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Legitimacy of the stochastic Michaelis–Menten approximation

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Abstract: Michaelis–Menten kinetics are commonly used to represent enzyme-catalysed reactions in biochemical models. The Michaelis–Menten approximation has been thoroughly studied in the context of traditional differential equation models. The presence of small concentrations in biochemical systems, however, encourages the conversion to a discrete stochastic representation. It is shown that the Michaelis–Menten approximation is applicable in discrete stochastic models and that the validity conditions are the same as in the deterministic regime. The authors then compare the Michaelis–Menten approximation to a procedure called the slow-scale stochastic simulation algorithm (ssSSA). The theory underlying the ssSSA implies a formula that seems in some cases to be different from the well-known Michaelis–Menten formula. Here those differences are examined, and some special cases of the stochastic formulas are confirmed using a first-passage time analysis. This exercise serves to place the conventional Michaelis–Menten formula in a broader rigorous theoretical framework.

1 Introduction

Enzyme-catalysed reactions are ubiquitous in biochemical systems. The enzyme–substrate reaction set

$$E + S \underset{c_2}{\overset{c_1}{\rightleftharpoons}} ES \overset{c_3}{\longrightarrow} E + P \qquad (1)$$
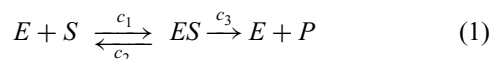
is a common model for such a system. Reactions R_1 and R_2 describe the reversible binding of an enzyme E to a substrate S. In reaction R_3 the intermediate complex ES reacts to form E and product P. The net result is the conversion of substrate to product. Modellers frequently use Michaelis–Menten kinetics to describe the rate of product formation in reaction set (1) [1, 2]. Michaelis–Menten kinetics effectively reduces the model from the three reactions in (1) to a single reaction. The Michaelis–Menten form is particularly convenient because the parameters are often easier to measure experimentally than the kinetic parameters c_i.

The Michaelis–Menten formula does not capture the dynamics of reactions (1) exactly. It is based on assumptions that, it is hoped, are approximately valid. Michaelis–Menten kinetics are derived from the ordinary differential equation (ODE) representation of reaction set (1), and in a deterministic setting, these assumptions have been well studied. However, biochemical systems often feature chemical species that are present in small populations where stochastic effects can play an important role. This leads many modellers to want to convert their ODE models into discrete stochastic models. Converting an ODE model to a stochastic model is straightforward if the ODEs describe

elementary reactions, but there is no general, theoretically rigorous method for converting Michaelis–Menten terms.

Gonze *et al.* [3] compared the output of a stochastic model that used Michaelis–Menten terms and a corresponding model decomposed into elementary reactions and found no significant differences in simulation results. Rao and Arkin [4] verified the equivalence of the deterministic and stochastic Michaelis–Menten approximations under a restricted set of initial conditions. Mastny *et al.* [5] derived a closed-form approximation similar to the Michaelis–Menten formula for reaction set (1) that is applicable in a different region of parameter space. We review and utilise the results of Rao and Arkin and Mastny *et al.* in Section 3. Cao *et al.* [6, 7] applied a method known as the slow-scale stochastic simulation algorithm (ssSSA) to reaction set (1) which significantly reduced the simulation time when $c_2 \gg c_3$.

The ssSSA also functions as a form of model reduction, similar to the Michaelis–Menten approximation. However, the theory underlying the ssSSA implies a formula for the enzyme–substrate reaction set (1) that seems in some cases to be different from the traditional Michaelis–Menten rate. Both approximations offer important benefits. But we need to be aware, in any specific circumstance, of both the benefits and drawbacks of describing the three reactions (1) with a reduced model. A recent paper by some of the present authors [8] thoroughly analysed model reduction in a stochastic context for the simplified reaction set

$$S_1 \underset{c_2}{\overset{c_1}{\rightleftharpoons}} S_2 \overset{c_3}{\longrightarrow} S_3 \qquad (2)$$
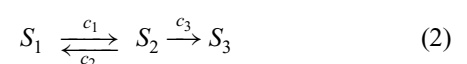
Some of the analysis of reaction set (2) can be applied to the enzyme–substrate reaction set (1).

In this paper we consider reaction set (1) and the Michaelis–Menten approximation from two perspectives. First, we address the problem of converting an ODE model with Michaelis–Menten terms to a stochastic model. Section 2 reviews the traditional deterministic Michaelis–Menten approximation. In Section 3 we justify the validity of using the Michaelis–Menten approximation in a stochastic setting, and in Section 4 we discuss some potential pitfalls. In the second part of the paper, Sections 5 and 6, we consider the Michaelis–Menten and ssSSA procedures in the case where all three rate constants in reaction set (1) are known. Differences between the Michaelis–Menten and ssSSA approximations are examined with a strong focus on simulation efficiency, and some special cases of the stochastic formulas are confirmed using a first-passage time analysis. This exercise serves to place the conventional Michaelis–Menten formula in a broader rigorous theoretical framework.

2 Michaelis–Menten kinetics in ODE models

Reaction set (1) leads to the ODE model

$$\frac{dS}{dt} = -c_1 S \times E + c_2 ES \qquad (3a)$$

$$\frac{dE}{dt} = -c_1 S \times E + (c_2 + c_3)ES \qquad (3b)$$

$$\frac{dES}{dt} = c_1 S \times E - (c_2 + c_3)ES \qquad (3c)$$

$$\frac{dP}{dt} = c_3 ES \qquad (3d)$$

$$S(0) = S_0, \quad E(0) = E_0, \quad ES(0) = ES_0, \quad P(0) = P_0$$

where the species populations are typically represented in units of concentration and the parameters c_i are the associated deterministic kinetic constants. To simplify the exposition, we will use deterministic and stochastic rate constants interchangeably; their meaning should be obvious from the context (see Appendix).

When reaction set (1) is considered in isolation, one can use the algebraic relations

$$E(t) + ES(t) = E_T \qquad (4a)$$

$$P(t) = P_0 + S_0 - (S(t) + ES(t)) \qquad (4b)$$

to reduce (3) to a set of two ODEs [Note 1]. However, inclusion of additional reactions or chemical species will add additional terms and equations and may preclude this reduction.

The derivation of Michaelis–Menten kinetics is based on the (deterministic) quasi-steady-state assumption (QSSA) [1, 2]. By assuming that the intermediate complex ES is in quasi-steady-state, we set dES/dt to zero and solve (3c) for ES. Substituting the result into (3d) and utilising the conservation relation (4a) leads to the Michaelis–Menten

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Note 1: In some related literature relation (4a) is often equated to E_0, which holds under the initial condition $ES(0) = 0$. To avoid ambiguity, we will use E_T for the enzyme conservation relation (4a) and E_0 for the initial value of E.

rate equation

$$\frac{dP}{dt} \simeq \frac{V_{\max} S}{K_m + S} \qquad (5)$$

where $V_{\max} = c_3 E_T$ and $K_m = (c_2 + c_3)/c_1$. $V_{\max}$ is the maximum rate of product formation under substrate saturation ($S \gg K_m$) and K_m is the substrate concentration at which the product formation rate is $V_{\max}/2$. This result is equivalent to assuming that the intermediate complex ES remains approximately equal to $E_T S/(K_m + S)$. One can also view the Michaelis–Menten approximation as a model reduction that eliminates species E and ES and replaces the full system (1) with the reduced model

$$S \xrightarrow{c_{MM}} P \qquad (6)$$

with rate

$$c_{MM} = \frac{V_{\max}}{K_m + S} \qquad (7)$$

Note that the rate c_{MM} in (7) varies based on the current amount of substrate in the system.

The Michaelis–Menten rate is an approximation. It is only valid in a particular region of parameter space. Segel and Slemrod [9] presented a detailed derivation of the Michaelis–Menten formula (5) and used singular perturbation analysis to establish the following validity criterion for the deterministic Michaelis–Menten approximation

$$E_T \ll S_0 + K_m \qquad (8)$$

When condition (8) holds, a separation exists between a fast 'pre-steady state' timescale and a slower 'steady state' timescale [9]. The solution of the Michaelis–Menten approximation closely tracks the solution of (3) on the slow timescale. Fig. 1 shows how closely the Michaelis–Menten approximation captures the behaviour of the full deterministic model when condition (8) is satisfied, except during the pre-steady-state transient period. When condition (8) is more strongly satisfied, the Michaelis–Menten rate becomes an even better approximation.

