On the validity of the stochastic quasi-steady-state approximation in open enzyme catalyzed reactions: *Timescale separation or singular perturbation?*  

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Abstract  

The quasi-steady-state approximation is widely used to develop simplified deterministic or stochastic models of enzyme catalyzed reactions. In deterministic models, the quasi-steady-state approximation can be mathematically justified from singular perturbation theory. For several closed enzymatic reactions, the homologous extension of the quasi-steady-state approximation to the stochastic regime, known as the *stochastic* quasi-steady-state approximation, has been shown to be accurate under the analogous conditions that permit the quasi-steady-state reduction of the deterministic counterpart. However, it was recently demonstrated that the extension of the stochastic quasi-steady-state approximation to an open Michaelis–Menten reaction mechanism is only valid under a condition that is far more restrictive than the qualifier that ensures the validity of its corresponding deterministic quasi-steady-state approximation. In this paper, we suggest a possible explanation for this discrepancy from the lens of geometric singular perturbation theory. In so doing, we illustrate a misconception in the application of the quasi-steady-state approximation: timescale separation does not imply singular perturbation.  

*Keywords:* Singular perturbation, stochastic process, quasi-steady-state approximation, Michaelis–Menten reaction mechanism, Langevin equation, linear noise approximation, slow scale linear noise approximation, Fenichel theory, Tikhonov’s theorem
1. Introduction

The quasi-steady-state approximation (QSSA) is a simple but powerful model reduction technique in the field of mathematical biochemistry. In a reduced model of an enzyme catalyzed reaction, the QSSA decreases the dimensionality of the full model in both state and parameter space which, consequently, increases the tractability of quantifying enzyme activity from experimental data or the modeling of the enzyme catalyzed reactions [1, 2]. Furthermore, as a course-grained model, the QSSA eases the computational burden of numerical simulations by omitting the impact of negligible changes in state that take place over extremely small timescales [3, 4, 5].

Depending on the specific application, the QSSA comes in two varieties: deterministic and stochastic. Regardless of whether or not the deterministic or stochastic approximation is employed, the challenge always resides in justifying its use in an application. Historically, the deterministic QSSA has been derived from the careful application of singular perturbation analysis [6, 7, 8], also known as Tikhonov theory [9] in Eastern Europe, or Fenichel theory [10] in the Western hemisphere. The existing link between the QSSA and singular perturbation theory is favorable: since singular perturbation theory has been developed quite extensively over the past several decades [11, 12], there is a rich mathematical foundation that supports the legitimization of the QSSA.

In contrast to its deterministic cousin, the justification of stochastic QSSA is significantly more difficult, especially in regimes far from the thermodynamic limit where the chemical master equation (CME) prevails as the physically relevant model. The stochastic multiscale method has provided a rigorous justification of the stochastic QSSA [13, 14, 15]; other justifications have leveraged scaling methodologies and singular perturbation methods [16, 17]. Regardless of how the stochastic QSSA is justified, there is a developed interest in deriving techniques capable of ascertaining, a priori, when the stochastic QSSA will generate accurate statistical moments.

Presently, there are several methodologies that rely on the linear noise approximation (LNA) that are very good at determining the reliability of the stochastic QSSA [4, 18, 19, 20, 21, 22]. In the particular case of Michaelis–Menten (MM) type reactions, the stochastic QSSA has been shown to be very accurate when the reaction is closed, meaning that it is void of any influx (or outflux) of the pertinent chemical species. Perhaps most surprisingly, the conditions that support the validity of the stochastic QSSA have been shown to be the same conditions for the validity of deterministic QSSA [3]. On the other hand, for open MM-type reactions, the proposed qualifier that establishes the accuracy of the stochastic QSSA is far more restrictive than the criterion that certifies the effectiveness of the corresponding deterministic QSSA [20, 23]. The natural question that follows is: Why?

The answer may at least partially hinge on the mathematical theory that ratifies the legitimacy of the deterministic QSSA. Although it is commonly assumed that the reliability of the deterministic QSSA is due to singular perturbation theory, recent analyses have clarified that this is not always the case [24, 25, 26]. In fact, in the instance of the open MM reaction mechanism, Eilertsen et al. [27] demonstrate that there are in fact two mathemat-

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3The use of “same” here is slightly abusive: deterministic and stochastic rate constants differ in terms of their units, and the state space is discrete–as opposed to continuous–in the realm of the CME.
ical mechanisms that legalize the QSSA: near invariance of the QSS manifold, and singular perturbation reduction. Hence, the validity of the deterministic QSSA in the open MM reaction mechanism is not necessarily attributable to a singular perturbation. However, when the MM reaction mechanism is closed, the QSSA is the result of a singular perturbation. Thus, adding influx to the MM reaction mechanism can blur the boundary between singular and non-singular perturbation scenarios. This observation is a possible small step in understanding why the stochastic QSSA works so well for the closed MM reaction mechanism, but is apparently only favorable under more restrictive conditions when open.

In what follows, we re-examine the role of competing QSSA mechanisms and the possible impact this has on the validity of the stochastic QSSA for the open MM reaction mechanism. Specifically, we show that the conditions that guarantee the accuracy of the stochastic QSSA for the open MM reaction mechanism in the linear noise regime are in agreement with a singular perturbation scenario in the corresponding deterministic limit.

2. The deterministic formation of the open Michaelis–Menten reaction mechanism

In this paper, we analyze the MM reaction mechanism supplied with a constant influx of substrate, which was first examined by Stoleriu et al. [28]. Let S denote a substrate molecule, E an enzyme molecule, C an enzyme-substrate complex, and P a product molecule. The reaction mechanism is

\[ \emptyset \xrightarrow{k_0} S, \quad S + E \xrightleftharpoons{k_1}{k_{-1}} C \xrightarrow{k_2} E + P, \]  

which is known as the open MM reaction mechanism. In contrast, the closed MM reaction mechanism is void of additional substrate influx, and corresponds to the special case when \( k_0 \) is identically zero.

