fast internal states of an individual group. The Markov chain describing the system split into groups is shown in Fig. 3. Our task is then to find (i) the equilibrium probabilities $p_{i,m}^{qe}$ of being in each fast internal state i (considering only the reactions between the internal states in group m) and (ii) to find the time-dependent probability $p_m^g(t)$ of being in group m . Knowledge of both (i) and (ii) will allow us to approximate the distribution of interest, $P(n; t)$.

We begin by finding the probabilities $p_{i,m}^{qe}$ and revise its definition for the multiple enzyme case: $p_{i,m}^{qe}$ is the equilibrium probability of having $M - i$ free enzymes in the case of a reduced system involving only the reactions among the fast internal states contained in group m . Now, finding $p_{i,m}^{qe}$ for any group $0 \leq m < (N - 1)$ is more complicated than was the case for a single enzyme system since there we had only two fast internal states in each group. To proceed, we consider the following Markovian dynamics of a system with $L + 1$ possible microstates,

$$0 \xrightleftharpoons[k_{1,0}]{k_{0,1}} 1 \xrightleftharpoons[k_{2,1}]{k_{1,2}} \dots \xrightleftharpoons[k_{L,L-1}]{k_{L-1,L}} L. \quad (24)$$

One can then write the master equation for this dynamical system in the matrix form,

$$\partial_t \underline{P}_t = \mathcal{M} \cdot \underline{P}_t, \quad (25)$$

where $\underline{P}_t = (P_t(0), P_t(1), \dots, P_t(L))$, $P_t(i)$ is the probability of being in microstate i at time t , and

FIG. 3. Illustration showing the transitions between the discrete microstates of enzyme system (1) with initially M enzymes and N substrate molecules where $N \geq M$. Microstates that are in rapid equilibrium with each other are aggregated together, with each set of such fast internal states corresponding to a group. Namely, the label “group m ” denotes the set of microstates that exist when there are m product molecules, given that there are no product molecules initially. Groups $0 \leq m \leq N - M$ have $M + 1$ fast internal states, whereas groups $N - M < m \leq N$ have $N - m + 1$ fast internal states. Note that as $t \rightarrow \infty$, we are guaranteed to be in the absorbing state $(0, M)$.

$$\mathcal{M} = \begin{pmatrix} -k_{0,1} & k_{1,0} & & & \\ k_{0,1} & -(k_{1,0} + k_{1,2}) & k_{2,1} & & \\ & k_{1,2} & -(k_{2,1} + k_{2,3}) & k_{3,2} & \\ & & \ddots & \ddots & \ddots \\ & & & k_{L-1,L} & -k_{L,L-1} \end{pmatrix}. \quad (26)$$

Enforcing the quasi-equilibrium condition, $\partial_t(\cdot) = 0$, converts the system of $L + 1$ ODEs in Eq. (25) into a system of $L + 1$ simultaneous equations in the equilibrium microstate probabilities $P(i)$, given by $\mathcal{M} \cdot \underline{P} = 0$. One can explicitly solve this set of simultaneous equations under the constraint $\sum_i P(i) = 1$, yielding the probabilities

$$P(i) = \frac{(\prod_{j=1}^i k_{j-1,j}) \times (\prod_{j=i+1}^L k_{j,j-1})}{\sum_{i=0}^L [(\prod_{j=1}^i k_{j-1,j}) \times (\prod_{j=i+1}^L k_{j,j-1})]}. \quad (27)$$

Note that due to the definition of the empty product being equal to 1, when we have either $i = 1$ or $i = L$, the numerator of Eq. (27) is equal to 1. Further note that one could also utilize the King–Altman method^{48,49} to arrive at Eq. (27). Using this result, we can find the quasi-equilibrium probabilities for each group shown in Fig. 3. First, we consider the groups $0 \leq m \leq N - M$, each with $M + 1$ fast internal states as these groups contain more (or the same number) free substrate molecules than enzymes. Taking the specific example of group $m = 0$, we see that we have a total of $M + 1$ microstates, i.e., $L = M$, $k_{j-1,j} = k_0(N - (j - 1))(M - (j - 1))$, and $k_{j,j-1} = jk_1$, with $1 \leq j \leq M$. Identifying $p_{i,0}^{qe}$ with $P(i)$ in Eq. (27), we find that

$$p_{i,0}^{qe} = \frac{k_0^i k_1^{M-i} \{ \prod_{j=1}^i (N - (j - 1))(M - (j - 1)) \} \times \{ \prod_{j=i+1}^M j \}}{\sum_{i=0}^M [k_0^i k_1^{M-i} \{ \prod_{j=1}^i (N - (j - 1))(M - (j - 1)) \} \times \{ \prod_{j=i+1}^M j \}]}. \quad (28)$$

The result can be easily generalized for groups $0 \leq m \leq N - M$ and $0 \leq i \leq M$,

$$p_{i,m}^{qe} = \frac{k^{-i} \{ \prod_{j=1}^i ((N - m) - (j - 1))(M - (j - 1)) \} \times \{ \prod_{j=i+1}^M j \}}{\sum_{i=0}^M [k^{-i} \{ \prod_{j=1}^i ((N - m) - (j - 1))(M - (j - 1)) \} \times \{ \prod_{j=i+1}^M j \}]}, \quad (29)$$

where we have re-introduced $k = k_1/k_0$. The dynamics of groups $N - M < m \leq N$ are slightly different as they contain fewer substrate molecules than enzymes. These groups correspondingly have $N - m + 1$ fast internal states, i.e., $0 \leq i \leq N - m$. This leads to quasi-equilibrium probabilities of the form

$$p_{i,m}^{qe} = \frac{k^{-i} \{ \prod_{j=1}^i ((N - m) - (j - 1))(M - (j - 1)) \} \times \{ \prod_{j=i+1}^{N-m} j \}}{\sum_{i=0}^{N-m} [k^{-i} \{ \prod_{j=1}^i ((N - m) - (j - 1))(M - (j - 1)) \} \times \{ \prod_{j=i+1}^{N-m} j \}]}. \quad (30)$$

Finally, by defining

$$g(m) = \Theta(m - (N - M)) \times (m - (N - M)), \quad (31)$$

where $\Theta(m - (N - M))$ is the Heaviside step function, we can write down a joint expression for all groups $0 \leq m \leq N$ and $0 \leq i \leq M - g(m)$,

$$p_{i,m}^{qe} = \frac{z_{i,m}}{\mathcal{Z}_m} \quad (32)$$

with

$$z_{i,m} = k^{-i} \left\{ \prod_{j=1}^i ((N - m) - (j - 1))(M - (j - 1)) \right\} \times \left\{ \prod_{j=i+1}^{M-g(m)} j \right\}, \quad (33)$$

$$\mathcal{Z}_m = \sum_{i=0}^{M-g(m)} z_{i,m}. \quad (34)$$

We now proceed to calculate $p_m^g(t)$. From Fig. 3, we observe that the transitions between the groups are described by the master equation identical in form to Eq. (9). However, the transition rates a_m in this case are different, as the group m can be reached from any of the $M - g(m) - 1$ microstates in the group $m - 1$ (excluding only the microstate with M free enzymes), and we must also take into account the quasi-equilibrium probabilities of being in the corresponding microstate. It follows that the transition rates can be defined as

$$a_m = \sum_{n=1}^{M-g(m-1)} n p_{n,m-1}^{qe} = -k \partial_k (\ln(Z_{m-1})), \quad 1 \leq m \leq N+1,$$

$$= \begin{cases} -M \times \left(\frac{k {}_1F_1(1-M, -m-M+N+3; -k)}{(-m-M+N+2) {}_1F_1(-M, -m-M+N+2; -k)} - 1 \right), & m \leq N-M+1, \\ -(N-m+1) \times \left(\frac{k {}_1F_1(m-N, m+M-N+1; -k)}{(m+M-N) {}_1F_1(m-N-1, m+M-N; -k)} - 1 \right), & m > N-M+1, \end{cases} \quad (35)$$

where ${}_1F_1(a, b; c)$ is the confluent hypergeometric function, a result that we prove in Appendix C. As the dynamics between the groups are identical to the single enzyme case, $p_m^g(t')$ has exactly the same form as Eq. (20) but with the eigenvalues of $\mathcal{Q}$ being given by $\lambda_i = -a_i$, where a_i are now defined in Eq. (35).