3 Converting ODE models with Michaelis–Menten terms to discrete stochastic models

Biochemical models often begin as a system of coupled ODEs describing the rates of change in chemical concentrations, as in (3a)–(3d). However, when some chemical species are present in small concentrations, a discrete stochastic representation is often more appropriate. For the discrete stochastic model, molecular concentrations are converted into populations and deterministic kinetic parameters are converted to stochastic kinetic parameters (see Appendix). The reaction rate equations are replaced with propensity functions that describe the probability of a reaction occurring in the next infinitesimal time interval. When the rate equations in the ODE model describe only elementary reactions, conversion to a stochastic model is straightforward and can be done automatically by introducing a volume parameter. However, when the ODE model contains Michaelis–Menten rate expressions, the appropriate conversion, or whether there is an appropriate conversion, is unclear.
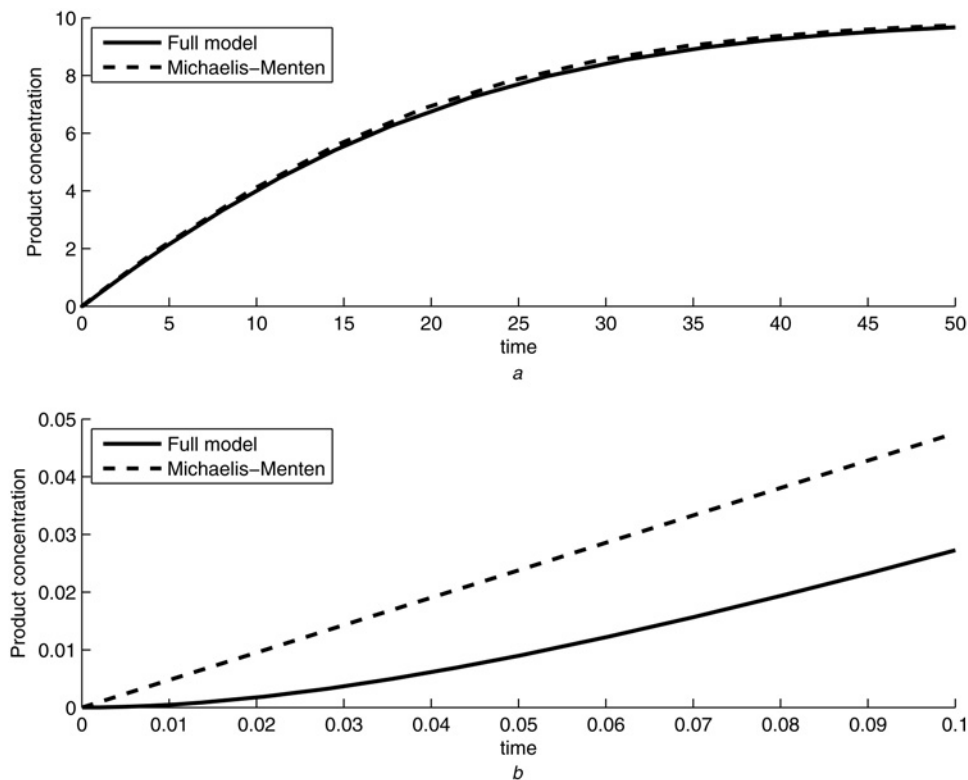
Fig. 1 *Deterministic trajectories of product concentration for the full model (solid curves) and the Michaelis–Menten approximation (dashed curves) on different time scales for parameters $S_0 = 10$, $E_0 = 1$, $ES_0 = P_0 = 0$, $c_1 = 1$, $c_2 = 10$, $c_3 = 1$. We note that validity condition (8) holds as $1 = E_T \ll S_0 + K_m = 21$*

a On the timescale of substantial product formation, the trajectory of the full model and the Michaelis–Menten approximation match closely

b Fast timescale: the Michaelis–Menten approximation fails to capture the behaviour of the full model during the pre-steady-state period

It is not possible to simply 'unpack' the Michaelis–Menten rate expression into the underlying elementary reactions. The two parameters V_{max} and K_m are comprised of the four unknown parameters E_T, c_1, c_2 and c_3 from the original system (1). Additional knowledge of the values of these four unknowns is required to fully determine all the parameters of the elementary reactions.

Since the Michaelis–Menten approximation effectively replaces reaction set (1) with the pseudo-unimolecular reduced mechanism (6), it is natural to consider whether the Michaelis–Menten rate can be converted directly to a stochastic propensity in the same way that a deterministic reaction rate can be converted to a propensity for a unimolecular reaction. Rao and Arkin [4] used a stochastic QSSA to show that in the limit of $E_T/S_0 \to 0$, the rate of product formation in a stochastic model approaches the deterministic Michaelis–Menten rate. Therefore an SSA simulation of the reduced mechanism (6) using effective propensity function

$$a_3(\boldsymbol{x}) \simeq \frac{V_{max} S}{K_m + S} \qquad (9)$$

will closely approximate the solution to the full stochastic model of reaction set (1) if

$$E_T \ll S_0 \qquad (10)$$

We note that the rate in (9) is essentially equal to the deterministic Michaelis–Menten rate (5), differing only in that the species populations are discrete molecule counts

rather than concentrations and that K_m is comprised of stochastic kinetic rates (see Appendix). The QSSA in a stochastic context is based on the assumption that the distribution of ES molecules remains approximately constant on the timescale of interest. Rao and Arkin [4] suggest that validity conditions for applying the QSSA in stochastic models may be the same as the conditions for deterministic models, but the validity criterion (10) is obviously different from (weaker than) the deterministic condition (8).

Mastny *et al.* [5], using a procedure they call the stochastic quasi-steady-state approximation singular perturbation analysis (sQSPA), show that the effective propensity function in the reduced stochastic model is given by

$$a_3(\boldsymbol{x}) \simeq \frac{V_{max} S}{K_m} \qquad (11)$$

when K_m is 'large' and the intermediate complex is 'small'. Mastny *et al.* [5] do not provide a more specific validity criterion for this result. However, analysis of their sQSPA procedure suggests that the validity condition is

$$E_T + S_0 \ll K_m \qquad (12)$$

Careful consideration of the results of Rao and Arkin [4] and those of Mastny *et al.* [5] leads to two important observations. First, the stochastic Michaelis–Menten rate is the same as the deterministic Michaelis–Menten rate (see Appendix). And, second, the condition for validity of the stochastic Michaelis–Menten rate is the same as the deterministic

case. To see that those conclusions are justified, first observe that combining validity conditions (10) and (12) covers the entire valid parameter range (8) of Segel and Slemrod [9]. That is, if condition (8) holds, then either condition (10) holds or condition (12) holds (or both hold). When (10) holds, Rao and Arkin [4] showed that the stochastic and deterministic Michaelis–Menten rates are equivalent. When condition (12) holds, rate (11) of Mastny *et al.* [5] is approximately equal to the Michaelis–Menten rate, because the missing S in the denominator is very small compared to K_m. Therefore we conclude that the deterministic validity criterion (8) of Segel and Slemrod [9] is sufficient for ensuring validity of the stochastic Michaelis–Menten approximation. As in the deterministic case, the stochastic Michaelis–Menten approximation fails to accurately capture the behaviour of the system during the pre-steady-state transient period. If a modeller wishes to study the behaviour on the fast timescale, the Michaelis–Menten approximation should be abandoned and replaced with the full model (1).

4 Some caveats

In the preceding section we discussed the apparent validity of the stochastic Michaelis–Menten approximation. We now take a step back and review some potential pitfalls of converting a deterministic model to a stochastic model with a particular emphasis on issues specific to models with Michaelis–Menten terms.