In the thermodynamic limit, the open MM reaction mechanism (1) is accurately modelled by the following set of rate equations derived from the law of mass action:

\[
\dot{s} = k_0 - k_1(e_T - c)s + k_{-1}c, \tag{2a}
\]

\[
\dot{c} = k_1(e_T - c)s - (k_{-1} + k_2)c. \tag{2b}
\]

The parameters \( k_1, k_2 \) and \( k_{-1} \) are deterministic rate constants, \( k_0 \) is the rate of substrate influx, and lowercase \( s, c, p \) denote the respective concentrations of substrate, complex, and product. The concentration of E, \( e \), can be determined a posteriori from the conservation law,

\[ e_T = e + c, \tag{3} \]

where \( e_T \) is the total enzyme concentration, a conserved quantity. Likewise, the concentration of P, \( p \), can be determined solely from the time course solution of \( c \):

\[ \dot{p} = k_2c. \tag{4} \]

Since \( c \leq e_T \), the limiting rate of the reaction is \( v := k_2 e_T \). Furthermore, there exists a stable fixed point in the first quadrant, \( (s,c) := (\gamma, \nu) \),

\[ \gamma := \frac{\alpha K_M}{1 - \alpha}, \quad \nu := \alpha e_T, \quad \alpha := \frac{k_0}{v} \tag{5} \]
whenever \( k_0 < v \) and the Michaelis constant, \( K_M := (k_{-1} + k_2)/k_1 \), is bounded.

To derive the deterministic QSSA from the system (2), one assumes that after an initial transient \( \dot{c} \approx 0 \), so that the algebraic relationship,

\[
c = \frac{e_T s}{K_M + s},
\]

is approximately valid. The curve defined by (6) is identical to the \( c \)-nullcline, but we will refer to it as the QSS manifold.\(^4\) Insertion of (6) into (2a) yields

\[
\dot{s} = k_0 - \frac{v s}{K_M + s},
\]

which is known as the standard QSSA (sQSSA).

The relevant question in the application of the sQSSA is always: Under what conditions does (7) prevail as a reliable approximation to the full system (2)? On the deterministic end of the thermodynamic spectrum, the answer to this question for both the open and closed MM reaction mechanisms is well-established. As carefully discussed in [29], the accuracy of (7) is holds as long as\(^5\)

\[
\varepsilon_{SS} := \frac{e_T}{K_M} \ll 1.
\]

Colloquially, (8) is known as the timescale separation condition. The condition (8) was originally justified by Palsson [30, 31] via linearization of the mass action equations, but the phrase “timescale separation” follows from the work of Segel [7] and Segel & Slemrod [8]. Following Segel, but using a more rigorous scaling, Eilertsen and Schnell [29] demonstrated that the sQSSA for the closed MM reaction is valid as long as the timescale that accounts for the fast transient, \( t_C := 1/(k_{-1} + k_2) \), is much shorter than the timescale required for a significant amount of substrate to deplete, \( t_S := 1/k_1 e_T \):

\[
\varepsilon_{SS} := \frac{t_C}{t_S} = \frac{e_T}{K_M}.
\]

3. The stochastic formulation of the open Michaelis–Menten reaction mechanism

The deterministic mass action equations (2) are only valid in the thermodynamic limit, in which both the number of molecules, \( n \), and system volume, \( \Omega \), approach infinity in a way that leaves the respective concentrations of each species finite. Far from the thermodynamic limit, where molecular copy numbers are finite, stochastic fluctuations persist. In this regime, the master equation, whose solution gives the probability of finding the system in state \( X \)

\(^4\)From this point onward, we often will refer to the curve defined by (6) as the QSS manifold.

\(^5\)Locally, near a specific value of \( s \), the metric \( e_T/(K_M + s) \ll 1 \) is often sufficient to validate the sQSSA. For more details, please consult [7, 8, 29].
at time $t$, is the appropriate model to employ. The specific CME that corresponds to (1) is

$$\frac{\partial}{\partial t} P(n_S, n_C, t) = \left[ \frac{k_1}{\Omega} (E_S^{-1} E_C^{-1} n_S (E_T - n_C) + \Omega k_0 (E_S^{-1} - 1) 
 + k_1 (E_C^{-1} - 1) n_C + k_2 (E_C^{-1} - 1) n_C \right] P(n_S, n_C, t). \quad (10)$$

In (10), $P(n_S, n_C, t)$ is the probability of finding the system with $n_S$ substrates molecules and $n_C$ complex molecules at time $t$, $\Omega$ is the volume of the system, and $E_X^{\pm j}$ are step operators:

$$E_X^{\pm j} f(X, Y, Z) = f(X \pm j, Y, Z). \quad (11)$$

The corresponding stochastic sQSSA is given by

$$\frac{\partial}{\partial t} P(n_S, t) = \left[ \Omega k_0 (E_S^{-1} - 1) - (E_S^{+1} - 1) \frac{k_2 e_T n_S}{K_M + n_S / \Omega} \right] P(n_S, t). \quad (12)$$

A formal derivation of the stochastic QSSA for the closed MM reaction can be found in [16]; the open stochastic QSSA (12) can be found in [20]. In either case, the implicit assumption made in the application of the stochastic sQSSA is that the propensity function, $a(n_S)$, that gives the probability that a product molecule is formed within the infinitesimal window $[t, t + dt)$ is identically

$$P(n_S - 1, t + dt|n_S, t) := a(n_S) dt = \frac{k_2 e_T n_S}{K_M + n_S / \Omega} dt. \quad (13)$$

Hence, the propensity function for the depletion of substrate is adopted directly from the non-elementary rate equation of the deterministic sQSSA. The challenge that emerges is thus to determine the specific conditions that permit the use of (13) in the Gillespie algorithm.

