Accuracy of the Michaelis–Menten approximation when analysing effects of molecular noise

Michael J. Lawson¹, Linda Petzold³ and Andreas Hellander²

¹Department of Cell and Molecular Biology, and ²Department of Information Technology, Uppsala University, Box 337, Uppsala 75105, Sweden
³Department of Computer Science, University of California Santa Barbara, Santa Barbara, CA 93106-5070, USA

Quantitative biology relies on the construction of accurate mathematical models, yet the effectiveness of these models is often predicated on making simplifying approximations that allow for direct comparisons with available experimental data. The Michaelis–Menten (MM) approximation is widely used in both deterministic and discrete stochastic models of intracellular reaction networks, owing to the ubiquity of enzymatic activity in cellular processes and the clear biochemical interpretation of its parameters. However, it is not well understood how the approximation applies to the discrete stochastic case or how it extends to spatially inhomogeneous systems. We study the behaviour of the discrete stochastic MM approximation as a function of system size and show that significant errors can occur for small volumes, in comparison with a corresponding mass-action system. We then explore some consequences of these results for quantitative modelling. One consequence is that fluctuation-induced sensitivity, or stochastic focusing, can become highly exaggerated in models that make use of MM kinetics even if the approximations are excellent in a deterministic model. Another consequence is that spatial stochastic simulations based on the reaction–diffusion master equation can become highly inaccurate if the model contains MM terms.

1. Introduction

As the effectiveness of quantitative modelling in biology, on scales ranging from molecular dynamics to well-mixed deterministic models, results in its widespread use, questions have arisen about how to accurately translate a given model from one scale to another [1]. An important finding in molecular systems biology has been the significant role of noise that arises from the discrete and sometimes small number of molecules in intracellular systems. A simplifying model that has been essential in quantifying enzymatic activity is the Michaelis–Menten (MM) approximation [2], which is so fundamental that it is taught as basic biochemistry theory [3].

As an example of the possible effects of molecular noise, it has been shown that the concavity of the MM propensity function (or convexity of its inhibitory form) can lead to deviation of the mean of a stochastic system from the deterministic signal, an effect referred to as stochastic focusing [4].

Given a deterministic model, it is often of interest to translate it to a discrete stochastic setting to study the potential effects of molecular fluctuations. The Markov process is a popular modelling framework on the discrete stochastic level. Models of stochastic chemical kinetics in this framework can be simulated using the stochastic simulation algorithm (SSA) or one of its many optimized or approximate variants [5]. The original formulation of the model allows for second order mass-action (MA) kinetics, but due to the omnipresence of MM terms in models, recent efforts have gone toward deriving conditions for the MM approximation’s validity in the discrete stochastic setting [6–8], with the conclusion that the MM approximation is accurate in the stochastic case provided that the deterministic validity conditions are satisfied. Some work has gone into comparing more generalized approximations [9], but these lack the interpretation and wide use of the MM approximation.
In the classical analysis of MM kinetics, one considers a pool of substrate molecules, whose concentration does not change appreciably during the timescale of the simulation, being converted to a product. In this work, we present an analysis of a slightly different situation: we consider the flux through a substrate conversion reaction with a Poissonian influx of substrate and an exponential decay of product. This framework closely captures the modelling situation of an enzymatic step embedded in a larger biochemical network. We then focus on the scenario where a modeller begins with a deterministic model in concentration form and translates it to a discrete stochastic setting. In doing so, it is necessary to introduce a new model parameter, the system volume, in order to convert from concentrations to discrete molecular populations. We show that the choice of this volume, in relation to the other model parameters, can be critical to the accuracy of the simulations. Both the importance of noise, as quantified by the stochastic focusing effect, and the size of the error due to the MM approximations grow quickly with small volume size. In the first part of this paper, we use moment equations to derive an expression for this error as a function of the system size. As we will see, the error introduced by using the MM approximation is only small if the system size is large enough, or equivalently, if the impact of noise on the network is small enough.

We then consider the next logical model progression: the translation from a discrete stochastic well-mixed model to a spatial stochastic model via the reaction–diffusion master equation (RDME). We demonstrate that the dependence of the MM approximation error on volume has important implications for its use in this setting. Spatial inhomogeneity and the importance of locality of reactions in cellular systems often require that the effective reaction volume be very small. Thus the error in the MM approximation can have very large consequences for both diffusion limited and reaction limited systems. Notably, the error depends strongly on the discretization and increases rapidly with increased spatial resolution. For this reason, the use of MM kinetics in RDME models is highly questionable.

