Bulletin of Mathematical Biology manuscript No.
(will be inserted by the editor)

Quasi-steady-state approximations derived from the stochastic model of enzyme kinetics

Hye-Won Kang · **Wasiur R. KhudaBukhsh** ·
Heinz Koeppl · **Grzegorz A. Rempała**

Received: date / Accepted: date

Abstract The paper outlines a general approach to deriving quasi steady-state approximations (QSSAs) of the stochastic reaction networks describing the Michaelis-Menten enzyme kinetics. In particular it explains how different sets of assumptions about chemical species abundance and reaction rates lead to the standard QSSA (sQSSA), the total QSSA (tQSSA), and the reverse QSSA (rQSSA) approximations. These three QSSAs have been widely studied in the literature in deterministic ordinary differential equation (ODE) settings and several sets of conditions for their validity have been proposed. With the help of the multiscaling techniques introduced in [4, 27] it is seen that the conditions for deterministic QSSAs largely agree (with some exceptions) with the ones for stochastic QSSAs in the large volume limits. The paper also illustrates how the stochastic QSSA approach may be extended to more complex stochastic kinetic networks like, for instance, the Enzyme-Substrate-Inhibitor system.

Keywords Michaelis-Menten kinetics · Stochastic reaction network · Multiscale approximation · QSSA

Mathematics Subject Classification (2000) 60J27 · 60J28 · 34E15 · 92C42 · 92B25 · 92C45

Hye-Won Kang and Wasiur R. KhudaBukhsh made equal contributions. Therefore, they are joint first authors.

Hye-Won Kang
Department of Mathematics and Statistics,
University of Maryland Baltimore County, USA
E-mail: hwkang@umbc.edu

Wasiur R. KhudaBukhsh orcid.org/0000-0003-1803-0470
Department of Electrical Engineering and Information Technology,
Technische Universität Darmstadt, Germany
E-mail: wasiur.khudabukhsh@bcs.tu-darmstadt.de

Heinz Koeppl
Department of Electrical Engineering and Information Technology,
Technische Universität Darmstadt, Germany
E-mail: heinz.koeppl@bcs.tu-darmstadt.de

Grzegorz A. Rempała orcid.org/0000-0002-6307-4555
Division of Biostatistics and Mathematical Biosciences Institute,
The Ohio State University, USA
E-mail: rempala.3@osu.edu

1 Introduction

In chemistry and biology, we often come across chemical reaction networks where one or more of the species exhibit a different intrinsic time scale and tend to reach the equilibrium state faster than the others. The quasi steady state approximation (QSSA) is a commonly used tool to simplify the description of dynamics of such systems. In particular, QSSA has been widely applied to the important class of chemical reaction networks known as the Michaelis-Menten models of enzyme kinetics [15, 26, 48].

Traditionally, the enzyme kinetics has been studied using systems of ordinary differential equations (ODEs). The ODE model allows one to analyze various aspects of the enzyme dynamics such as asymptotic stability. However, it ignores the fluctuations of the enzyme reaction network due to intrinsic noise and instead focuses on the averaged dynamics. If accounting for the intrinsic noise is desirable then the use of an alternative stochastic model of reaction network may be more appropriate, especially when some of the species have low copy numbers. It is well-known that the molecular fluctuations in the species with small copy numbers, and stochasticity in general, can lead to interesting dynamics. For instance, in a recent paper [41], Perez et al. gave an account of how the intrinsic noise controls the dynamics and steady state of a morphogen-controlled bistable genetic switches. In a recent paper [1], Anderson et al. show that, in general, the behaviors of the deterministic system and the stochastic system can be vastly different with regards to the possibility of an explosion. In particular, they provide examples of an explosive stochastic system whose deterministic counterpart admits bounded solutions, and also non-explosive stochastic models whose deterministic counterpart suffers a blow-up. It is also worthwhile to note that there are stochastic reaction networks whose associated stochastic process explodes but the chemical master equation (Kolmogorov equation) still admits a stationary solution. Along similar lines, David McQuarrie showed in the paper [36] that the expectation for finite volumes do not match the reaction rate equation in general for reactions of high arity (see [36, Figure 3], for example). All these cases point to the fact that studying only the behavior of the deterministic models of enzyme kinetics or reaction networks in general may be often inadequate. Indeed, stochastic models have been recently strongly advocated by many authors [3, 8, 10, 11, 38, 39]. In this paper, we consider the stochastic models of enzyme kinetics specifically in the context of various stochastic QSSAs with the goal of relating them to the deterministic ones reported previously in the chemical physics literature. In order to illustrate how the probabilistic tools discussed here can be used to derive various QSSAs for more general enzyme kinetics than the basic Michaelis-Menten reaction network, we also briefly consider a fully competitive enzyme-substrate-inhibitor (ESI) system later in the paper.

The studying of QSSAs is practically useful, as they not only help reduce model complexity, but also may relate it better to experimental observables by averaging out unobservable or difficult-to-measure model components (see, for instance, [14] for a recent discussion in the context of enzyme kinetics). A substantial body of work has been published to justify such QSSA reductions in deterministic models, typically by means of perturbation theory [6, 20, 35, 45, 47, 50, 52]. In contrast to this approach, we derive here the QSSA reductions using stochastic multiscaling techniques [4, 27]. While perturbation analysis often agrees with the stochastic QSSAs[1], they fail to capture certain limiting behaviors (for instance, when the limit itself is stochastic). We shall make this point precise later in Section 4. Although our approach is applicable more generally, we focus below on the three well established Michaelis-Menten enzyme kinetics QSSAs, namely the standard QSSA (sQSSA), the total QSSA (tQSSA), and the reverse QSSA (rQSSA). To illustrate more complicated kinetics we also briefly consider a fully competitive enzyme-inhibitor-substrate system and show that sQSSA and tQSSA can be derived there as well. In all cases considered here the QSSAs are obtained as a consequence of the (Poisson) law of large numbers applied to the stochastic reaction network under different scaling regimes. A similar approach has been recently taken in [30] with respect to a particular type of QSSA (tQSSA, see Section 2 below). However, our current derivation is different in that it entirely avoids a spatial averaging argument used in [30]. Such an argument requires additional assumptions that are difficult to verify in practice.

[1] The agreement is meant in the sense that it gives a reduced ODE model whose propensity functions are analogs of those in the stochastic QSSA.

The paper is organized as follows. We first review the Michaelis-Menten enzyme kinetics in the deterministic setting and discuss the corresponding QSSAs in Section 2. The stochastic setting is introduced in Section 3, where we also briefly describe the multiscale approximation technique proposed in [27]. Following this, we derive the Michaelis-Menten (deterministic) sQSSA, tQSSA and rQSSA approximations from the stochastic model analysis in Sections 5, 6, and 7 respectively. In Section 8, we extend our approach to more complicated enzyme networks and derive sQSSA, and tQSSA for the ESI system. We conclude with a short discussion in Section 9.

2 QSSAs for deterministic Michaelis-Menten kinetics

The Michaelis-Menten enzyme-catalyzed reaction networks have been studied in depth over past several decades [15, 26, 48] and have been described in various forms. Although the methods discussed below certainly apply to more general networks of reactions describing enzyme kinetics, in this paper, we adopt the simplest (and minimal) description for illustration purpose. In its simplest form, the Michaelis-Menten enzyme-catalyzed network of reactions describes reversible binding of a free enzyme (E) and a substrate (S) into an enzyme-substrate complex (C), and irreversible conversion of the complex to the product (P) and the free enzyme. The enzyme-catalyzed reactions are typically schematically represented as

$$S+E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_2} P+E, \tag{2.1}$$

where k_1 and k_{-1} are the reaction rate constants for the reversible enzyme binding in the units of $M^{-1}s^{-1}$ and s^{-1} while k_2 is the rate constant for the product creation in the unit of s^{-1}. By applying the law of mass action to (2.1), temporal changes of the concentrations may be described by the following system of ODEs:

$$\begin{aligned}
\frac{d[S]}{dt} &= -k_1[S][E]+k_{-1}[C],\\
\frac{d[E]}{dt} &= -k_1[S][E]+k_{-1}[C]+k_2[C],\\
\frac{d[C]}{dt} &= k_1[S][E]-k_{-1}[C]-k_2[C],\\
\frac{d[P]}{dt} &= k_2[C],
\end{aligned} \tag{2.2}$$

where the bracket notation $[\cdot]$ denotes species concentration. In this closed system, there are two conservation laws for the total amount of enzyme and substrate

$$\begin{aligned}
[E_0] &\equiv [E]+[C],\\
[S_0] &\equiv [S]+[C]+[P].
\end{aligned} \tag{2.3}$$

These conservation laws not only reduce (2.2) to two equations, but also play an important role in the analysis of the reaction network given in (2.1). It is worth mentioning that some authors also consider an additional reversible reaction in the form of binding of the product (P) and the free enzyme (E) to produce the enzyme-substrate complex (C), i.e., $P+E \rightharpoonup C$. We remark that should we expand the model in (2.1) to include such a reaction, our discussion in later sections would remain largely the same requiring only simple modifications.

In early 20th century Leonor Michaelis and Maud Menten investigated the enzymatic kinetics in (2.1) and proposed a mathematical model for it in [37]. They suggested an approximate solution for the initial speed of the enzyme inversion reaction in terms of the substrate concentrations. Following their work, numerous attempts have been made to obtain approximate solutions of (2.2) under various quasi-steady-state assumptions. Several conditions on the rate constants have also been proposed to ensure validity of such approximations. For example in 1920's Briggs and Haldane derived a simplified version of the Michaelis-Menten equation, commonly referred to now as the sQSSA [12]. The sQSSA is based on the assumption that the complex

reaches its steady state quickly after a transient time, i.e., $d[C]/dt \approx 0$ [50]. This approximation is found to be accurate only when the enzyme concentration is small compared to that of the substrate. The condition for the validity of the sQSSA was first suggested as $[E_0] \ll [S_0]$ by Laidler [34], and a more general condition was derived as $[E_0] \ll [S_0] + K_M$ by Segel [49] and Segel and Slemrod [50], where $K_M \equiv (k_2 + k_{-1})/k_1$ is the so-called Michaelis-Menten constant.

Borghans et al. later extended the sQSSA to the case with an excessive amount of enzyme and derived the so-called tQSSA by introducing a new variable for the total substrate concentration [9]. In the tQSSA, one assumes that the total substrate concentration changes on a slow time scale and that the complex reaches its steady state quickly after a transient time when $d[C]/dt \approx 0$. Then, the complex concentration $[C]$ is found as a solution of the quadratic equation. Approximating $[C]$ in a simple way, they proposed a necessary and sufficient condition for the validity of tQSSA as

$$([E_0] + [S_0] + K_M)^2 \gg K[E_0], \tag{2.4}$$

where $K = k_2/k_1$ is the so-called Van Slyke-Cullen constant [56]. Later, Tzafriri [55] revisited the tQSSA and derived another set of sufficient conditions for the validity of the tQSSA as $\varepsilon \equiv (K/(2[S_0]))\, f(r([S_0])) \ll 1$ where $f(r) = (1-r)^{-1/2} - 1$ and $r([S_0]) = 4[E_0][S_0]/([E_0] + [S_0] + K_M)^2$. He argued that this sufficient condition was always roughly satisfied by showing ε was less than $1/4$ for all values of $[E_0]$ and $[S_0]$. The tQSSA was later improved by Dell'Acqua and Bersani [19] at high enzyme concentrations when (2.4) is satisfied.

The rQSSA was first suggested as an alternative to the sQSSA by Segel and Slemrod [50]. In the rQSSA, the substrate, instead of the complex, was assumed to be at steady state, $d[S]/dt \approx 0$, and the domain of the validity of the rQSSA was suggested as $[E_0] \gg K$. Then, Schnell and Maini showed that at high enzyme concentration, the assumption $d[S]/dt \approx 0$ was more appropriate in the rQSSA than the assumption $d[C]/dt \approx 0$ used in the sQSSA or tQSSA due to possibly large error during the initial stage of the reactions [46]. They derived necessary conditions for the validity of the rQSSA as $[E_0] \gg K$ and $[E_0] \gg [S_0]$. In the following sections, we will provide alternative derivations of these different conditions arriving at them in a more natural way. To this end let us start with the stochastic description of (2.1).

3 Multiscale stochastic Michaelis-Menten kinetics

Let X_S, X_E, X_C, and X_P denote the copy numbers of molecules of the substrates (S), the enzymes (E), the enzyme-substrate complex (C), and the product (P) respectively. We assume the evolution of these copy numbers is governed by a Markovian dynamics given by the following stochastic equations (see, for instance, [2]):

$$\begin{aligned}
X_S(t) &= X_S(0) - Y_1\left(\int_0^t \kappa_1' X_S(s)X_E(s)\,ds\right) + Y_{-1}\left(\int_0^t \kappa_{-1}' X_C(s)\,ds\right),\\
X_E(t) &= X_E(0) - Y_1\left(\int_0^t \kappa_1' X_S(s)X_E(s)\,ds\right) + Y_{-1}\left(\int_0^t \kappa_{-1}' X_C(s)\,ds\right) + Y_2\left(\int_0^t \kappa_2' X_C(s)\,ds\right),\\
X_C(t) &= X_C(0) + Y_1\left(\int_0^t \kappa_1' X_S(s)X_E(s)\,ds\right) - Y_{-1}\left(\int_0^t \kappa_{-1}' X_C(s)\,ds\right) - Y_2\left(\int_0^t \kappa_2' X_C(s)\,ds\right),\\
X_P(t) &= X_P(0) + Y_2\left(\int_0^t \kappa_2' X_C(s)\,ds\right),
\end{aligned} \tag{3.1}$$

where Y_1, Y_{-1} and Y_2 are independent unit Poisson processes and $t \geq 0$. The quantities $\kappa_1', \kappa_{-1}', \kappa_2'$ are the stochastic reaction rate constants. They can be related to the deterministic reaction rate constants by means of the Avogadro's number. We shall make this point precise in Section 5. We denote $X_{E_0} \equiv X_E(t) + X_C(t)$ and $X_{S_0} \equiv X_S(t) + X_C(t) + X_P(t)$, and as in the deterministic model (2.2) in previous section assume that the total substrate and enzymes copy numbers, X_{S_0} and X_{E_0}, are conserved in time. As shown in [4, 27], the representation (3.1) is especially helpful in analyzing systems with multiple time scales or involving species

with abundances varying over different orders of magnitude. Unlike the chemical master equations, (3.1) explicitly reveals the relations between the species abundances and the reaction rates.