**Remark 1.** In what follows we will be interested in understanding the validity of the stochastic sQSSA near the deterministic equilibrium point (5). The reason for this is that, from a computational perspective, we can view the open MM reaction (2) as being embedded in a possibly larger chemical network. Thus, there is utility in minimizing the computational cost of simulation via Gillespie’s algorithm by applying the stochastic sQSSA.

In the linear noise regime, the legitimacy of (12) in the neighborhood of the deterministic stationary point (5) was rigorously analyzed by Thomas et al. [20]. These authors discovered that timescale separation is necessary, but not sufficient for the validity of (12) near $(s, c) = (\gamma, \nu)$. Specifically, they demonstrated that the variance in the number of substrate molecules under steady–state conditions obtained from the full CME (10) can differ from the variance of substrate under steady–state conditions computed from the stochastic sQSSA (12) by as much as 30%. Given the success of stochastic reductions for the closed reaction [3, 32], this result is somewhat surprising.

In order to derive a qualifier that yields a useful a priori indication of the accuracy of (12), Thomas et al. [20] analyzed the open MM reaction mechanism in the linear noise regime.
Performing a $\Omega$-expansion on (10) and (12) and discarding terms that are $o(\Omega^{-1/2})$ yields their respective LNAs [33]. From the LNAs, expressions for the substrate variance under steady–state conditions can computed by solving a Lyapunov equation. From Thomas et al. [20], these are:

$$
\sigma_\text{full}^2 = \frac{\gamma}{\Omega} \left( 1 + \frac{\gamma}{K_M} \cdot \frac{K_S + \gamma}{K_M + \gamma} \cdot \frac{1}{1 + \varepsilon} \right), \quad K_S := k_{-1}/k_1, 
$$

(14a)

$$
\sigma_\text{red.}^2 = \frac{\gamma}{\Omega} \left( 1 + \frac{\gamma}{K_M} \right)
$$

(14b)

where $\varepsilon$ is given by: $\varepsilon := \left( e_T - \nu \right)/(K_M + \gamma) \leq \varepsilon_{SS}$, $\sigma_\text{full}^2$ is the substrate variance under steady–state conditions obtained from the LNA to full CME (10), and $\sigma_\text{red.}^2$ is the substrate variance under steady–state conditions obtained from the LNA to (12). By equating $\varepsilon$ with zero in (14a), Thomas et al. [20] found that, for $0 \leq \alpha < 1$, it is also necessary that

$$
\left| \frac{\sigma_\text{full}^2 - \sigma_\text{red.}^2}{\sigma_\text{full}^2} \right| = \frac{(1 - \alpha)\alpha\beta}{1 + \beta [1 - \alpha (1 - \alpha)]} \ll 1, \quad \beta := \frac{k_2}{k_{-1}}, \quad \alpha := \frac{k_0}{k_2 e_T} \equiv \frac{k_0}{v} 
$$

(15)

hold in order to ensure the accuracy of the stochastic sQSSA near the deterministic stationary point (5). From inspection of (15), we observe that $\sigma_\text{red.}^2$ is a good approximation to $\sigma_\text{full}^2$ when $0 \leq \alpha \ll 1$ or as $\alpha \to 1$. However, when $\alpha \sim 1/2$, it is necessary that $\beta \ll 1$; see Figure 1. Note that these restrictions are in addition to, and distinct from, the timescale separation condition $\varepsilon_{SS} \ll 1$. Furthermore, while (15) was derived under the assumption that the reaction is occurring in a region within the thermodynamic spectrum where LNA is valid (i.e., very close to the thermodynamic limit), numerical simulations suggest that, when combined with timescale separation, (15) is a good a priori indicator of the accuracy of (12); again, see Figure 1. With that said, let us state the following:

**Remark 2.** We are not suggesting that the conditions $\varepsilon_{SS} \ll 1$ and (15) are sufficient for the validity of (12), as they were obtained by analysing the LNAs and not the CME. However, based on reports in the literature [34], the validity of the reduced LNA does appear to yield a good a priori indication of the accuracy of (12); again, see Figure 1. With that said, let us state the following:

The additional constraint defined by (15) lies in stark contrast to validity of the stochastic QSSA applied to the closed MM reaction mechanism (i.e., when $k_0$ is identically zero). Earlier studies demonstrated, with great clarity, that the stochastic sQSSA for the closed MM reaction is valid under the same conditions that ensure the accuracy of the deterministic sQSSA [3]. Moreover, analyses of the deterministic open MM equations indicate that the timescale separation condition (8) is also sufficient for the validity of the open sQSSA [28, 27]. But, why does the addition of one elementary reaction (i.e., substrate influx) give rise to a more restrictive set of qualifiers for the validity of the stochastic sQSSA? After all, the qualifier $e_T/k_M \ll 1$ is independent of the amount of substrate present in the system, and thus it seems reasonable to assume that the addition of substrate should not compromise the integrity of the stochastic QSSA. Nevertheless, as Thomas et al. [20] have clearly shown, it does. Ultimately, the answer to this question may partially lie in the application of singular perturbation theory, the basics of which are reviewed in Section 4.1.
Figure 1: **Accuracy of the stochastic sQSSA for the open Michaelis–Menten reaction mechanism.** If $\alpha \sim 1/2$, then $\beta \ll 1$ is required to ensure the accuracy of (12). In both panels, $\Omega = 1$, the total number of enzyme molecules, $n_E$, is 10, $k_1 = 1.0$, $k_0 = 0.5v$ and $k_{-1}, k_2$ are varied so that $k_{-1}, k_2 \in [10, 100, 1000]$ with $\beta = 0.01, 0.1, 1.0, 10.0$ and 100.0. Moreover, in each simulation, parameters are chosen so that $e_T/K_M = 0.01$ is constant. All units are arbitrary. **TOP:** In this panel, $\log_{10}\beta$ is plotted versus the numerically-approximated mean of $n_S$, $\mu$, the number of substrate molecules, under steady-state conditions. The green diamonds are the means estimated from (10) via $10^7$ realizations produced by Gillespie algorithm; the blue crosses are the means estimated from (12) via $10^7$ realizations generated Gillespie’s algorithm. **BOTTOM:** In this panel, $\log_{10}\beta$ is plotted versus the numerically-approximated standard deviation of $n_S$, $\sigma$, under steady-state conditions. Green diamonds are standard deviations recovered via realizations of Gillespie’s algorithm applied to (10). Blue crosses are standard deviations obtained from realizations generated by Gillespie’s algorithm applied to (12). Clearly, $\beta \ll 1$ is necessary to recover accurate standard deviations when $\alpha = 1/2$. 
4. Singular perturbations, near invariance, and the validity of the stochastic quasi-steady-state approximation