In summary, this paper paints a rather negative picture of the use of MM kinetics for quantitative modelling on the discrete stochastic level. In a well-mixed system, as the importance of noise on the network increases, so does the error, making any interpretation of the effects of noise uncertain, especially when the motif is embedded in a larger network that can respond non-linearly to the introduced errors. When transitioning the well-mixed model to a spatial setting, this same effect shows up as a discretization artefact that is likely to render the simulation output highly inaccurate compared with the corresponding MA system, irrespective of whether the system is reaction or diffusion limited.

2. Results

2.1. Michaelis–Menten kinetics

We consider the following simple model of enzyme kinetics where substrate $S$ is being created with rate $\mu$ and converted to product $P$ by enzyme $C$.

\[
\begin{align*}
\emptyset & \overset{\mu}{\rightarrow} S, \\
C + S & \overset{k_1}{\underset{k_2}{\rightarrow}} CS \overset{k_2}{\rightarrow} C + P \\
&D \overset{k_4}{\rightarrow} \emptyset,
\end{align*}
\]

and

\[
\begin{align*}
\emptyset & \overset{\mu}{\rightarrow} S, \\
S & \overset{V_{\text{max}}[S]}{\rightarrow} P \\
P & \overset{k_4}{\rightarrow} \emptyset
\end{align*}
\]

where $q = 1/(k_m + [S]), K_m = (k_2 + k_3)/k_1$ and $V_{\text{max}} = k_3[C_1]$. The MM approximation, motivated mathematically by the quasi-steady-state assumption [10], effectively reduces the three reactions in the enzymatic conversion step to a single unimolecular reaction. While this can be advantageous from a computational standpoint [11], a more central reason for its widespread use is the relative ease with which one can interpret and determine (in vitro) values for $K_m$ and $V_{\text{max}}$. In contrast, determining the rate constants $k_1$, $k_2$ and $k_3$ of the full model (2.1) can be quite challenging.

Provided $V_{\text{max}} > \mu$, the deterministic ODE models corresponding to (2.1) and (2.2) (see electronic supplementary material, equations (S1) and (S2)) have unique steady-state solutions that agree for all combinations of the parameter values

\[
[S] = \frac{\mu K_m}{\alpha}
\]

where

\[
\alpha = V_{\text{max}} - \mu, \quad \alpha > 0.
\]

For $\alpha < 0$ the inflow rate of substrate is greater than the maximal conversion rate at saturation, so the substrate pool will grow unboundedly. Even though the steady state for the two models agree for all values of the parameters, the MM approximation does not necessarily provide an accurate representation of the transient kinetics of (2.1). In the deterministic case, a validity condition that guarantees a good approximation also for the transient behaviour can be formulated as [8,10]:

\[
[C_1] \ll [S_0] + K_m
\]

where $[C_1]$ is the total enzyme concentration and $[S_0]$ is the initial substrate concentration.

The MM model was originally developed to study enzyme kinetics in test tube conditions. An important observation in cellular biology is that in vitro kinetics on this scale are more accurately described by discrete stochastic models [6–8]. A natural question then becomes whether the MM approximation can be accurately carried over to well-mixed...
stochastic simulations. This issue was addressed recently by Sanft et al. [8], who suggested that under the same validity condition (2.5) with concentrations replaced by copy number and the macroscopic rates properly converted to mesoscopic rates, the MM approximation to (2.1) with \( \mu = 0 \) and \( k_d = 0 \) holds also in well-mixed stochastic simulations.

Introducing the system volume \( V \), the propensity function for the conversion reaction \( S \rightarrow P \) in the well-mixed discrete stochastic case can be written

\[
a(s) = \frac{v_{max}S}{K_{m} + S/V},
\]

where \( V \) is the system volume. Throughout the paper all rate constants will have the ODE form and will be converted to reaction propensity constants by explicitly multiplying or dividing by \( V \) as appropriate. The question of how the stochastic MM steady-state solution of (2.2) compares with the ODE solution in small reaction volumes was addressed in Grimma et al. [8], who suggested that under the same validity condition (2.5) with concentrations replaced by copy number and the macroscopic rates properly converted to mesoscopic rates, the MM approximation to (2.1) with \( \mu = 0 \) and \( k_d = 0 \) holds also in well-mixed stochastic simulations.

2.2. The accuracy of stochastic Michaelis–Menten approximation decreases for small system volumes

As we have seen, the steady-state concentration of \( S \) (2.3) is the same for both the MM and MA systems in the continuum ODE model, independent of the system volume. This is no longer the case in a discrete stochastic setting. Figure 1 shows steady-state concentrations based on solutions of the chemical master equation (CME) (see electronic supplementary material, equation (S3)) (solid blue and dashed red lines) for varying system volumes \( V \). In the thermodynamic limit, \( V \rightarrow \infty \) both CME solutions converge to the ODE solution. By reducing the volume, the system is made increasingly noisy relative to the mean. As can be seen, the MM and MA solutions diverge as \( V \) is reduced. As expected, both the stochastic MA and stochastic MM steady-state levels increase relative to the ODE solution, but they do not increase at the same rate.