The derivation of the Michaelis–Menten rate considered reaction set (1) in isolation. Care must be taken when using the Michaelis–Menten approximation if reaction set (1) is embedded in a larger network of reactions. The

approximation is accurate only on the slow timescale. If S appears as a reactant in other fast reactions, the Michaelis–Menten approximation should not be applied in either the deterministic or stochastic case. Consider coupling reaction set (1) with the additional reaction

$$ S \xrightarrow{c_4} X \qquad (13) $$

If rate c_4 is large, the population of S will be decaying on a fast timescale via reaction (13) and the Michaelis–Menten approximation will not be valid, as seen in Figs. 2a and b. If, on the other hand, c_4 is small, then the Michaelis–Menten approximation can be applied to (1) and coupled with reaction (13) with minimal loss of accuracy (on the slow timescale) as shown in Figs. 2c and d. But how does one determine if the timescale of an additional reaction channel such as (13) is sufficiently slow? The appropriate comparison is that the characteristic timescale of the additional reaction channels should be much slower than the (fast) pre-steady-state timescale in the Michaelis–Menten approximation. Segel and Slemrod [9] estimate the pre-steady-state timescale as

$$ t_{\text{fast}} \simeq \frac{1}{c_1(S_0 + K_m)} \qquad (14) $$

Evaluating (14) for the parameters given in Fig. 2, namely $S_0 = 10$, $E_0 = 1$, $ES_0 = P_0 = 0$, $c_1 = 1$, $c_2 = 10$, $c_3 = 1$, we obtain an estimate of $t_{\text{fast}} \simeq 1/(1 \times (10 + 11)) = 1/21$. Comparing that to the timescale of reaction (13), which is $\simeq 1/(c_4 S)$, we deduce that the addition of reaction (13)
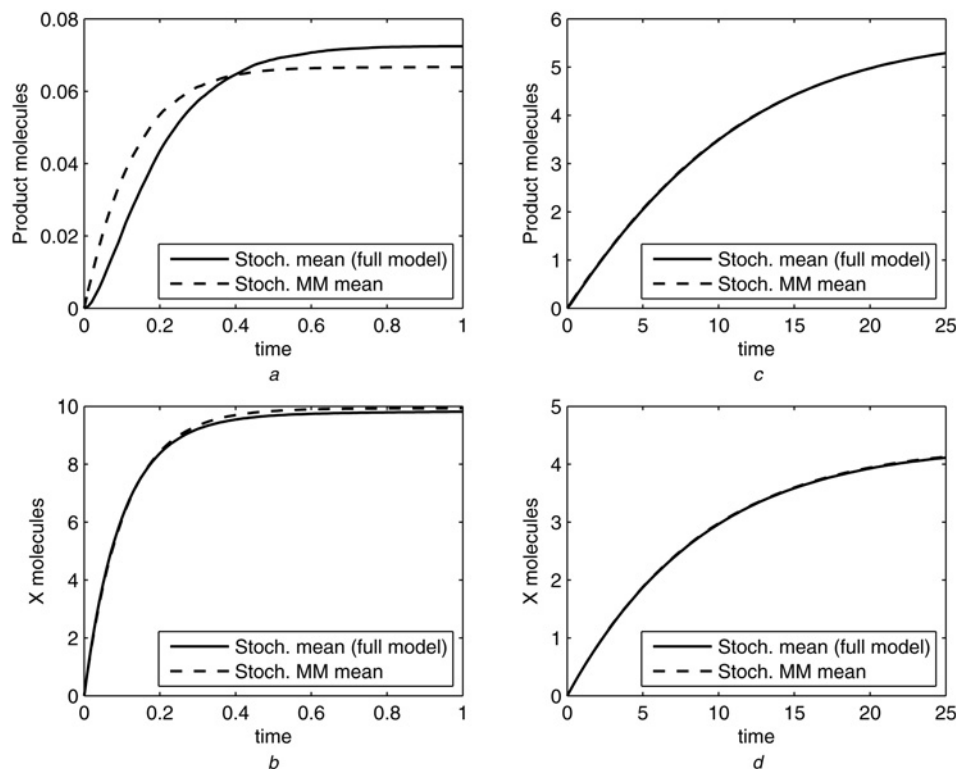

Fig. 2 *Effects of coupling reaction set (1) with reaction (13) when (13) is fast (a, b) and slow (c, d)*

a and *b* Means of P and X for the full stochastic model and stochastic Michaelis–Menten approximation when reactions (1) and (13) are coupled with c_4 large ($S_0 = 10$, $E_0 = 1$, $ES_0 = P_0 = 0$, $c_1 = 1$, $c_2 = 10$, $c_3 = 1$, $c_4 = 10$). Since reaction (13) introduces important dynamics on a fast timescale, the Michaelis–Menten approximation (which is valid on a slow timescale) is inaccurate
c and *d* When c_4 is sufficiently small [$c_4 = 1/20$ in this case, with other parameters the same as in (*a, b*)], reaction (13) is slow and can be coupled with the Michaelis–Menten approximation with minimal loss of accuracy

for the example in Fig. 2 will be valid if $c_4 \ll 2$. When that separation of timescales does not hold, the Michaelis–Menten approximation should not be used. It is important to recognise that in practice the situation is often more complicated than the example in Fig. 2. In most models containing a Michaelis–Menten term, the precise value of c_1 is generally not known (as c_1 only appears as part of composite variable K_m in the Michaelis–Menten rate), thus a direct evaluation of the timescale in (14) is not possible. One could compare the propensities of the additional reactions with the Michaelis–Menten rate, but that is a comparison with the slow timescale of the Michaelis–Menten approximation, not the fast pre-steady-state timescale. When adding a reaction such as (13) to a model with valid Michaelis–Menten terms, comparing the propensity to the Michaelis–Menten rate provides a conservative criterion for validity. That is, if the Michaelis–Menten approximation was properly applied for reactions (1), one can safely add a substrate-consuming reaction such as (13) if it is slower than the Michaelis–Menten rate. However, if the additional reaction is faster than the Michaelis–Menten rate, the modeller is forced to estimate the fast timescale by either making an assumption about the magnitude of c_1 or by determining the value of c_1 via biological experimentation. It is worth noting that if Michaelis–Menten terms are coupled with additional reactions appropriately in an ODE model, then direct conversion to a stochastic model is valid.

In Section 2 it was stated that the Michaelis–Menten approximation is essentially a model reduction that eliminates species ES and E but that it implies the effective population of species ES

$$ES_{\text{effective}} = \frac{E_T S}{K_m + S} \qquad (15)$$

$ES_{\text{effective}}$ can be used to incorporate additional slow reaction channels that consume ES or E (using $ES_{\text{effective}} = E_T - ES_{\text{effective}}$), but this requires knowledge of the value of E_T. It also typically requires explicitly tracking the evolution of E_T since reactions that create or consume ES or E will modify the value of E_T (i.e. E_T may no longer be constant). For example, the reaction

$$ES \xrightarrow{c_5} Y \qquad (16)$$

would have effective propensity $c_5 ES_{\text{effective}} = c_5 E_T S/(K_m + S)$ and since ES is not tracked, the stoichiometry of the reaction would be treated as if the reaction consumed an S molecule and an E_T 'molecule'

$$S + E_T \rightarrow Y \qquad (17)$$

In general, if E_T is known and the proper stoichiometries are applied, coupling additional slow reaction channels with a Michaelis–Menten approximation is possible. Equation (15) also suggests a way to refine the propensities of other reactions in which species S is a reactant. Since S molecules that are bound to enzymes are not available as reactants in other reactions, propensities that include species S can be improved by substituting the effective unbound S population

$$S_{\text{effective}} = S - ES_{\text{effective}} \qquad (18)$$

This value of $S_{\text{effective}}$ was used in place of S for the propensity

of reaction (13) in Fig. 2. However, a subtle implication of the Michaelis–Menten approximation is that whenever validity condition (8) holds, the mean of ES is very small compared to the mean of S and, hence, $S_{\text{effective}} \simeq S$. Therefore this correction leads to a small improvement in accuracy.

In [8] some of the present authors showed that a model reduction that replaces a more complex reaction set with a single reaction can be accurate only if the time to the product-forming reaction in the full model is approximately exponentially distributed [8]. Consider reaction set (1) with parameters

$$S_0 = 100, E_0 = E_T = 1, c_1 = 10^{-2}, c_2 = 0, c_3 = 1 \qquad (19)$$

Validity condition (8) strongly holds as $1 = E_T \ll S_0 + K_m = 200$. But by inspection the expected time to the first product-forming reaction is the sum of two exponential distributions with equal means of one. The sum of two exponentials with equal means is a case of the well-known gamma distribution. The mean and variance are $\mu = 2$ and $\sigma^2 = 2$, respectively. The Michaelis–Menten approximation would replace the full system with a single reaction with propensity $V_{\text{max}} S/(K_m + S) = 1/2$. The time to the first product forming reaction is then exponentially distributed with $\mu = 2$ and $\sigma^2 = 4$. The Michaelis–Menten approximation captures the mean, but the variance is doubled. As the simulation of (19) progresses and the substrate is consumed, the time to the next product formation in the full system gets closer to being exponentially distributed and the accuracy of the Michaelis–Menten approximation improves. Fig. 3 compares the full model to the Michaelis–Menten approximation for parameter set (19). Over the full simulation, the variance of the Michaelis–Menten model appears only slightly larger than in the full model as shown in Fig. 3a, but Fig. 3b demonstrates the severity of the error in the variance and shows that the error persists beyond the fast pre-steady-state timescale. It is important to be aware of this possible overestimate of the variance. We discuss accuracy issues further in Section 6.