In this section we review the basics of Fenichel theory, and demonstrate how QSSAs can be recovered from slow manifold projection.

4.1. Singular perturbation theory: The basics

A perturbed dynamical system may be expressed in the general form

\[ \dot{z} = w(z) + \varepsilon G(z, \varepsilon) \]  

(16)

where \(0 < \varepsilon \ll 1\) is a parameter that is proportional to the ratio of fast and slow timescales. The type of perturbation, i.e., regular or singular, is determined by the number of stationary points in the unperturbed vector field, \(w(z)\). If, in the singular limit that coincides with \(\varepsilon = 0\), there exists a compact and differentiable manifold, \(M\), consisting of non-isolated stationary solutions, \(M \subseteq S := \{z \in \mathbb{R}^n : w(z) = 0\}\), then the perturbation is singular, and \(M\) is referred to as the critical manifold. Moreover, if the critical manifold is normally hyperbolic (and attracting so that the real parts of the non-zero eigenvalues of the Jacobian at \(z, Dw(z)\), are strictly less than zero) such that

\[ \mathbb{R}^n = T_zM \oplus N_z, \quad T_zM = \ker Dw(z), \quad \text{with rank } Dw(z) = n - \dim M, \quad \forall z \in M, \]  

(18)

where \(T_zM\) is the tangent space of \(M\) and \(N_z\) is complementary\(^6\) to \(T_zM\), then Fenichel theory \([10]\) says that the perturbed dynamical system (16) is approximated via projection of the perturbation, \(G(z,0)\), onto \(T_zM\):

\[ \Pi^M : \mathbb{R}^n \mapsto T_zM \quad \forall z \in M, \]  

(19a)

\[ \dot{z} = \Pi^M G(z,0). \]  

(19b)

We refer the reader to Appendix A as well as \([26, 12, 27]\) for details regarding the explicit construction of \(\Pi^M\), which is straightforward to compute for the open MM reaction.

4.2. Tikhonov–Fenichel Parameter Values

In order to justify the application of any form of the QSSA\(^7\) from singular perturbation theory, it imperative to identify the specific conditions under which the vector field of interest possesses a critical manifold. Tikhonov–Fenichel parameter values (TFPV) provide the necessary conditions for the existence of a normally hyperbolic critical manifold in chemical systems \([26]\). For the open MM reaction mechanism, let \(\pi \in \mathbb{R}_+^5\) denote the parameter vector: \(\pi := [k_0 \ e_T \ k_1 \ k_2 \ k_{-1}]^T\). The interesting TFPVs and their corresponding critical manifolds, \(M\), are as follows:

\[ \pi_1 = [0 \ 0 \ k_1 \ k_2 \ k_{-1}], \quad \& \quad M_1 := \{(s, c) \in \mathbb{R}^2 : c = 0\}, \]  

(20a)

\[ \pi_2 = [0 \ e_T \ 0 \ k_2 \ k_{-1}], \quad \& \quad M_2 = M_1 := \{(s, c) \in \mathbb{R}^2 : c = 0\}, \]  

(20b)

\[ \pi_3 = [0 \ e_T \ k_1 \ 0 \ k_{-1}], \quad \& \quad M_3 := \{(s, c) \in \mathbb{R}^2 : c = k_1 e_T s/(k_{-1} + k_1 s)\}. \]  

(20c)

\(^6\) \(N_z\) is the range, \(R_z\), of the Jacobian, \(Dw(z)\), for \(z \in M\).

\(^7\) For a review describing the different forms of the QSSA, we invite the reader to consult \([35, 29]\)
A small perturbation to a TFPV results in the validity of a specific QSSA. For example, the TFPV $\pi_1$ corresponds to both $e_T$ and $k_0$ vanishing in the singular limit. Requiring $e_T$ and $k_0$ to be small by defining $e_T \mapsto \varepsilon e_T^*$ and $k_0 \mapsto \varepsilon k_0^*$ with $0 < \varepsilon \ll 1$ allows us to recast (2) into the perturbation form,

$$\begin{pmatrix} \dot{s} \\ \dot{c} \end{pmatrix} = \begin{pmatrix} k_1 s + k_{-1} \\ -k_1 s - k_{-1} - k_2 \end{pmatrix} c + \varepsilon \begin{pmatrix} k_0^* - k_1 e_T^* s \\ k_1 e_T^* s \end{pmatrix},$$

with $w(s, c)$ given by

$$w(s, c) := \begin{pmatrix} k_1 s + k_{-1} \\ -k_1 s - k_{-1} - k_2 \end{pmatrix} c,$$

and $G(s, c, \varepsilon)$ give by

$$\varepsilon G(s, c, \varepsilon) := \varepsilon \begin{pmatrix} k_0^* - k_1 e_T^* s \\ k_1 e_T^* s \end{pmatrix}.$$