The fact that the steady state of the discrete stochastic MM system deviates from that of the continuous MM system for small volumes, and hence for a large noise level relative to the mean, should not be surprising. We illustrate the effect intuitively in figure 2 where the black solid line shows the reaction propensity (2.6) as a function of \( S \). Replacing \( S \) in (2.6) with \( E[S] \) (blue square), i.e. using a deterministic input, the propensity function takes the value \( a(E[S]) \) (blue diamond). But for a stochastic system the average rate of the conversion of \( S \) depends on the shape of the probability distribution of \( S \) (solid blue) and the mean value of the propensity. Hence the average conversion rate of substrate (red diamond) is not the same as the rate obtained by using the mean of \( S \). This effect has previously been described as stochastic focusing [4] in the systems biology context. Mathematically, it is explained by Jensen's
inequality applied to random variables
\[ E[a(S)] \leq aE[S], \quad (2.7) \]
where \( a \) is a concave function (the inverse relationship holds for convex functions).

Clearly, the same type of focusing effect will occur also in the corresponding discrete stochastic MA system (2.1), but not necessarily to the same extent. For both MA and MM the strength of this focusing effect depends on the system size. While the discrepancy between the stochastic MA system (solid blue) and the deterministic system (magenta line) in figure 1 can be viewed as a focusing effect caused by molecular noise, the deviation between the stochastic MM (red curve) and the stochastic MA system (blue curve) represents the modelling error due to the MM approximation. Next we seek analytical estimates for this error.

### 2.3. Michaelis–Menten approximation error

In order to understand this observed discrepancy between the MM and MA models, we write equations for the first and second moments of \( S \) [14] (see electronic supplementary material, equation (S4)). The steady-state solution of electronic supplementary material, equation (S4) is given by
\[
E[S]^{\text{MM}} = \frac{\Omega K_m \mu}{\alpha + \mu} \quad \text{and} \quad \sigma^2[S] = \frac{\mu(\mu + K_m)\Omega V_{\max}^2}{\Omega K_m V_{\max} \alpha^2}, \quad (2.8)
\]
where the expected values for the discrete stochastic system are expressed in molecular populations. The solution is shown in figure 1 (red squares) and it agrees well with the corresponding CME solution. Note that the expected value of substrate may be far from that of the continuum model for small system volumes. As an immediate consequence of (2.8), in order for the mean steady-state levels in the discrete stochastic case to be close to those in the ODE case, we must require that
\[
\Omega K_m \gg 1. \quad (2.9)
\]

Note the relationship between this condition and the expression for the range of deviations between the discrete stochastic MM system at steady state and the deterministic counterpart derived by Grima based on the LNA in [12, eqn 13]. There, it is shown that the deviation \( \Delta \) from the ODE solution behaves like \( 1 < \Delta < 1 + (\Omega K_m)^{-1} \).

Equations for the first and second moments of the full MA system (2.1) are given in (electronic supplementary material, equation (S5)). The steady-state solution for the expected value of substrate \( S \) for the full MA system is given by
\[
E[S]^{\text{full}} = \frac{\Omega K_m \mu}{2\alpha - \Omega V_{\max} K_m} + \frac{\alpha C_T}{2V_{\max}} \quad \text{and} \quad \sigma^2[S] = \Omega \alpha V_{\max} \left( \frac{2V_{\max}^2}{\Omega} \right) \quad \text{as} \quad \Omega \rightarrow \infty.
\]

It is instructive to study the error in the limits of large and small system sizes. In the large system limit when \( \Omega \rightarrow \infty \), the two discrete stochastic models agree with each other and with the ODE model
\[
\lim_{\Omega \rightarrow \infty} \frac{E[S]^{\text{MM}}}{\Omega} = \lim_{\Omega \rightarrow \infty} \frac{E[S]^{\text{full}}}{\Omega} = \frac{\mu K_m}{\alpha}. \quad (2.12)
\]