We can now obtain the probability distribution $P(n; t)$, which requires us to find all microstates in the system containing n free substrate molecules. From Fig. 3, we see that for substrate numbers n , where $0 \leq n \leq N-M$, there are $M+1$ corresponding microstates given by $(n, 0), (n, 1), \dots, (n, M)$, which, respectively, belong to groups $(N-M) - n, (N-M) - n + 1, \dots, N - n$. Therefore, the distribution has the form

$$P(n; t') = \sum_{j=0}^M p_{j, N-(n+j)}^{qe} p_{N-(n+j)}^g(t') \quad \text{where } 0 \leq n \leq N-M. \quad (36)$$

In the case of $N-M < n \leq N$, there are $N - (n-1)$ microstates containing n substrate molecules, explicitly defined as $(n, M - (N-n)), (n, M - (N-n) + 1), \dots, (n, M)$ and associated with groups $0, 1, \dots, N-n$, respectively. Hence, we have

$$P(n; t') = \sum_{j=0}^{N-n} p_{j, N-(n+j)}^{qe} p_{N-(n+j)}^g(t') \quad \text{where } N-M < n \leq N. \quad (37)$$

Finally, using the function $g(m)$ previously defined in Eq. (31), we obtain

$$P(n; t') = \sum_{j=0}^{M-g(n)} p_{j, N-(n+j)}^{qe} p_{N-(n+j)}^g(t') \quad \text{where } 0 \leq n \leq N, \quad (38)$$

which fully describes the time-dependent solution for the multiple enzyme system $N \geq M$ with the initial condition $p_m^g(0) = \delta_{m,0}$. Note that the solution can also be extended to a more general initial distribution in the same way as was done for the single enzyme system in Sec. III A. The equations for the mean number of the substrate, $\langle n(t') \rangle$, and standard deviation, $\sigma(t')$, at rescaled time t' are the same as in Eqs. (22) and (23), but where $P(n; t')$ is now given by Eq. (38).

Now, consider a multiple enzyme system that initially contains fewer free substrate molecules than enzymes, i.e., $M \geq N$. The Markov chain describing the transitions between the microstates of this system, shown in Fig. 4, has similarities to that for the system with $N \geq M$. Specifically, if we replace N by M in groups 0 to N in the $M \geq N$ case of Fig. 4, then we exactly recover groups $N-M$ to N in the $N \geq M$ case of Fig. 3. This mapping implies that the dynamics of the system with $M \geq N$ are correctly described by Eq. (38) due to the utility of $g(m)$. Therefore, Eq. (38) is a valid solution for any positive integer values of N and M .

As for the single enzyme case, we can make the initial statement that k_2 must be sufficiently small for the derivation to hold, more precise. Suppose we are in the microstate (n, n_e) . There are then three possible reactions that can occur: (i) $(n, n_e) \rightarrow (n, n_e + 1)$ with rate $k_2(M - n_e)$, (ii) $(n, n_e) \rightarrow (n + 1, n_e + 1)$ with rate $k_1(M - n_e)$, and (iii) $(n, n_e) \rightarrow (n - 1, n_e - 1)$ with rate $k_0 n n_e$. Only the first reaction leads to a transition out of the current group of microstates (since its associated with the product formation step), and hence, the probability of exiting the current group is $k_2(M - n_e)/((k_1 + k_2)(M - n_e) + k_0 n n_e)$. It is easy to prove that the latter is always less than $k_2/(k_1 + k_2)$. Hence, quasi-equilibrium of microstates in each group is possible when $k_2/(k_1 + k_2) \ll 1$. In other words, generally, the closed-form solution for the distribution of substrate numbers will be accurate for all times, provided that $k_1 \gg k_2$.

FIG. 4. Illustration showing the transitions between the discrete microstates of enzyme system (1) with initially M enzymes and N substrate molecules where $M \geq N$. As before, fast internal states are aggregated together into groups. The dynamics of the groups 0 to N can be mapped onto the dynamics of groups $N-M$ to N in the system with $N \geq M$ (shown in Fig. 3). See text for discussion.

In Figs. 5(A-i)–5(A-iii) and 5(B-i)–5(B-iii), we show agreement between $P(n; t')$ from Eq. (38) and the SSA where $k_1 \gg k_2$ is enforced, over times ranging between the initial time when the number of the substrate is $n = N$ and the absorbing state at $n = 0$ for large times, for cases $M \geq N$ and $N \geq M$, respectively. In Figs. 5(A-iv) and 5(B-iv), we plot the mean and standard deviation of our analytical distribution ($\langle n \rangle$, σ), the deterministic mean $\langle n \rangle_d$, and the mean predicted by the SSA for $M \geq N$ and $N \geq M$, respectively. The SSA prediction of the mean is shown to be in exact correspondence with $\langle n \rangle$ when the QEA holds. The discrepancy previously seen in Fig. 2(B-iv) between $\langle n \rangle$ and $\langle n \rangle_d$ at a low molecule number is no longer observed in Fig. 5(A-iv) where $M = \mathcal{O}(N)$, highlighting that the discrepancy seen in Fig. 2(B-iv) originates from the molecular discreteness of the enzyme species. We additionally note the presence of transient bimodality in Fig. 5(B-ii) similar to that seen in the single enzyme case from Sec. III A; note that the parameter set

chosen for Figs. 5(A-i)–5(A-iii) does not exhibit transient bimodality. The parameter space of transient bimodality is explored later in more detail in Sec. III B 2. In Fig. 6, we demonstrate using stochastic simulations that, as predicted by our theory, the requirement for the stochastic QEA to be a good approximation relies only on satisfying the condition $k_1 \gg k_2$ and does not require any additional constraint on the value of k_0 .

1. Time-dependent solution for the probability distribution of enzyme molecules

Having solved the master equation for the group dynamics, it is relatively straightforward to extract the time-dependent probability distribution for the number of free enzyme molecules, $P(n_E; t')$, and, hence, the distribution for the number of enzyme–substrate complexes, $P(n_C; t')$. As previously stated, we begin by considering

FIG. 5. Comparison of the closed-form time-dependent probability distribution of substrate molecules, for enzyme reaction (1) with multiple enzyme molecules M and initial substrate molecules N , to the distribution obtained from the SSA. Note that the closed-form solution is given by Eq. (38). In (A-i)–(A-iii), $N = 15$, $M = 20$, and $k = 10^2$, and we simulate the SSA using $k_0 = 1$, $k_1 = 10^2$, and $k_2 = 1$; the theory (green lines) agrees with the SSA since the quasi-equilibrium assumption is justified, i.e., $k_1/k_2 \gg 1$. In (B-i)–(B-iii), $N = 60$, $M = 10$, and $k = 10^{-1}$, and we simulate the SSA using $k_0 = 10^3$, $k_1 = 10^2$, and $k_2 = 1$; again, the theory is in agreement with the SSA since quasi-equilibrium is justified. Note that these results show that the theory accurately describes both the $N \geq M$ and $M \geq N$ cases. In (A-iv) and (B-iv), we show the corresponding plots of the time-evolution of the mean $\langle n \rangle$ and of the standard deviation σ of the distributions of substrate molecules, as predicted by our theory; these are compared with the mean calculated from the SSA and the corresponding mean $\langle n \rangle_d$ obtained from the numerical solution of the deterministic rate equations given by Eq. (3). The parameter set in (B) is shown to be transiently bimodal in (B-ii), whereas for the parameter set describing (A), transient bimodality is not observed. Each SSA probability distribution here is constructed from 10^5 individual reaction trajectories.