We will refer to (22) as the unperturbed vector field associated with the sQSSA, and to (23) as its associated perturbation. Projecting the perturbation, $G(s, c, 0)$, onto the tangent space of the critical manifold $M_1$, $T_z M_1$, yields the sQSSA (again, see Appendix A for details):

$$\dot{s} = k_0 - \frac{v s}{s + K_M}.$$  

In a similar manner, defining both $k_1$ and $k_0$ to be small ($k_1 \mapsto \varepsilon k_1^*$ and $k_0 \mapsto \varepsilon k_0^*$), or both $k_2$ and $k_0$ to be small ($k_2 \mapsto \varepsilon k_2^*$ and $k_0 \mapsto \varepsilon k_0^*$) leads to either the small-$s$ linear limit of the sQSSA (25)

$$\dot{s} = k_0 - \frac{v s}{K_M},$$

or the quasi-equilibrium approximation (QEA),

$$\dot{s} = (k_{-1} + k_1 s) \cdot \frac{k_0(k_{-1} + k_1 s) - k_2 k_1 e_T s}{k_1 k_{-1} e_T + (k_{-1} + k_1 s)^2},$$

respectively. Again, see Appendix A of this manuscript, as well as [27] for details.

4.3. Near invariance: A minimal requirement for the justification of the quasi-steady-state approximation

Singular perturbation theory is not the only way to justify a QSSA. In fact, as first shown by Schauer and Heinrich [36], the “near invariance” of the corresponding QSS manifold (again, usually the $c$-nullcline) is often sufficient to justify a QSSA. For example, consider the resulting set of equations that emerge from (2) when $e_T = 0$:

$$\begin{align*}
\dot{s} &= k_0 + k_1 cs + k_{-1} c, \\
\dot{c} &= -k_1 cs - (k_{-1} + k_2) c.
\end{align*}$$

In the limiting vector field defined by (27), the manifold $c = 0$ is invariant: any trajectory starting on $c = 0$ stays on it for all time. Moreover, $\dot{c}$ is identically zero on $c = 0$. Thus,
it is heuristically\(^8\) reasonable to assume that, as long as \(e_T\) is sufficiently small, the sQSSA should provide a reasonably good approximation to the full system (2), since \(\dot{c}\) will be close to zero when \(e_T\) is nearly zero. Schauer and Heinrich [36], Nothen and Walcher [24], Goeke et al. [26] and Eilertsen et al. [27] have shown that the \(c\)-nullcline is nearly invariant when \(e_T\) is sufficiently small, which means that the sQSSA (7) is almost\(^9\) a solution to the full system (2).

While small \(e_T\) is sufficient for the validity of the sQSSA, it is insufficient for the application of singular perturbation theory. Taking \(e_T \to 0\) does not result in the formation of a critical manifold of stationary points. Hence, one cannot attribute the validity of the sQSSA to singular perturbation theory in regions where only (8) stands, since the vector field does not contain a critical manifold in the singular limit (again, see [27], Section 4 for details). Hence,

\[
\text{near invariance } \not\Rightarrow \text{ singular perturbation.} \tag{28}
\]

**Remark 3.** The observation that small \(e_T\) does not necessarily lead to a singular perturbation scenario (i.e., a vector field with a critical set) for the open MM reaction mechanism (2) is in stark contrast to that of the closed MM reaction. The closed form of the reaction corresponds to \(k_0 = 0\). Hence, the manifold \(c = 0\) is filled with non-isolated stationary points when \(e_T = 0\). Consequently, small \(e_T\) is enough to justify the sQSSA from singular perturbation theory when the reaction is closed.

From the practical point of view, we must ask: Does the precise mathematical justification of the sQSSA’s validity really matter? In other words, is it really important if the sQSSA derives its justification from near invariance of the QSS manifold or from a singular perturbation reduction? In the deterministic regime, the answer appears to be no. However, at this point it is not clear if same answer holds in the stochastic realm if one tries to extend the form of the non-elementary rate equations (from the deterministic QSS reduction) to propensity functions.

In conclusion of this section, we emphasize that the reliability of the deterministic sQSSA (7) hinges upon the relative “smallness” of a specific parameter. At the moment, the term “small” has only been defined generically in terms of an abstract parameter “\(\varepsilon\).” However, we will clarify the notion of what it means to be small in Sections 5 and 6.

### 5. The validity of the stochastic QSSA: intimations from Fenichel theory

From the perspective of singular perturbation theory, the fact that timescale separation “\(\varepsilon_{SS} \ll 1\)” does not completely ensure the validity of the stochastic sQSSA is now obvious: taking \(e_T\) to be exceptionally small is not sufficient to justify the sQSSA from singular perturbation theory; it is also necessary that \(k_0\) be small. After all, both \(e_T\) and \(k_0\) must vanish in the singular limit in order to recover the appropriate unperturbed vector field (22) and the critical manifold \(c = 0\). Of course, the notion of small only makes sense from a

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\(^8\)In fact, one can rigorously make this claim by analyzing the Lie derivative and demanding that the vector field be nearly tangent to the QSS manifold; see [36, 25, 26, 27].

\(^9\)We say “almost” because a solution would require the QSS manifold to be perfectly invariant.
relative perception. Concentrations are small in comparison to other other concentrations, and rates are small in comparison to other rates. The total enzyme concentration, $e_T$, is defined to be small whenever $e_T \ll K_M$. Thus, $e_T$ is only “small” when it is much less than $K_M$. On the other hand, $k_0$ may be designated as small if, for example, it is much less than the limiting rate, $v$. Thus, we have that

$$\varepsilon_{SS} \ll 1 \quad \text{and} \quad \alpha \ll 1$$

(29)

comprise a pair of qualifiers that are at least consistent with a singular perturbation scenario, since (29) defines a region in parameter space that is close to the TFPV defined by (20a).