In the small volume limit \( \Omega \rightarrow 0 \), we can expand (2.10) around \( \Omega \) in a Taylor series, truncate higher-order terms and obtain an asymptotic expression for the error (see electronic supplementary material, equation (S8)). However, an issue with this limit in the discrete stochastic context is that for some positive \( \Omega \), \( C_T < 1 \). This clearly does not make sense in a discrete stochastic model. Indeed, the volumes in figure 1 are chosen such that we always have integer numbers of \( C_T \), with the smallest value corresponding to \( C_T = 1 \). However, one observation we can make from electronic supplementary material, equation (S8) is that since the absolute error tends to a constant in the limit \( \Omega \rightarrow 0 \) while the concentration \( E[S]^{\text{MM}}/\Omega \) tends to infinity, the relative error
\[
e^\text{rel} = \frac{E[S]^{\text{MM}} - E[S]^{\text{full}}}{E[S]^{\text{full}}}, \quad (2.13)
\]
tends to zero. This is also the case in the large volume limit. Hence, the relative error will take a maximal value for some \( \Omega > 0 \). By maximizing the relative error (2.13), we find that the maximum relative error
\[
e^\text{rel}_{\text{max}} = \frac{\alpha C_T}{4K_m V_{\max} \mu} + \frac{\alpha}{4\mu}, \quad (2.14)
\]
occurs when
\[
\Omega_{\text{max}} = \frac{K_m V_{\max}^2}{\alpha C_T + 2V_{\max}} + \frac{K_m V_{\max}}{\mu}. \quad (2.15)
\]

Given equations (2.14) and (2.15), we can ask whether there are conditions on the model parameters for which the maximum relative error will remain small independent of the system size \( \Omega \). The result (see electronic supplementary material, §S1.5) is that no such condition exists, since we arrive at the contradiction
\[
1 \ll K_m \Omega_{\text{max}} \approx 1. \quad (2.16)
\]

We note briefly that the first inequality in equation (2.16) resembles the condition (2.9), thus it makes sense that (2.16) does not hold because we would not expect to find the maximum error when the MM approximation agrees nicely with the ODE. We can conclude that for every set of parameters, \( e^\text{rel}_{\text{max}} \) will be significant, i.e. the error owing to the MM approximation will never be uniformly small in \( \Omega \). In what follows, we will elaborate on this and ask whether this error will be relevant under the same conditions for which the effects of molecular noise are significant.

### 2.4. Michaelis–Menten error and stochastic focusing

In this section, we will discuss two interesting questions for quantitative modelling; first, whether the error introduced by the MM approximation is significant under the same conditions for which the effects of noise in the system are significant, and second, if the volumes \( \Omega \) for which the approximation is a substantial source of error represent relevant biological length scales. After all, elucidating the potential effects of noise is why we want to consider a
Figure 2. Effect of Michaelis–Menten (MM) approximation on stochastic input. The black line shows the reaction propensity [13] as a function of substrate population $S$, for a MM system (2.2) with $K_m = 2$ and $V_{\text{max}} = 1$. The substrate population $S$ is defined by a Poisson distribution with rate $\lambda = 2$, shown in blue. The mean, $E[S]$, of this population is 2 (blue square) and the reaction velocity for the mean input is 0.5. However, the expected value of the reaction velocity is approximately 14% lower, leading to higher steady-state values of $S$ for the stochastic version of the system (2.2) compared with the deterministic version.

stochastic model in the first place. To quantify the effects of noise we consider the stochastic focusing, here defined as the relative increase in the expected value of the substrate in the stochastic MA model compared with the deterministic model

$$\delta_{SF} = \frac{E[S]^{\text{full}} - E[S]^{\text{ODE}}}{E[S]^{\text{ODE}}}.$$

It is clear from the above that in the small volume limit, $\delta_{SF} \to \infty$ and that in the large volume limit $\delta_{SF} \to 0$. Hence, by comparing $\delta_{SF}$ to $\epsilon^{\text{vol}}$ from equation (2.13), we can immediately conclude that in the small volume limit, the true stochastic effects will dominate over the error due to the MM approximation. In the large volume limit, both the error and the true effect of noise will be small.

An important question is what happens between these two limits: how large is the focusing effect for the system volume at which the error takes its maximal value? By plugging (2.15) into the definition (2.17), we find that $\delta_{SF}(\Omega_{\text{max}}) = 1$. That is, when the stochastic focusing effect results in a mean value that is double the ODE value the error is at its maximum and hence, by equation (S13), significant. Figure 3 shows an example of this behaviour for a representative set of biologically relevant parameters.

As can be seen in figure 3, the behaviour of the MM error and the true effect of noise can roughly be divided into three regions. First, to the far right, the relative error is much larger than the stochastic focusing effect, but both of those effects are very small. Here, all solutions are very close to the deterministic ODE solution. To the far left, the relative effect of stochastic focusing is much greater than the relative error due to MM. In between, we have a large range of volumes where the effect of stochastic focusing results in a significant deviation from the deterministic mean, but where the relative error due to MM is even greater. What are the length scales for which the MM error will cause unreliable simulations? As an example, consider the rightmost point where the volume is 1 $\mu$m$^3$, roughly the volume of an Escherichia coli cell. With the parameters as in the figure caption, a system of that size will contain approximately 20 enzyme molecules, a realistic number for many proteins in the living cell. As the figure shows, for this volume and these parameters the relative error (and also the stochastic focusing) is very small. However, for volumes less than $1 \times 10^{-21}$ m$^3$ (1000 nm$^3$), the MM error is substantial. This volume range would be relevant for reactions that become highly localized on a subcellular scale. A relevant example is the limited dispersion of mRNA in E. coli [15], resulting in highly localized translational events.