FIG. 6. Testing the conditions necessary for the accuracy of the stochastic QEA. The three panels (i)–(iii) show how the accuracy of the closed-form time-dependent solution changes with time as we vary k_0/k_2 and k_1/k_2 while keeping $k = k_1/k_0$ fixed to 10^2 for the initial substrate number $N = 10^2$ and the total number of enzyme molecules equal to $M = 25$. The green line denotes the stochastic QEA solution from Eq. (38); SSA 1 (black dots) denotes the SSA prediction with parameters $k_0/k_2 = 1$, $k_1/k_2 = 10^2$ calculated over 10^4 trajectories; SSA 2 (red blocked region) denotes the SSA prediction with parameters $k_0/k_2 = 10^{-2}$, $k_1/k_2 = 1$ calculated over 10^5 trajectories; SSA 3 (blue blocked region) denotes the SSA prediction with parameters $k_0/k_2 = 10$, $k_1/k_2 = 10^3$ calculated over 10^3 trajectories. It is clear that SSA 2 is poorly predicted by $P(n; t)$, which is expected as $k_1 = \mathcal{O}(k_2)$. Since $P(n; t)$ is in equally good agreement with SSA 1 and SSA 3, it can be seen that the only requirement is $k_1 \gg k_2$, without requiring additional constraints on k_0 .

the $N \geq M$ system depicted in Fig. 3. We observe that the groups $0 \leq m \leq N - M$ all contain a microstate with n_E free enzyme molecules, where $0 \leq n_E \leq M$, as enzymes are saturated with the substrate. However, for groups $N - M < m \leq N$, free enzymes become more abundant than free substrate molecules so that microstates containing $0 < n_E \leq M$ enzymes are found only in groups $N - M < m \leq N - (M - n_E)$. Note that the quasi-equilibrium probability of having n_E free enzymes in group m is $p_{M-n_E, m}^{qe}$, given by Eq. (32), and the group probabilities $p_m^g(t')$ are identical to the ones defined for the distribution of the substrate number in Eq. (38). Therefore, the

distribution of free enzymes takes the form

$$P(n_E; t') = \sum_{j=0}^{N-(M-n_E)} p_{M-n_E, j}^{qe} p_j^g(t'), \quad 0 \leq n_E \leq M. \quad (39)$$

This expression is valid for any positive integer values of N and M again due to the mapping between the Markov chains of $N \geq M$ and $M \geq N$ systems, described above. Moreover, for the $N \leq M$ system, the definition of an empty sum as zero ensures that non-physical values of n_E are not allowed, i.e., the number of bound enzymes cannot be larger than N , given the chosen initial conditions, so that $P(n_E; t') = 0$ for $n_E < M - N$. Finally, as $n_C = M - n_E$, the probability distribution of the enzyme–substrate complex follows trivially

$$P(n_C; t') = \sum_{j=0}^{N-n_C} p_{n_C, j}^{qe} p_j^g(t'), \quad 0 \leq n_C \leq M. \quad (40)$$

In Figs. 7(A-i)–7(A-iii) and 7(B-i)–7(B-iii), we confirm that $P(n_C; t')$ from Eq. (40) and the SSA are in good agreement for enzyme systems with $M \geq N$ and $N \geq M$, respectively, over the whole time range from near the initial condition to the absorbing state, where again $k_1 \gg k_2$ is enforced (using the same parameters as in Fig. 5). Note that the transient bimodality is seemingly not manifest in $P(n_C; t')$ at the points in the parameter space where it is observed for the distribution of the substrate number [see Figs. 5(B-ii) and 7(B-ii)]. In Figs. 7(A-iv) and 7(B-iv), we plot the mean and standard deviation of our analytical distribution for the enzyme–substrate complexes ($\langle n_C \rangle$ and σ_C), the mean predicted by the SSA, and the mean number of complex molecules $\langle n_C \rangle_d$ obtained from the numerical solution of the deterministic rate equations given by Eq. (3) for $M \geq N$ and $N \geq M$, respectively. The SSA prediction of the mean matches $\langle n_C \rangle$ for all times further validating our solution, given that the QEA condition holds.

2. Bimodality

In Figs. 8(A-i)–8(A-iii), we further explore the transient bimodality observed in Figs. 2(A-ii), 2(B-iii), and 5(B-ii). Namely, we investigate how the strength of the bimodality varies with the parameters N , M , and k using the stochastic QEA solution from Eq. (38). Each point on the heatmaps in Figs. 8(A-i)–8(A-iii) shows, for a particular parameter set, the maximum of the strength of bimodality calculated over the entire time course from $t' = 0$ to a time near the absorbing state of $n = 0$. We utilize the measure of bimodality strength introduced in Ref. 41, which is explicitly given by

$$\kappa = \frac{H_{\text{low}} - H_{\text{valley}}}{H_{\text{high}}}, \quad (41)$$

where H_{low} and H_{high} are the heights of the smallest and largest magnitude modes, respectively, and H_{valley} is the height of the valley between the modes. For bimodal distributions, κ has a value between 0 (no bimodality) and 1 (maximum bimodality), and for monomodal distributions, it is defined as zero. This definition of bimodality strength considers the “most bimodal” distributions to have modes of equal height with a deep valley between them. In order to produce each heatmap, we devised a simple algorithm as follows. For each parameter set $\{N, M, k\}$,

FIG. 7. Comparison of the closed-form time-dependent probability distribution of enzyme-substrate complexes, for enzyme reaction (1) with multiple enzyme molecules M and initial substrate molecules N , to the distribution obtained from the SSA. Note that the closed-form solution is given by Eq. (40). In (A-i)–(A-iii), $N = 15$, $M = 20$, and $k = 10^2$, and we simulate the SSA using $k_0 = 1$, $k_1 = 10^2$, and $k_2 = 1$; in (B-i)–(B-iii), $N = 60$, $M = 10$, and $k = 10^{-1}$, and we simulate the SSA using $k_0 = 10^3$, $k_1 = 10^2$, and $k_2 = 1$ (parameters are the same as in Fig. 5). In both cases, the theory (green lines) agrees with the SSA since the quasi-equilibrium assumption is justified, i.e., $k_1/k_2 \gg 1$. In (A-iv) and (B-iv), we show the corresponding plots of the time-evolution of the mean $\langle n_C \rangle$ and of the standard deviation σ_C of the distributions of enzyme-substrate complex, as predicted by our theory; these are compared with the mean calculated from the SSA and the mean $\langle n_C \rangle_d$ obtained from the numerical solution of the deterministic rate equations given by Eq. (3). Each SSA probability distribution here is constructed from 10^5 individual reaction trajectories.

1. Calculate the estimated time to reach the absorbing state, which provides us with the time range, T_a , over which the transient bimodality search will be conducted. In order to avoid additional computational burdens of finding the absorption time using stochastic simulations, we use a much simpler but reasonably accurate estimate obtained from the deterministic QEA mean, given by solving Eq. (5) for $t' = k_2 t$,

$$T_a = \frac{N}{M} - \frac{k}{M} \log \left(\frac{\langle n \rangle_a e^{\frac{\langle n \rangle_a}{k}}}{N} \right), \quad (42)$$

where we set $\langle n \rangle_a = 10^{-2}$, which was chosen small enough such that transient bimodality for all parameter sets was accounted for.

2. Choose the number of iterations, I , over which to check if the distribution is bimodal. In our case, we chose $I = 400$. This

gives the set of times over which we check for bimodality as $t_i = iT_a/I$ for $1 \leq i \leq I$.

3. Define a variable denoting the maximum bimodality measure κ_0 , which is initially set to zero. For each t_i , find the number of peaks in the distribution given by Eq. (38) for the stochastic QEA, and if two peaks are detected, calculate the bimodality strength κ from Eq. (41). If $\kappa > \kappa_0$, then set $\kappa_0 = \kappa$. Do for all t_n .
4. Once all iterations of this process are complete, the value of κ_0 will denote the largest value of the transient bimodality measure for all probability distributions at $t \in t_i$. We take κ_0 as the largest value of transient bimodality encountered on the time course.

The results obtained using this algorithm are summarized by the three heatmaps in Figs. 8(A-i)–8(A-iii). The distribution of

FIG. 8. Heatmaps elucidating the regions of parameter space where transient bimodality is observed using the stochastic QEA solution [(A-i)–(A-iii)] from Eq. (38) and the discrete stochastic MM approximation [(B-i)–(B-iii)] given by Eq. (46). Note that κ_0 is a measure of how bimodal is the distribution of substrate molecules across the time course of the reaction (see text for details). Three parameter regimes are considered: N vs M with $k = 1$ (left), N vs k with $M = 5$ (middle), and M vs k with $N = 30$ (right). The plots (C-i)–(C-iii) show the closed-form distributions of the stochastic QEA, $P(n; t')$, and the discrete stochastic MM approximation, $P(n; t')^{(M)}$, at the times when the stochastic QEA exhibits maximum bimodality, for cases with $k = 1$, $N = 80$ and (i) $M = 1$, (ii) $M = 5$, and (iii) $M = 15$ [highlighted on the heatmap (A-i) as the points a , b , and c , respectively]. The corresponding SSA predictions with $k_0/k_2 = 10^2$ and $k_1/k_2 = 10^2$ are also included (constructed from 10^5 individual reaction trajectories). Note that the two distributions (discrete stochastic MM approximation and stochastic QEA) are almost identical in (C-i), but the difference becomes more pronounced in (C-ii) and (C-iii) with an increase in M .

substrate molecules corresponding to the time at which the maximal bimodality strength κ_0 occurs for points a , b , c in Fig. 8(A-i) are shown by the blue solid lines in Figs. 8(C-i)–8(C-iii), respectively. Note that the bimodality is most pronounced in (C-i), less in (C-ii), and least in (C-iii), in accordance with the value of κ_0 in Fig. 8(A-i); this validates the use of Eq. (41) as an accurate measure of the strength of bimodality. From Figs. 8(A-i)–8(A-iii), it is clear that bimodality is most pronounced when the initial number of substrate

molecules N is significantly larger than the total enzyme number M and also when k is small, i.e., when the frequency of enzyme–substrate binding is much larger than the frequency of complex dissociation into an enzyme and substrate. Note that, generally, the frequency of enzyme–substrate binding is inversely proportional to the volume of the compartment⁶ in which the bimolecular reaction occurs, and hence, the transient bimodality is likely observable inside cells.