In the reaction system (2.1), various scales can exist in the species numbers and reaction rate constants, which determine time scales of the species involved. In order to relate these scales to each other, we introduce a scaling parameter N (note that N is not necessarily the system size) and express the orders of magnitude of species copy numbers and rate constants as powers of N[2]. Denoting scaling exponents for the ith species and the kth rate constant by α_i and β_k respectively, we express unscaled species copy numbers and rate constants in powers of N as

$$\begin{aligned} X_i(t) &= N^{\alpha_i} Z_i^N(t), \text{ for } i = S,E,C,P \\ \text{and } \kappa_k' &= N^{\beta_k}\kappa_k, \text{ for } k = 1,-1,2, \end{aligned} \tag{3.2}$$

so that the scaled variables and constants, $Z_i^N(t)$ and κ_k, are approximately of order 1 (denoted as $O(1)$). In Z_i^N, the superscript represents the dependence of the scaled species numbers on N. Note that to express different time scales as powers of N, we may apply a time change by replacing t with $N^\gamma t$. The scaled species number after such the time change is denoted by

$$X_i(N^\gamma t) = N^{\alpha_i} Z_i^N(N^\gamma t) \equiv N^{\alpha_i} Z_i^{N,\gamma}(t).$$

With this change of variables $\{Z^{N,\gamma}\} \equiv \left\{ \left(Z_S^{N,\gamma}, Z_E^{N,\gamma}, Z_C^{N,\gamma}, Z_P^{N,\gamma} \right) \right\}$ becomes a parametrized family of stochastic processes satisfying, according to (3.1),

$$\begin{aligned}
Z_S^{N,\gamma}(t) &= Z_S^N(0) + N^{-\alpha_S}\left[-Y_1\left(\int_0^t N^{\rho_1+\gamma}\kappa_1 Z_S^{N,\gamma}(s) Z_E^{N,\gamma}(s)\,ds \right) + Y_{-1}\left(\int_0^t N^{\rho_{-1}+\gamma}\kappa_{-1} Z_C^{N,\gamma}(s)\,ds \right) \right], \\
Z_E^{N,\gamma}(t) &= Z_E^N(0) + N^{-\alpha_E}\left[-Y_1\left(\int_0^t N^{\rho_1+\gamma}\kappa_1 Z_S^{N,\gamma}(s) Z_E^{N,\gamma}(s)\,ds \right) + Y_{-1}\left(\int_0^t N^{\rho_{-1}+\gamma}\kappa_{-1} Z_C^{N,\gamma}(s)\,ds \right) \right. \\
&\qquad \left. + Y_2\left(\int_0^t N^{\rho_2+\gamma}\kappa_2 Z_C^{N,\gamma}(s)\,ds \right) \right], \\
Z_C^{N,\gamma}(t) &= Z_C^N(0) + N^{-\alpha_C}\left[Y_1\left(\int_0^t N^{\rho_1+\gamma}\kappa_1 Z_S^{N,\gamma}(s) Z_E^{N,\gamma}(s)\,ds \right) - Y_{-1}\left(\int_0^t N^{\rho_{-1}+\gamma}\kappa_{-1} Z_C^{N,\gamma}(s)\,ds \right) \right. \\
&\qquad \left. - Y_2\left(\int_0^t N^{\rho_2+\gamma}\kappa_2 Z_C^{N,\gamma}(s)\,ds \right) \right], \\
Z_P^{N,\gamma}(t) &= Z_P^N(0) + N^{-\alpha_P} Y_2\left(\int_0^t N^{\rho_2+\gamma}\kappa_2 Z_C^{N,\gamma}(s)\,ds \right),
\end{aligned} \tag{3.3}$$

where $\rho_1 \equiv \alpha_S + \alpha_E + \beta_1$, $\rho_{-1} \equiv \alpha_C + \beta_{-1}$, and $\rho_2 \equiv \alpha_C + \beta_2$. As seen from (3.3), the values of ρ's, α's and γ's determine the temporal dynamics of the scaled random processes. For example, consider the limiting behavior of the scaled process for the first reaction in the equation for S,

$$N^{-\alpha_S} Y_1\left(\int_0^t N^{\rho_1+\gamma}\kappa_1 Z_S^{N,\gamma}(s) Z_E^{N,\gamma}(s)\,ds \right). \tag{3.4}$$

Assuming that $Z_S^{N,\gamma}$ and $Z_E^{N,\gamma}$ are $O(1)$ on the time scale of interest, the limiting behavior of the scaled process depends upon ρ_1, α_S, and γ. If the $\rho_1 + \gamma < \alpha_S$, the scaled process vanishes to zero as N goes to infinity. This means that the number of occurrences of the first reaction is outweighed by the order of magnitude of the

[2] We note that $1/N$ plays a similar role as the expansion parameter (usually denoted by ε) in the singular perturbation analysis of deterministic models [23,50]. In this approach, the time scales are often separated by introducing, in addition to the time variable t, a new slow time scale $\tau = \varepsilon t$, where ε is assumed small and eventually sent to zero. This allows one to reformulate the system of differential equations into the Tikhonov standard form [23]. Alternatively, especially in case of perturbation analysis of chemical reaction networks, one often scales the reaction rates instead to separate the fast reactions from the assumed slow ones. For instance, a reaction with rate εk_1 will correspond to a slow reaction compared to a reaction with rate k_2. See [23, Section 2.4 Examples] for some examples.

species copy number for S. When $\rho_1+\gamma=\alpha_S$, the number of occurrences of the first reaction is comparable with the order of magnitude of the species copy number for S. Then, using the law of large numbers for the Poisson process[3], the limiting behavior of (3.4) is approximately the same as that of

$$\int_0^t \kappa_1 Z_S^{N,\gamma}(s) Z_E^{N,\gamma}(s)\, ds. \tag{3.5}$$

Lastly, when $\rho_1+\gamma>\alpha_S$, the first reaction occurs so frequently that the scaled process in (3.4) tends to infinity. The limiting behaviors of other scaled processes are determined similarly. Using the scaled processes involving the reactions where S is produced or consumed, we can choose γ so that $Z_S^{N,\gamma}(t)$ becomes $O(1)$. Therefore, we have $\alpha_S=\max(\rho_1+\gamma,\rho_{-1}+\gamma)$, and the *time scale* of S is given by

$$\gamma=\alpha_S-\max(\rho_1,\rho_{-1}). \tag{3.6}$$

Note that the time scales of the species numbers and their limiting behaviors are decided by the scaling exponents for species numbers and reactions, that is, they are dictated by the choice of α's and β's.

In order to prevent the system from vanishing to zero or exploding to infinity in the scaling limit, the parameters α's and β's must satisfy what are known as the balance conditions [27]. Essentially, these conditions ensure that the scaling limit is $O(1)$. Intuitively, the largest order of magnitude of the production of species i should be the same as that of consumption of species i. In the Michaelis-Menten reaction network described in Section 2, balance for the substrate S can be achieved in two ways. First, through the equation $\rho_1=\rho_{-1}$, which *balances* the binding and unbinding of the enzyme and the substrate; and second, by making α_S large enough so that the imbalance between the occurrences of the reversible binding of the enzyme to substrate can be nullified. This gives a restriction on the time scale γ as $\gamma+\max(\rho_1,\rho_{-1})\le\alpha_S$. Combining the conditions for all species, we get

$$\begin{aligned}
\rho_1=\rho_{-1} \quad &\text{or} \quad \gamma\le\alpha_S-\max(\rho_1,\rho_{-1}),\\
\rho_1=\max(\rho_{-1},\rho_2) \quad &\text{or} \quad \gamma\le\alpha_E-\max(\rho_1,\rho_{-1},\rho_2),\\
\rho_1=\max(\rho_{-1},\rho_2) \quad &\text{or} \quad \gamma\le\alpha_C-\max(\rho_1,\rho_{-1},\rho_2),\\
\rho_2+\gamma=0 \quad &\text{or} \quad \gamma\le\alpha_P-\rho_2.
\end{aligned} \tag{3.7}$$

In addition to (3.7) further conditions are also required for each linear combination of species, to balance their collective production and consumption rate. These additional conditions are

$$\begin{aligned}
\rho_2+\gamma=0 \quad &\text{or} \quad \gamma\le\max(\alpha_S,\alpha_C)-\rho_2,\\
\rho_1=\rho_{-1} \quad &\text{or} \quad \gamma\le\max(\alpha_C,\alpha_P)-\max(\rho_1,\rho_{-1}),
\end{aligned} \tag{3.8}$$

and are obtained by comparing collective production and consumption rates of $S+C$ and $C+P$, respectively.

4 Discrepancy between stochastic and deterministic QSSAs

The multiscaling technique allows one to produce a wide range of approximations by tuning the scaling exponents suitably to reflect different regimes of time-scale separation and species abundance. While the main purpose of the paper is to show how the sQSSA, the tQSSA and the rQSSA can be derived directly from the stochastic description by means of an appropriate choice of the scaling exponents, the multiscaling method may also be used to derive other approximations, which have a quasi-steady state flavor and are not directly derivable from the deterministic systems. To illustrate this point, we furnish two simple examples.

[3] The strong law of large numbers states that, for a unit Poisson process Y, $\frac{1}{N}Y(Nu)\to u$ almost surely as $N\to\infty$, (see [21]).

4.1 Michaelis-Menten kinetics with extremely large enzyme concentration

Consider the Michaelis-Menten kinetics (2.1) with the enzyme E in much greater abundance compared to the other species. We also assume that all reaction constants have the same order of magnitude. In order to model such a pathological case in the deterministic setting, one could assume the enzyme concentration does not change over time, i.e., $d[E]/dt \approx 0$ and consider the following reduced model:

$$\frac{d[S]}{dt} = -k_1[E][S] + k_{-1}[C], \quad \text{and} \quad \frac{d[C]}{dt} = k_1[E][S] - (k_{-1}+k_2)[C], \tag{4.9}$$

where $[E]$ is no longer a function of time t, and is treated as a constant (i.e., $[E] = [E_0]$ at all times). Note that here we do not assume the conservation law $[E] + [C] \equiv [E_0]$. A similar setup under the assumption of the conservation law will be discussed later in Section 7. By virtue of the constancy of $[E]$, the quantity $k_1[E]$ can be interpreted as an effective reaction rate in the reduced system

$$S \underset{k_{-1}}{\overset{k_1[E]}{\rightleftharpoons}} C \xrightarrow{k_2} \emptyset \tag{4.10}$$

where we assume that k_1, k_{-1}, and k_2 are all $O(1)$. Since $k_1[E]$ is much greater than the other two reaction rate constants, the above system qualitatively predicts rapid decay in substrate concentration and an initial growth in complex concentration. However, such a prediction does not provide insights into the inherent time scales of the different species.

Now, let us consider the deterministic approximation derived from the stochastic framework. The idea is to study the stochastic system in the (possibly distinct) intrinsic time scales of the species, while matching the assumptions about the abundances (and the reaction rates) made in the deterministic setup. Therefore, setting $\alpha_S = \alpha_C = \alpha_P = 1$, $\alpha_E = 2$, and $\beta_1 = \beta_{-1} = \beta_2 = 0$ we see that the reaction propensities are given by $\rho_1 = 3, \rho_{-1} = \rho_2 = 1$. This ensures that the stochastic system is indeed comparable to the deterministic system in (4.9). The choice of $\alpha_E = 2$ reflects the high abundance of the enzyme. The time scales of S, E, and C are given by

$$\begin{aligned}
\gamma_S &= \alpha_S - \max\{\rho_1, \rho_{-1}\} = -2, \\
\gamma_E &= \alpha_E - \max\{\rho_1, \rho_{-1}, \rho_2\} = -1, \\
\gamma_C &= -2,
\end{aligned}$$

revealing two different intrinsic time scales. The advantage of the multiscaling approach is that we may study the reaction system separately in each one.

