The total quasi-steady state assumption: its justification by singular perturbation and its application to the chemical master equation

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Abstract

Deterministic models of enzymatic reactions based on the quasi-steady state assumption (QSSA) and total quasi-steady state assumption (tQSSA) have been used successfully in the past. This is surprising as the QSSA and tQSSA can neither be verified mathematically nor by experiment for most cases of interest. Here, we discuss an approach using singular perturbation theory to justify the approximation obtained by tQSSA. In addition, we extend the application of tQSSA to the stochastic model to deal with stiff differential equations originating from the chemical master equation.

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1 Introduction

One of the most well-known enzymatic reactions is the so-called Michaelis–Menten mechanism:

$$\mathsf{E} + \mathsf{S} \underset{\kappa_{-1}}{\overset{\kappa_1}{\rightleftharpoons}} \mathsf{C} \xrightarrow{\kappa_2} \mathsf{E} + \mathsf{R} \tag{1}$$

which is an irreversible conversion of the substrate, S, into the product, R, through the formation of an intermediate species named complex, C, catalyzed by the enzyme, E. The forward and backward rate constants, here κ_1, κ_2 and κ_{-1}, are used for description of the reaction kinetics (see equation (2) and (3)).

Michaelis and Menten [7] proposed that the amount of complex is negligible compared to the amount of substrate in the system (1) where the amount of enzyme present is relatively small compared to the substrate [8].

Briggs and Haldane [3] then extended this idea by postulating that the concentration of the complex remains constant in the enzymatic reaction. This approximation is known as the quasi-steady state approximation (QSSA). By utilizing such an assumption, the fast dynamics of the complex are eliminated from the system and thus the model complexity and computational cost are reduced.

2 Total quasi-steady state approximation

In some cases, the enzyme concentration is virtually the same or greatly exceeds the substrate concentration in the actual biochemical environment. Thus, the standard QSSA breaks down in the circumstance where there is an excess of enzyme level [2]. In dealing with the invalidity of the QSSA, Borghans and collaborators [2] proposed a new approach, the total quasi-steady state approximation (tQSSA), by introducing a lumped variable, the total substrate concentration $[\bar{S}] = [S] + [C]$, to replace the free substrate concentration $[S]$ in classical QSSA. We use $[X]$ to denote the concentration of species X as a function of time and $X(t)$ to denote the value of this function at time t.

The rate equations for the reactions (1) in the tQSSA framework are [2]

$$\frac{d[\bar{S}]}{dt} = -\kappa_2[C]\,, \tag{2}$$

$$\frac{d[C]}{dt} = \kappa_1(E_0 - [C])([\bar{S}] - [C]) - (\kappa_{-1} + \kappa_2)[C]\,, \tag{3}$$

with initial conditions $\bar{S}(0) = S_0$ and $C(0) = 0$. The concentrations $[E]$ and $[P]$ are obtained from the conservation laws $[E]+[C] = E_0$ and $[S]+[C]+[P] = S_0$ where E_0 is the total enzyme concentration and S_0 is the total substrate concentration [9]. The association (κ_1) and dissociation (κ_2 and κ_{-1}) rate constants carry the units $nM^{-1}min^{-1}$ and min^{-1} respectively.

An inspection of the phase plane for the system of rate equations and further analysis of equations (2) and (3) reveals that a steady state exists at $([\bar{S}], [C]) = (0, 0)$ and that the complex concentration $[C]$ evolves in two stages:

1. the transient stage or pre-steady state characterised by a rapid increase of $[C]$;
2. the quasi-steady state during which the complex concentration slowly decays towards the steady state.