However, the story does not end with small $\alpha$. Close inspection of (15) reveals that $\alpha \ll 1$ is sufficient, but not necessary for the accuracy of (12) since, as $\alpha$ increases with $\varepsilon_{SS}$ remaining small, the stochastic sQSSA is only valid if $\beta \ll 1$. For example, the function $\alpha(1 - \alpha)$ is maximal when $\alpha = 1/2$, and so the accuracy of the stochastic QSSA (12) requires $k_2 \ll k_{-1}$ in this case. This is because, at the stationary point where $c := \nu = \alpha e_T$, the influx rate, $k_0$, is small compared to the maximal disassociation rate,

$$k_0 = \alpha k_2 e_T \ll k_{-1} e_T.$$

(30)

The restriction on the size of $k_0$ when $\alpha \sim 1/2$ is also evident from (15). Rewriting the numerator of (15) as

$$(1 - \alpha)\alpha \beta = (1 - \alpha)\lambda, \quad \lambda := \frac{k_0}{k_{-1} e_T},$$

it becomes clear that one way to ensure (15) is small when $\alpha \sim 1/2$ is to demand $\lambda \ll 1$. Thus, if $\alpha \sim 1/2$, then (12) will hold if $k_0$ is much less than the maximum disassociation rate: $k_{-1} e_T$. Hence, we can interpret the additional qualifier (15) as a restriction on the relative size of $k_0$,

$$k_0 \ll \max\{k_2 e_T, k_{-1} e_T\},$$

(31)

which, together with timescale separation, suggests that the stochastic QSSA (12) is accurate as long as both $k_0$ and $e_T$ are be comparatively small. And, a restriction on the relative sizes of $k_0$ and $e_T$ is perhaps more consistent with a singular perturbation scenario than a near-invariance scenario in the deterministic regime.

Finally, let us point out that there is yet another way to interpret the requirement that $\beta \ll 1$ when $\alpha \sim 1/2$. Note that if $k_0$ and $k_2$ are small then presumably, in parameter space, we are close to the TFPV given by (20c), as opposed to (20a). As a result, the QEA (26) is
valid, since it is now $k_0$ and $k_2$ that are “small.” Carefully rewriting the QEA yields

$$\dot{s} = (k_{-1} + k_1 s) \cdot \frac{k_0 (k_{-1} + k_1 s) - k_1 k_2 e_T s}{k_1 k_{-1} e_T + (k_{-1} + k_1 s)^2},$$

(32a)

$$= (k_{-1} + k_1 s)^2 \cdot \frac{k_0 - \frac{k_1 k_2 e_T s}{(k_{-1} + k_1 s)}}{k_1 k_{-1} e_T + (k_{-1} + k_1 s)^2},$$

(32b)

$$= \frac{k_0 - \frac{k_1 v s}{(k_{-1} + k_1 s)}}{k_1 k_{-1} e_T / (k_{-1} + k_1 s)^2 + 1}.$$  

(32c)

Moreover, if $\varepsilon_{SS} \ll 1$ and $\beta \ll 1$, then

$$k_1 e_T \ll k_{-1},$$

(33)

and it follows from (32c) that the QEA is approximately

$$\dot{s} = k_0 - \frac{v s}{K_S + s}.$$  

(34)

Consequently, we recover a special case of the QEA when $\varepsilon_{SS} \ll 1$ and $\beta \ll 1$, which is equivalent to the sQSSA when $k_2 \ll k_{-1}$. A parameter range in which $\alpha \sim 1/2$ and $\beta \sim 1$ would then be the equivalent of “no man’s land”, since we are presumably somewhere between the TFPVs (20a) and (20c).

The last component of (15) concerns the case in which $\alpha \to 1$. However, this is a special case, and justification concerning the validity of the (12) in this region is relatively intuitive. Since the steady-state substrate concentration tends to infinity as $\alpha \to 1$, the enzyme molecules are, practically speaking, perpetually bound to substrate molecules due to the overwhelming disparity between the populations of their respective copy numbers and concentrations. Direct approximation of the invariant slow manifold and its proximity to the (non-invariant) QSS manifold also supports the validity of the sQSSA when $\alpha \to 1$; see Section 5 in [27].

In summary, our analysis indicates that, in order to ensure the accuracy of the stochastic sQSSA (12), it is absolutely necessary to operate in a parameter regime where both $k_0$ is small and $\varepsilon_{SS} \ll 1$. Again, the requirement that both (15) and (8) hold is compatible with a singular perturbation scenario in the thermodynamic limit.

6. It’s all relative: The utility of dimensionless parameters

The difficulty that resides in interpreting (15) arises from the fact that it is necessary to demand that two parameters be small. In [27], we pointed out that singular perturbation theory is really only applicable along rays in parameter space. For example, one can fix $k_0 = \alpha k_2 e_T$. By invoking such a constraint, it ensures that both $k_0$ and $e_T$ vanish by setting
$e_T = 0$. This also allows one to sort of make the claim that $k_0 \sim \mathcal{O}(e_T)$ and vice versa. However, it is important to understand that in practical applications, what we really want know is: how close are we, in parameter space, to the associated TFPV of interest, which can be very difficult to ascertain. Almost all of the theoretical analyses of the closed MM reaction mechanism are implicitly trying to answer this question with respect to only $e_T$. For the closed reaction there are four parameters, $\pi \in \mathbb{R}^4 := [e_T, k_1, k_2, k_{-1}]^T$, and the validity of the closed sQSSA requires $\pi$ to be sufficiently close to the TFPV $\pi_{\text{stand}} := [0, k_1, k_2, k_{-1}]^T$. Thus, the closed sQSSA requires $e_T$ to be small, but what does that mean? Well, the answer to this question required a significant amount of research [30, 7, 8, 29, 6], the corpus of which indicates that $e_T/k_M \ll 1$ is sufficient for the validity of the closed sQSSA. The important takeaway from the literature is that the “smallness” of $e_T$ is measured in a comparative sense: $e_T$ is small if it is much less than $K_M$, and when this holds we can make the argument $\pi$ is close to $\pi_{\text{stand}}$. Thus, the proximity of $\pi$ to $\pi_{\text{stand}}$ is defined by the magnitude of a dimensionless parameter, and it is this distinguishing feature is crucial. As an example, consider a reaction in which $e_T$ is small (in an arbitrary unit), but with a much smaller $K_M$ (in the same unit). The nearest point in parameter space would be $\pi = \pi_{\text{rev}} := [e_T, k_1, 0, 0]$. The corresponding vector field,