Let us begin with the time scale of S, i.e., set $\gamma = \gamma_S = -2$. Following the scaling notations introduced earlier in this section, we obtain $\left(Z_S^{(-2)}, Z_C^{(-2)}, Z_P^{(-2)}\right)$ as a scaling limit of $\left(Z_S^{N,-2}, Z_C^{N,-2}, Z_P^{N,-2}\right)$ as N goes to infinity. Then, the scaling limit of S satisfies

$$Z_S^{(-2)}(t) = Z_S^{(-2)}(0) - \int_0^t \kappa_1 Z_S^{(-2)}(s) Z_E(0)\, ds, \tag{4.11}$$

where E is approximated as its initial value since the time scale of E ($\gamma_E = -1$) is later than that of S ($\gamma_S = -2$). There are two things of note here. First, the above QSSA for S derived from the stochastic system in the time scale of S is different from the reduced model in the coupled ODE system (4.9). Second, the above equation (4.11) allows us to make a quantitative statement about the decay rate of S. Indeed, $Z_S^{(-2)}$ decays exponentially with a decay rate $\kappa_1 Z_E(0)$ depending solely on κ_1 and the initial enzyme concentration, i.e.,

$$Z_S^{(-2)}(t) = Z_S^{(-2)}(0) \exp\left(-\kappa_1 Z_E(0) Z_S^{(-2)}(t)\right).$$

Now, let us look at the time scale of the enzyme, i.e., set $\gamma = \gamma_E = -1$. In this time scale, we do not get a functional limit for $Z_S^{N,-1}$ and $Z_C^{N,-1}$ immediately. However, the term in (3.4) with our choice of scaling

exponents is in the time scale of $\gamma = -1$. Since $\rho_1 + \gamma = 2$, which is greater than $\alpha_S = 1$, the substrates S will be rapidly converted into complex molecules C (see the parametrized family (3.3)). As a consequence, the substrates and the complexes will be averaged out in the limit as $N \to \infty$. In this case, we can still approximate the average values of the fast species (S and C) by showing that the occupational random measures of S and C converge to a limiting measure as $N \to \infty$ (as done in [27, 30, 33]). If we denote a limit point of $Z_E^{N,-1}$ by $Z_E^{(-1)}$, then expectedly, the limit $Z_E^{(-1)}$ satisfies $Z_E^{(-1)}(t) = Z_E(0)$. Moreover, from the limiting measure (of the occupational measures), we can get the averaged behaviors of S and C as

$$\overline{Z_S^{(-1)}}(t) \equiv \int Z_S^{(-1)} d\psi = 0, \text{ and also, } \overline{Z_C^{(-1)}}(t) = 0, \tag{4.12}$$

where ψ is the *conditional equilibrium* or the *local-averaging* distribution (see [27, 30, 33] for similar examples). Therefore, in the time scale of the enzyme, there is no dynamic behavior (time evolution) at all in the limit. Note that (4.12) does not depend on the values of the reaction rate constants. One can obtain such a behavior from the system (4.9) by *additionally* assuming $d[S]/dt \approx 0$ and $d[C]/dt \approx 0$, which renders the ODE system completely trivial and our assumptions about the initial species abundances irrelevant. On the other hand, the averaged behavior (4.12) is a direct implication of the multiscale approximation in the time scale of the enzyme, rather than an additional assumption.

4.2 Three species kinetics

Consider the following three-species example introduced by Anderson et al. [1]

$$\begin{aligned} \emptyset \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E_{in}, \\ 2C \xrightarrow{1} 3C, \\ 3C + E \xrightarrow{1} 2C + E \end{aligned} \tag{4.13}$$

where E and E_{in} represent active and inactive enzymes. The authors in [1] considered the case when $k_{\pm 1} = k_{\pm 2} = 1$ and showed that if the initial copy number of C is greater or equal to 2, the species C always explodes to infinity in the stochastic model while its concentration always converges to the steady state 1 in the corresponding ODE model.

For the purpose of our current discussion we have modified the parameter values to consider the following two cases. Case 1: $k_{\pm 1} = 1$, $k_{\pm 2} = 100$ and Case 2: $k_{\pm 1} = 100$, $k_{\pm 2} = 1$. In the ODE model for Case 1, the species E_{in} evolves fast. Therefore, we define a new slow variable $E_T \equiv E + E_{in}$ and assume $d[E_{in}]/dt \approx 0$. This gives $[E_{in}] \approx k_2[E_T]/(k_2 + k_{-2})$, $[E] \approx k_{-2}[E_T]/(k_2 + k_{-2})$ leading to the reduced ODE model:

$$\frac{d[E_T]}{dt} = k_1 - \frac{k_{-1}k_{-2}}{k_2 + k_{-2}}[E_T], \quad \text{and} \quad \frac{d[C]}{dt} = [C]^2 - \frac{k_{-2}}{k_2 + k_{-2}}[C]^3[E_T]. \tag{4.14}$$

Now, let us consider the system approximation for Case 1 using the stochastic multiscaling framework. Setting the stochastic and deterministic rate constants to be equal ($\kappa_i' = k_i$), let $\alpha_E = \alpha_{E_{in}} = \alpha_C = 0$, $\beta_{\pm 1} = 0$, and $\beta_{\pm 2} = 1$ with $N = 100$. It follows that the reaction propensities are given by $\rho_{\pm 1} = \rho_3 = \rho_4 = 0$ and $\rho_{\pm 2} = 1$. The choice of $\beta_{\pm 2} = 1$ reflects the fast conversion between active and inactive enzymes. As $N \to \infty$, the scaling limits of E_T and C give the following reduced model

$$\begin{aligned} Z_{E_T}(t) &= Z_{E_T}(0) + Y_1(\kappa_1 t) - Y_{-1}\left(\int_0^t \frac{\kappa_{-1}\kappa_{-2}}{\kappa_2 + \kappa_{-2}} Z_{E_T}(s)\, ds\right) \\ Z_C(t) &= Z_C(0) + Y_3\left(\int_0^t Z_C(s)(Z_C(s) - 1)\, ds\right) \\ &\quad - Y_4\left(\int_0^t \frac{\kappa_{-2}}{\kappa_2 + \kappa_{-2}} Z_C(s)(Z_C(s) - 1)(Z_C(s) - 2) Z_{E_T}(s)\, ds\right). \end{aligned} \tag{4.15}$$

The above model is stochastic and the copy numbers of E_T and C are $O(1)$, i.e., $\alpha_E = \alpha_{E_{in}} = \alpha_C = 0$. Note that the propensity functions of the ODE (4.14) and the stochastic reduced models (4.15) are slightly different since the reactants are dimers or trimers of C. Observe further that in (4.15) C explodes which is an important feature of the full dynamics of (4.13) lost in the reduced ODE model (4.14).

Next, consider the ODE model for Case 2. The species E_T evolves fast in this case. Therefore, we assume $d[E_T]/dt \approx 0$, which gives $[E_T] \approx [E_{in}] + k_1/k_{-1}$, $[E] \approx k_1/k_{-1}$ and results in the following reduced ODE model:

$$\frac{d[E_{in}]}{dt} = \frac{k_1 k_2}{k_{-1}} - k_{-2}[E_{in}], \quad \text{and} \quad \frac{d[C]}{dt} = [C]^2 - \frac{k_1}{k_{-1}}[C]^3. \tag{4.16}$$

As for Case 1, consider now the approximation of Case 2 using the stochastic multiscaling framework. Set $\alpha_E = \alpha_{E_{in}} = \alpha_C = 0$, $\beta_{\pm 1} = 1$, and $\beta_{\pm 2} = 0$ with $N = 100$. The reaction propensities are given by $\rho_{\pm 1} = 1$ and $\rho_{\pm 2} = \rho_3 = \rho_4 = 0$. As $N \to \infty$, the scaling limit of E_{in} and C gives the following reduced stochastic model:

$$\begin{aligned} Z_{E_{in}}(t) &= Z_{E_{in}}(0) + Y_2\left(\frac{\kappa_1 \kappa_2}{\kappa_{-1}} t\right) - Y_{-2}\left(\int_0^t \kappa_{-2} Z_{E_{in}}(s)\, ds\right) \\ Z_C(t) &= Z_C(0) + Y_3\left(\int_0^t Z_C(s)(Z_C(s)-1)\, ds\right) \\ &\quad - Y_4\left(\int_0^t \frac{\kappa_1}{\kappa_{-1}} Z_C(s)(Z_C(s)-1)(Z_C(s)-2)\, ds\right), \end{aligned} \tag{4.17}$$

Interestingly, note that in (4.17) C fluctuates with a low copy number (about 2-10 molecules) when we start with two molecules of C. In contrast, in the ODE system (4.16) the concentration of C attains steady state. As in Case 1, the propensity functions in the reduced models (4.16) and (4.17) are slightly different due to the discrepancy between propensities of higher order reactions for stochastic and deterministic equations.

The three-species example shows that the reduced stochastic and the ODE models may have different dynamics depending on the choice of the parameter values. In Case 1, the species C explodes in the stochastic model while it does not in the corresponding ODE model. In Case 2, though there is no explosion of C, C fluctuates randomly but never goes below 2 molecules in the stochastic model with two molecules of C initially, while C converges to the steady state 1 in the corresponding ODE model.

It is of importance to note that in both reaction system examples discussed in this section the stochastic QSSA techniques allowed us to derive reduced stochastic models exhibiting important aspects of the original dynamics that the corresponding ODE approximations failed to capture. It is often possible to use multiscaling techniques on the deterministic system directly (for instance, as done in [35]) and they often agree with the stochastic QSSAs (in the sense that it gives a reduced ODE model whose propensity functions are analogs of those in the stochastic QSSA), but the stochastic averaging in (4.12) and the stochastic limits in the example described above can not be obtained from deterministic systems. Having thus demonstrating the usefulness of the stochastic QSSA we now return to the discussion of the sQSSA, tQSSA and rQSSA approximations for the stochastic Michaelis-Menten kinetics (3.1).

5 Standard quasi-steady-state approximation (sQSSA)

In the deterministic sQSSA, one assumes that the substrate-enzyme complex C reaches its steady-state quickly after a brief transient phase while the other species are still in their transient states. Therefore, by setting $d[C]/dt \approx 0$, one approximates the steady state concentration of the complex. The steady state equation of the complex in (2.2) and the conservation of the total enzyme concentration in (2.3) give

$$[C] = \frac{[E_0][S]}{K_M + [S]}, \tag{5.1}$$

where $K_M = (k_{-1}+k_2)/k_1$. The substrate concentration is then given by

$$\frac{d[S]}{dt} = -\frac{k_2[E_0][S]}{K_M+[S]}. \tag{5.2}$$

The corresponding differential equations for $[E]$ and $[P]$ can be written similarly. This approximation is known as the sQSSA of the Michaelis-Menten kinetics (2.1) under the deterministic setting.

Now, we use stochastic equations for the species copy numbers in (3.1) and apply the multiscale approximation to derive an analogue of (5.1)-(5.2). Equations like (5.2) have been previously derived from the stochastic reaction network [16, 17]. It was also revisited specifically using the multiscale approximation method in [2,27]. However, for the sake of completeness, we furnish a brief description below. Assuming that E and C are on the faster time scale than S and P, consider the scaled processes in (3.3) with the following scaling exponents:

$$\begin{aligned} &\alpha_S = \alpha_P = 1, \qquad \alpha_E = \alpha_C = 0, \\ &\beta_1 = 0, \qquad \beta_{-1} = \beta_2 = 1, \end{aligned} \tag{5.3}$$

that is, $\rho_1 = \alpha_S + \alpha_E + \beta_1 = 1$, $\rho_{-1} = \alpha_C + \beta_{-1} = 1$, and $\rho_2 = \alpha_C + \beta_2 = 1$. Note that when $\gamma = 0$, the above corresponds to assuming the abundances of the substrate and the product are order N while those of the enzyme and the enzyme-substrate complex are order 1. We are interested in the time scale of S given in (3.6). Plugging in the scaling exponent values in (5.3), the time scale of S we are interested in corresponds to $\gamma = 0$. Setting $\gamma = 0$ in the scaled stochastic equations in (3.3) and writing Z_i^N instead of $Z_i^{N,\gamma}$ for $i = S,E,C,P$ one obtains from (5.3). Define $M \equiv Z_E^N(t) + Z_C^N(t)$ and

$$\mathbb{Z}_C^N(t) \equiv \int_0^t Z_C^N(s)\,ds = Mt - \int_0^t Z_E^N(s)\,ds.$$

Note that $M = Z_E^N(0) + Z_C^N(0) = X_E(0) + X_C(0)$, and that M does not depend on the scaling parameter N. As done in [2, 27], assume that $Z_S^N(0) \to Z_S(0)$. The scaled variables Z_S^N and $\mathbb{Z}_C^N$ are bounded so they are relatively compact in the finite time interval $[0,\mathscr{T}]$, where $0 < \mathscr{T} < \infty$. Then, $\left(Z_S^N, \mathbb{Z}_C^N\right)$ converges to $(Z_S, \mathbb{Z}_C)$ as $N \to \infty$ and satisfies for every $t > 0$,

$$\begin{aligned} Z_S(t) &= Z_S(0) - \int_0^t \kappa_1 Z_S(s)\left(M - \dot{\mathbb{Z}}_C(s)\right) ds + \int_0^t \kappa_{-1}\dot{\mathbb{Z}}_C(s)\,ds, \\ 0 &= \int_0^t \kappa_1 Z_S(s)\left(M - \dot{\mathbb{Z}}_C(s)\right) ds - \int_0^t (\kappa_{-1}+\kappa_2)\,\dot{\mathbb{Z}}_C(s)\,ds. \end{aligned} \tag{5.4}$$

Note that we get (5.4) by dividing the equation for $Z_C^N(t)$ in (3.3) by N and taking the limit as $N \to \infty$. From (5.4), we get

$$\begin{aligned} \dot{Z}_S(t) &= -\frac{\kappa_2 M Z_S(t)}{\kappa_M + Z_S(t)}, \\ \dot{\mathbb{Z}}_C(t) &= \frac{M Z_S(t)}{\kappa_M + Z_S(t)}, \end{aligned} \tag{5.5}$$

where $\kappa_M = (\kappa_{-1}+\kappa_2)/\kappa_1$, which is precisely the sQSSA.

Note that we only use the Poisson law of large numbers and the conservation law to derive (5.5). In Figure 1, we compare the limit $Z_S(t)$ in (5.5) with the scaled substrate copy number $Z_S^N(t)$ in (3.3), obtained from 1000 realizations of the stochastic simulation (using the Gillespie algorithm [22]). Figure 1 shows the excellent agreement between the scaled process $Z_S^N(t)$ and its limit $Z_S(t)$.