For spatial stochastic models, where a subcellular resolution is introduced via a discretization in subcompartments, this behaviour also results in numerical artefacts. This will be discussed in greater detail in the next section.

2.5. Michaelis–Menten, enzyme locality and the reaction–diffusion master equation

In the modelling process, the next logical step in the refinement of the model beyond a well-mixed discrete stochastic setting is to consider a discrete spatial stochastic model. The results of the previous section show that when the reaction volume of a well-mixed system becomes small, the true effect of noise in the system becomes more pronounced but so does the error introduced by the MM approximation. As we will illustrate in this section, this is a point of great concern for spatial stochastic models in the RDME framework (see electronic supplementary material, §S1.6 for a brief description of the RDME).

In principle, the conversion of the stochastic well-mixed MM system (2.2) to a spatial stochastic model is straightforward; we simply use the propensity function (2.6) in each of the voxels, but with the total system volume $\Omega$ replaced...
by the local voxel volumes $\Omega_i$. Apart from the shape of the domain (which is not important for the considerations here), the modeller has to select the voxel volumes. In this section, for notational simplicity and without loss of generality, we assume that the whole domain is of unit volume, $\Omega = 1$. We can then write the local (to voxels) MM propensity functions $a_{i}(S_i)$

$$a_{ui} = \frac{V_{\text{max}} S_i}{K_m + S_i / \Omega_i}, \quad (2.18)$$

with $S_i$ being the copy number of substrate in voxel $v_i$.

Apart from the substrate and product which are diffusing in the domain, we need to specify how to handle the implicit enzyme species in the spatial model. In order to directly correspond to the well-mixed model reduction and to avoid reintroducing the enzyme explicitly, we are here regarding $V_{\text{max}}$ as a given parameter of the model and assume that $k_3$ and $E_T$ are not explicitly known. This means that the enzyme is considered globally (i.e. in the whole domain and not only in the voxels) uniformly distributed. As a consequence, we can effectively end up with fractions of enzyme molecules in the individual voxels. Clearly, this is not a realistic assumption for low enzyme densities, at least not in cases where there are reasons to believe that the spatial distribution of enzyme matters for the system dynamics [1]. Already in this light, it is questionable whether MM kinetics should be applied in a spatial stochastic context in cases where the enzyme population is not macroscopically large.

In order to compare the results of spatial simulations with MM kinetics to simulations with the MA model, we must decide how to treat the enzyme in the MA model. Unlike in the well-mixed case, the models will no longer exactly correspond to each other if the enzyme is allowed to diffuse in the MA model. Hence, we first need to make assumptions to enforce a well-mixed enzyme in the MA simulations. In principle, there are two ways we can achieve this. First, the enzyme can be made to be uniformly distributed over the voxels and prevented from diffusing, emulating a statically localized enzyme. Second, if the enzyme is diffusing freely with a large diffusion constant such that any reaction in which it participates is highly reaction limited, the enzyme can be considered effectively well mixed in the global sense. The second scenario is in line with the usual and conventional interpretation of well mixedness, while the first scenario corresponds precisely to the setting in the spatial MM model. Intuitively, it might be natural to think that both these scenarios should result in more or less the same simulation dynamics, but as we illustrate next, this is not the case.

Both scenarios are illustrated in figure 4, where we use the same total system size (unity) and parameters as in figure 1 and vary the diffusion constant of the substrate to range from very fast to very slow. As the diffusion constant of $S$ decreases, both the MM (blue, cross) and MA (red, cross) systems approach the steady-state levels of a well-mixed system of volume equal to the voxel volume (dashed), rather than the volume of the whole simulation domain (solid). This can be understood from the results of the previous section, since in the limit of zero diffusion constant the global system effectively consists of $K$ smaller, independent subsystems of size $\Omega_v$. In the limit of fast diffusion, such that the substrate can also be considered well-mixed, the MA model approaches the well-mixed counterpart for the total system volume but the MM system does not. The parameter regime where a MM approximation of the system (2.1) in a spatial setting