5 ssSSA as an alternative to Michaelis–Menten when all parameters are known

One important benefit of the Michaelis–Menten approximation is that it requires only two parameters, V_{max} and K_m, which are often easier to obtain experimentally than are accurate estimates of E_T, c_1, c_2 and c_3. However, when a full description of all parameters in reaction set (1) is available, this consideration is no longer an issue. Another benefit of the Michaelis–Menten approximation is the reduced complexity of the model achieved by removing two species and two reactions. Considering that models are manipulated and simulated using computer software, this advantage is also of limited benefit except when the reduction leads to a substantial increase in simulation efficiency.

5.1 Simulation efficiency

Stochastic models are simulated using the well-known SSA [10, 11]. The SSA produces exact trajectories of a model, but since it simulates every reaction event and an ensemble of trajectories is required for reliable statistics, the SSA is computationally expensive.
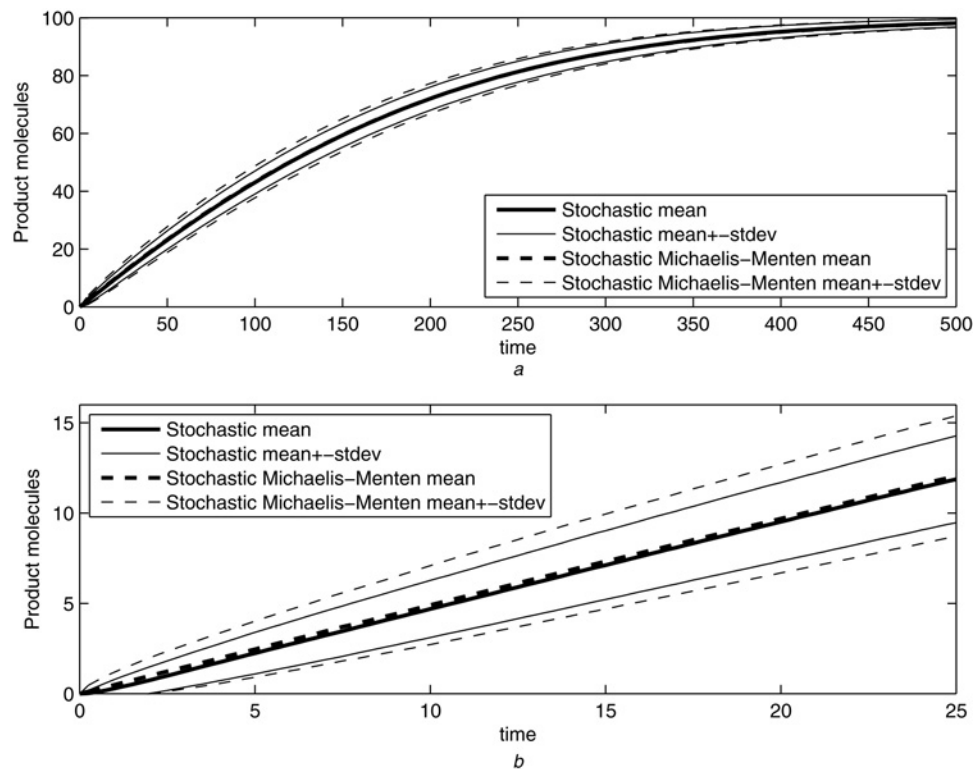
Fig. 3 *Means (heavy lines) and means $\pm$ standard deviation (light lines) of product formation for 10 000 realisations of the full model (solid curves) and the Michaelis–Menten approximation (dashed curves) for $S_0 = 100$, $E_T = E_0 = 1$, $c_1 = 10^{-2}$, $c_2 = 0$, $c_3 = 1$. Validity condition (8) holds as $1 = E_T \ll S_0 + K_m = 200$. Since $c_2 = 0$, the SSA had to simulate exactly two reaction events for each substrate to product conversion compared to one for the Michaelis–Menten approximation*

a On the scale of total substrate to product conversion, the means are practically indistinguishable but the standard deviation of the Michaelis–Menten approximation is slightly greater than the true standard deviation
b Early mismatch in the standard deviation of the Michaelis–Menten approximation persists over a longer time interval than the pre-steady-state transient in the mean

Replacing reactions (1) with a single reaction reduced system such as (6) means that a product molecule would be formed at each reaction event. It turns out that such a reduction will lead to a significant speedup only when

$$c_2 \gg c_3 \qquad (20)$$

This result was derived using a different model [8] but the reasoning is also applicable to reaction set (1). Since an ES molecule has probability $c_3/(c_2 + c_3)$ of producing a P molecule when it decays, then on average, $(c_2 + c_3)/c_3$ ES molecules must be created, and then annihilated, in order to produce one P molecule. Therefore on average

$$2(c_2 + c_3)/c_3 \equiv n_{\text{avg}} \qquad (21)$$

reaction events have to be simulated by the SSA in order to convert one S molecule into a P molecule via reactions (1). The value of n_{avg} represents the expected simulation speedup of replacing reactions (1) with a single reaction reduced system. Thus, only if $c_2 \gg c_3$ will such a reduction lead to substantial computational savings. In contrast, if $c_2 \ll c_3$, the SSA will have to simulate an average of about two reaction events in order to convert an S molecule into a P molecule via reactions (1). And if $c_2 \simeq c_3$, the SSA will need to simulate an average of about four reaction events to accomplish that conversion. Unless condition (20) holds, the gain in simulation speed that would result from applying the SSA to any single reaction reduction of

reactions (1) would be so modest that it would likely not offset the benefit that comes from directly simulating reactions (1) with the SSA, namely, that the behaviours of all species are exactly rendered.

5.2 ssSSA approach

A stochastic alternative to the SSA for reactions (1) which is specifically tailored for condition (20) is the ssSSA [6, 7]. We now summarise the ssSSA procedure presented in [6, 7] for simulation of reactions (1) under condition (20). The ssSSA essentially eliminates the two 'fast' reactions R_1 and R_2

$$E + S \underset{c_2}{\overset{c_1}{\rightleftharpoons}} ES \qquad (22)$$

and simulates only the 'slow' product-forming reaction R_3. However, instead of using the R_3 propensity function $a_3(x) = c_3 ES$, the ssSSA uses the effective R_3 propensity function

$$\bar{a}_3(\boldsymbol{x}) = c_3 \langle \widehat{ES}(\infty) \rangle \qquad (23)$$

where $\langle \widehat{ES}(\infty) \rangle$ is the mean of the steady-state distribution of the enzyme–substrate complex evolving under only the two fast reactions R_1 and R_2, given the state $\boldsymbol{x} = (E, S, ES, P)$. What assures us that this is a legitimate tactic is the so-called slow-scale approximation lemma [6]. The

slow-scale approximation lemma states that when a system has a stable virtual fast process with a relaxation time that is fast compared to the slow reactions, the propensity functions of the slow reactions can be well approximated by replacing the actual populations of the fast species with the mean values of the steady-state distribution of the virtual fast process [6]. When $c_2 \gg c_3$, reactions R_1 and R_2 reach a stable equilibrium distribution on a scale that is fast compared to reaction R_3. Therefore condition (20) is sufficient for the validity of that lemma (and hence the ssSSA) for reaction set (1) [7]. We note that this condition differs from, and thus corrects, a condition given in [7]. In [7], it was stated that the ssSSA validity condition $c_2 \gg c_3$ could be refined [7, (26)]. This refinement was incorrect because it was comparing a single-walker timescale with a many-walker timescale to estimate the separation between the relaxation time of the virtual fast process and the expected time to the next slow reaction. We note that model reductions of reaction set (1) are possible under a wider range of conditions, but unless $c_2 \gg c_3$ one can efficiently generate exact trajectories of all species using the SSA.