Conditions for sQSSA in the deterministic system. We have shown that the scaling exponents (5.3) indeed yielded the sQSSA. We now show how the conditions (5.3) are related to the conditions proposed in the literature for the validity of the deterministic sQSSA. First, we consider a general condition derived by Segel [49] and Segel and Slemrod [50],

$$[E_0] \ll [S_0] + K_M, \tag{5.6}$$

where $K_M = (k_{-1} + k_2)/k_1$ is the Michaelis-Menten constant. We rewrite (5.6) in terms of the species copy numbers and the stochastic reaction rate constants. The stochastic and the deterministic reaction rates are related as follows

$$(k_1, k_{-1}, k_2) = \left(V\kappa_1', \kappa_{-1}', \kappa_2'\right), \tag{5.7}$$

where V is the system volume multiplied by the Avogadro's number [32]. We also use the relation between molecular numbers and molecular concentrations as

$$[i] = X_i(t)/V, \quad i = S, E, C, P. \tag{5.8}$$

Combining (5.7) and (5.8) with (5.6), and canceling out V, we get

$$X_{E_0} \ll X_{S_0} + \frac{\kappa_{-1}' + \kappa_2'}{\kappa_1'}. \tag{5.9}$$

Plugging in our choice of the scaled variables and rate constants given in (3.2) and (5.3) into (5.9) gives

$$Z_E^N(t) + Z_C^N(t) \ll N\left(Z_S^N(t) + Z_P^N(t)\right) + Z_C^N(t) + \frac{N(\kappa_{-1} + \kappa_2)}{\kappa_1}. \tag{5.10}$$

Since $Z_i^N(t) \approx O(1)$ and $\kappa_k \approx O(1)$, the left and the right sides of (5.10) become of order 1 and N, respectively. We see that our choice of the scaling in the stochastic model is in agreement with the conditions for the validity of the sQSSA in the deterministic model (5.6).

Note that the choice of scaling exponents in (5.3) is, in general, not unique. We now derive more general conditions on the scaling exponents, α's and β's, leading to the sQSSA limit (5.5). Note that for (5.5) to hold the time scale of C should be faster than that of S, so that we can obtain (5.4) from the equation of C, i.e.,

$$\alpha_C - \max(\rho_1, \rho_{-1}, \rho_2) < \alpha_S - \max(\rho_1, \rho_{-1}), \tag{5.11}$$

which is an analogue of $d[C]/dt \approx 0$. Moreover, for E to be expressed in terms of C and retained in the limit, the species copy number of C has to be greater than or equal to that of E in the conservation equation of the total enzyme

$$\alpha_E \leq \alpha_C. \tag{5.12}$$

Finally, all reaction propensities are of the same order so that all the terms are present in (5.5)

$$\rho_1 = \rho_{-1} = \rho_2. \tag{5.13}$$

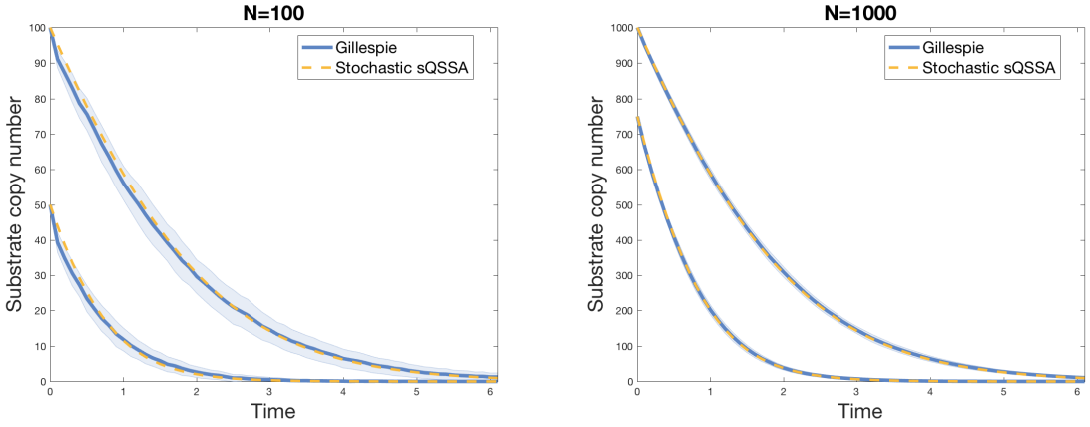
Combining (5.11), (5.12), and (5.13) together, we get the following conditions

$$\begin{aligned} &\alpha_E \leq \alpha_C < \alpha_S, \\ &\alpha_S + \beta_1 = \beta_{-1} = \beta_2. \end{aligned} \tag{5.14}$$

The second condition in (5.14) can be rewritten as $\alpha_S = \beta_{-1} - \beta_1 = \beta_2 - \beta_1$ and so (5.14) implies

$$\begin{aligned} &X_{E_0} \ll X_{S_0}, \\ &X_{E_0} \ll \frac{\kappa_{-1}'}{\kappa_1'} \approx \frac{\kappa_2'}{\kappa_1'}, \end{aligned}$$

which is comparable to the general condition (5.6) for the sQSSA in the deterministic system.



(a) (b)

Fig. 1: Michaelis-Menten kinetics with sQSSA derived from the stochastic system. The scaling limit of the substrate copy number, drawn in yellow dotted line, is compared with the mean substrate copy number (in blue) obtained from the direct simulations of the stochastic system (3.1). The light blue shaded region represents one standard deviation from the mean. Different choices of initial conditions are used to reflect the fact that convergence can be achieved under varying values of the conservation constant M. Simulation settings: **(a)** $N = 100$, $(X_S^N(0), X_E^N(0), X_C^N(0), X_P^N(0)) = (100, 10, 0, 0)$ for the upper curve and $(50, 20, 0, 0)$ for the lower curve; and **(b)** $N = 1000$, $(X_S^N(0), X_E^N(0), X_C^N(0), X_P^N(0)) = (1000, 10, 0, 0)$ for the upper curve and $(750, 20, 0, 0)$ for the lower curve. The reaction rate constants are $(\kappa_1, \kappa_{-1}, \kappa_2) = (1, 1, 0.1)$ in both **(a)** and **(b)**.

6 Total quasi-steady-state approximation (tQSSA)

In the tQSSA we introduce a new variable, namely the total substrate concentration $[T] \equiv [S] + [C]$. The idea behind the tQSSA is to get an accurate approximation of (2.2) for a wider range of dynamics (for example, for both high and low enzyme concentrations). Assuming that $[T]$ changes on the slow time scale, the equations (2.2)-(2.3) give the following reduced model [9,55]

$$\begin{aligned} \frac{d[T]}{dt} &= -k_2[C], \\ \frac{d[C]}{dt} &= k_1\left\{([T]-[C])([E_0]-[C]) - K_M[C]\right\}, \end{aligned} \tag{6.1}$$

where $K_M = (k_{-1} + k_2)/k_1$. Assuming that $d[C]/dt \approx 0$ and using $[C] \leq [E_0]$, the unique solution is found as the positive root of the quadratic equation

$$[C] = \frac{([E_0] + K_M + [T]) - \sqrt{([E_0] + K_M + [T])^2 - 4[E_0][T]}}{2}, \tag{6.2}$$

and the evolution of the total substrate concentration obeys

$$\frac{d[T]}{dt} = -k_2 \frac{([E_0] + K_M + [T]) - \sqrt{([E_0] + K_M + [T])^2 - 4[E_0][T]}}{2}. \tag{6.3}$$

The above approximation is the tQSSA of the Michaelis-Menten kinetics (2.1) derived from the deterministic equation (2.2).

Now, consider the stochastic model (3.1). We will apply the multiscale approximation with the appropriate scaling so that (6.3) is obtained as the limit of the stochastic system (3.3) for $N \to \infty$. We assume that S, E, and C are on the faster time scale than P. Then our choice of scaling is

$$\begin{aligned} &\alpha_S = \alpha_E = \alpha_C = \alpha_P = 1, \\ &\beta_1 = \beta_2 = 0, \quad \beta_{-1} = 1, \end{aligned} \tag{6.4}$$

that is, $\rho_1 = \alpha_S + \alpha_E + \beta_1 = 2$, $\rho_{-1} = \alpha_C + \beta_{-1} = 2$, and $\rho_2 = \alpha_C + \beta_2 = 1$. We are interested in behavior of the stochastic model in the time scale of T. Adding unscaled equations for S and C and dividing by $N^{\max(\alpha_S,\alpha_C)}$ from (3.3) we have

$$\begin{aligned} \frac{N^{\alpha_S} Z_S^{N,\gamma}(t) + N^{\alpha_C} Z_C^{N,\gamma}(t)}{N^{\max(\alpha_S,\alpha_C)}} = &\frac{N^{\alpha_S} Z_S^N(0) + N^{\alpha_C} Z_C^N(0)}{N^{\max(\alpha_S,\alpha_C)}} \\ &- \frac{1}{N^{\max(\alpha_S,\alpha_C)}} Y_2 \left(\int_0^t N^{\rho_2+\gamma} \kappa_2 Z_C^{N,\gamma}(s)\, ds \right). \end{aligned}$$

Thus, the time scale of T is given by

$$\gamma = \max(\alpha_S, \alpha_C) - \rho_2. \tag{6.5}$$

Using (6.4) gives $\gamma = 0$. We denote $Z_i^{N,\gamma}$ as Z_i^N for $i = S, E, C, P$ as we did in Section 5.

Define the new slow variable

$$Z_T^N(t) \equiv Z_S^N(t) + Z_C^N(t),$$

which satisfies

$$Z_T^N(t) = Z_T^N(0) - \frac{1}{N} Y_2 \left(\int_0^t N \kappa_2 Z_C^N(s)\, ds \right). \tag{6.6}$$

We have two conservation laws for the total amount of substrate and enzyme, $m^N \equiv Z_E^N(t) + Z_C^N(t)$ and $k^N \equiv Z_T^N(t) + Z_P^N(t)$, and we denote their limits as $N \to \infty$ by m and k, respectively. We also define

$$\mathbb{Z}_C^N(t) \equiv \int_0^t Z_C^N(s)\, ds = m^N t - \int_0^t Z_E^N(s)\, ds.$$

Since $Z_T^N(t) \le k^N \to k$ and $\mathbb{Z}_C^N(t) \le m^N t \to mt$, Z_T^N and $\mathbb{Z}_C^N$ are bounded, they are also relatively compact in the finite time interval $t \in [0, \mathscr{T}]$ where $0 < \mathscr{T} < \infty$. Since the law of large numbers implies that $Z_T^N(0) \to Z_T(0)$ as $N \to \infty$ then $\left(Z_T^N, \mathbb{Z}_C^N\right)$ (possibly along a subsequence only) converges to $(Z_T, \mathbb{Z}_C)$ which satisfies

$$\begin{aligned} Z_T(t) &= Z_T(0) - \int_0^t \kappa_2 \dot{\mathbb{Z}}_C(s)\, ds, \\ 0 &= \int_0^t \kappa_1 \left(Z_T(s) - \dot{\mathbb{Z}}_C(s) \right) \left(m - \dot{\mathbb{Z}}_C(s) \right) ds - \int_0^t \kappa_{-1} \dot{\mathbb{Z}}_C(s)\, ds. \end{aligned} \tag{6.7}$$

Note that (6.7) is the limit as $N \to \infty$ when we divide the equation for the scaled variable of C in (3.3) by N. Hence, we obtain

$$\dot{\mathbb{Z}}_C(t) = \frac{(m + \kappa_D + Z_T(t)) - \sqrt{(m + \kappa_D + Z_T(t))^2 - 4mZ_T(t)}}{2}, \tag{6.8}$$

$$\dot{Z}_T(t) = -\kappa_2 \frac{(m + \kappa_D + Z_T(t)) - \sqrt{(m + \kappa_D + Z_T(t))^2 - 4mZ_T(t)}}{2}, \tag{6.9}$$

where $\kappa_D \equiv \kappa_{-1}/\kappa_1$. The equations (6.8) and (6.9) are analogous to (6.2) and (6.3), respectively. Note that we only have κ_D in (6.8)-(6.9) instead of $K_M = (k_{-1} + k_2)/k_1$ in (6.2)-(6.3). The reaction rate κ_2 disappears,

since the propensity of the second reaction is of order of N, which is slower than the other two reactions whose propensities are of order N^2 as shown in (3.3). In Figure 2, we compare the limit $Z_T(t)$ in (6.9) and the scaled total substrate copy number $Z_T^N(t)$ in (6.6), obtained from 1000 realizations of the stochastic simulation using Gillespie's algorithm [22]. The plot indicates close agreement between the scaled process $Z_T^N(t)$ and its proposed approximation $Z_T(t)$.

Conditions for tQSSA in the deterministic system. To derive tQSSA from (6.1), it is assumed that the total substrate concentration changes in the slow time scale and that the complex reaches its steady state quickly after some transient time, that is, $d[C]/dt \approx 0$. The complex concentration $[C]$ is then found as the nonnegative solution of a quadratic equation. As mentioned earlier, Borghans et al. [9] approximated $[C]$ in a form simpler than the exact solution in (6.2) and found a necessary and sufficient condition for the validity of the tQSSA as

$$K[E_0] \ll ([E_0]+[S_0]+K_M)^2, \tag{6.10}$$

where $K = k_2/k_1$ and $K_M = (k_{-1}+k_2)/k_1$. The benefit of tQSSA over sQSSA is that (6.10) is always roughly valid [40, 55]. The condition (6.10) is equivalent to

$$1 \ll \left(1+\frac{[E_0]+[S_0]}{K}+\frac{k_{-1}}{k_2}\right)\left(1+\frac{[S_0]+K_M}{[E_0]}\right) \tag{6.11}$$

and is implied by any one of the following

$$\begin{aligned} &K \ll [E_0]+[S_0], \\ &k_2 \ll k_{-1}, \\ &[E_0] \ll [S_0]+K_M. \end{aligned} \tag{6.12}$$

We convert concentrations and deterministic rate constants to molecular numbers and stochastic rate constants using (5.7)-(5.8). After simplification, the condition in (6.10) becomes

$$\frac{\kappa_2'}{\kappa_1'}X_{E_0} \ll \left(X_{E_0}+X_{S_0}+\frac{\kappa_{-1}'+\kappa_2'}{\kappa_1'}\right)^2, \tag{6.13}$$

by using the same argument as in (5.9). Plugging our choice of the scaled variables and rate constants as specified in (3.2) and (6.4) yields

$$\frac{\kappa_2}{\kappa_1}N\left(Z_E^N(t)+Z_C^N(t)\right) \ll \left(N\left(Z_E^N(t)+Z_C^N(t)\right)+N\left(Z_S^N(t)+Z_C^N(t)+Z_P^N(t)\right)+\frac{N\kappa_{-1}+\kappa_2}{\kappa_1}\right)^2.$$

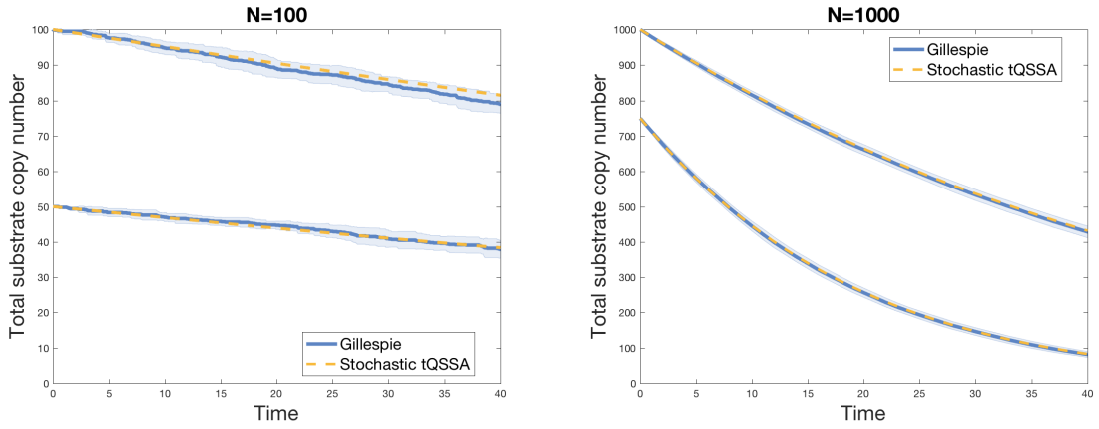
Since in the above expression the term on the left is $O(N)$ and the term on the right is $O(N^2)$, our choice of scaling in the stochastic model is in agreement with the condition (6.10) for the validity of the tQSSA in the deterministic model.