IV. THE DISCRETE STOCHASTIC MICHAELIS-MENTEN APPROXIMATION

We next consider how the analytical solution that we obtained for reaction system (1) using a combination of averaging and linear algebra techniques in Sec. III B is compared with the solution of a commonly used reduced CME for enzyme kinetics.

The reduced CME for single substrate enzyme kinetics can be heuristically justified as follows (for a derivation, see Ref. 13). Under the QEA approximation, from the deterministic analysis in Sec. II, it follows that the rate equation describing the time-evolution of the substrate concentration is given by

$$\frac{d[S(t)]}{dt} = -\frac{V_{\max}[S(t)]}{k + [S(t)]}. \quad (43)$$

Note that $V_{\max} = k_2M$, where M is the total number of enzyme molecules. Hence, species S can be seen as changing into P by means of an effective first-order decay reaction with the rate given by the right-hand side of Eq. (43). One common way to approximately describe the enzyme reaction stochastically consists of writing down an effective propensity describing the decay of the substrate, i.e., we postulate that if there are m substrate molecules at time t , then

the probability that a reaction $S \rightarrow P$ occurs somewhere in a unit volume in the time interval $[t, t + dt]$ is approximately given by $a_m dt$ where $a_m = V_{\max}m/(k + m)$. This is the discrete stochastic Michaelis-Menten (MM) approximation. Hence, if we choose an initial condition of N substrate molecules, it follows that a corresponding effective CME is given by

$$\partial_t P_{N-m}(t) = a_{m+1}P_{N-(m+1)}(t) - a_m P_{N-m}(t), \quad (44)$$

where $P_{N-m}(t)$ is the probability that there are m substrate molecules at time t ($0 \leq m \leq N$). This CME can be conveniently written as

$$\partial_t \underline{P}(t) = \underline{Q} \cdot \underline{P}(t) \quad (45)$$

where $\underline{P}(t) = (P_0(t), P_1(t), \dots, P_N(t))$ and $\underline{Q}$ is a $(N + 1) \times (N + 1)$ lower-bidiagonal matrix whose only non-zero elements are $Q_{i,i} = -a_{N-(i-1)} = -\frac{(N-(i-1))V_{\max}}{k+(N-(i-1))}$ for $1 \leq i \leq N + 1$ and $Q_{i+1,i} = a_{N-(i-1)} = \frac{(N-(i-1))V_{\max}}{k+(N-(i-1))}$ for $1 \leq i \leq N$. Using the method in Ref. 35 that was used to solve the master equation for the group dynamics for the single enzyme, the solution is found to be given by Eq. (20), modified to take into account the fact that P_{N-n} is equivalent to the probability of being in the group $N - n$,

$$P_{N-n}(t')^{(M)} = \begin{cases} 0, & n > N, \\ e^{\lambda_1^{(M)} t'}, & n = N, \\ \left\{ (-1)^{N-n} \prod_{k=1}^{N-n} \lambda_k^{(M)} \right\} \times \left\{ \sum_{k=1}^{N-n+1} \frac{e^{\lambda_k^{(M)} t'}}{\prod_{j=1, j \neq k}^{N-n+1} (\lambda_k^{(M)} - \lambda_j^{(M)})} \right\}, & 0 \leq n < N. \end{cases} \quad (46)$$

Note the superscript (M) specifying that the solution is for CME (45) resulting from the discrete stochastic MM approximation. Here, we have again rescaled the time $t' = k_2 t$, and $\lambda_m^{(M)}$ are the eigenvalues of $\underline{Q}$, which are simply given by the diagonal elements,

$$\lambda_m^{(M)} = -\frac{M(N - (m - 1))}{k + N - (m - 1)}, \quad 1 \leq m \leq N + 1. \quad (47)$$

We shall denote the time-dependent mean and standard deviation of the distribution equation (46) by $\langle n(t') \rangle^{(M)}$ and $\sigma(t')^{(M)}$, respectively. Note that the distributions for the number of free enzymes/enzyme-substrate complexes cannot be obtained under the discrete stochastic MM approximation as the enzyme number fluctuations are not taken into account, in contrast to the stochastic QEA from which enzyme/enzyme-substrate complex distributions can be obtained (see Sec. III B 1).

A. Comparison with the stochastic QEA

We used the algorithm described in Sec. III B 2 [with the difference that in step 3, we use Eq. (46) instead of Eq. (38)] to explore the regions of parameter space where the discrete stochastic MM approximation predicts the distribution of substrate molecules to

be bimodal. The results are summarized by the three heatmaps in Figs. 8(B-i)–8(B-iii). By comparison to the heatmaps generated using the stochastic QEA in Figs. 8(A-i)–8(A-iii), it is clear that the discrete stochastic MM approximation tends to predict bimodality where in reality, there is none. Notably, the bimodality predicted by the discrete stochastic MM approximation is independent of M [see Figs. 8B(i) and 8B(iii)] since M only acts to scale the eigenvalues representing the system's relaxation timescales in Eq. (47); in contrast, the stochastic QEA predicts bimodality, which is strongly dependent on M [Figs. 8(A-i) and 8(A-iii)]. These issues with the discrete stochastic MM approximation are also clearly discernible in Figs. 8(C-i)–8(C-iii), where we compare the distribution of substrate molecule numbers predicted by this approximation (green line) with that predicted by the SSA (dots) and the stochastic QEA (blue line).

A different way to contrast the discrete stochastic MM approximation and the stochastic QEA involves comparing the eigenvalues of the transition matrix. In the single enzyme case where $M = 1$, one observes that the eigenvalues predicted by Eq. (47) exactly match the eigenvalues predicted by averaging for the group dynamics in the single enzyme case from Eq. (10). However, note that the group dynamics is not precisely the same as the substrate dynamics, which is determined by two microstates in different groups. For example,

the averaging technique implies that there are two microstates that contain n substrate molecules: $(n, 0)$ and $(n, 1)$ associated with groups $N - (n + 1)$ and $N - n$, respectively. However, this subtlety is not important if $N \gg 1$, and hence, the CME resulting from the discrete stochastic MM approximation will practically lead to the same results as averaging for most cases of interest.

The comparison is more complicated in the case of multiple enzymes ($M > 1$) and abundant substrate $N \gg 1$, which we explore in Fig. 9 (for $N = 100$ and $k = 1$), showing how the discrete stochastic MM approximate solution differs to that from averaging as the ratio M/N is increased. We first consider the case where $M/N = 1/20$, and we see that $\langle n \rangle^{(M)}$ in Fig. 9(A-i) is a good approximation of $\langle n \rangle$ for the time range of interest, i.e., from the initial state at $N = 100$ to a time $t' = 30$ where both $\langle n \rangle^{(M)}$ and $\langle n \rangle$ are small quantities. Note that the error in the standard deviation for this parameter set, shown in Fig. 9(A-ii), is also small. The slight difference in the relaxation dynamics is corroborated by small differences in the eigenspectra of λ_m [given in Eq. (35) again noting that $\lambda_i = -a_i$] and $\lambda_m^{(M)}$ [given by Eq. (47)], which can be appreciated in Fig. 9(A-iii). We additionally plot the deterministic mean as predicted by Eq. (5), which clearly shows that the relaxation dynamics of $\langle n \rangle_a$ accurately approximates $\langle n \rangle$ for short times only.