Figure 3. Relative error and stochastic focusing. The relative effect of stochastic focusing $\delta_{SF}$ (red), the error introduced by the MM approximation $e_{\text{rel}}$ (green) and the quotient between them (blue). As can be seen, the behaviour can roughly be divided into three regions. First, to the far right, the relative effect is much larger than the stochastic focusing effect, but both of those effects are very small. Here, all solutions are very close to the deterministic ODE solution. In between, we have a large range of volumes where the effect of stochastic focusing results in a significant deviation from the deterministic mean, but where the relative error due to MM is even greater. The parameters have been chosen to illustrate this point, and not in cases where there are reasons to believe that the spatial distribution of enzyme matters for the system dynamics [1]. Here, all solutions are very close to the deterministic ODE solution. To the far left, the relative effects are even smaller than the stochastic focusing effect, but both of those effects are very small. Here, all solutions are very close to the deterministic ODE solution.
would be motivated from a biophysical perspective is to the far right of figure 4a, where the system is strongly reaction limited. However, even in this regime the RDME may not provide accurate simulations due to the artificial focusing effect demonstrated here.

For the case of a diffusing enzyme (red, circles), in the limit of slow diffusion $E[S]/[E]$ diverges (only three points are shown in the figure for visualization purposes). This is not caused by an artificial focusing effect, but rather by the locality effect discussed in Mahmutovic et al. [1]. S is still made constitutively across the domain, but $P$ formation will be highly diffusion limited. The effect is that slow diffusion will result in long periods, on the timescale of $S$ birth events, during which $S$ in some voxels does not have access to enzyme $C$. The result is that the growth of $S$ is checked only by its diffusion out of the voxel (which is also slow on the timescale of $S$ birth events) until an $C$ molecule diffuses in again. We note that this behaviour has nothing to do with the inaccuracies that can occur for small voxels and bimolecular reactions in MA kinetics in the diffusion limited case [13,16], as we conducted the simulations in figure 4 in one dimension where those effects are not observed. What we demonstrate here would only be more pronounced in higher dimensions since the spurious focusing effect depends only on the voxel volumes.

We also note that diffusion can play a role in reducing the stochastic focusing effect by reducing the variance of $S$ (figure 4b). In effect, diffusion is smoothing the spatial distribution of $S$ by averaging between neighbouring voxels. This effect is limited by the noise induced by diffusion, and thus the variance will not converge to zero. Comparing figure 4b,c makes it clear that this reduction in variance results in a clear reduction in stochastic focusing (defined here as $100(E[Sq(S)] - E[Sq(S)])/E[Sq(S)]$), note that at steady-state $E[Sq(S)] = \Omega c / V_{\text{max}}$ in all cases) for both systems. This follows the intuition given in figure 2 that narrowing the distribution will reduce the stochastic focusing effect.

In the well-mixed case and for biologically realistic parameters, the system volume for which the error introduced by the MM approximation becomes a pressing issue might be very small and may imply mean enzyme populations below unity. As an example, consider the parameter set in figure 3 where the rightmost point corresponds approximately to 20 enzyme molecules in 1 $\mu$m$^3$, roughly the volume of an $E. coli$ cell. As the figure implies, for some parameter sets, significant error will only be observed when we consider sub-cellular resolution. This is the aim of the RDME. Indeed, in a spatial stochastic simulation, we can quickly run into problematic volume regimes in individual voxels, leading to an artificial dependence on voxel size. Figure 5 illustrates this point. We simulate the MM and MA systems in a cube of volume $10^{-18}$ $m^3$ (i.e. corresponding to the rightmost point in figure 3) in three dimensions using the same biologically realistic parameter values. We used a typical diffusion constant $D = 1 \mu$m$^2$ s$^{-1}$ for which the system is rather reaction limited. When varying the mesh resolution, the MA solution (red, cross) stays close to the well-mixed solution (red, solid) for all voxel sizes, as expected. However, we see that the steady-state values of substrate are highly mesh dependent when using MM (blue, cross), with a large error even for modest

Figure 4. Steady-state levels as a function of substrate mobility. RDME simulations of MM and MA systems for the case where the enzyme in the MA systems is distributed uniformly over the voxels and is stationary. The mesh resolution is chosen such that there is an integer number of enzyme molecules in each voxel. (a) As the diffusion constant becomes small, $E[S]/[E]$ approaches the corresponding well-mixed value for the voxel volumes (dashed lines) from figure 1 for the MA (red, crosses) and MM (blue, crosses) systems respectively, rather than the value determined by the total system volume $\Omega$ (solid lines). This is to be expected because at zero diffusion each voxel become its own independent well-mixed system, and the global sum of substrate molecules simply becomes the sum of those subsystems. Fast diffusion lowers the variance of zero diffusion each voxel become its own independent well-mixed system, and the global sum of substrate molecules simply becomes the sum of those subsystems.