As in case of the sQSSA, we may again derive more general conditions on the scaling exponents, α's and β's, which will lead to tQSSA limit in (6.9). To this end note that the time scale of C is faster than that of T so that we can derive an analogue of $d[C]/dt \approx 0$ in (6.7)

$$\alpha_C - \max(\rho_1, \rho_{-1}, \rho_2) < \max(\alpha_S, \alpha_C) - \rho_2. \tag{6.14}$$

Moreover, the species copy number of C has an order greater than or equal to that of S, since otherwise C would disappear in the limit of T. Similarly, the species copy number of C has an order greater than or equal to that of E so that the limit for E can be expressed in terms of a conservation constant and C. Therefore, we have

$$\max(\alpha_S, \alpha_E) \le \alpha_C. \tag{6.15}$$



(a) (b)

Fig. 2: Michaelis-Menten kinetics with tQSSA. The scaling limit of the total substrate copy number, drawn in yellow dotted line, is compared with the mean total substrate copy number (in blue), obtained from direct simulations of the system (3.1). The light blue shaded region represents one standard deviation from the mean. Different choices of initial conditions are used to reflect the fact that convergence can be achieved under varying values of the conservation constant m. Simulation settings: **(a)** $N = 100$, $(X_S^N(0), X_E^N(0), X_C^N(0), X_P^N(0)) = (100, 10, 0, 0)$ for the upper curve and $(50, 10, 0, 0)$ for the lower curve; and **(b)** $N = 1000$, $(X_S^N(0), X_E^N(0), X_C^N(0), X_P^N(0)) = (1000, 10, 0, 0)$ for the upper curve and $(750, 25, 0, 0)$ for the lower curve. The reaction rate constants are $(\kappa_1, \kappa_{-1}, \kappa_2) = (1, 4, 1)$ in both **(a)** and **(b)**.

Finally, to obtain a quadratic equation with a square root solution in the limit, the enzyme binding reaction rate should be equal to the unbinding reaction rate. That is,

$$\rho_1 = \rho_{-1}. \tag{6.16}$$

Combining (6.14), (6.15), and (6.16), we get the following conditions

$$\begin{aligned} &\max(\alpha_S, \alpha_E) \leq \alpha_C, \\ &\beta_2 < \beta_{-1} = \alpha_C + \beta_1. \end{aligned} \tag{6.17}$$

Note that due to $\beta_2 < \beta_{-1}$ in (6.17), we have the discrepancy between κ_D in (6.9) and K_M in (6.3). In other words, the reason behind this discrepancy is that the propensity of the second reaction (product formation) is of order of N, which is slower than the other two reactions whose propensities are of order N^2 as shown in (3.3). Therefore, the reaction rate κ_2 disappears. This minor discrepancy essentially shows that even though the scaling exponents in the stochastic system are in agreement with those in the deterministic setting, the resultant limiting system may differ. Note that, the condition (6.17) implies

$$\begin{aligned} &X_{S_0} \approx X_{E_0}, \\ &\frac{\kappa_2'}{\kappa_1'} \ll \frac{\kappa_{-1}'}{\kappa_1'} \approx X_{E_0}, \end{aligned} \tag{6.18}$$

which is consistent with the condition $k_2 \ll k_{-1}$ in (6.12) that was also suggested for the stochastic system tQSSA in [5].

7 Reverse quasi-steady-state approximation (rQSSA)

In the deterministic rQSSA, it is assumed that the enzyme is in high concentration. In this approximation, two time scales are considered. Starting with an initial condition $([S],[E],[C],[P]) = ([S_0],[E_0],0,0)$ in (2.2), the enzyme concentration is $[E] \approx [E_0]$ during the initial transient phase. Since there is almost no complex during this time, we get an approximate model as

$$\begin{aligned} \frac{d[S]}{dt} &= -k_1[E_0][S], \\ \frac{d[C]}{dt} &= k_1[E_0][S]. \end{aligned} \tag{7.1}$$

After the initial transient phase, the substrate is depleted. Therefore, we assume that $d[S]/dt \approx 0$ in (2.2) and obtain

$$[S] = \frac{k_{-1}[C]}{k_1([E_0]-[C])}, \tag{7.2}$$

so that the differential equation for the complex becomes

$$\frac{d[C]}{dt} = -k_2[C]. \tag{7.3}$$

We refer to the approximation of the system (2.2) by (7.1)-(7.3) as the rQSSA of the Michaelis-Menten kinetics in the deterministic setting.

As in the previous sections, let us consider the stochastic equations for the Michaelis-Menten kinetics given by (3.1) and apply another multiscale approximation with time change, to derive the rQSSA in (7.1)-(7.3) as the limit of stochastic system. Assuming that S and C are on faster time scale than E and P, the following scales are chosen

$$\begin{aligned} &\alpha_S = \alpha_C = \alpha_P = 1, \quad \alpha_E = 2, \\ &\beta_1 = 0, \quad \beta_{-1} = \beta_2 = 1, \end{aligned} \tag{7.4}$$

that is, $\rho_1 = \alpha_S + \alpha_E + \beta_1 = 3$, $\rho_{-1} = \alpha_C + \beta_{-1} = 2$, and $\rho_2 = \alpha_C + \beta_2 = 2$. Note that this choice of scaling does not satisfy the balance equations introduced in (3.7). The inequalities for S and C give $\gamma \le -2$ and those for E and P give $\gamma \le -1$. These conditions suggest the first and the second time scales as $\gamma = -2$ when S and C become $O(1)$ and $\gamma = -1$ when E and P are $O(1)$. Define the following conservation constants

$$\begin{aligned} m^N &\equiv Z_E^{N,\gamma}(t) + \frac{1}{N} Z_C^{N,\gamma}(t), \\ k^N &\equiv Z_S^{N,\gamma}(t) + Z_C^{N,\gamma}(t) + Z_P^{N,\gamma}(t), \end{aligned} \tag{7.5}$$

which we assume to converge to some limiting values m and k as $N \to \infty$, respectively. In this setting, $Z_S^{N,\gamma}$, $Z_E^{N,\gamma}$, $Z_C^{N,\gamma}$, and $Z_P^{N,\gamma}$ are bounded so that they are relatively compact for $t \in [0, \mathscr{T}]$, where $0 < \mathscr{T} < \infty$. In the first time scale when $\gamma = -2$, the scaled species for E and P converge to their initial conditions, $Z_E^{N,-2}(t) \to Z_E(0)$ and $Z_P^{N,-2}(t) \to Z_P(0)$ as $N \to \infty$, since the scaling exponents in the propensities are greater than those of species copy numbers in this time scale. Therefore $\left(Z_S^{N,-2}, Z_C^{N,-2}\right)$ converges to $\left(Z_S^{(-2)}, Z_C^{(-2)}\right)$ satisfying

$$\begin{aligned} Z_S^{(-2)}(t) &= Z_S(0) - \int_0^t \kappa_1 Z_S^{(-2)}(s) Z_E(0)\, ds, \\ Z_C^{(-2)}(t) &= Z_C(0) + \int_0^t \kappa_1 Z_S^{(-2)}(s) Z_E(0)\, ds. \end{aligned} \tag{7.6}$$

Since $Z_C^{N,-2}(t)$ is bounded by k^N from (7.5), the remaining reaction terms for the unbinding of the complex and for the product production vanish as $N \to \infty$. The equations (7.6) are seen as the integral version of (7.1), that is, the rQSSA for the first (transient) time scale.

Next, consider the second time scale when $\gamma = -1$. Plugging $\gamma = -1$ in the equation for S in (3.3), and applying the law of large numbers, we obtain

$$Z_S^{N,-1}(t) \approx Z_S^N(0) - \int_0^t \left(N\kappa_1 Z_S^{N,-1}(s) Z_E^{N,-1}(s) - \kappa_{-1} Z_C^{N,-1}(s) \right) ds. \tag{7.7}$$

Using (7.7), the equations for E and C in (3.3) become

$$Z_C^{N,-1}(t) \approx Z_C^N(0) + Z_S^N(0) - Z_S^{N,-1}(t) - \int_0^t \kappa_2 Z_C^{N,-1}(s)\, ds, \tag{7.8}$$

$$Z_E^{N,-1}(t) \approx Z_E^N(0) - \int_0^t \kappa_1 Z_S^{N,-1}(s) Z_E^{N,-1}(s)\, ds, \tag{7.9}$$

since the remaining reaction terms are asymptotically equal to zero. Dividing (7.7) by N, we obtain

$$\int_0^t \kappa_1 Z_S^{N,-1}(s) Z_E^{N,-1}(s)\, ds \to 0, \tag{7.10}$$

as $N \to \infty$, since all other terms vanish asymptotically. Due to (7.9) and (7.10), $Z_E^{N,-1}(t) \to Z_E(0)$ as $N \to \infty$. Defining $\mathbb{Z}_S^{N,-1}(t) \equiv \int_0^t Z_S^{N,-1}(s)\, ds$ and using (7.10) and (7.8), we conclude that $\left(\mathbb{Z}_S^{N,-1}, Z_C^{N,-1} \right)$ converges to $\left(\mathbb{Z}_S^{(-1)}, Z_C^{(-1)} \right)$ satisfying

$$\begin{aligned} 0 &= \int_0^t \kappa_1 \dot{\mathbb{Z}}_S^{(-1)}(s) Z_E(0)\, ds, \\ Z_C^{(-1)}(t) &= Z_C(0) + Z_S(0) - \dot{\mathbb{Z}}_S^{(-1)}(t) - \int_0^t \kappa_2 Z_C^{(-1)}(s)\, ds. \end{aligned} \tag{7.11}$$

Therefore,

$$\begin{aligned} \dot{\mathbb{Z}}_S^{(-1)}(t) &= 0, \\ \dot{Z}_C^{(-1)}(t) &= -\kappa_2 Z_C^{(-1)}(t), \end{aligned} \tag{7.12}$$

which is the analogue of the rQSSA in the second time scale (7.2)-(7.3) as derived from the deterministic model.

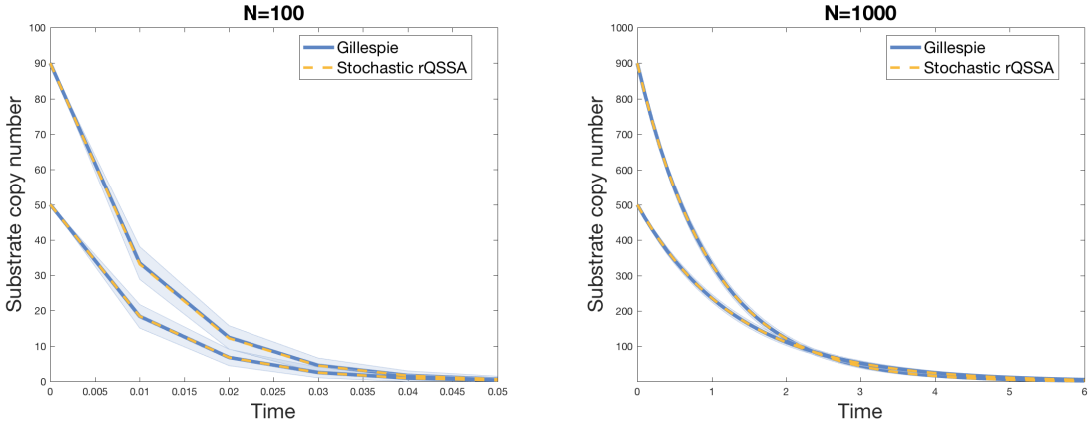
We illustrate the quality of rQSSA in the stochastic Michaelis-Menten system with some simulations. In Figure 3, we compare the limit $Z_S^{(-2)}(t)$ in (7.6) and the scaled substrate copy number $Z_S^{N,-2}(t)$ in (3.3) using 1000 runs of the Gillespie's algorithm. In Figure 4, we compare the limit $Z_C^{(-1)}(t)$ in (7.12) and the scaled complex copy number $Z_C^{N,-1}(t)$ in (3.3) using 10000 runs of the Gillespie's algorithm. Note that the initial condition of $Z_C^{(-1)}(t)$ is $Z_C(0) + Z_S(0)$ in (7.11). However, this does not affect since $Z_S(0) = 0$ in our simulation in Figure 4. In both time scales, the scaled processes are in close agreement with the proposed limits.