The mean of $\widehat{ES}(\infty)$ that appears in (23) can be computed as follows. Let E_T and S_T denote the total numbers of enzyme units and substrate units in state $\boldsymbol{x}$, that is

$$E + ES \equiv E_T \quad \text{and} \quad S + ES \equiv S_T \qquad (24)$$

Note that both E_T and S_T are conserved under the two fast reactions R_1 and R_2. The steady-state master equation for reactions (22) in isolation yields the moment relation $\langle c_1 \hat{E}(\infty)\hat{S}(\infty)\rangle = \langle c_2 \widehat{ES}(\infty)\rangle$, and with (24) this becomes

$$\left\langle c_1(E_T - \widehat{ES}(\infty))(S_T - \widehat{ES}(\infty))\right\rangle = \left\langle c_2\widehat{ES}(\infty)\right\rangle \qquad (25)$$

Upon expanding the left side and replacing the term $\langle \widehat{ES}^2(\infty)\rangle$ with the statistically equivalent $\langle \widehat{ES}(\infty)\rangle^2 + \text{var}\{\widehat{ES}(\infty)\}$, we obtain a simple quadratic equation for $\langle \widehat{ES}(\infty)\rangle$ whose solution is

$$\left\langle \widehat{ES}(\infty)\right\rangle = \frac{1}{2}\left\{\left(E_T + S_T + \frac{c_2}{c_1}\right)\right.$$
$$\left. - \sqrt{\left(E_T + S_T + \frac{c_2}{c_1}\right)^2 - 4\left(E_T S_T + \text{var}\{\widehat{ES}(\infty)\}\right)}\right\} \qquad (26)$$

Formula (26) is exact but it involves the variance of $\widehat{ES}(\infty)$. Since the standard deviation of $\widehat{ES}(\infty)$ is typically on the order of $\sqrt{\langle \widehat{ES}(\infty)\rangle}$, then $\text{var}\{\widehat{ES}(\infty)\}$ will usually be on the order of $\langle \widehat{ES}(\infty)\rangle$. And since ES is bounded above by $\min(E_T, S_T)$, which in turn is usually much smaller than $E_T S_T$, it will usually be permissible to drop the variance term in (26) and approximate $\langle \widehat{ES}(\infty)\rangle \simeq \overline{ES}$, where $\overline{ES}$ is given by (26) with $\text{var}\{\widehat{ES}(\infty)\} = 0$

$$\overline{ES} \equiv \frac{1}{2}\left\{\left(E_T + S_T + \frac{c_2}{c_1}\right) - \sqrt{\left(E_T + S_T + \frac{c_2}{c_1}\right)^2 - 4E_T S_T}\right\} \qquad (27)$$

The result of this approximation is that $\bar{a}_3(\boldsymbol{x})$ in (23) gets

approximated by

$$\bar{a}_3(\boldsymbol{x}) = \frac{c_3}{2}\left\{\left(E_T + S_T + \frac{c_2}{c_1}\right) - \sqrt{\left(E_T + S_T + \frac{c_2}{c_1}\right)^2 - 4E_T S_T}\right\} \qquad (28)$$

Approximation (28) can in some cases be improved by using in (26) a better estimate of $\text{var}\{\widehat{ES}(\infty)\}$ than zero. Analysis of (26) reveals that the variance term can be significant only if E_T and S_T are both small. Under these conditions, it is possible to improve the accuracy of the ssSSA by using a recurrence relation derived in [7] to exactly calculate the $\widehat{ES}(\infty)$ state probabilities. The mean of $\widehat{ES}(\infty)$ can then be computed and used directly in (23) to determine the effective propensity $\bar{a}_3(\boldsymbol{x})$. In general, the algorithm for the recursion calculation requires a loop from $\min(E_T, S_T)$ down to zero, but that becomes a fast calculation when E_T and S_T are both small. In an ensemble simulation, values calculated via the recursion relation can be stored in a table for immediate lookup in subsequent realisations. In practice, formula (28) provides sufficient accuracy if the population of either E_T or S_T is around 10 or more. However, if E_T and S_T are both small the recursion calculation can yield significant gains in accuracy as shown in Fig. 4. Even with small molecular populations, if $c_2 \gg c_3$ the ssSSA will still produce substantial gains in simulation speed over the exact SSA (see Section 5.1).

As with the Michaelis–Menten approximation (see Section 3), care must be taken when using the ssSSA if reaction set (1) is embedded in a larger network of reactions. The addition of slow reaction channels does not pose a problem and can be implemented using a procedure similar to that described in Section 3 for the Michaelis–Menten approximation (see [7] for details). However, the addition of fast reaction channels typically requires repartitioning the system into different fast and slow subsets [7]. The new 'virtual fast process' might be considerably more complicated than the two-reaction set (22).

6 Verifying accuracy using first-passage time analysis

The slow-scale SSA and the stochastic Michaelis–Menten formula both approximate the rate at which reaction R_3 is firing, and hence the rate at which product molecules are being formed. We should therefore expect a close connection between the ssSSA's formula (28) for $\bar{a}_3(\boldsymbol{x})$ and the Michaelis–Menten rate (5). But at first inspection, (28) seems to bear little resemblance to (5). The ssSSA and Michaelis–Menten approximations are valid in different regions of parameter space, as depicted in Fig. 5. It is hoped that the two approximations agree when both conditions $E_T \ll S_0 + K_m$ and $c_2 \gg c_3$ are satisfied, as in the intersecting region in Fig. 5.

Evaluating the ssSSA rate (28) under condition (10) considered by Rao and Arkin [4], namely $S_0 \gg E_T$, with $S_0 = S_T$ gives

$$\bar{a}_3(\boldsymbol{x}) \simeq \frac{c_3}{2}\left\{(S_T + (c_2/c_1)) - \sqrt{(S_T + (c_2/c_1))^2 - 4E_T S_T}\right\}$$
$$\simeq \frac{c_3}{2}(S_T + (c_2/c_1))\left\{1 - \left(1 - \frac{1}{2}\frac{4E_T S_T}{(S_T + (c_2/c_1))^2}\right)\right\}$$
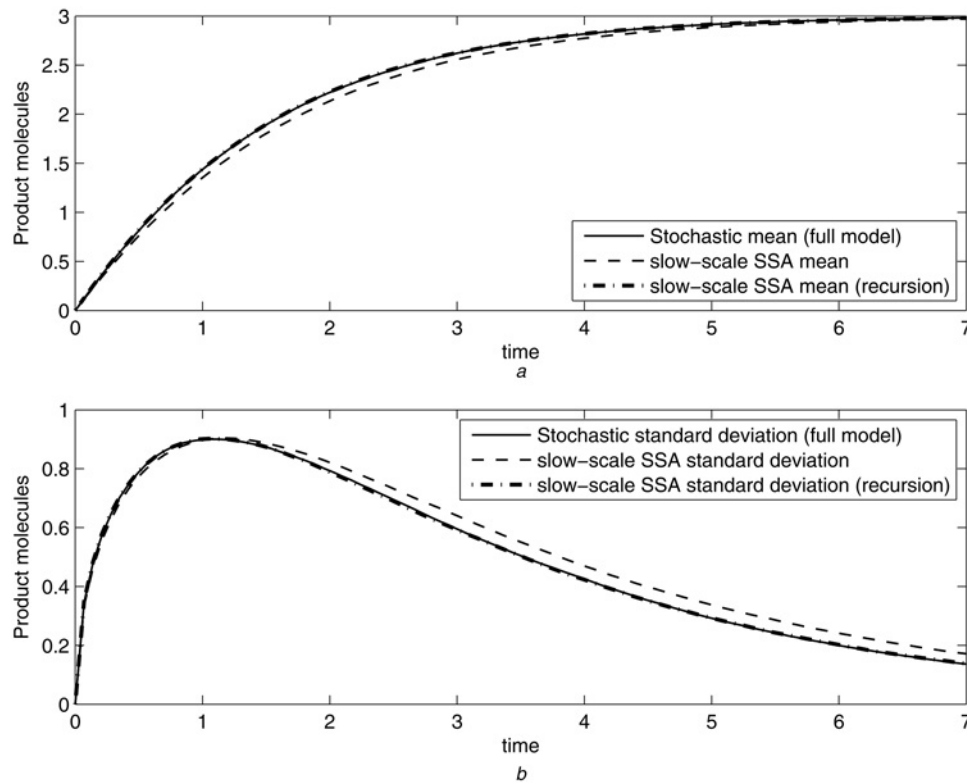
Fig. 4 *SSA mean (a) and standard deviation (b) of reaction set (1) against the ssSSA formula (28) and the improved ssSSA formula achieved by exactly solving for the mean of $\widehat{ES}(\infty)$ under conditions $S_0 = 3$, $E_T = E_0 = 3$, $c_1 = 100$, $c_2 = 100$, $c_3 = 1$*

