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

$$E + S \xrightarrow{c_1} c_2 ES \xrightarrow{c_3} E + P$$  \hspace{1cm} (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

$$S_1 \xrightarrow{c_1} c_2 S_2 \xrightarrow{c_3} S_3$$  \hspace{1cm} (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

\[ \frac{dS}{dt} = -c_1 S \times E + c_2 ES \]  
\[ \frac{dE}{dt} = -c_1 S \times E + (c_2 + c_3)ES \]  
\[ \frac{dES}{dt} = c_1 S \times E - (c_2 + c_3)ES \]  
\[ \frac{dP}{dt} = c_3 ES \]  

(3a) (3b) (3c) (3d)

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

\[ E(t) + ES(t) = E_T \]  
\[ P(t) = P_0 + S_0 - (S(t) + ES(t)) \]  

(4a) (4b)

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

\[ E_T \ll S_0 + K_m \]  

(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.
It is not possible to simply ‘unpack’ the Michaelis–Menten rate expression into the underlying elementary reactions. The two parameters $V_{\text{max}}$ 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 $E_T/S_0 \rightarrow 0$, 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

$$a_3(x) \simeq V_{\text{max}}S/K_m + S$$

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

$$E_T \ll S_0$$

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

$$a_3(x) \simeq \frac{V_{\text{max}}S}{K_m}$$

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

$$E_T + S_0 \ll K_m$$

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 condition of Eq. (8).

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.
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

$$S + c_4 \rightarrow X$$

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

$$t_{fast} \approx \frac{1}{c_1(S_0 + K_m)}$$

Evaluating (14) for the parameters given in Fig. 2, namely $S_0 = 10$, $E_0 = 1$, $E_0S_0 = P_0 = 0$, $c_1 = 1$, $c_2 = 10$, $c_3 = 1$, we obtain an estimate of $t_{fast} \approx 1/(1 \times (10 + 11)) = 1/21$. Comparing that to the timescale of reaction (13), which is $\approx 1/(c_4S)$, we deduce that the addition of reaction (13)
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 $ES$

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

$ES_{\text{effective}}$ 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

$$ES \xrightarrow{c_3} Y \quad (16)$$

would have effective propensity $c_3 ES_{\text{effective}} = c_3 E_T S / (K_m + S)$ 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’

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

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

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

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

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

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, $V_{\text{max}}$ 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.
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 $c_2 \gg c_3$ (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

$$2(c_2 + c_3)/c_3 = n_{\text{avg}}$$ (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 $n_{\text{avg}}$ 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 $c_2 \approx c_3$, 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 $R_2$ 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

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

where $\langle ES(\infty) \rangle$ is the mean of the steady-state distribution of the enzyme–substrate complex evolving under both the two fast reactions $R_1$ and $R_2$, given the state $x = (E, S, ES, P)$. 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 \(c_2 \gg c_1\), 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 \(c_2 \gg c_1\) one can efficiently generate exact trajectories of all species using the SSA.

The mean of \(\mathcal{E}\mathcal{S}(\infty)\) 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 \(x\), that is

\[
E + ES = E_T \quad \text{and} \quad S + ES = S_T \quad (24)
\]

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 \(\langle c_1 \mathcal{E}(\infty) \mathcal{S}(\infty) \rangle = \langle c_2 \mathcal{E}\mathcal{S}(\infty) \rangle\), and with (24) this becomes

\[
\langle c_1 (E_T - \mathcal{E}(\infty))(S_T - \mathcal{S}(\infty)) \rangle = \langle c_2 \mathcal{E}\mathcal{S}(\infty) \rangle \quad (25)
\]

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

\[
\langle \mathcal{E}\mathcal{S}(\infty) \rangle = \frac{1}{2} \left\{ (E_T + S_T + \frac{c_2}{c_1}) - \sqrt{(E_T + S_T + \frac{c_2}{c_1})^2 - 4(E_T S_T + \text{var}\{\mathcal{E}\mathcal{S}(\infty)\})} \right\} \quad (26)
\]