Conditions for rQSSA in the deterministic system. Consider the general condition for the validity of the rQSSA at high enzyme concentrations suggested by Schnell and Maini [46],

$$K \ll [E_0] \quad \text{and} \quad [S_0] \ll [E_0], \tag{7.13}$$

where $K = k_2/k_1$. Rewriting (7.13) in terms of molecular copy numbers and stochastic rate constants using (5.7)-(5.8) gives

$$\frac{\kappa_2'}{\kappa_1'} \ll X_{E_0} \quad \text{and} \quad X_{S_0} \ll X_{E_0}, \tag{7.14}$$



(a) (b)

Fig. 3: Michaelis-Menten kinetics with rQSSA in the first time scale $\gamma = -2$: The scaling limit of the substrate copy number, drawn in yellow dotted line, is compared with the mean substrate copy number (in blue) obtained from direct simulations of the system (3.1). The light blue shaded region represents one standard deviation from the mean. Simulation settings: **(a)** $N = 100$, $(X_S^N(0), X_E^N(0), X_C^N(0), X_P^N(0)) = (90, 10^6, 10, 0)$ for the upper curve and $(50, 10^6, 10, 0)$ for the lower curve; and **(b)** $N = 1000$, $(X_S^N(0), X_E^N(0), X_C^N(0), X_P^N(0)) = (900, 10^6, 10, 0)$ for the upper curve and $(500, 75 \cdot 10^4, 110, 0)$ for the lower curve. The reaction rate constants are $(\kappa_1, \kappa_{-1}, \kappa_2) = (1, 1, 0.1)$ in both **(a)** and **(b)**. Given the scaling assumptions, the convergence is not sensitive to the exact values of the initial conditions. The only purpose of the two different sets of initial conditions is to illustrate convergence under varying values of the conservation constant m.

since V's all cancel out. Using our choice of scaling in (3.2) and (7.4), the conditions (7.14) become

$$\begin{aligned} &\frac{N\kappa_2}{\kappa_1} \ll \left(N^2 Z_E^{N,\gamma}(t) + N Z_C^{N,\gamma}(t)\right) \quad \text{and} \\ &N\left(Z_S^{N,\gamma}(t) + Z_C^{N,\gamma}(t) + Z_P^{N,\gamma}(t)\right) \ll \left(N^2 Z_E^{N,\gamma}(t) + N Z_C^{N,\gamma}(t)\right). \end{aligned} \tag{7.15}$$

Since the inequalities in (7.15) hold for large N, our choice of scaling is seen to satisfy the conditions (7.13).

As in the previous sections, we may also derive more general conditions on the scaling exponents, α's and β's, leading to (7.6) and (7.12). In the first scaling, the time scales of S and C are the same and faster than the time scale of E. Therefore it follows that

$$\alpha_S - \max(\rho_1, \rho_{-1}) = \alpha_C - \max(\rho_1, \rho_{-1}, \rho_2) < \alpha_E - \max(\rho_1, \rho_{-1}, \rho_2). \tag{7.16}$$

Since the binding reaction rate of the enzyme is faster than the rates of the other two reactions as we see in the limit (7.6), we have

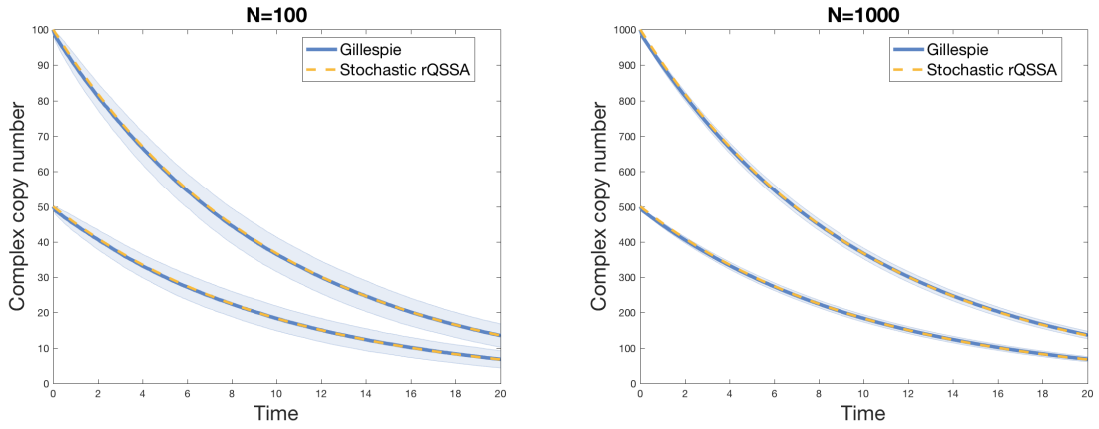
$$\max(\rho_{-1}, \rho_2) < \rho_1. \tag{7.17}$$

Combining (7.16) and (7.17), the conditions in the first time scale are

$$\begin{aligned} &\alpha_S = \alpha_C < \alpha_E, \\ &\max(\beta_{-1}, \beta_2) < \alpha_E + \beta_1. \end{aligned} \tag{7.18}$$

Then, the condition in (7.18) implies

$$\begin{aligned} &X_{S_0} \ll X_{E_0}, \\ &\max\left(\frac{\kappa'_{-1}}{\kappa'_1}, \frac{\kappa'_2}{\kappa'_1}\right) \ll X_{E_0}, \end{aligned} \tag{7.19}$$



(a) (b)

Fig. 4: Michaelis-Menten kinetics with rQSSA in the second time scale $\gamma = -1$: The scaling limit of the complex copy number, drawn in yellow dotted line, is compared with the mean complex copy number (in blue) obtained from direct simulations of the system (3.1). The light blue shaded region represents one standard deviation from the mean. Simulation settings: **(a)** $N = 100$, $(X_S^N(0), X_E^N(0), X_C^N(0), X_P^N(0)) = (0, 10^4, 100, 0)$ for the upper curve and $(0, 7500, 50, 0)$ for the lower curve; and **(b)** $N = 1000$, $(X_S^N(0), X_E^N(0), X_C^N(0), X_P^N(0)) = (0, 10^5, 10^3, 0)$ for the upper curve and $(0, 75 \cdot 10^3, 500, 0)$ for the lower curve. The reaction rate constants are $(\kappa_1, \kappa_{-1}, \kappa_2) = (1, 1, 0.1)$ in both **(a)** and **(b)**. Given the scaling assumptions, the convergence is not sensitive to the exact values of the initial conditions. The only purpose of the two different sets of initial conditions is to illustrate convergence under varying values of the conservation constant m.

which is comparable to (7.13).

Next, consider the second time scale and the condition on the scaling exponents that yields (7.12). Note that the conditions (7.16)-(7.17) are already sufficient to derive the limiting process in the second time scale. The condition (7.16) implies the time scales of S and C are the same. Since $\rho_2 < \rho_1$ as in (7.17), the time scale of $S + C$ is slower than that of S. Setting the time scale of $S + C$ as the reference one, we see that on that timescale S will be rapidly depleted and then approximated by zero in view of the discrepancy between the consumption and production rates of S, due to $\rho_{-1} < \rho_1$ in (7.17). Therefore, the conditions in (7.16)-(7.17) are sufficient to obtain the limit in (7.12) on the second time scale as well. Finally, note that the stochastic Michaelis-Menten system with (7.18) does not provide an analogue equation for S in (7.2) due to the condition, $\rho_{-1} < \rho_1$, as shown in (7.17). Assuming $\rho_{-1} = \rho_1$ will balance production and consumption of S, but in this case we can no longer claim the relative compactness of S.

8 Enzyme-Substrate-Inhibitor System

Our multiscaling approach to deriving QSSAs for enzyme kinetics may be extended to more realistic reaction networks. To illustrate this, consider the following known as ESL and described in [15]. Inhibitors are compounds that diminish the rate of enzyme-catalyzed reactions. They form complexes with the enzymes that exhibit a wide variety of catalytic properties. The most common type of inhibition is the competitive inhibition, where the inhibitor *competes* with the substrate in that it binds to the same site on the enzyme as the substrate. Interestingly, similar inhibitory behavior is also observed when more substrates that bind with the same enzyme are present [15, Chapter 4]. This is commonly observed in many industrial applications. Each substrate competes with other substrates for the same catalytic site and inhibit each others' enzymatic reactions. Note that there are also other variants of ESI system depending on the nature of competitiveness, such

as the uncompetitive inhibition system, mixed inhibition system, substrate inhibition system etc [15, Chapter 4]. However, since the approach to derive QSSAs remains the same across these variants, for the purpose of illustration we only consider here the fully competitive ESI system described by the following set of chemical reactions [49]:

$$\begin{aligned} S+E &\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_1 \xrightarrow{k_2} P_1+E, \\ I+E &\underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_2 \xrightarrow{k_4} P_2+E, \end{aligned} \tag{8.1}$$

where C_1, C_2 are, respectively, the substrate-enzyme and the inhibitor-enzyme complexes; P_1, and P_2 are the respective products; and S, E denote, as before, the substrate and free enzyme. Classically, the enzyme-substrate-inhibitor system has been studied using ODEs for the concentrations of the various species, as was done for the Michaelis-Menten system. In order to specify our stochastic model, let X_S, X_I, X_E, X_{C_1}, X_{C_2}, X_{P_1}, and X_{P_2} denote the copy numbers of molecules of the substrates S, the inhibitors I, the enzymes E, the enzyme-substrate complex C_1, the inhibitor-enzyme complex C_2, and the products P_1, P_2 respectively. As done in Section 3, we assume a Markovian dynamics for these copy numbers along with law of mass-action. We also introduce the scaled processes and the necessary exponents:

$$\begin{aligned} X_i(t) = N^{\alpha_i} Z_i^N(t), \text{ for } i = S,I,E,C_1,C_2,P_1,P_2, \\ \text{and } \kappa_k' = N^{\beta_k}\kappa_k, \text{ for } k = 1,-1,3,-3,2,4, \end{aligned} \tag{8.2}$$

Standard QSSA (sQSSA) for the ESI system The standard QSSA (sQSSA) for the ESI system is analogous to that for the Michaelis-Menten enzyme kinetics described in Section 5. Here, one assumes both the enzyme-substrate complex C_1 and the inhibitor-enzyme complex C_2 reach a steady-state quickly after a brief transient phase while the other species still remain transient. Therefore, one sets $d[C_1]/dt \approx 0$ and $d[C_2]/dt \approx 0$. We use the following scaling exponents:

$$\begin{aligned} &\alpha_S = \alpha_I = \alpha_{P_1} = \alpha_{P_2} = 1, \qquad \alpha_E = \alpha_{C_1} = \alpha_{C_2} = 0, \\ &\beta_1 = \beta_3 = 0, \qquad \beta_{-1} = \beta_{-3} = \beta_2 = \beta_4 = 1, \end{aligned} \tag{8.3}$$

to obtain the stochastic sQSSA, which is analogous to its deterministic counterpart [40, 49]:

$$\dot{Z}_S(t) = -\frac{\kappa_2 M Z_S(t)}{\kappa_M^{(1)}\left(1+\frac{Z_I(t)}{\kappa_M^{(2)}}\right)+Z_S(t)}, \tag{8.4}$$

$$\dot{Z}_I(t) = -\frac{\kappa_4 M Z_I(t)}{\kappa_M^{(2)}\left(1+\frac{Z_S(t)}{\kappa_M^{(1)}}\right)+Z_I(t)}, \tag{8.5}$$

where $M \equiv Z_E^N(t)+Z_{C_1}^N(t)+Z_{C_2}^N(t)$, $\kappa_M^{(1)} = (\kappa_{-1}+\kappa_2)/\kappa_1$ and $\kappa_M^{(2)} = (\kappa_{-3}+\kappa_4)/\kappa_3$. For the sake of brevity the detailed calculations are omitted as they largely follow the familiar pattern from previous sections. With regards to the validity of the sQSSA, the following conditions were proposed by [49]:

$$[E_0] \ll \kappa_M^{(2)}\left(1+\frac{[S_0])}{\kappa_M^{(1)}}\right)+[I_0] \quad \text{and} \quad [E_0] \ll \kappa_M^{(1)}\left(1+\frac{[I_0])}{\kappa_M^{(2)}}\right)+[S_0], \tag{8.6}$$

where $[E_0] \equiv [E]+[C_1]+[C_2]$, $[S_0] \equiv [S]+[C_1]+[P_1]$ and $[I_0] \equiv [I]+[C_2]+[P_2]$ describe the conservation laws in the system. As done in Section 5, rewriting (8.6) in terms of the species copy numbers, we see that the left hand sides of both inequalities in (8.6) correspond to $Z_E+Z_{C_1}+Z_{C_2}$, which is order 1. On the other hand, $\kappa_M^{(2)}\left(1+\frac{[S_0])}{\kappa_M^{(1)}}\right)+[I_0]$ simplifies to $N\left(\frac{\kappa_{-3}+\kappa_4}{\kappa_3}+\frac{(\kappa_{-3}+\kappa_4)\kappa_1}{\kappa_3(\kappa_{-1}+\kappa_2)}(Z_S+Z_{P_1})+(Z_I+Z_{P_2})\right)+\frac{(\kappa_{-3}+\kappa_4)\kappa_1}{\kappa_3(\kappa_{-1}+\kappa_2)}Z_{C_1}+$

Z_{C_2}, which is of order N. In a similar fashion, the quantity $\kappa_M^{(1)}\left(1+\frac{[I_0])}{\kappa_M^{(2)}}\right)+[S_0]$ can be simplified to $N\left(\frac{\kappa_{-1}+\kappa_2}{\kappa_1}+\frac{(\kappa_{-1}+\kappa_2)\kappa_3}{\kappa_1(\kappa_{-3}+\kappa_4)}(Z_I+Z_{P_2})+(Z_S+Z_{P_1})\right)+\frac{(\kappa_{-1}+\kappa_2)\kappa_3}{\kappa_1(\kappa_{-3}+\kappa_4)}Z_{C_2}+Z_{C_1}$, which is also of order N. Therefore, the condition (8.6) is included in the validity region for the stochastic sQSSA.