In Figs. 9(B-i) and 9(C-i), we see that as M/N increases to $1/5$ and $1/2$, respectively, $\langle n \rangle^{(M)}$ becomes a worse approximation of $\langle n \rangle$, with $\langle n \rangle^{(M)}$ tending more to $\langle n \rangle_a$ than $\langle n \rangle$. The corresponding error in the standard deviation, as shown in Figs. 9(B-ii) and 9(C-ii), also

follows that of the mean, increasing with M/N . There are two main reasons for this disagreement:

1. If M is comparable to N , then initially there will be large fluctuations in the number of enzyme molecules, which are taken into account by the averaging solution (since it allows for switching between microstates in each group) but not by the CME resulting from the discrete stochastic MM approximation (since the total number of enzymes only appears as a constant through V_{max}). This is most clearly seen in Fig. 9(C-i) where we observe a large discrepancy between $\langle n \rangle$ and $\langle n \rangle^{(M)}$ at $t' = t'_c \ll 1$ (where t'_c is the time over which the initial transient occurs and is indistinguishable from $t' = 0$ in Fig. 9(C-i)).
2. Where $M/N \approx \mathcal{O}(1)$, the eigenspectra λ_m and $\lambda_m^{(M)}$ show a large disagreement [see Figs. 9(B-iii) and 9(C-iii)]. This leads to the misprediction of the relaxation dynamics of $\langle n \rangle^{(M)}$, which better represents the dynamics predicted by $\langle n \rangle_a$ rather than of $\langle n \rangle$, for both the small and large times. This is due to the fact that the effective Michaelis-Menten propensity in reduced CME (45) is of the same form as the effective rate from the deterministic rate equation given by Eq. (43).

In summary, the solution of the CME obtained by the discrete stochastic MM approximation is a good approximation to the solution of the CME derived by averaging, provided that $N \gg 1$ and $N/M \gg 1$.

FIG. 9. Comparison of the discrete stochastic MM approximation and the exact result from averaging in the quasi-equilibrium limit. (A-i), (B-i), and (C-i) show log-scale plots of $\langle n \rangle$, $\langle n \rangle^{(M)}$, and $\langle n \rangle_a$ [from Eq. (5)] for $N = 100$, $k = 1$, and $M = 5$ (i.e., $M/N = 1/20$), $M = 20$ (i.e., $M/N = 1/5$), and $M = 50$ (i.e., $M/N = 1/2$), respectively. The corresponding SSA results with $k_0/k_2 = 10^2$ and $k_1/k_2 = 10^2$ are also included (constructed from 10^5 individual reaction trajectories). (A-ii), (B-ii), and (C-ii) are the corresponding plots of the standard deviations $\sigma(t')$, $\sigma(t')^{(M)}$, and that of SSA. (A-iii), (B-iii), and (C-iii) show the eigenspectra for each differing M/N ; each symbol corresponds to an individual eigenvalue (since the spectra are discrete), and the dashed lines are only present to aid the reader.

V. MULTI-SUBSTRATE MECHANISMS

Thus far, we have considered the simple enzyme mechanism shown in (1) where an enzyme can catalyze a single type of substrate. However, in nature, it is common for one enzyme species to be able to catalyze multiple substrates.⁵⁰ Multi-substrate reactions follow various mechanisms that describe how substrates bind and in what sequence. One such common mechanism is that of ternary complex formation, whereby two substrates bind sequentially to an enzyme to form a complex with three molecules. An example is the following mechanism involving two substrate species A and B and two corresponding reaction products, P and Q ,⁵⁰

Note that here we have assumed an ordered binding mechanism, in the sense that binding of A must precede that of B . An alternative is a random binding mechanism, wherein either A or B could first bind the enzyme. We assume that both the enzyme-substrate binding reactions and the steps subsequent to complex formation are fast such that we can consider the simpler reaction scheme,

Note that ordered or random binding mechanisms cannot be distinguished within this reaction scheme. We assume that there are initially N_A molecules of substrate A , N_B molecules of substrate B , where $N_A \geq N_B$, and M free enzymes. There exists a relation between the number of species A and B , denoted as n_A and n_B , respectively,

which we can write as $n_A - n_B = N_A - N_B \equiv \Delta_{AB}$. Hence, each microstate of the system is fully specified by (n_B, n_E) . Again, the group dynamics where $k_1 \gg k_2$ are given by Eq. (20), but the eigenvalues λ_m specific to this mechanism are given by

$$\lambda_m = - \sum_{n=1}^{M-g(m)} n p_{n,m-1}^{qe} = k \partial_k (\ln(\mathcal{Z}_{m-1})), \quad 1 \leq m \leq N_B + 1, \quad (50)$$

where we have now defined

$$p_{i,m}^{qe} = \frac{z_{i,m}}{\mathcal{Z}_m}, \quad (51)$$

$$g(m) = \Theta(m - (N_B - M)) \times (m - (N_B - M)), \quad (52)$$

$$z_{i,m} = k^{-i} \left\{ \prod_{j=1}^i ((N_A - m) - (j - 1)) \times ((N_B - m) - (j - 1))(M - (j - 1)) \right\} \times \left\{ \prod_{j=i+1}^{M-g(m)} j \right\}, \quad (53)$$

$$\mathcal{Z}_m = \sum_{i=0}^{M-g(m)} z_{i,m}. \quad (54)$$

Using the results for the group dynamics and quasi-equilibrium probabilities, we can then find the probability distribution for the substrate molecules,

FIG. 10. Comparison of the analytic distribution of two types of substrate species A and B , involved in the reaction mechanism (49), against the distributions obtained using the SSA. Note that SSA A and SSA B denote the SSA predictions for species A (red dots) and species B (green dots), respectively. In the panels (i)–(iv), we plot the probability distributions $P(n_A, t')$ [red line; from Eq. (57)] and $P(n_B, t')$ [green line; from Eq. (56)] for four different time points from near the initial condition (i) to near the absorbing state (iv) (time is non-dimensional as in Figs. 2 and 5–9). The initial number of substrate molecules is $N_A = 60$ and $N_B = 40$, and the number of enzyme molecules is $M = 5$; the rates are $k_0/k_2 = k_1/k_2 = 10^3$, which enforce the QEA. The analytic distributions are in good agreement with the respective SSA distributions. Note that the absorbing point of A is $n_A = 20$, while that of B is $n_B = 0$; this is dictated by the difference between the initial number of substrate molecules $N_A - N_B = 20$. Each SSA probability distribution is constructed from 10^5 individual reaction trajectories.

$$P(n_A, n_B; t') = \delta_{n_A - \Delta_{AB}, n_B} \times \left(\sum_{j=0}^{M-g(n_B)} p_{j, N_B - (n_B + j)}^{qe} p_{N_B - (n_B + j)}^g(t') \right), \quad (55)$$

where $\delta_{i,j}$ is the Kronecker delta symbol. This allows us to find the marginal distributions,

$$P(n_B; t') = \sum_{n_A} P(n_A, n_B; t') = \sum_{j=0}^{M-g(n_B)} p_{j, N_B - (n_B + j)}^{qe} p_{N_B - (n_B + j)}^g(t'), \quad (56)$$

$$P(n_A; t') = P(n_B + \Delta_{AB}; t'). \quad (57)$$

In Fig. 10, we compare the analytic marginal distributions against the SSA, and as expected, we find very good agreement when the rate parameters are consistent with the QEA. As previously stated, for the single substrate mechanism, the distributions of *A* and *B* molecules display bimodality at intermediate times.

VI. DISCUSSION

In summary, we have shown using averaging that in the limit of quasi-equilibrium between the substrate and the enzyme, it is possible to reduce the two variable stochastic description of the MM reaction to that of an effective one-variable master equation describing the slow transitions between groups of microstates. This master equation is subsequently solved exactly, using methods from the linear algebra and complex analysis, to obtain closed-form solutions for the time-dependent marginal distributions of substrate and enzyme numbers. We have shown theoretically and verified by means of stochastic simulations that the solutions for the time-dependent marginal distributions are accurate for all times, provided that the probability of complex decay into a substrate and enzyme is much larger than the probability of complex decay into a product and enzyme. To our knowledge, this is the first systematically derived approximate closed-form solution for the MM reaction for an arbitrary initial number of substrate and enzyme molecules; previous work treated a similar problem but using a heuristic approach¹¹ or derived closed-form solutions for the case of a single enzyme molecule^{9,10} or else considered reactions with multiple enzyme molecules focusing on deriving expressions for the turnover rate.^{26,31,34} We have also shown how the same procedure can be used to obtain the solution of more complex enzyme mechanisms such as those involving the catalysis of multiple types of substrate by the same enzyme species.

For the MM reaction, we have compared our closed-form solution with that obtained by the solution of the CME reduced by means of the widely used discrete stochastic MM approximation,¹³ where the propensity for substrate decay has a hyperbolic dependence on the number of substrate molecules. If the initial substrate number *N* is not much larger than the total enzyme number *M*, but the rate constants satisfy the inequality $k_1 \gg k_2$, then the enzyme numbers fluctuations can be large, even though the rapid equilibrium approximation is valid. In this case, we show that the distribution predicted by the CME reduced using the discrete stochastic MM approximation is significantly different from the one obtained from stochastic

simulations, whereas the solution provided by our theory accurately matches the simulations.