Formula (26) is exact but it involves the variance of \(\mathcal{E}\mathcal{S}(\infty)\). Since the standard deviation of \(\mathcal{E}\mathcal{S}(\infty)\) is typically on the order of \(\sqrt{\mathcal{E}\mathcal{S}(\infty)}\), then \(\text{var}\{\mathcal{E}\mathcal{S}(\infty)\}\) will usually be on the order of \((\mathcal{E}\mathcal{S}(\infty))^2\). And since \(\mathcal{E}\mathcal{S}\) 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 \(\mathcal{E}\mathcal{S}(\infty) \approx \mathcal{E}\mathcal{S}\), where \(\mathcal{E}\mathcal{S}\) is given by (26) with \(\text{var}\{\mathcal{E}\mathcal{S}(\infty)\} = 0\)

\[
\mathcal{E}\mathcal{S} = \frac{1}{2} \left\{ E_T + S_T + \frac{c_2}{c_1} - \sqrt{(E_T + S_T + \frac{c_2}{c_1})^2 - 4E_T S_T} \right\} \quad (27)
\]

The result of this approximation is that \(\tilde{a}_2(x)\) in (23) gets approximated by

\[
\tilde{a}_2(x) = \frac{c_1}{2} \left\{ (E_T + S_T + \frac{c_2}{c_1}) - \sqrt{(E_T + S_T + \frac{c_2}{c_1})^2 - 4E_T S_T} \right\} \quad (28)
\]

Approximation (28) can in some cases be improved by using in (26) a better estimate of \(\text{var}\{\mathcal{E}\mathcal{S}(\infty)\}\) 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 \(\mathcal{E}\mathcal{S}(\infty)\) state probabilities. The mean of \(\mathcal{E}\mathcal{S}(\infty)\) can then be computed and used directly in (23) to determine the effective propensity \(\tilde{a}_2(x)\). 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 realizations. 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 \(\tilde{a}_2(x)\) 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 \(E_T \ll S_T + c_2\) and \(c_2 \gg c_1\) 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

\[
\tilde{a}_2(x) \simeq \frac{c_1}{2} \left\{ (S_T + (c_2/c_1)) - \sqrt{(S_T + (c_2/c_1))^2 - 4E_T S_T} \right\} \quad (29)
\]

\[
\simeq \frac{c_1}{2} \left( S_T + (c_2/c_1) \right) \left\{ 1 - \frac{4E_T S_T}{2(S_T + (c_2/c_1))^2} \right\} \quad (29)
\]
whence

\[ \overline{a}(x) \simeq \frac{c_1 E_T S_T}{S_T + (c_2/c_1)} \ (S_T \gg E_T, c_2 \gg c_3) \]  

(29)

Under \( c_2 \gg c_3 \), we have \( c_2/c_1 \simeq (c_2 + c_1)/c_1 = K_m \); 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 \( S_0 \approx S \), indeed, since (28) is symmetric in \( E_T \) and \( S_0 \), its approximate form in that case can be inferred simply by interchanging those two variables in (29)

\[ \overline{a}(x) \simeq \frac{c_1 E_T S_T}{S_T + (c_2/c_1)} \ (S_T \ll E_T, c_2 \gg c_3) \]  

(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 \( \overline{a}(x) \), is mathematically equivalent to asserting that the time to the next \( R_3 \) reaction is an exponential random variable with mean \( 1/\overline{a}(x) \) [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 \( x = (E, S, ES, P) \), the time \( T(x) \) 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...
\[ \Omega(x) | x' \rightarrow S_T = S + ES \]. 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 \( S_3 \)

\[
S_1 \xrightarrow{c_1} S_2 \xrightarrow{c_3} S_3
\]  

(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 \( (x_1, x_2) \) until the first of the walkers reaches state \( S_3 \) is a random variable with probability density function

\[
P(t; x_1, x_2) = x_1 x_2 P(2, t|1, 0)(P(1, t|1, 0) + P(2, t|1, 0))^{x_1-1} 
\times (P(1, t|2, 0) + P(2, t|2, 0))^{x_2-1} 
\]

(32)

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

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

(33a)

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

(33b)

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

(33c)

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

(33d)

where

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

(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 \( \lambda_+ \approx c_1 + c_2 \) and \( \lambda_- \approx c_1 c_3/(c_1 + c_2) \ll \lambda_+ \), and that (32) then approximates to the exponential form

\[
P(t; x_1, x_2) \simeq \frac{c_1 c_3 (x_1 + x_2)}{c_1 + c_3} e^{-(c_1 c_3 (x_1 + x_2)/(c_1 + c_3))^t}, \quad (c_2 \gg c_3, t \gg (c_1 + c_2)^{-1})
\]

(35)

Similarly, when

\[
c_3 \gg c_1
\]

(36)

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

\[
P(t; x_1, x_2) \simeq \frac{c_1 c_3 (x_1 + x_2)}{c_2 + c_3} e^{-(c_1 c_3 (x_1 + x_2)/(c_2 + c_3))^t}, \quad (c_3 \gg c_1, t \gg (c_2 + c_3)^{-1})
\]