Total QSSA (tQSSA) for the ESI system In [40], the authors propose the tQSSA for the ESI system. Following [9], they define two new total substrates as follows:

$$T_1 \equiv S+C_1, \quad \text{and} \quad T_2 \equiv I+C_2. \tag{8.7}$$

Applying the usual quasi-steady state approximation $d[C_1]/dt \approx 0$ and $d[C_2]/dt \approx 0$, and assuming $[C_1] < [T_1], [C_2] < [T_2]$, one can rewrite the system of ODEs in terms of T_1 and T_2. This yields the total QSSA for the ESI system [40]. To obtain the stochastic tQSSA, we apply the following scalings:

$$\begin{aligned} &\alpha_S = \alpha_I = \alpha_{P_1} = \alpha_{P_2} = \alpha_E = \alpha_{C_1} = \alpha_{C_2} = 1, \\ &\beta_1 = \beta_3 = \beta_2 = \beta_4 = 0, \qquad \beta_{-1} = \beta_{-3} = 1. \end{aligned} \tag{8.8}$$

In order to specify the conservation laws, let $m^N \equiv Z_E^N(t)+Z_{C_1}^N(t)+Z_{C_2}^N(t)$, and $l_1^N \equiv Z_{T_1}^N(t)+Z_{P_1}^N(t), l_2^N \equiv Z_{T_2}^N(t)+Z_{P_2}^N(t)$. We assume $m^N \to m, l_1^N \to l_1, l_2^N \to l_2$ as $N \to \infty$. Now, define the cumulative processes:

$$\mathbb{Z}_{C_1}^N(t) \equiv \int_0^t Z_{C_1}^N(s)\,ds \quad \text{and} \quad \mathbb{Z}_{C_2}^N(t) \equiv \int_0^t Z_{C_2}^N(s)\,ds,$$

so that $\mathbb{Z}_{C_1}^N(t)+\mathbb{Z}_{C_2}^N(t) = m^N t - \int_0^t Z_E^N(s)\,ds$. Then, given the constants $\kappa_D^{(1)} \equiv \kappa_{-1}/\kappa_1$, and $\kappa_D^{(2)} \equiv \kappa_{-3}/\kappa_3$, the stochastic tQSSA is given by

$$\dot{\mathbb{Z}}_{C_1} = m - \left(m-\dot{\mathbb{Z}}_{C_1}(t)\left(1+\frac{\kappa_D^{(1)}}{Z_{T_1}(t)-\dot{\mathbb{Z}}_{C_1}(t)}\right)\right)\left(1+\frac{\kappa_D^{(2)}}{Z_{T_2}(t)-m-\dot{\mathbb{Z}}_{C_1}(t)\left(1+\frac{\kappa_D^{(1)}}{Z_{T_1}(t)-\dot{\mathbb{Z}}_{C_1}(t)}\right)}\right),$$

which implies the steady-state concentration of the substrate-enzyme complex is found by solving the following cubic equation for a positive root:

$$\begin{aligned} p_3^{(1)}(\dot{\mathbb{Z}}_{C_1}) \equiv &-(\kappa_D^{(1)}-\kappa_D^{(2)})(\dot{\mathbb{Z}}_{C_1})^3 + \left((m+\kappa_D^{(1)}+Z_{T_1}(t))(\kappa_D^{(1)}-\kappa_D^{(2)}) - (Z_{T_1}(t)\kappa_D^{(2)}+Z_{T_2}(t)\kappa_D^{(1)})\right)(\dot{\mathbb{Z}}_{C_1})^2 \\ &+\left(-m(\kappa_D^{(1)}-\kappa_D^{(2)})+\kappa_D^{(2)}(m+\kappa_D^{(1)})+(Z_{T_1}(t)\kappa_D^{(2)}+Z_{T_2}(t)\kappa_D^{(1)})\right)Z_{T_1}(t)\dot{\mathbb{Z}}_{C_1} - m\kappa_D^{(2)}(Z_{T_1}(t))^2. \end{aligned} \tag{8.9}$$

An analogous third degree polynomial can be written for $\dot{\mathbb{Z}}_{C_2}$. Finally, the tQSSA for the totals is expressed as follows:

$$\dot{Z}_{T_1}(t) = -\kappa_2\dot{\mathbb{Z}}_{C_1}, \quad \text{and} \quad \dot{Z}_{T_2}(t) = -\kappa_4\dot{\mathbb{Z}}_{C_2}, \tag{8.10}$$

where $\dot{\mathbb{Z}}_{C_1}$, and $\dot{\mathbb{Z}}_{C_2}$ satisfy their respective cubic equations [40].

Interestingly, when the substrate and the inhibitor have identical affinity towards the enzyme in that $\kappa_D^{(1)} = \kappa_D^{(2)} = \kappa_D$, the third degree polynomial $p_3^{(1)}$ reduces to a second degree polynomial, allowing for simpler computations. In that case, the tQSSA limiting ODEs are given by

$$\begin{aligned} \dot{Z}_{T_1}(t) &= -\kappa_2\frac{Z_{T_1}(t)\,(Z_T(t)+\kappa_D+m)}{2Z_T(t)}\left(1-\sqrt{1-\frac{4mZ_T(t)}{(Z_T(t)+\kappa_D+m)^2}}\right), \\ \dot{Z}_{T_2}(t) &= -\kappa_4\frac{Z_{T_2}(t)\,(Z_T(t)+\kappa_D+m)}{2Z_T(t)}\left(1-\sqrt{1-\frac{4mZ_T(t)}{(Z_T(t)+\kappa_D+m)^2}}\right), \end{aligned} \tag{8.11}$$

where $Z_T(t) \equiv Z_{T_1}(t) + Z_{T_2}(t)$ is the total of the substrate, the inhibitor, the substrate-enzyme complex and the inhibitor-enzyme complex. Note that the limiting equations given in (8.10) and (8.11) are analogous to their deterministic counterparts with the exception that we have $\kappa_D^{(1)}, \kappa_D^{(2)}$ instead of the Michaelis-Menten type constants $\kappa_M^{(1)}, \kappa_M^{(2)}$. The reason behind this discrepancy is that the propensities of the product formations are of order N, which are slower than the other reactions leading to the disappearance of the constants κ_2 and κ_4.

Regarding the validity of the tQSSA, the following sufficient condition was proposed by [40]:

$$\max\{\frac{k_2 C_1([T_0^{(1)}],[T_0^{(2)}])}{[T_0^{(1)}]}, \frac{k_4 C_2([T_0^{(1)}],[T_0^{(2)}])}{[T_0^{(2)}]}\} \max\{\frac{C_1([T_0^{(1)}],[T_0^{(2)}])}{k_1[E_0][T_0^{(1)}]}, \frac{C_2([T_0^{(1)}],[T_0^{(2)}])}{k_3[E_0][T_0^{(2)}]}\} \ll 1, \qquad (8.12)$$

where, as before, $[E_0] \equiv [E] + [C_1] + [C_2]$, $[T_0^{(1)}] \equiv [S] + [C_1]$ and $[T_0^{(2)}] \equiv [I] + [C_2]$, and $C_1([T_0^{(1)}],[T_0^{(2)}])$, and $C_2([T_0^{(1)}],[T_0^{(2)}])$ are the steady-state concentrations of the substrate-enzyme complex and the inhibitor-enzyme complex treated as functions of the initial conditions $[T_0^{(1)}],[T_0^{(2)}]$. Since the quantities C_1, C_2 are to be obtained as a positive root to a cubic equation analogous to (8.9), a direct comparison with the stochastic validity conditions is cumbersome. However, for the special case of identical affinity, we can simplify the equations and do a qualitative comparison. When the substrate and the inhibitor exhibit the same affinity, the quantities C_1 and C_2 admit the following relatively simpler expressions [40]:

$$\begin{aligned} C_1([T_0^{(1)}],[T_0^{(2)}]) &= \frac{[T_0^{(1)}]\,([T_0]+K_D+[E_0])}{2[T_0]}\left(1-\sqrt{1-\frac{4[E_0][T_0]}{([T_0]+K_D+[E_0])^2}}\right), \\ C_2([T_0^{(1)}],[T_0^{(2)}]) &= \frac{[T_0^{(2)}]\,([T_0]+K_D+[E_0])}{2[T_0]}\left(1-\sqrt{1-\frac{4[E_0][T_0]}{([T_0]+K_D+[E_0])^2}}\right), \end{aligned} \qquad (8.13)$$

where $K_D = k_{-1}/k_1$, $[T_0] \equiv [T_0^{(1)}] + [T_0^{(2)}]$. Then, the sufficient condition proposed by [40] in (8.12) can be rewritten as

$$[E_0] \gg \frac{k_2}{k_1}\frac{([T_0]+K_D+[E_0])^2}{4[T_0]^2}\left(1-\sqrt{1-\frac{4[E_0][T_0]}{([T_0]+K_D+[E_0])^2}}\right)^2,$$

which allows for a direct comparison with our stochastic system. As done in Section 6, if we convert the concentrations appearing above to molecular species copy numbers in our stochastic system, we can immediately see that the left hand side of the above inequality is of order N. On the other hand, the right hand side is of order 1. To see this, note that the quantity $\frac{([T_0]+K_D+[E_0])^2}{4[T_0]^2}$ corresponds to $\frac{(Z_T(t)+\kappa_D+m)^2}{(Z_T(t))^2}$ and therefore, is of order 1. Similarly, the quantity under the square root sign is also of order 1 for our choice of the scaling exponents. Therefore, the inequality above is satisfied in our stochastic setup and the validity region for the stochastic system tQSSA is therefore included in the validity region for the deterministic system tQSSA.

9 Summary and Discussion

In this paper, we derived the popular s- t- and rQSSA for the Michaelis-Menten model of enzyme kinetics from general stochastic equations describing mass-action interactions between enzyme, substrate and enzyme-substrate complex in terms of a jump Markov process. We have shown that these various QSSAs are consequences of the Poisson law of large numbers under the appropriately chosen scaling regimes. Our derivations relied on the general multiscale approximation approach [4, 27] for stochastic enzyme kinetics network that could be also applied to identify similar QSSAs in other more complicated chemical reactions models. For illustration, we briefly outlined derivation of two QSSAs in the enzyme-substrate-inhibitor system of [49]. We remark that another important class of models with enzyme-type dynamics where our QSSA derivations could be potentially applicable are the networks of protein phosphorylation in signaling transduction, such as the mitogen-activated protein kinase (MAPK) pathway [7, 18, 24]. In MAPK signaling pathway,

Conditions on	sQSSA	tQSSA	rQSSA
stochastic scaling	$\alpha_E \le \alpha_C < \alpha_S$ $\alpha_S = \beta_{-1} - \beta_1 = \beta_2 - \beta_1$	$\max(\alpha_S, \alpha_E) \le \alpha_C$ $\beta_2 < \beta_{-1} = \alpha_C + \beta_1$	$\alpha_S = \alpha_C < \alpha_E$ $\max(\beta_{-1}, \beta_2) < \alpha_E + \beta_1$
stochastic abundance	$X_{E_0} \ll X_{S_0}$ $X_{E_0} \ll \frac{\kappa'_{-1}}{\kappa'_1} \approx \frac{\kappa'_2}{\kappa'_1}$	$X_{E_0} \approx X_{S_0}$ $\frac{\kappa'_2}{\kappa'_1} \ll \frac{\kappa'_{-1}}{\kappa'_1} \approx X_{E_0}$	$X_{S_0} \ll X_{E_0}$ $\max\left(\frac{\kappa'_{-1}}{\kappa'_1}, \frac{\kappa'_2}{\kappa'_1}\right) \ll X_{E_0}$
deterministic abundance	$[E_0] \ll [S_0] + K_M$	$K[E_0] \ll ([E_0] + [S_0] + K_M)^2$	$K \ll [E_0]$ and $[S_0] \ll [E_0]$

Table 1: Comparison of conditions for the quasi-steady-state approximations in the stochastic and deterministic Michaelis-Menten kinetics. The X_i's denote the species copy numbers in the stochastic setting. In the deterministic setting, $[\cdot]$ is used to denote the species concentrations. Recall that the α's correspond to the scaling of species abundance ($X_i \approx O(N^{\alpha_i})$) while the β's correspond to the scaling of the reaction rate ($\kappa'_k \approx O(N^{\beta_k})$). The parameters are $K = k_2/k_1$ and $K_M = (k_{-1} + k_2)/k_1$.

the product of one level of the cascade may act as the enzyme at the next level, with different Michaelis-Menten QSSAs found to be appropriate at different levels [7, 18, 24, 44].

In order to summarize the general results on the s- t- and r-QSSAs discussed in the paper, we list in Table 1, the conditions for validity of different QSSAs in terms of their scaling exponents and the stochastic and deterministic species abundances. We note that the conditions for the stochastic scalings presented in the first row of the table clearly separate the range of parameter values into three regimes. As can bee seen, in case of the sQSSA the exponent α_S should be greater than the other species scaling exponents, while for rQSSA the exponent α_E should be greater. In the tQSSA, α_C needs to be greater than or equal to the other exponents. For the sQSSA and the rQSSA, the stochastic species abundance conditions (listed in the second row) are seen to also imply the deterministic abundance conditions (listed in the third row). However, the necessary condition for the tQSSA derived from the stochastic model is slightly different then the corresponding deterministic model condition, as it requires similar order of magnitude for the total amount of enzyme and the total amount of substrate. Note, however, that the condition on the deterministic rates $k_2 \ll k_{-1}$, which is an analog of the stochastic rates condition $\kappa'_2 \ll \kappa'_{-1}$, implies both the deterministic and the stochastic abundance conditions for the tQSSA.