$$
\dot{s} = -k_1(e_T - c)s, \\
\dot{c} = k_1(e_T - c)s,
$$

is equipped with the critical set, $\mathcal{S}$:

$$
\mathcal{S} := \mathcal{S}_1 \cup \mathcal{S}_2 = \{(s, c) \in \mathbb{R}^2 : c = e_T\} \cup \{(s, c) \in \mathbb{R}^2 : s = 0\},
$$

but $\mathcal{S}$ is notably different than $M_1$. Furthermore, $\mathcal{S}$ is not even a manifold. It is possible to define compact submanifolds of $\mathcal{S}$ that do not contain the intersection $\mathcal{S}_1 \cap \mathcal{S}_2$, and in fact the reverse QSSA of the closed MM reaction,

$$
\begin{pmatrix}
\dot{s} \\
\dot{c}
\end{pmatrix} = \begin{pmatrix}
0 \\
-k_2c_2
\end{pmatrix},
$$

is recovered by projecting the appropriate perturbation onto $T_zM_{10}$.

$$
M \subset \mathcal{S}_2 := \{(s, c) \in \mathbb{R}^2 : s = 0, 0 \leq c \leq e_T - \delta\}, \quad \text{for some} \quad 0 < \delta < e_T.
$$

However, (37) is very different from the closed sQSSA, even though the dimensional magnitude of $e_T$ might be small. Thus, the dimensional magnitude of parameter is not necessarily sufficient to determine the validity of a QSS reduction.

The need for a qualifier in the form of a dimensionless parameter is not limited to chemical kinetics. The “$\varepsilon$” of Tikhonov and Fenichel theory is colloquially defined as a dimensionless timescale ratio. Moreover, mathematical timescale ratios are ultimately eigenvalue ratios, and the ratio will be dimensionless, even if the eigenvalues obtained from the Jacobian of a

\[^{10}\text{The “$\delta$” is included in the definition on $\mathcal{M}$ so that the compactness requirement of Fenichel theory is satisfied.}\]
physical application carry units. Thus, by basing the size of a parameter on a dimensional magnitude, we can easily find ourselves in a situation where: (i) there is not a sufficient gap in the eigenspectrum, or (ii) our vector field is not close to the TFPV that corresponds to the particular QSSA of interest. Hence the need for an appropriate dimensionless parameter.

In the case of the open MM reaction mechanism (1), the question is more complex, since there is an additional parameter, $k_0$. Thus, one is tasked with ensuring that both $e_T$ and $k_0$ are sufficiently small but, small compared to what? In the deterministic regime, we do not really have to answer the question pertaining to what $k_0$ must be smaller than to determine when the sQSSA is valid, and this is both a pro and a con. It is a pro since all we need to do is vary one parameter to ensure the validity of the sQSSA; the accuracy of the open sQSSA is not necessarily penalized if we wander too far away from $\pi_1$ in parameter space. It is a con because it is difficult to establish why, mathematically, the sQSSA is valid. Of course, one can constrain $k_0$ and $e_T$ to lie on an appropriate parametric curve, but in applications the reaction unfolds at a single point in parameter space, and to really attribute the validity of the sQSSA to either a singular perturbation scenario or a near invariance scenario, it seems necessary to have some idea about the proximity of $\pi$ to $\pi_1$. As we have suggested, the notion of nearness, as it pertains to points in parameter space, is best defined in terms of suitable dimensionless ratios.

7. Discussion

In this paper we reiterated how, in the deterministic regime, the validity of the sQSSA of the open MM reaction mechanism is attributable to (i) the near invariance of the QSS manifold, or (ii) a singular perturbation reduction. To determine the mathematical justification of the sQSSA, it is necessary to have a metric — defined in terms of a dimensionless parameter — that determines the juxtaposition to the TFPV $\pi_1$. A singular perturbation scenario for the open MM reaction mechanism requires both $e_T$ and $k_0$, the influx rate, to be small. But, small compared to what? Since the validity of the LNA to the stochastic QSSA appears to require $k_0 \ll \max\{v, k_1 e_T\}$, a condition which places an upper bound on the relative size of $k_0$, we speculate that this restriction is consistent with a singular perturbation scenario. Thus, singular perturbation scenarios in the deterministic realm may facilitate a more favorable environment for the validity of QSS reductions in the stochastic regime. Let us stress however, that we are by no means suggesting that a singular perturbation scenario automatically leads the validity of a stochastic QSSA. Several counterexamples that disprove this hypothesis already exist in the literature; see [37]. Furthermore, we wish to emphasize that our observation that singular perturbation scenarios may create more favorable conditions for the accuracy of the stochastic QSSA (as opposed to just near invariance of the QSS manifold) is certainly not the final answer concerning the general validity of stochastic

\[11 \text{Fenichel theory} [10] \text{ ensures the eventual validity of (7) as } e_T \text{ goes to zero with } k_0 \text{ constrained to lie along the ray } k_0 = \alpha e_T k_2, \text{ provided initial conditions are sufficiently close to the critical manifold and all other parameters are bounded away from zero. However, neither TFPV theory nor Fenichel theory indicate how small } e_T \text{ must be to obtain an accurate QSS reduction. Moreover, Fenichel applies only to compact, normally hyperbolic critical manifolds.} \]
reductions. However, our suggestion that the type of mathematical mechanism that permits the deterministic reduction may have ramifications concerning the validity of homologous reductions in stochastic regime deserves further investigation.