- **(a)** $E[S]/[E]$ (steady-state) in mm well mixed, $\Omega = 0.1$.
- **(b)** $\text{Var}(S)$ as a function of $D$.
- **(c)** Stochastic focusing (%).

Steady-state levels as a function of substrate mobility. RDME simulations of MM and MA systems for the case where the enzyme in the MA systems is distributed uniformly over the voxels and is stationary. The mesh resolution is chosen such that there is an integer number of enzyme molecules in each voxel. (a) As the diffusion constant becomes small, $E[S]/[E]$ approaches the corresponding well-mixed value for the voxel volumes (dashed lines) from figure 1 for the MA (red, crosses) and MM (blue, crosses) systems respectively, rather than the value determined by the total system volume $\Omega$ (solid lines). This is to be expected because at zero diffusion each voxel become its own independent well-mixed system, and the global sum of substrate molecules simply becomes the sum of those subsystems. Fast diffusion lowers the variance of zero diffusion each voxel become its own independent well-mixed system, and the global sum of substrate molecules simply becomes the sum of those subsystems.

- **(a)** $E[S]/[E]$ (steady-state) in mm well mixed, $\Omega = 0.1$.
- **(b)** $\text{Var}(S)$ as a function of $D$.
- **(c)** Stochastic focusing (%).
diffusion is relatively fast and we would expect solutions close to the well-mixed value (solid red) for all voxel sizes. This is indeed the case when using the MA system (red, cross) but the steady-state value of $E[S]$ increases rapidly with decreasing voxel size for MM (blue, circles). The inset shows a snapshot of a simulation of the MA system using PyURDME (www.pyurdme.org), where the mesh is shown in green and the coloured spheres depicts S molecules (blue), C molecules (red) and CS complexes (yellow). The mesh resolution is here 20 voxels per axis, giving voxel volumes of $1.25 \times 10^{-21}$ m$^3$, placing it in the far left region of volumes in figure 3. (Online version in colour.)

3. Discussion
We have investigated the viability of progressing the MM approximation from a well-mixed deterministic ODE model of enzyme kinetics to a well-mixed discrete stochastic model and finally to a spatial stochastic model. As we have shown, care must be exercised in interpreting effects of noise in the system if the MM approximation is used, since there will be ranges of volumes where the error introduced by the MM approximation will be significant in relation to the stochastic effects that would be observed using the full MA model. Our analysis in the well-mixed case provides a quantitative measure of the deviation from the MA solution, and shows that the error will be significant whenever the stochastic focusing effect in the substrate is significant. In fact, a small error implies that both the stochastic MM and MA solutions are close to the deterministic solution.

What is clear from figure 4a is that there are two true contributions to amplification of $E[S]$ above the deterministic level in spatial models with enzyme kinetics. The first contribution is illustrated when we assume that $E$ is uniformly distributed and does not diffuse. This removes the issue of $E$ not overlapping with location of creation of $S$. In this case there is still an issue of locality due to slow diffusion of substrate $S$, which becomes apparent as the diffusion coefficient goes to zero. In this limit small voxels are effectively isolated, thus voxel size becomes the effective system size. Not surprisingly, in this limit $E[S]/\Omega$ goes to the well-mixed equivalent for a system with volume equal to that of one of the voxels. This is the stochastic focusing effect, and in the limit of

mesh resolutions. The rightmost point corresponds to 5 voxels in each axis (a very coarse mesh) and the leftmost point to 20 voxels. So even though the error that would be incurred by MM in a well-mixed simulation in the total system volume is small, by discretizing space, the small volumes of the voxels result in a large error in the RDME solution. In fact, for these voxel sizes, we span the peak of the relative error in figure 3. Since the finer mesh resolutions would be necessary in order to resolve internal structures of the cells and to accurately resolve boundaries introduced by membranes, this greatly restricts the applicability of RDME simulations when the model contains MM motifs.

To further illustrate this point, we conclude by considering a model of a two-stage MAPK pathway [17,18]. The model definition and parameter values are found in electronic supplementary material, §§S1.7. To illustrate the potential caveats of MM modelling, as a starting point we considered the parameter values used in Takahashi et al. [18]. For those values, the condition (2.5) is not strongly satisfied, and indeed, the authors rely on MA kinetics. However, small changes in parameters (no parameter is allowed to vary more than a factor of 10 from its original value, and they are only allowed to take biophysically plausible values), in line with what would be a minimal level of sensitivity analysis, result in a regime where the second step of the cascade appears amenable to MM model reduction (that is, condition (2.5) is satisfied). We note that when using the MM approximation it is essential to continuously monitor the validity condition, however, in this case it is always met and the result is not due to its violation. Figure 6 shows the response of the system (the expected value of doubly phosphorylated MAPKpp) as a function of time when the cascade is simulated by the RDME in a cubic box of volume 1 $\mu$m$^3$. As can be seen, the MA and MM simulations agree well when there are only a small number of voxels (close to a well-mixed system), but as the resolution increases, the MM system displays an artificial amplification over the MA system due to small-volume effects. These effects in the MM system could easily be misinterpreted as, e.g. spatial effects caused from short-timescale spatiotemporal correlations (which in a MA system may need a high mesh resolution to be captured [18]).
slow diffusion, the error due to the MM approximation can be understood qualitatively from the analysis in the previous section. However, we see that as the diffusion coefficient is increased there is a reduction in this focusing effect (figure 4c). This is because diffusion provides a smoothing or averaging between neighbours, and thus effectively narrows the distribution of $S$ (figure 4b).