The recursion formula is beneficial only for very small populations. When the value of S_T or E_T is around 10 or larger, the simple ssSSA formula (28) usually provides sufficient accuracy
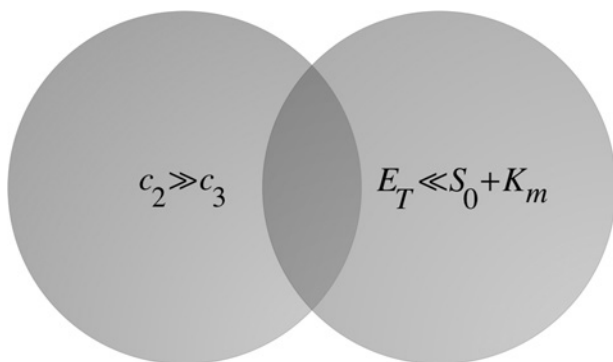

Fig. 5 *Illustration of the validity regions for the ssSSA ($c_2 \gg c_3$) and Michaelis–Menten ($E_T \ll S_0 + K_m$) approximations for reaction set (1)*

The two approximations are essentially equivalent in the (shaded) overlapping region. Only when $c_2 \gg c_3$ will either approximation lead to significant simulation efficiency gains over an exact SSA simulation of the full model

whence

$$\bar{a}_3(\boldsymbol{x}) \simeq \frac{c_3 E_T S_T}{S_T + (c_2/c_1)} \quad (S_T \gg E_T, c_2 \gg c_3) \quad (29)$$

Under $c_2 \gg c_3$, we have $c_2/c_1 \simeq (c_2 + c_3)/c_1 = K_m$; thus we see that the ssSSA result (28) does indeed agree with the Michaelis–Menten formula (5) when $S_0 \gg E_T$. However, (28) does not appear to be easily reducible to the Michaelis–Menten formula in the remainder of the overlapping region between the ssSSA condition (20) and

the validity region (8) of Segel and Slemrod [9]. Interestingly, (28) can also be simplified in the case $S_0 = S_T \ll E_T$; indeed, since (28) is symmetric in E_T and S_T, its approximate form in that case can be inferred simply by interchanging those two variables in (29)

$$\bar{a}_3(\boldsymbol{x}) \simeq \frac{c_3 E_T S_T}{E_T + (c_2/c_1)} \quad (S_T \ll E_T, c_2 \gg c_3) \quad (30)$$

In what follows, we corroborate the stochastic Michaelis–Menten formula and the ssSSA rate (28) by presenting independent derivations of some special cases including the limiting forms (29) and (30).

The assertion of the slow-scale approximation lemma, that under condition (20) reaction R_3 occurs according to the propensity function $\bar{a}_3(\boldsymbol{x})$, is mathematically equivalent to asserting that the time to the next R_3 reaction is an exponential random variable with mean $1/\bar{a}_3(\boldsymbol{x})$ [8, Section 2 and Appendix A]. Similarly, the stochastic Michaelis–Menten formula effectively approximates the next R_3 reaction as an exponential random variable with mean $(K_m + S)/V_{max}S$, the inverse of the Michaelis–Menten rate. Accuracy of the stochastic Michaelis–Menten approximation and the ssSSA can in principle be tested by making a first-passage time type of analysis, but there are some subtleties in doing that properly.

The signature effect of an R_3 event is the reduction by 1 of the total number of substrate units (free and bound). Therefore given that the system is currently in state $\boldsymbol{x} = (E, S, ES, P)$, the time $T(\boldsymbol{x})$ to the next R_3 reaction can be most generally defined as the time required for the system, evolving according to reactions (1), to first exit the state space region

$\Omega(\boldsymbol{x})\{\boldsymbol{x}' \| S_T = S + ES\}$. This first-exit problem appears to be quite difficult to solve in general. But it can be solved approximately in some cases, provided we are clever in what we take to be the 'random walker'.

6.1 Generic first-passage time result

We summarise here a generic result in Markov process theory that will be useful in our analysis. For a derivation of this result, see [8, Appendix B]. Suppose a 'random walker' executes the following transitions among three 'states', S_1, S_2 and S_3

$$S_1 \underset{c_2}{\overset{c_1}{\rightleftarrows}} S_2 \overset{c_3}{\longrightarrow} S_3 \qquad (31)$$
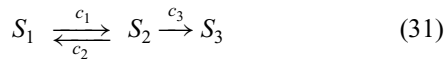
Here c_j is a constant, and $c_j dt$ gives the probability that the walker, if currently in the state at the tail of the arrow, will jump to the state at the head of the arrow in the next infinitesimal time interval dt. For this random walker, the following result has been proved [8]: If there are currently x_1 walkers in state S_1 and x_2 walkers in state S_2, and if all these walkers move independently, then the time $T(x_1, x_2)$ until the first of the walkers reaches state S_3 is a random variable with probability density function

$$\begin{aligned}
P(t; x_1, x_2) &= x_1 c_3 P(2, t|1, 0)(P(1, t|1, 0) + P(2, t|1, 0))^{x_1-1} \\
&\times (P(1, t|2, 0) + P(2, t|2, 0))^{x_2} \\
&+ x_2 c_3 P(2, t|2, 0)(P(1, t|1, 0) + P(2, t|1, 0))^{x_1} \\
&\times (P(1, t|2, 0) + P(2, t|2, 0))^{x_2-1}
\end{aligned}$$
$$(32)$$

Here, $P(n, t|\alpha, 0)$ is the probability that a walker, in state S_α at time 0, will be in state S_n at time t, and it is given explicitly by

$$P(1, t|1, 0) = \frac{1}{(\lambda_+ - \lambda_-)}[(c_1 - \lambda_-)e^{-\lambda_+ t} - (c_1 - \lambda_+)e^{-\lambda_- t}] \qquad (33a)$$

$$P(2, t|1, 0) = \frac{(c_1 - \lambda_+)(c_1 - \lambda_-)}{c_2(\lambda_+ - \lambda_-)}[e^{-\lambda_+ t} - e^{-\lambda_- t}] \qquad (33b)$$

$$P(1, t|2, 0) = \frac{c_2}{(\lambda_+ - \lambda_-)}[e^{-\lambda_- t} - e^{-\lambda_+ t}] \qquad (33c)$$

$$P(2, t|2, 0) = \frac{1}{(\lambda_+ - \lambda_-)}[(c_1 - \lambda_-)e^{-\lambda_- t} - (c_1 - \lambda_+)e^{-\lambda_+ t}] \qquad (33d)$$

where

$$\lambda_\pm \equiv \frac{1}{2}\left[(c_1 + c_2 + c_3) \pm \sqrt{(c_1 + c_2 + c_3)^2 - 4c_1 c_3}\right] \qquad (34)$$

This result (32) is exact, but does not describe an exponential distribution. However, under condition (20), it can be shown [8] from (34) that $\lambda_+ \simeq c_1 + c_2$ and $\lambda_- \simeq c_1 c_3/(c_1 + c_2) \ll \lambda_+$, and that (32) then approximates

to the exponential form

$$P(t; x_1, x_2) \simeq \left(\frac{c_1 c_3 (x_1 + x_2)}{c_1 + c_2}\right)e^{-(c_1 c_3 (x_1 + x_2)/(c_1 + c_2))t},$$
$$(c_2 \gg c_3; \, t \gg (c_1 + c_2)^{-1}) \qquad (35)$$

Similarly, when

$$c_3 \gg c_1 \qquad (36)$$

it can be shown [8] that (32) approximates to

$$P(t; x_1, x_2) \simeq \left(\frac{c_1 c_3 (x_1 + x_2)}{c_2 + c_3}\right)e^{-(c_1 c_3 (x_1 + x_2)/(c_2 + c_3))t},$$
$$(c_3 \gg c_1; \, t \gg (c_2 + c_3)^{-1}) \qquad (37)$$