Using the closed-form solution for the time-dependent marginal probability distribution for the substrate number, we have found that, unexpectedly for a delta function (unimodal) initial condition, the distribution of substrate numbers can be bimodal at intermediate times if the initial number of substrate molecules is significantly larger than the total number of enzyme molecules and provided that the rate of complex decay into a substrate and enzyme is much less than the rate of substrate and enzyme binding. We note that the latter rate in the CME formulation is inversely proportional to the compartment volume (since the encounter rate of two molecules decreases with an increase in volume⁶), and hence, our results imply that in the limit of small volumes (taken at a constant initial number of substrate and enzyme molecules), bimodality of the distribution of substrate molecules is observable. This result is of particular relevance in understanding enzyme dynamics inside cells where the volume is very small. Our system with the initial conditions used can then be interpreted as modeling the enzyme-mediated decay of substrate molecules, following the production (via translation) of a short burst of substrate molecules *N* at time *t* = 0, provided that there is not another burst of substrate expression before the substrate decays; these conditions are common for many cells where protein production occurs sporadically in bursts of short duration.^{51,52} We emphasize that the presence of transient bimodality in the MM reaction system is particularly interesting since it has no deterministic counterpart.

AUTHORS' CONTRIBUTIONS

J.H. and A.S. contributed equally to this work.

ACKNOWLEDGMENTS

This work was supported by a BBSRC EASTBIO Ph.D. studentship for J.H., the Alan Turing Institute Doctoral Studentship for A.S (EPSRC grant EP/N510129/1), and a Leverhulme Trust grant (Grant No. RPG-2018-423) for R.G.

APPENDIX A: EXACT TIME-DEPENDENT SOLUTION OF SINGLE ENZYME SYSTEM

The master equation for a single enzyme molecule [given by Eq. (6)] was first solved by Arányi and Tóth.⁹ As the original paper is rather difficult to find, we present the solution here. The authors used marginal probability generating functions,

$$G_{n_E}(z, t) = \sum_{n=0}^{N-1+n_E} z^n P(n, n_E, t) \quad (n_E = 0, 1; t \geq 0) \quad (A1)$$

to transform Eq. (6) into the following first-order partial differential equations:

$$\begin{cases} \frac{\partial G_0(z, t)}{\partial t} = -(k_1 + k_2)G_0(z, t) + k_0 \frac{\partial G_1(z, t)}{\partial z}, \\ \frac{\partial G_1(z, t)}{\partial t} = -k_0 z \frac{\partial G_1(z, t)}{\partial z} + k_1 z G_0(z, t) + k_2 G_0(z, t). \end{cases} \quad (A2)$$

By a simple substitution, one can prove that the solutions have the form

$$G_0(z, t) = \bar{\Gamma} e^{-\frac{k_1}{k_0}(z-1)} e^{-k_2 t} + \bar{\Gamma} \frac{k_1 + k_2}{k_1 z + k_2} e^{-(k_0 + k_2)t} + \sum_{i=1}^2 \sum_{m=0}^{\infty} \Gamma_i^{(m)} \left[\frac{k_2 - (k_2 + \lambda_i^{(m)})z}{-\lambda_i^{(m)}} \right]^{q_m} e^{\lambda_i^{(m)} t}, \quad (\text{A3})$$

$$G_1(z, t) = \Gamma^{(-1)} - \bar{\Gamma} e^{-\frac{k_1}{k_0}(z-1)} e^{-k_2 t} - \bar{\Gamma} e^{-(k_0 + k_2)t} - \sum_{i=1}^2 \sum_{m=0}^{\infty} \Gamma_i^{(m)} \left[\frac{k_2 - (k_2 + \lambda_i^{(m)})z}{-\lambda_i^{(m)}} \right]^{q_m+1} e^{\lambda_i^{(m)} t} \quad (\text{A4})$$

where

$$\lambda_i^{(m)} \neq -k_2, \quad q_m = -\frac{(\lambda_i^{(m)})^2 + (k_1 + k_0 + k_2)\lambda_i^{(m)} + k_0 k_2}{k_0(k_2 + \lambda_i^{(m)})}, \quad i = 1, 2. \quad (\text{A5})$$

Since G_0 and G_1 are generating functions of a system with a finite state space, i.e., the numbers of substrate and enzyme are bounded quantities ($n \in [0, N]$, $n_E \in [0, 1]$), they must be polynomials of a finite degree in z . Hence, the summations in Eqs. (A3) and (A4) must contain a finite number of terms only, meaning that $\bar{\Gamma} = \bar{\Gamma} = 0$ (if $k_1 \neq 0$). By the same reasoning, q_m must be positive integers, i.e., $0 \leq q_m \leq N - 1$, ($q_m = m$); then, $\lambda^{(m)}$ are the roots of a quadratic equation,

$$(\lambda^{(m)})^2 + [k_1 + k_0(m+1) + k_2]\lambda^{(m)} + k_0 k_2(m+1) = 0 \quad (\text{A6})$$

$$(m = 0, 1, \dots, N-1).$$

The constants Γ can be determined from the initial conditions,

$$\begin{aligned} G_0(1, t) + G_1(1, t) &= 1, \\ G_0(z, 0) &= 0, \\ G_1(z, 0) &= z^N. \end{aligned} \quad (\text{A7})$$

The first constraint implies that $\Gamma^{(-1)} = 1$, while the remaining two lead to a linear algebraic system for $\Gamma_i^{(m)}$ by enforcing the constraints

explicitly on each coefficient of the polynomials G_0 and G_1 for each power of z . However, solving for $\Gamma_i^{(m)}$ becomes computationally expensive for larger values of N .

To summarize, the solution has the form

$$\begin{aligned} G_0(z, t) &= \sum_{i=1}^2 \sum_{m=0}^{N-1} \Gamma_i^{(m)} \left[\frac{k_2 - (k_2 + \lambda_i^{(m)})z}{-\lambda_i^{(m)}} \right]^m e^{\lambda_i^{(m)} t}, \\ G_1(z, t) &= 1 - \sum_{i=1}^2 \sum_{m=0}^{N-1} \Gamma_i^{(m)} \left[\frac{k_2 - (k_2 + \lambda_i^{(m)})z}{-\lambda_i^{(m)}} \right]^{m+1} e^{\lambda_i^{(m)} t}, \end{aligned} \quad (\text{A8})$$

where

$$\begin{aligned} \lambda_1^{(m)} &= -\frac{k_0(m+1) + k_1 + k_2}{2} \\ &\quad + \frac{\sqrt{[k_0(m+1) + k_1 + k_2]^2 - 4k_0 k_2(m+1)}}{2}, \\ \lambda_2^{(m)} &= -\frac{k_0(m+1) + k_1 + k_2}{2} \\ &\quad - \frac{\sqrt{[k_0(m+1) + k_1 + k_2]^2 - 4k_0 k_2(m+1)}}{2}. \end{aligned} \quad (\text{A9})$$

Finally, the probabilities can be calculated from the generating functions according to

$$P(n, n_E, t) = \frac{1}{n!} \left. \frac{\partial^n G_{n_E}(z, t)}{\partial z^n} \right|_{z=0}. \quad (\text{A10})$$

APPENDIX B: FIGURE SHOWING THE INITIAL TRANSIENT

Exhibition of the initial transient seen explicitly from the SSA (Fig. 11).