(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 \( T(x) \) 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

\[
F \xrightarrow{c_1 S_T} B \xrightarrow{c_3} 3
\]

(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 \( c_1 S_T \) 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 \( S \approx S_T \). Then each enzyme unit will be performing the random walk

\[
F \xrightarrow{c_1 S_T} B \xrightarrow{c_3} 3 \quad (E_T \ll S_T)
\]

(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

\[
c_1 \rightarrow c_1 S_T, \quad x_1 \rightarrow E, \quad x_2 \rightarrow ES
\]

(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

\[
P_x(t) \simeq \left( \frac{c_1S_Tc_3(E + ES)}{c_1S_T + c_2} \right) e^{-\left( c_1S_Tc_3(E + ES)/(c_1S_T + c_2) \right)t} \tag{41}
\]

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

\[
P_x(t) \simeq \left( \frac{c_3S_TE_T}{S_T + (c_2/c_1)} \right) e^{-\left( c_3S_TE_T/(S_T + (c_2/c_1)) \right)t} \tag{42}
\]

which is valid under the conditions

\[ E_T \ll S_T, \quad c_2 \gg c_3, \quad t \gg (c_1S_T + c_2)^{-1} \tag{43} \]

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 \( dt > (c_1S_T + c_2)^{-1} \) is

\[
\left( \frac{c_3S_TE_T}{S_T + (c_2/c_1)} \right) dt \tag{44}
\]

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 \( 100 = c_2 \gg c_1 = 1 \), 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

\[
F \xrightarrow{c_1} E \xrightarrow{c_2} B \xrightarrow{c_3} 3 \tag{45}
\]

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

\[
P_x(t) \simeq \left( \frac{c_3E_TS_T}{E_T + (c_2/c_1)} \right) e^{-\left( c_3E_TS_T/(E_T + (c_2/c_1)) \right)t} \tag{46}
\]

which holds when

\[
S_T \ll E_T, \quad c_2 \gg c_3, \quad t \gg (c_3E_T + c_2)^{-1} \tag{47}
\]

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

\[
E_T, S_T \ll K_m \tag{48}
\]

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
since $K_68\approx(32)$ as

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

$$P_s(t) \approx \left( \frac{c_3E_7c_3S}{c_2 + c_3} \right)^{n}E_1(c_3+c_3)S^n$$

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

$$a_s(x) \approx \frac{c_3E_7S}{(c_2 + c_3)/c_1} = \frac{V_{\max}S}{k_{m}}$$

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 QSPA 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 $S_0 \gg E_7$ 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 $E_7 \ll S_0 + K_{m}$. 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 $E_7$, $S_0$ 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 provides 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

The authors thank Y. Cao, Sotiria Lampoudi, and Min Roh for helpful discussions. The authors gratefully acknowledge financial support as follows: K.S. and L.P. were supported by Grant No. R01EB007511 from the National Institute of Biomedical Imaging and Bioengineering. Pfizer Inc., DOE Contract No. DE-FG02-04ER25621, NSF Contract No. IGERT DG02-21715, and the Institute for Collaborative Biotechnologies through Grant No. DFR3A-8-447850-23002 from the US Army Research Office. K.S. was also supported by a National Science Foundation Graduate Research Fellowship. D.G. was supported by the California Institute of Technology through Consulting Agreement No. 102-1080890 pursuant to Grant No. R01GM078992 from the National Institute of General Medical Sciences, and through Contract No. 82-1083250 pursuant to Grant No. R01EB007511 from the National Institute of Biomedical Imaging and Bioengineering, and also from the University of California at Santa Barbara under Consulting Agreement No. 054281A20 pursuant to funding from the National Institutes of Health.

9 References

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 $\text{t}^{-1}$ in both deterministic and stochastic models. However, bimolecular kinetic constants typically have units $\text{M}^{-1} \text{t}^{-1}$ in deterministic models against units of molecules$^{-1} \text{t}^{-1}$ in stochastic models. Converting a deterministic bimolecular kinetic constant $k$ into a stochastic kinetic constant $c$ requires a system volume parameter $\Omega$:

$$c = k/(N\Omega),$$

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 $V_{\text{max}}S/(K_m + S)$, which implies an effective unimolecular kinetic ‘constant’ $c_{\text{MM}} = V_{\text{max}}/(K_m + S)$ with the single reactant species $S$. The kinetic parameter $c_{\text{MM}}$ has units $\text{t}^{-1}$, consistent with a unimolecular rate constant.