Appendix A. Fenichel theory: projecting onto a slow manifold

In this brief appendix, we briefly describe how to construct $\Pi^M$, the projection operator. For more technical details, we invite the reader to consult [12, 26, 38]. Following Wechselberger [12], we begin with the perturbation form

$$\dot{z} = w(z) + \varepsilon G(z, \varepsilon), \quad z \in \mathbb{R}^n.$$  

and let $M$ be a compact subset of $w(z) = 0$ that forms a $k$-dimensional manifold ($k < n$) comprised of stationary points such that:

- For all $z \in M$, the algebraic and geometric multiplicities of the zero eigenvalues of $Dw(z)$ are equal with $T_zM = \ker Dw(z)$ and $\dim \ker Dw(z) = k$,

- If $\lambda_j$ is a nonzero eigenvalue of $Dw(z)$, then $\Re(\lambda_j) < 0 \quad \forall z \in M$,

then, $M$ is locally attracting and there exists a splitting

$$\mathbb{R}^n := T_zM \oplus N_z \quad \forall z \in M,$$  

where $T_zM$ is the tangent space of $M$ at $z$ given by $\{x \in \mathbb{R}^n : x \in \ker Dw(z)\}$, and $N_z$ is the complement to $T_zM$ and coincides with range of the Jacobian, $\mathcal{R}(Dw(z))$. The objective from the point forward will be to exploit the splitting (A.1) and construct an oblique\(^{12}\) projection operator:

$$\Pi^M : \mathbb{R}^n \mapsto T_zM, \quad I - \Pi^M : \mathbb{R}^n \mapsto N_z.$$

To construct $\Pi^M$, we invoke the factorization

$$w(z) = P(z)f(z),$$  

where $P(z)$ is a rectangular matrix function, and the zero level set of $f(z)$ coincides with the critical manifold, $M$. The derivative of $f(z)$, $Df(z)$, has full rank $\forall z \in M$ and:

- The columns of $P(z)$ span the range of the Jacobian $\mathcal{R}(Dw(z)) = N_z$, for all $z \in M$.

- The rows of $Df(z)$ span the orthogonal complement of $\ker Dw(z) = (T_zM)^\perp$ for all $z \in M$.

\(^{12}\)The projection operator $\Pi^M$ is oblique since the $Dw(z)$-invariant subspaces, $N_z = \text{range } Dw(z), \ z \in M, \ \ker Dw(z) = T_zM, \ z \in M$, are not necessarily orthogonal to one another.
It follows that since \( T_z M \) and \( N_z \) are complementary subspaces, the matrix
\[
\Pi^M := I - P(DfP)^{-1}Df
\]
(A.3)
defines the oblique projection onto \( T_z M \). To leading order in \( \varepsilon \), the reduced flow on the slow manifold is
\[
\dot{z} = \Pi^M G(z, 0), \quad (A.4)
\]
which is the QSS reduction obtained from Fenichel theory [10].

**Remark 4.** The critical manifold is said to be normally hyperbolic if the matrix \( DfP \) (the eigenvalues of which are the non-trivial eigenvalues of \( Dw(z) \)) is hyperbolic \( \forall z \in M \), meaning that \( \Re(\lambda_i) \neq 0 \). In most applications, we are interested in attracting critical manifolds, and thus we often require \( \Re(\lambda_i) < 0 \). However, normally hyperbolic critical manifolds are repelling if \( \Re(\lambda_i) > 0 \), or of saddle type if the real parts of the eigenvalues of \( DfP \) are both positive and negative.

As an example, we will explicitly compute the sQSSA for the open MM reaction mechanism. In standard form, we have
\[
\begin{pmatrix}
\dot{s} \\
\dot{c}
\end{pmatrix} = \begin{pmatrix}
\frac{k_{-1} + k_1 s}{-k_{-1} - k_2 - k_1 s}
\end{pmatrix} c + \varepsilon \begin{pmatrix}
\frac{k^*_0 - k_1 e^*_T s}{k_1 e^*_T s}
\end{pmatrix}
\]
(A.5)
so that \( f(s, c) = c \) and
\[
P \equiv \begin{pmatrix}
\frac{k_{-1} + k_1 s}{-k_{-1} - k_2 - k_1 s}
\end{pmatrix}.
\]
(A.6)
The derivative of \( f(s, c) \) is \([0 \ 1]\), and thus \((DfP)^{-1}\) is
\[
\frac{1}{k_1(K_M + s)},
\]
(A.7)
which is of course a scalar. The product, \( PDf \), is given by
\[
\begin{pmatrix}
0 & k_1(K_S + s) \\
0 & -k_1(K_M + s)
\end{pmatrix}, \quad K_S := \frac{k_{-1}}{k_1}, \quad K_M := \frac{k_{-1} + k_2}{k_1}.
\]
(A.8)
Computing \( \Pi^M \) from (A.3) yields
\[
\Pi^M := \begin{pmatrix}
1 & \frac{K_S + s}{K_M + s} \\
0 & 0
\end{pmatrix}.
\]
(A.9)

To recover the sQSSA, we simply project the perturbation onto the tangent space of the critical manifold:
\[
\begin{pmatrix}
\dot{s} \\
\dot{c}
\end{pmatrix} = \left. \begin{pmatrix}
\frac{K_S + s}{K_M + s} \\
0
\end{pmatrix} \begin{pmatrix}
\frac{k^*_0 - k_1 e^*_T s}{k_1 e^*_T s}
\end{pmatrix} \right|_{c=0} = \begin{pmatrix}
\frac{k^*_0 - k_1 k_2 e^*_T s}{k_{-1} + k_2 + k_1 s} \\
0
\end{pmatrix}.
\]
(A.10)
The QEA (26) that corresponds to small \( k_0 \) and small \( k_2 \) is computed in a similar manner. For more details see [27].
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