The QSSAs for the stochastic Michaelis-Menten kinetics lead to ODE systems where reaction propensities follow the rational or square-root functions. Such non-mass-action propensity functions based on the QSSAs are often used as intensity functions in the random time change representation of the approximating Poisson processes in order to help build more accurate reduced stochastic models. See, for instance, Grima et al.[25], Chow et al. [13], as well as others [28, 54] who have applied this idea to construct approximate, stochastic Michaelis-Menten kinetic networks or even gene regulatory networks [51]. However, in some cases such approximations have been also found to perform poorly. Indeed, the authors investigating this issue recently in [53] found the relative error of the approximation using Michaelis-Menten propensities to be as high as 30% for certain reaction networks. Similarly, Kim [29, 31] considered accuracy of the related heuristic stochastic simulation scheme where additionally the fast reactions were omitted to improve computational efficiency and concluded that the approach was reasonably accurate under tQSSA but not under other types of QSSAs. As some of the current authors argued in their recent joint work with Kim ([30]), such approximations often perform better when one simultaneous re-scales the reaction rates and chemical species. It is hoped that our derivations presented here could be used to suggest such re-scalings, at least for networks satisfying certain parametric restrictions like, for instance, those presented in Table 1 [29, 42, 43].

The approach to QSSAs for the stochastic Michaelis-Menten enzyme kinetics often presented in the chemical physics literature relies on the two-step procedure. First, one applies the large volume van Kampen limit (also known as the system size expansion) to obtain the limiting ODE system, and then one derives the QSSAs for the deterministic system by applying various scaling relations. Since the ODE approximation to the stochastic system is typically not uniform with respect to the rate parameters, this two step approximation may be correct or not, depending the specific scalings used and the values of the reaction rates. In contrast to

the two-step approach, we derive here the QSSAs directly from the stochastic system as a scaling limit of a subset of the reaction rates and the chemical species. The scaling exponents in our derivations are chosen so that the resultant scaling limits match those derived from the ODEs, thereby providing further insights into the validity of these approximations across ranges of species and parameter values. Such approach is relevant not only when one needs to estimate the parameters of the Michaelis-Menten kinetics (see, for instance, [13] for application to the tQSSA), but also when the reaction rates vary over several orders of magnitude, which is often the case in biochemical network models.

The multiscaling approach as outlined in Section 3 may be used to derive various QSSAs in the presence of intrinsic noise, and also for modeling the intrinsic noise itself. Although we do not discuss such applications here, let us just briefly remark that our techniques could yield diffusion approximations under various scaling relationships among species and reaction rates. Indeed, under scaling regimes satisfying certain technical requirements, one can apply the functional central limit theorem (or the so-called linear noise approximation) to the stochastic enzyme kinetic reaction system (3.1) and approximate not only the mean (as we did here) but also higher order moments, which are often of interest in applications. Note that our present derivations do not suggest any such approximation of the higher order moments, since we rely solely on the first order considerations via the Poisson law of large numbers. For higher order analysis the QSSA approximations necessarily need to be more subtle, as discussed recently in [30]. Building upon some of the QSSA results discussed here, we hope to be able to pursue this topic in our future work.

Acknowledgements This work has been co-funded by the German Research Foundation (DFG) as part of project C3 within the Collaborative Research Center (CRC) 1053 – MAKI (WKB) and the National Science Foundation under the grants RAPID DMS-1513489 (GR) and DMS-1620403 (HWK). This research has also been supported in part by the University of Maryland Baltimore County under grant UMBC KAN3STRT (HWK). This work was initiated when HWK and WKB were visiting the Mathematical Biosciences Institute (MBI) at the Ohio State University in Winter 2016-17. MBI is receiving major funding from the National Science Foundation under the grant DMS-1440386. HWK and WKB acknowledge the hospitality of MBI during their visits to the institute.

References

[1] D. F. Anderson, D. Cappelletti, M. Koyama, and T. G. Kurtz, *Non-explosivity of stochastically modeled reaction networks that are complex balanced*, ArXiv e-prints (20178), available at 1708.09356.
[2] D. F. Anderson and T. G. Kurtz, *Continuous time markov chain models for chemical reaction networks*, Design and analysis of biomolecular circuits, 2011, pp. 3–42.
[3] M. Assaf and B. Meerson, *WKB theory of large deviations in stochastic populations*, J. Phys. A **50** (2017), no. 26, 263001.
[4] K. Ball, T. G. Kurtz, L. Popovic, and G. A. Rempala, *Asymptotic analysis of multiscale approximations to reaction networks*, Ann. Appl. Probab. **16** (2006), no. 4, 1925–1961.
[5] D. Barik, M. R. Paul, W. T. Baumann, Y. Cao, and J. J. Tyson, *Stochastic simulation of enzyme-catalyzed reactions with disparate timescales*, Biophys. J. **95** (2008), no. 8, 3563–3574.
[6] A. M. Bersani and G. Dell'Acqua, *Asymptotic expansions in enzyme reactions with high enzyme concentrations*, Math. Methods Appl. Sci. **34** (2011), no. 16, 1954–1960.
[7] A. M. Bersani, M. G. Pedersen, E. Bersani, and F. Barcellona, *A mathematical approach to the study of signal transduction pathways in MAPK cascade*, Ser. Adv. Math. Appl. Sci. **69** (2005), 124.
[8] T. Biancalani and M. Assaf, *Genetic toggle switch in the absence of cooperative binding: exact results*, Phys. Rev. Lett. **115** (2015), 208101.
[9] J. A. M. Borghans, R. J. De Boer, and L. A. Segel, *Extending the quasi-steady state approximation by changing variables*, Bull. Math. Biol. **58** (1996), no. 1, 43–63.
[10] P. C. Bressloff, *Stochastic switching in biology: from genotype to phenotype*, J. Phys. A **50** (2017), no. 13, 133001.
[11] P. C. Bressloff and J. M. Newby, *Metastability in a stochastic neural network modeled as a velocity jump markov process*, SIAM J. Appl. Dyn. Syst. **12** (2013), no. 3, 1394–1435.
[12] G. E. Briggs and J. B. S. Haldane, *A note on the kinetics of enzyme action*, Biochem. J. **19** (1925), no. 2, 338.
[13] B. S. Choi, G. A. Rempala, and J. Kim, *Beyond the Michaelis-Menten equation: Accurate and efficient estimation of enzyme kinetic parameters*, Vol. 7, 2017.
[14] Boseung Choi, Grzegorz A Rempala, and Jae Kyoung Kim, *Beyond the michaelis-menten equation: Accurate and efficient estimation of enzyme kinetic parameters*, Scientific reports **7** (2017), no. 1, 17018.
[15] A. Cornish-Bowden, *Fundamentals of enzyme kinetics*, Portland Press, 2004.
[16] T. A. Darden, *A pseudo-steady-state approximation for stochastic chemical kinetics*, Rocky Mt. J. Math. **9** (1979), no. 1, 51–71.
[17] ———, *Enzyme kinetics: stochastic vs. deterministic models*, Instabilities, bifurcations, and fluctuations in chemical systems, 1982, pp. 248–272.

[18] G. Dell'Acqua and A. M. Bersani, *Quasi-steady state approximations and multistability in the double phosphorylation-dephosphorylation cycle*, International joint conference on biomedical engineering systems and technologies, 2011, pp. 155–172.
[19] ———, *A perturbation solution of Michaelis–Menten kinetics in a "total" framework*, J Math Chem. **50** (2012), no. 5, 1136–1148.
[20] J. W. Dingee and A. B. Anton, *A new perturbation solution to the Michaelis-Menten problem*, AlChE J. **54** (2008), no. 5, 1344–1357.
[21] S. N. Ethier and T. G. Kurtz, *Markov processes: characterization and convergence*, Vol. 282, John Wiley & Wiley, 1986.
[22] D. T. Gillespie, *Exact stochastic simulation of coupled chemical reactions*, J. Phys. Chem. **81** (1977), no. 25, 2340–2361.
[23] A. Goeke and S. Walcher, *A constructive approach to quasi-steady state reductions*, J. Math. Chem. **52** (2014), no. 10, 2596–2626.
[24] C. A. Gómez-Uribe, G. C. Verghese, and L. A. Mirny, *Operating regimes of signaling cycles: statics, dynamics, and noise filtering*, PLoS Comput. Biol. **3** (2007), no. 12, e246.
[25] R. Grima, D. R. Schmidt, and T. J. Newman, *Steady-state fluctuations of a genetic feedback loop: An exact solution*, J. Chem. Phys. **137** (2012), no. 3, 035104, available at `http://dx.doi.org/10.1063/1.4736721`.
[26] G. Hammes, *Enzyme catalysis and regulation*, Elsevier, 2012.
[27] H.-W. Kang and T. G. Kurtz, *Separation of time-scales and model reduction for stochastic reaction networks*, Ann. Appl. Probab. **23** (2013), no. 2, 529–583.
[28] H. Kim and E. Gelenbe, *Stochastic gene expression modeling with hill function for switch-like gene responses*, IEEE/ACM Trans. Comput. Biol. Bioinform. **9** (2012), no. 4, 973–979.
[29] J. K. Kim, K. Josić, and M. R. Bennett, *The relationship between stochastic and deterministic quasi-steady state approximations*, BMC Syst. Biol. **9** (2015), no. 1, 87.
[30] J. K. Kim, G. A. Rempala, and H.-W. Kang, *Reduction for stochastic biochemical reaction networks with multiscale conservations*, arXiv preprint arXiv:1704.05628 (2017).
[31] Jae Kyoung Kim, Krešimir Josić, and Matthew R Bennett, *The validity of quasi-steady-state approximations in discrete stochastic simulations*, Biophysical journal **107** (2014), no. 3, 783–793.
[32] T. G. Kurtz, *The relationship between stochastic and deterministic models for chemical reactions*, J. Chem. Phys. **57** (1972), no. 7, 2976–2978.
[33] ———, *Averaging for martingale problems and stochastic approximation*, Applied stochastic analysis, 1992, pp. 186–209.
[34] K. J. Laidler, *Theory of the transient phase in kinetics, with special reference to enzyme systems*, Can. J. Chem. **33** (1955), no. 10, 1614–1624.
[35] C. C. Lin and L. A. Segel, *Mathematics Applied to Deterministic Problems in the Natural Sciences*, SIAM, 1988.
[36] Donald A McQuarrie, *Stochastic approach to chemical kinetics*, Journal of applied probability **4** (1967), no. 3, 413–478.
[37] L. Michaelis and M. L. Menten, *Die kinetik der invertinwirkung*, Biochem. Z. **49** (1913), no. 333-369, 352.
[38] J. M. Newby, *Isolating intrinsic noise sources in a stochastic genetic switch*, Phys. Biol. **9** (2012), no. 2, 026002.
[39] ———, *Bistable switching asymptotics for the self regulating gene*, J. Phys. A **48** (2015), no. 18, 185001.
[40] Morten Gram Pedersena, Alberto M. Bersanib, and Enrico Bersanic, *The total quasi-steady-state approximation for fully competitive enzyme reactions*, Bulletin of Mathematical Biology **69** (2006Jul), no. 1, 433.
[41] R. Perez-Carrasco, P. Guerrero, J. Briscoe, and K. M. Page, *Intrinsic noise profoundly alters the dynamics and steady state of morphogen-controlled bistable genetic switches*, PLoS Comput. Biol. **12** (2016), no. 10, 1–23.
[42] C. V. Rao and A. P. Arkin, *Stochastic chemical kinetics and the quasi-steady-state assumption: application to the Gillespie algorithm*, J. Chem. Phys. **118** (2003), no. 11, 4999–5010.
[43] K. R. Sanft, D. T. Gillespie, and L. R. Petzold, *Legitimacy of the stochastic Michaelis–Menten approximation*, IET Syst. Biol. **5** (2011), no. 1, 58–69.
[44] H. M. Sauro and B. N. Kholodenko, *Quantitative analysis of signaling networks*, Prog. Biophys. Mol. Biol. **86** (2004), no. 1, 5–43.
[45] K. R. Schneider and T. Wilhelm, *Model reduction by extended quasi-steady-state approximation*, J. Math. Biol. **40** (2000), no. 5, 443–450.
[46] S. Schnell and P. K. Maini, *Enzyme kinetics at high enzyme concentration*, Bull. Math. Biol. **62** (2000), no. 3, 483–499.
[47] S. Schnell and C. Mendoza, *Closed form solution for time-dependent enzyme kinetics*, J. Theor. Biol. **187** (1997), no. 2, 207–212.
[48] I. H. Segel, *Enzyme kinetics*, Vol. 360, Wiley, New York, 1975.
[49] L. A. Segel, *On the validity of the steady state assumption of enzyme kinetics*, Bull. Math. Biol. **50** (1988), no. 6, 579–593.
[50] L. A. Segel and M. Slemrod, *The quasi-steady-state assumption: a case study in perturbation*, SIAM Rev. **31** (1989), no. 3, 446–477.
[51] S. Smith, C. Cianci, and R. Grima, *Analytical approximations for spatial stochastic gene expression in single cells and tissues*, J. Royal Soc. Interface **13** (2016), no. 118.
[52] M. Stiefenhofer, *Quasi-steady-state approximation for chemical reaction networks*, J. Math. Biol. **36** (1998), no. 6, 593–609.
[53] Philipp Thomas, Arthur V. Straube, and Ramon Grima, *Communication: Limitations of the stochastic quasi-steady-state approximation in open biochemical reaction networks*, The Journal of Chemical Physics **135** (2011), no. 18, 181103, available at `https://doi.org/10.1063/1.3661156`.
[54] T. Tian and K. Burrage, *Stochastic models for regulatory networks of the genetic toggle switch*, Proc. Natl. Acad. Sci. U.S.A. **103** (2006), no. 22, 8372–8377.
[55] A. R. Tzafriri, *Michaelis-Menten kinetics at high enzyme concentrations*, Bull. Math. Biol. **65** (2003), no. 6, 1111–1129.
[56] D. D. Van Slyke and G. E. Cullen, *The mode of action of urease and of enzymes in general*, J. Biol. Chem. **19** (1914), no. 2, 141–180.