APPENDIX C: DERIVATION OF EQ. (35)

In this the appendix, we prove the results stated in Eq. (35) of the main text. First, consider the sum that defines $\mathcal{Z}_{m-1}$ explicitly,

FIG. 11. Exhibition of the initial transient seen explicitly from the SSA. (i) Comparison of two differing SSA means for the same value of $k = 0.1$ against $\langle n \rangle$ from Eq. (22). SSA 1 was simulated with parameters $N = 5$, $M = 1$, $k_0 = 10^3$, and $k_1 = 10^2$, and SSA 2 with $N = 5$, $M = 1$, $k_0 = 10^2$, and $k_1 = 10$. For most of the time course, the SSA means agree with the mean predicted analytically from Eq. (22), aside from the initial transient very close to $t' = 0$. (ii) Zoomed in area around the initial transient. There exists some critical time t'_c for both SSA 1 and SSA 2, denoted by t'_c^1 and t'_c^2 , respectively, over which the mean predicted by the SSA relaxes to the mean value predicted by Eq. (22). In SSA 1, where k_0 and k_1 are a magnitude of 10 larger than the same parameters in SSA 2, one observes that the initial transient occurs over a much shorter time. This relaxation of the SSA means to the mean predicted by the quasi-equilibrium analysis is known as the initial transient. Dots of differing color, seen in the legend, show the means of the stochastic QEA and SSAs at $t' = 0$ (IC in the legend refers to initial conditions). The mean predicted by the stochastic QEA in Eq. (22) reaches the quasi-equilibrium instantaneously at $t' = 0$, unlike that seen in the SSA. In both cases, the SSA means were determined as an average over 10^5 individual reaction trajectories.

$$\begin{aligned} \mathcal{Z}_{m-1} &= \sum_{i=0}^{M-g(m-1)} z_{i,m-1} \\ &= \sum_{i=0}^{M-g(m-1)} k^{-i} \left(\prod_{j=1}^i ((N-m+1)-(j-1))(M-(j-1)) \right) \\ &\quad \times \left(\prod_{j=i+1}^{M-g(m-1)} j \right). \end{aligned} \quad (C1)$$

We now relabel $g(m-1) = Q$ for brevity and consider later the individual cases where $g(m-1) = 0$ for $m \leq N-M+1$ and $g(m-1) = (m-1) - (N-M)$ for $m > N-M+1$. Using the definition of the Pochhammer function, $(x)_n = \prod_{j=0}^{n-1} (x+j)$, one can re-write Eq. (C1) to give

$$\mathcal{Z}_{m-1} = \sum_{i=0}^{M-Q} k^{-i} (m-N-1)_i (-M)_i (i+1)_{M-Q-i}. \quad (C2)$$

We now utilize the relation between the Pochhammer function and the Gamma function, namely, $(x)_n = \Gamma(x+n)/\Gamma(x)$, which allows us to tactically write Eq. (C2) as

$$\mathcal{Z}_{m-1} = \frac{k^{-(M-Q)} \Gamma(-Q) \Gamma(m+M-N-Q-1)}{\Gamma(-M) \Gamma(m-N-1)} \times \mathcal{S}, \quad (C3)$$

where $\mathcal{S}$ is defined by the sum,

$$\mathcal{S} = \sum_{i=0}^{M-Q} k^{M-Q-i} \frac{\Gamma(m-N-1+i) \Gamma(i-M)}{\Gamma(m+M-N-Q-1) \Gamma(-Q)} (i+1)_{M-Q-i}. \quad (C4)$$

Our task is now to find an analytic function that is equal to the sum $\mathcal{S}$. Motivated by the Pochhammer and Gamma functions contained within the sum, we look to match this sum to the definition of a generalized hypergeometric function ${}_pF_q(\{\alpha_1, \alpha_2, \dots, \alpha_L\}, \{\beta_1, \beta_2, \dots, \beta_{L_2}\}, z)$ defined by

$${}_pF_q(\{\alpha_1, \alpha_2, \dots, \alpha_L\}, \{\beta_1, \beta_2, \dots, \beta_{L_2}\}; z) = \sum_{n=0}^{\infty} \left(\frac{\prod_{l=1}^{L_1} (\alpha_l)_n}{\prod_{l=1}^{L_2} (\beta_l)_n} \times \frac{z^n}{n!} \right). \quad (C5)$$

We begin by relabeling the summation index in Eq. (C4) by $j = M-Q-i$ and again utilizing the definition of the Pochhammer function in terms of Gamma functions, giving us

$$\mathcal{S} = \sum_{j=0}^{M-Q} k^j (M-Q+1-j)_j (m-N-1+M-Q)_{-j} (-Q)_{-j}. \quad (C6)$$

Consider now the latter two Pochhammer functions in the summand of Eq. (C6). Using the relation $(b)_{-n} = (-1)^n / (1-b)_n$, we find that

$$(m-N-1+M-Q)_{-j} \times (-Q)_{-j} = \frac{1}{(Q+1)_j (Q+N+2-m-M)_j}. \quad (C7)$$

Now, consider the first Pochhammer function in the summand of Eq. (C6). One can re-write this as

$$(M-Q+1-j)_j = (-1)^j (Q-M)_j. \quad (C8)$$

Note that $(Q-M)_j$ has the property $(Q-M)_{j>M-Q} = 0$, which is found trivially from the definition of the Pochhammer function. Using Eqs. (C7) and (C8) and the relation $j! = (1)_j$, one can then show that

$$\begin{aligned} \mathcal{S} &= \sum_{j=0}^{\infty} \left(\frac{(1)_j (Q-M)_j}{(Q+1)_j (Q+N+2-m-M)_j} \times \frac{(-k)^j}{j!} \right), \\ &= {}_2F_2(\{1, Q-M\}, \{Q+1, Q+N+2-m-M\}; -k), \end{aligned} \quad (C9)$$

using the definition of the generalized hypergeometric function in Eq. (C5). Note that one is able to extend the upper limit of the sum defining $\mathcal{S}$ to infinity due to the property $(Q-M)_{j>M-Q} = 0$. One finds that $\mathcal{Z}_{m-1}$ is now fully specified by Eqs. (C3) and (C9), and we can now return to our original problem of finding the group transition rates a_m in Eq. (35).

In order to find a_m , we must now compute $a_m = -k \partial_k (\ln(\mathcal{Z}_{m-1}))$, which using the chain rule and the differentiation rules for generalized hypergeometric functions gives

$$a_m = (M-Q) - \frac{k(Q-M) {}_2F_2(\{2, Q-M+1\}, \{Q+2, Q+N+3-m-M\}; -k)}{(Q+1)(Q+N+2-m-M) {}_2F_2(\{1, Q-M\}, \{Q+1, Q+N+2-m-M\}; -k)}. \quad (C10)$$

Where $m \leq N-M+1$, $Q=0$, and Eq. (C10) becomes

$$a_m = -M \times \left(\frac{k {}_1F_1(1-M, -m-M+N+3; -k)}{(-m-M+N+2) {}_1F_1(-M, -m-M+N+2; -k)} - 1 \right), \quad (C11)$$

noting that for $Q=0$, the ${}_2F_2(\dots)$ general hypergeometric function reduces to the ${}_1F_1(\dots)$ confluent hypergeometric function.

Finally, where $m > N-M+1$, $Q = (m-1) - (N-M)$, Eq. (C10) becomes

$$\begin{aligned} a_m &= -(N-m+1) \\ &\quad \times \left(\frac{k {}_1F_1(m-N, m+M-N+1; -k)}{(m+M-N) {}_1F_1(m-N-1, m+M-N; -k)} - 1 \right), \end{aligned} \quad (C12)$$

where again the ${}_2F_2(\dots)$ general hypergeometric function reduces to the ${}_1F_1(\dots)$ confluent hypergeometric function. This completes our derivation of Eq. (35) from the main text.

DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

REFERENCES

- ¹L. Michaelis and M. L. Menten, "Die kinetik der invertinwirkung," *Biochem. Z.* **49**, 333–369 (1913).
- ²A. Cornish-Bowden, "One hundred years of Michaelis–Menten kinetics," *Perspect. Sci.* **4**, 3–9 (2015).
- ³S. Schnell and P. K. Maini, "A century of enzyme kinetics: Reliability of the K_M and v_{max} estimates," *Comments Theor. Biol.* **8**, 169–187 (2003).
- ⁴S. Schnell and C. Mendoza, "Closed form solution for time-dependent enzyme kinetics," *J. Theor. Biol.* **187**(2), 207–212 (1997).
- ⁵A. F. Bartholomay, "A stochastic approach to statistical kinetics with application to enzyme kinetics," *Biochemistry* **1**(2), 223–230 (1962).
- ⁶D. T. Gillespie, "Stochastic simulation of chemical kinetics," *Annu. Rev. Phys. Chem.* **58**, 35–55 (2007).
- ⁷C. Gardiner, *Stochastic Methods* (Springer Berlin, 2009), Vol. 4.
- ⁸Y. Ishihama, T. Schmidt, J. Rappsilber, M. Mann, F. U. Hartl, M. J. Kerner, and D. Frishman, "Protein abundance profiling of the *Escherichia coli* cytosol," *BMC Genomics* **9**(1), 102 (2008).
- ⁹P. Arányi and J. Tóth, "A full stochastic description of the Michaelis–Menten reaction for small systems," *Acta Biochim. Biophys.* **12**(4), 375–388 (1977).
- ¹⁰D. Schnoerr, G. Sanguinetti, and R. Grima, "The complex chemical Langevin equation," *J. Chem. Phys.* **141**, 024103 (2014).
- ¹¹É. Dóka and G. Lente, "Stochastic mapping of the Michaelis–Menten mechanism," *J. Chem. Phys.* **136**(5), 054111 (2012).
- ¹²E. L. Haseltine and J. B. Rawlings, "Approximate simulation of coupled fast and slow reactions for stochastic chemical kinetics," *J. Chem. Phys.* **117**(15), 6959–6969 (2002).
- ¹³C. V. Rao and A. P. Arkin, "Stochastic chemical kinetics and the quasi-steady-state assumption: Application to the Gillespie algorithm," *J. Chem. Phys.* **118**(11), 4999–5010 (2003).
- ¹⁴M. R. Roussel and R. Zhu, "Reducing a chemical master equation by invariant manifold methods," *J. Chem. Phys.* **121**(18), 8716–8730 (2004).
- ¹⁵J. Goutsias, "Quasiequilibrium approximation of fast reaction kinetics in stochastic biochemical systems," *J. Chem. Phys.* **122**(18), 184102 (2005).
- ¹⁶P. Thomas, A. V. Straube, and R. Grima, "Communication: Limitations of the stochastic quasi-steady-state approximation in open biochemical reaction networks," *J. Chem. Phys.* **135**, 181103 (2011).
- ¹⁷K. R. Sanft, D. T. Gillespie, and L. R. Petzold, "Legitimacy of the stochastic Michaelis–Menten approximation," *IET Syst. Biol.* **5**(1), 58–69 (2011).
- ¹⁸P. Thomas, A. V. Straube, and R. Grima, "The slow-scale linear noise approximation: An accurate, reduced stochastic description of biochemical networks under timescale separation conditions," *BMC Syst. Biol.* **6**(1), 39 (2012).
- ¹⁹J. K. Kim, K. Josić, and M. R. Bennett, "The validity of quasi-steady-state approximations in discrete stochastic simulations," *Biophys. J.* **107**(3), 783–793 (2014).
- ²⁰J. K. Kim, K. Josić, and M. R. Bennett, "The relationship between stochastic and deterministic quasi-steady state approximations," *BMC Syst. Biol.* **9**(1), 87 (2015).
- ²¹H. Qian and L. M. Bishop, "The chemical master equation approach to nonequilibrium steady-state of open biochemical systems: Linear single-molecule enzyme kinetics and nonlinear biochemical reaction networks," *Int. J. Mol. Sci.* **11**(9), 3472–3500 (2010).
- ²²X. Kan, C. H. Lee, and H. G. Othmer, "A multi-time-scale analysis of chemical reaction networks: II. Stochastic systems," *J. Math. Biol.* **73**(5), 1081–1129 (2016).
- ²³D. Schnoerr, G. Sanguinetti, and R. Grima, "Approximation and inference methods for stochastic biochemical kinetics—A tutorial review," *J. Phys. A: Math. Theor.* **50**(9), 093001 (2017).
- ²⁴N. Herath and D. Del Vecchio, "Reduced linear noise approximation for biochemical reaction networks with time-scale separation: The stochastic tQSSA⁺," *J. Chem. Phys.* **148**(9), 094108 (2018).
- ²⁵H.-W. Kang, W. R. KhudaBukhsh, H. Koepl, and G. A. Rempala, "Quasi-steady-state approximations derived from the stochastic model of enzyme kinetics," *Bull. Math. Biol.* **81**(5), 1303–1336 (2019).
- ²⁶E. Levine and T. Hwa, "Stochastic fluctuations in metabolic pathways," *Proc. Natl. Acad. Sci. U. S. A.* **104**(22), 9224–9229 (2007).
- ²⁷M. O. Stefani, A. J. McKane, and T. J. Newman, "Single enzyme pathways and substrate fluctuations," *Nonlinearity* **18**(4), 1575 (2005).
- ²⁸B. P. English, W. Min, A. M. Van Oijen, K. T. Lee, H. G. Luo, B. J. Sun, S. C. Cherayil, and X. S. Kou, "Ever-fluctuating single enzyme molecules: Michaelis–Menten equation revisited," *Nat. Chem. Biol.* **2**(2), 87–94 (2006).
- ²⁹R. Grima, "Noise-induced breakdown of the Michaelis–Menten equation in steady-state conditions," *Phys. Rev. Lett.* **102**(21), 218103 (2009).
- ³⁰R. Grima, "Investigating the robustness of the classical enzyme kinetic equations in small intracellular compartments," *BMC Syst. Biol.* **3**(1), 101 (2009).
- ³¹R. Grima and A. Leier, "Exact product formation rates for stochastic enzyme kinetics," *J. Phys. Chem. B* **121**(1), 13–23 (2017).
- ³²D. Singh and S. Chaudhury, "Single-molecule kinetics of an enzyme in the presence of multiple substrates," *ChemBioChem* **19**(8), 842–850 (2018).
- ³³R. Grima, N. G. Walter, and S. Schnell, "Single-molecule enzymology à la Michaelis–Menten," *FEBS J.* **281**(2), 518–530 (2014).
- ³⁴S. Bo and A. Celani, "Multiple-scale stochastic processes: Decimation, averaging and beyond," *Phys. Rep.* **670**, 1–59 (2017).
- ³⁵S. Smith and V. Shahrezaei, "General transient solution of the one-step master equation in one dimension," *Phys. Rev. E* **91**(6), 062119 (2015).
- ³⁶G. E. Briggs and J. B. S. Haldane, "A note on the kinetics of enzyme action," *Biochem. J.* **19**(2), 338 (1925).
- ³⁷L. A. Segel and M. Slemrod, "The quasi-steady-state assumption: A case study in perturbation," *SIAM Rev.* **31**(3), 446–477 (1989).
- ³⁸J. J. Tyson, "Biochemical oscillations," in *Computational Cell Biology* (Springer, 2002), pp. 230–260.
- ³⁹L. A. Segel, "On the validity of the steady state assumption of enzyme kinetics," *Bull. Math. Biol.* **50**(6), 579–593 (1988).
- ⁴⁰N. G. van Kampen, *Stochastic Processes in Physics and Chemistry* (Elsevier, 1992), Vol. 1.
- ⁴¹C. Jia and R. Grima, "Dynamical phase diagram of an auto-regulating gene in fast switching conditions," *J. Chem. Phys.* **152**(17), 174110 (2020).
- ⁴²C. Jia, "Model simplification and loss of irreversibility," *Phys. Rev. E* **93**(5), 052149 (2016).
- ⁴³N. J. Higham, *Functions of Matrices: Theory and Computation* (SIAM, 2008), Vol. 104.
- ⁴⁴K. Emrah and P. Stanica, "The inverse of banded matrices," *J. Comput. Appl. Math.* **237**(1), 126–135 (2013).
- ⁴⁵J. W. Brown, R. V. Churchill *et al.*, *Complex Variables and Applications* (McGraw-Hill Higher Education, Boston, 2009).
- ⁴⁶G. Broggi, L. A. Lugiato, and A. Colombo, "Transient bimodality in optically bistable systems," *Phys. Rev. A* **32**(5), 2803 (1985).
- ⁴⁷M. Mierzejewski, J. Dajka, J. Łuczka, P. Talkner, and H. Peter, "Dynamical bimodality in equilibrium monostable systems," *Phys. Rev. E* **74**(4), 041102 (2006).
- ⁴⁸C. F. Lam and D. G. Priest, "Enzyme kinetics: Systematic generation of valid King–Altman patterns," *Biophys. J.* **12**(3), 248–256 (1972).
- ⁴⁹P. A. Sims, "An 'Aufbau' approach to understanding how the King–Altman method of deriving rate equations for enzyme-catalyzed reactions works," *J. Chem. Educ.* **86**(3), 385 (2009).
- ⁵⁰A. Cornish-Bowden, *Fundamentals of Enzyme Kinetics* (John Wiley & Sons, 2013).
- ⁵¹J. Yu, J. Xiao, X. Ren, K. Lao, and X. S. Xie, "Probing gene expression in live cells, one protein molecule at a time," *Science* **311**(5767), 1600–1603 (2006).
- ⁵²D. M. Suter, N. Molina, D. Gafeld, K. Schneider, U. Schibler, and F. Naef, "Mammalian genes are transcribed with widely different bursting kinetics," *Science* **332**(6028), 472–474 (2011).