6.2 First-passage time analysis of reactions (1)

If we focus on the individual enzyme units, both the free ones E and the bound ones ES, we can define $T(\boldsymbol{x})$ to be the time required for the first of those enzyme units to participate in the production of a product molecule via reaction R_3. Each individual enzyme unit will be performing the random walk

$$F \underset{c_2}{\overset{c_1 S}{\rightleftarrows}} B \overset{c_3}{\longrightarrow} 3 \qquad (38)$$
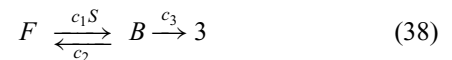
where F is the free-enzyme state, B the bound-enzyme state and 3 the state of an enzyme that has just assisted the conversion of some substrate unit into a product molecule. Two obstacles prevent (38) from being an instance of the generic random walk (31): First, the R_1 reaction probability rate $c_1 S$ in (38) depends on the constantly changing number of free substrate units S, and hence is not a constant. Second, the individual enzyme units do not evolve independently of each other; because, owing to (24), the dependence of the R_1 reaction rate on the number of free substrate units means that the fate of any enzyme unit depends on the number of enzyme–substrate complexes ES, and that in turn depends on the bound–unbound status of all the enzyme units.

But both of these obstacles go away, at least to a good approximation, in the special case $S_T \gg E_T$. Because then, since S must always be in the interval $[S_T - E_T, S_T]$, where the lower limit corresponds to all the enzyme units being bound and the upper limit corresponds to all the enzyme units being free, we will have to a very good approximation $S \simeq S_T$. Then each enzyme unit will be performing the random walk

$$F \underset{c_2}{\overset{c_1 S_T}{\rightleftarrows}} B \overset{c_3}{\longrightarrow} 3 \quad (E_T \ll S_T) \qquad (39)$$
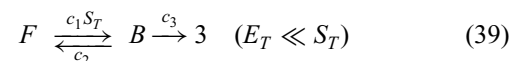
Here, all the reaction probability rates are constants (up to the moment of the first conversion), and the individual enzyme units will be executing this random walk independently of each other. Now we can apply the results for the random walk (31) to the random walk (39) simply by making the replacements

$$c_1 \to c_1 S_T, \quad x_1 \to E, \quad x_2 \to ES \qquad (40)$$

With these replacements, (32) becomes the probability

density function for the time $T(x)$ to the next R_3 reaction in state x. It shows that in general $T(x)$ is not exponentially distributed, and moreover will depend on how many of the enzyme units are free and how many are bound. But the restricted approximate result (35) shows that in the case $c_2 \gg c_3$ the probability density function of $T(x)$ reduces to the exponential form

$$P_x(t) \simeq \left(\frac{c_1 S_T c_3 (E + ES)}{c_1 S_T + c_2} \right) e^{-(c_1 S_T c_3 (E+ES)/(c_1 S_T + c_2))t} \quad (41)$$

With (24) and some simple algebraic rearrangements, this result can be written as

$$P_x(t) \simeq \left(\frac{c_3 S_T E_T}{S_T + (c_2/c_1)} \right) e^{-(c_3 S_T E_T/(S_T + (c_2/c_1)))t} \quad (42)$$

which is valid under the conditions

$$E_T \ll S_T, \quad c_2 \gg c_3, \quad t \gg (c_1 S_T + c_2)^{-1} \quad (43)$$

Equation (42) implies that, if the system is currently in state x, then under conditions (43), the probability that reaction R_3 will occur in the next 'infinitesimal' time $dt > (c_1 S_T + c_2)^{-1}$ is

$$\left(\frac{c_3 S_T E_T}{S_T + (c_2/c_1)} \right) dt \quad (44)$$

This is precisely the assertion of (29) for the case $E_T \ll S_T$. Noting again that $(c_2/c_1) \simeq K_m$ when $c_2 \gg c_3$, this also provides an independent proof of the agreement and correctness of the ssSSA and the result of Rao and Arkin [4] in the overlapping validity regions $((c_2 \gg c_3) \cap (S_0 \gg E_T))$. Fig. 6 shows an example corroborating the assertion that the Michaelis–Menten and ssSSA approximations are accurate in their overlapping validity regions. In that example $100 = c_2 \gg c_3 = 1$, so both approximations led to
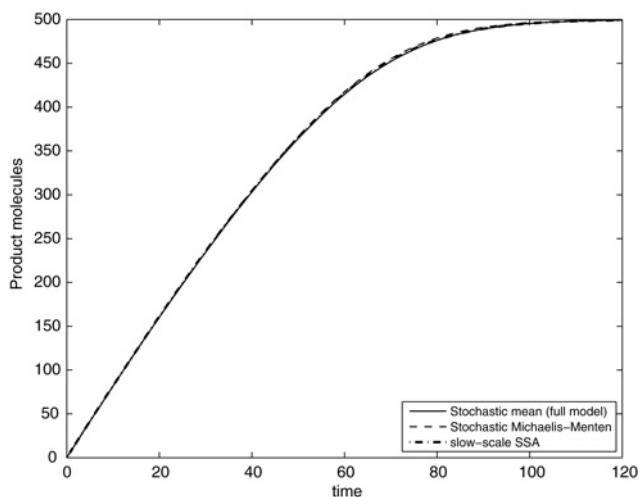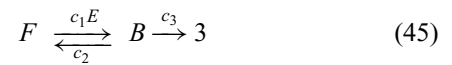
simulation speedups of about 200 times over the full SSA simulation of reaction set (1) as predicted by n_{avg} in (21).

To obtain a result for the opposite case $S_T \ll E_T$, we must focus our attention on the individual substrate units instead of the individual enzyme units. We thus consider each substrate unit to be independently executing the random walk

$$F \underset{c_2}{\overset{c_1 E}{\rightleftarrows}} B \overset{c_3}{\longrightarrow} 3 \quad (45)$$

where F is now the free-substrate state, B the bound-substrate state and 3 the state in which the substrate unit has just been converted into a product molecule. Repeating the above analysis with the roles of the enzyme and substrate units interchanged, we obtain a result analogous to (42), namely

$$P_x(t) \simeq \left(\frac{c_3 E_T S_T}{E_T + (c_2/c_1)} \right) e^{-(c_3 E_T S_T/(E_T + (c_2/c_1)))t} \quad (46)$$

which holds when

$$S_T \ll E_T, \quad c_2 \gg c_3, \quad t \gg (c_1 E_T + c_2)^{-1} \quad (47)$$

The exponential form (46) validates the prediction (30) of the slow-scale approximation lemma in the case $S_T \ll E_T$. Fig. 7 compares the ssSSA to the Michaelis–Menten approximation when condition (47) holds, but the Michaelis–Menten validity condition (8) does not hold.

We now consider the random walk (45) for the case

$$E_T, S_T \ll K_m \quad (48)$$

We can use the intuition underlying the results of Mastny et al. [5] to argue that under conditions (48), ES 'usually samples zero'. That is, whenever an E and S bind to form ES, the ES molecule quickly either decays back to E and S or produces a product. Therefore we can regard E and S as approximately independent and utilise the generic first-passage time result. The condition K_m large implies

Fig. 6 *Exact SSA, Michaelis–Menten approximation and ssSSA approximation mean values of product formed in reaction set (1) for $S_0 = 500$, $E_T = E_0 = 10$, $c_1 = 1$, $c_2 = 100$, $c_3 = 1$*

When $c_2 \gg c_3$ and $S_0 \gg E_T$ the Michaelis–Menten approximation and the ssSSA are essentially equivalent and the simulation results are indistinguishable from the exact solution. In this example, both approximations led to simulations that ran about 200 times faster than the full model
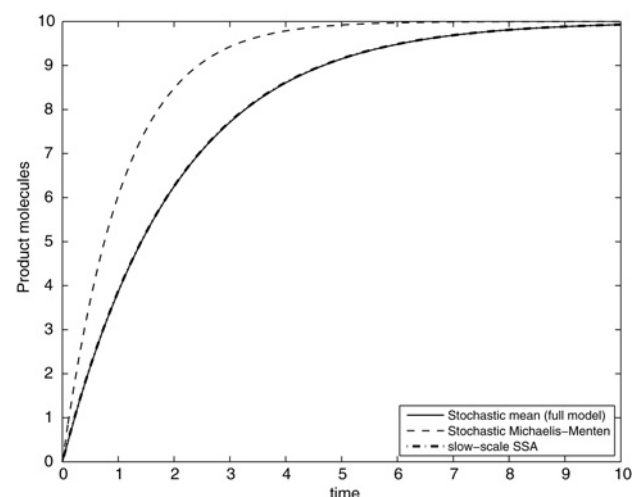