The second component to the spatial amplification is the locality effect discussed in [1]. In that work the authors introduce a system where a molecule $S$ is created by another molecule $X$ and degraded by an enzyme $E$. They note that in the case of slow diffusion there will be situations where $X$ and $E$ molecules are not sufficiently close, causing ‘concentration peaks’ around $X$ and valleys around $E$ molecules, resulting in slower effective degradation rates for $S$. In our system $S$ is constitutively created everywhere thus as $E$ is allowed to diffuse there will almost certainly be locations where there is no $E$ around to convert $S$ to $P$. The result is an explosion in $S$ for slower diffusion coefficients, as shown in figure 4a. This effect can never be captured by a system using the naive MM approximation. Apart from these difficulties, the fact that the accuracy of the MM solution depends so critically on the system volume means that it becomes highly sensitive to the mesh resolution in a RDME simulation, as illustrated in figure 5 and for the MAPK model in figure 6.

In conclusion, our results paint a picture of the discrete stochastic MM approximation as being naturally applicable only in a well-mixed setting, and in that case, is only guaranteed to be accurate when the effects of noise (here defined as the stochastic focusing of the mean value) are small. Hence, while there are parameter regimes where the MM approximation gives accurate results, a deterministic simulation should be applicable and preferable from a computational standpoint.

We note that another common approximation used frequently in systems biology to model, for example, cooperative binding of transcription factors to promoter regions is the Hill equation [3]:

$$\frac{[S]^n}{K_m^n + [S]^n}$$  \hspace{1cm} (3.1)

The Hill equation is understood as an approximation in the case of cooperative binding, where $\max(n)$ is the number of possible binding events, and $n$ takes that value only in the case of perfect cooperativity. Although it is awkward to talk about the cooperativity of one binding event, MM can be seen mathematically as a Hill equation with $n = 1$. The higher $n$, the stronger the nonlinearity, the steeper the curve and the greater the stochastic focusing effect. Therefore, the problems we have outlined for MM in both the well-mixed and spatial stochastic settings will likely be even more pronounced in models using the Hill equation. An alternative and more direct way to model transcription factor binding to a promoter without the recourse to Hill kinetics was demonstrated in Sturrock et al. [19] in a spatial stochastic setting.

As a final comment, in this paper, we have considered the errors introduced by the MM model reduction in the mean value in steady state. That this error is small in cases where stochastic effects on the mean are relevant should be seen as a minimal requirement from a modelling perspective. If e.g. the error in variances is considered, even more stringent conditions than those discussed here may apply. As an example, in Thomas et al. [20], it is shown that the error in the variance in steady state can be as large as 30% even in the regime where the stochastic MM, MA and ODE solutions should result in the same mean values.

Figure 6. Response of a two-stage MAPK cascade. Small volume effects cause an artificial amplification of MAPKpp in the stochastic MM system compared to the MA system for higher number of voxels in the mesh (dashed green and red lines). Here, the difference in steady-state levels between MA (solid lines) and MM is 23% for the finest mesh resolution, even though both models agree well for a close to well-mixed system with only 10 voxels (blue lines). Had the comparison with a corresponding MA system not been available, this amplification could easily have been misinterpreted as spatial effects in the system, something that may need high spatio-temporal resolution to resolve [18].
Disclaimer. The content of this paper is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

Data accessibility. Simulations have been conducted using the pyURDME software. It is publicly available from www.pyurdme.org.

Funding statement. This work was supported by the Swedish strategic research programme eSSENCE, the National Science Foundation (NSF) award no. DMS-1001012, ICB award no. W911NF-09-0001 from the US Army Research Office, NIBIB of the NIH under award no. R01-EB014877-01 and (US) Department of Energy (DOE) Award No. DE-SC0008975.

Author contributions. M.J.L. and A.H. designed and performed the research. M.J.L., L.P. and A.H. analysed data and wrote the paper.

Conflict of interests. We have no competing interests.

References