Fig. 7 *Exact SSA, Michaelis–Menten approximation and ssSSA mean values of reaction set (1) for $S_0 = 10$, $E_T = E_0 = 100$, $c_1 = 1$, $c_2 = 100$, $c_3 = 1$*

Under these conditions, the ssSSA is valid ($100 = c_2 \gg c_3 = 1$) whereas the Michaelis–Menten approximation is not ($100 = E_T \simeq S_0 + K_m = 111$). The ssSSA had to simulate exactly ten reactions per realisation compared to an average of about 2000 reactions for the SSA simulations of the full model

$c_2 + c_3 \gg c_1$. Since E_T is small, we can also write $c_2 + c_3 \gg c_1 E_T$. We can then further categorise condition (48) into subcases based on the magnitude of c_3 relative to $c_1 E_T$

$$c_3 \gg c_1 E_T \tag{49a}$$

$$c_3 \ll c_1 E_T \quad (\Rightarrow c_2 \gg c_3) \tag{49b}$$

$$c_3 \simeq c_1 E_T \quad (\Rightarrow c_2 \gg c_3) \tag{49c}$$

Considering, for example, condition (49a), we can use (37) to approximate (32) as

$$P_x(t) \simeq \left(\frac{c_1 E_T c_3 S}{c_2 + c_3}\right) e^{-(c_1 E_T c_3 S/(c_2 + c_3))t} \tag{50}$$

Writing the pdf (50) as a propensity function and rearranging, we have

$$a_3(\boldsymbol{x}) \simeq \frac{c_3 E_T S}{(c_2 + c_3)/c_1} = \frac{V_{\max} S}{K_m} \tag{51}$$

We recognise (51) as result (11) of Mastny *et al.* [5] Again, since $K_m \gg S$, this is approximately the Michaelis–Menten rate. Similar approximations can be made for conditions (49b) and (49c) by using (35). This serves as an independent proof of result (11) of Mastny *et al.* [5] under condition (48). It also shows that the ssSSA formula and the Michaelis–Menten approximation agree in the intersection of the ssSSA's validity region (20) and condition (48).

The exponential forms of (42) and (46) validate predictions (29) and (30) of the ssSSA. The first-passage time analysis provides independent verification of the stochastic QSSA of Rao and Arkin [4] and the sQSPA of Mastny *et al.* [5] in at least some portions of their respective validity regions, including the regions overlapping with the ssSSA. But first-passage time analysis cannot confirm the accuracy of the $S_0 \gg E_T$ validity condition of Rao and Arkin [4] in general, because of the gamma distribution that arises under the condition (19) example given in Section 4.

7 Summary and conclusions

In this paper, we have shown that the Michaelis–Menten approximation is applicable in stochastic simulation under the same validity conditions as in the deterministic case, namely the Segel and Slemrod [9] condition $E_T \ll S_0 + K_m$. This was justified by a careful analysis and application of previous results by Rao and Arkin [4] and Mastny *et al.* [5]. Thus, the conversion of an ODE model with Michaelis–Menten terms to a stochastic model can be achieved by converting the Michaelis–Menten rate directly to a propensity function. However, we did show that under some conditions the stochastic Michaelis–Menten approximation could lead to an estimate of the variance that is larger than the true variance of the underlying full model.

One important benefit of the Michaelis–Menten formula is that the two parameters $V_{\max}$ and K_m are often easier to determine experimentally than the rate constants c_i in (1). When all the parameters in reaction set (1) are known, the SSA gives exact trajectories for all species. Because the SSA can be computationally expensive, simulation efficiency concerns can encourage the use of approximate methods. But any gain in efficiency is justified only if the loss of accuracy is not too great. We showed that condition $c_2 \gg c_3$ is the only case where a model reduction can provide a substantial speedup over the SSA. In that case, the ssSSA procedure provides an efficient and accurate approximation. An advantage of the ssSSA is that it is valid for reaction set (1) whenever condition $c_2 \gg c_3$ holds, independent of the values of E, S and c_1.

Finally, our first-passage time analysis provides another method of assessing accuracy in model reductions such as the Michaelis–Menten approximation. When the rate of product formation can be described by an approximately exponential distribution, a single reaction model reduction may be possible. Using appropriate choices of the 'random walker', this analysis provided independent validation of the results of Rao and Arkin [4], Mastny *et al.* [5] and the ssSSA [6, 7] for the enzyme–substrate reaction set (1) in portions of their respective validity regions. While not able to provide a unified proof under all conditions, our first-passage time analysis helps to put the Michaelis–Menten approximation into a broader theoretical framework.

8 Acknowledgments

9 References

1 Nelson, D.L., Cox, M.M.: 'Lehninger principles of biochemistry' (Freeman, 2005, 4th edn.)
2 Michaelis, L., Menten, M.L.: 'Die kinetik der invertinwirkung', *Biochem. Z.*, 1913, **49**, pp. 333–369
3 Gonze, D., Halloy, J., Goldbeter, A.: 'Deterministic versus stochastic models for circadian rhythms', *J. Biol. Phys.*, 2002, **28**, pp. 637–653
4 Rao, C.V., Arkin, A.: 'Stochastic chemical kinetics and the quasi-steady-state assumption: application to the Gillespie algorithm', *J. Chem. Phys.*, 2003, **118**, pp. 4999–5010
5 Mastny, E.A., Haseltine, E.L., Rawlings, J.B.: 'Two classes of quasi-steady-state model reductions for stochastic kinetics', *J. Chem. Phys.*, 2007, **127**, article id 094106
6 Cao, Y., Gillespie, D.T., Petzold, L.R.: 'The slow-scale stochastic simulation algorithm', *J. Chem. Phys.*, 2005, **122**, article id 014116
7 Cao, Y., Gillespie, D.T., Petzold, L.R.: 'Accelerated stochastic simulation of the stiff enzyme–substrate reaction', *J. Chem. Phys.*, 2005, **123**, article id 144917
8 Gillespie, D.T., Cao, Y., Sanft, K.R., Petzold, L.R.: 'The subtle business of model reduction for stochastic chemical kinetics', *J. Chem. Phys.*, 2009, **130**, article id 064103
9 Segel, L.A., Slemrod, M.: 'The quasi-steady-state assumption: a case study in perturbation', *SIAM Rev.*, 1989, **31**, pp. 446–477
10 Gillespie, D.T.: 'Exact stochastic simulation of coupled chemical reactions', *J. Phys. Chem.*, 1977, **81**, pp. 2340–2361
11 Gillespie, D.T.: 'Stochastic simulation of chemical kinetics', *Annu. Rev. Phys. Chem.*, 2007, **58**, pp. 35–55

10   Appendix

10.1   Deterministic and stochastic kinetic constants

Deterministic and stochastic kinetic constants are often denoted k_i and c_i, respectively, to distinguish their types. Unimolecular kinetic constants typically have units t^{-1} in both deterministic and stochastic models. However, bimolecular kinetic constants typically have units $M^{-1} t^{-1}$ in deterministic models against units of molecules$^{-1} t^{-1}$ in stochastic models. Converting a deterministic bimolecular kinetic constant k into a stochastic kinetic constant c requires a system volume parameter Ω:

$c = k/(N\Omega)$, where N is Avogadro's constant. Also, note that the stochastic reaction rate is different from the kinetic constant (which is sometimes referred to as the kinetic rate). The reaction rate is described by the propensity function. The propensity function is equal to the kinetic constant multiplied by the population counts of the reactant species [10, 11]. Therefore the reaction rate (propensity) in the stochastic Michaelis–Menten approximation is $V_{max}S/(K_m + S)$, which implies an effective unimolecular kinetic 'constant' $c_{MM} = V_{max}/(K_m + S)$ with the single reactant species S. The kinetic parameter c_{MM} has units t^{-1}, consistent with a unimolecular rate constant.