In addition, the QSSA and tQSSA postulate that during the pre-steady state the concentrations $[S]$ (QSSA) and $[\bar{S}]$ (tQSSA) are approximately constant [9]. With this assumption, Borghans and collaborators [2] defined two time scales for the system: the fast transient period of the complex in the pre-steady state, t_c, and the slow time scale for the significant depletion of $[\bar{S}]$ after the transient period, $t_{\bar{S}}$. Specifically, they suggested

$$t_c = \frac{1}{\kappa_1(E_0 + S_0 + \kappa_m)} \quad \text{and} \quad t_{\bar{S}} = \frac{E_0 + S_0 + \kappa_m}{\kappa_2 E_0}$$

where $\kappa_m = (\kappa_{-1} + \kappa_2)/\kappa_1$ is the Michaelis–Menten constant.

3 Singular perturbation analysis of tQSSA

Here we present a mathematical justification for the tQSSA in terms of singular perturbation theory. In particular, we show how to include the initial condition $C(0) = 0$ which cannot be satisfied in the original tQSSA. We have the following steps:

1. In equations (2) and (3), we scale the variables (see Section 3.1 and 3.2) to yield the equivalent system

$$\frac{ds_O(T;\epsilon)}{dT} = f_1\big[s_O(T;\epsilon), c_O(T;\epsilon)\big],$$
$$\epsilon\frac{dc_O(T;\epsilon)}{dT} = f_2\big[s_O(T;\epsilon), c_O(T;\epsilon)\big].$$

   By a theorem of Tikhonov [10], the solution of this system converges to the solution of the differential algebraic equations (DAEs):

$$\frac{ds_O(T;0)}{dT} = f_1\big[s_O(T;0), c_O(T;0)\big], \quad 0 = f_2\big[s_O(T;0), c_O(T;0)\big] \qquad (4)$$

   in the outer region as $\epsilon \to 0$, for example, $s_O(T;\epsilon) \to s_O(T;0)$.

2. Rescaling the time differently by setting $\tau = T/\epsilon$ (see Section 3.3), we get another (equivalent) set of equations from system (2) and (3):

$$\frac{ds_I(\tau;\epsilon)}{d\tau} = \epsilon f_1\big[s_I(\tau;\epsilon), c_I(\tau;\epsilon)\big], \quad \frac{dc_I(\tau;\epsilon)}{d\tau} = f_2\big[s_I(\tau;\epsilon), c_I(\tau;\epsilon)\big].$$

   For $\epsilon \to 0$, this ia a (regularly) perturbed system and one can show that for bounded τ, $c_I(\tau;\epsilon) \to c_I(\tau;0)$ and $s_I(\tau;\epsilon) \to s_I(\tau;0)$ where $c_I(\tau;0)$ and $s_I(\tau,0)$ solve

$$\frac{ds_I(\tau;0)}{d\tau} = 0, \quad \frac{dc_I(\tau;0)}{d\tau} = f_2\big[s_I(\tau;0), c_I(\tau;0)\big].$$

3. The outer and inner solutions are matched at the edge of the boundary layer (see Section 3.4).

3.1 Scaling

For the two time periods we introduce two differently scaled dimensionless times:

$$\tau = \frac{t}{t_c} \quad \text{for the transient period;}$$

$$T = \frac{t}{t_{\bar{S}}} \quad \text{for the post-transient period.}$$

Likewise, the complex and total substrate concentrations are scaled by dividing them by their respective maxima:

$$c = \frac{[C]}{C_0}; \quad s = \frac{[\bar{S}]}{S_0}.$$

The maximum of [C], C_0, is derived by Borghans et al. [2] as

$$C_0 = E_0 S_0 / (E_0 + S_0 + \kappa_m).$$

3.2 Outer solution

Borghans et al. [2] found a necessary condition for the validity of the tQSSA, namely $t_c \leq t_{\bar{S}}$, or alternatively, that $0 < \epsilon \leq 1$ where

$$\epsilon = \frac{t_c}{t_{\bar{S}}} = \frac{\kappa_2 E_0}{\kappa_1 (E_0 + S_0 + \kappa_m)^2}.$$

This term is thus used as a small dimensionless parameter in the singular perturbation analysis.

In the outer region, equation (3) is nondimensionalized with the scaled variables c and T to give

$$\begin{aligned} \frac{dc}{dT} &= \frac{1}{C_0} \times \kappa_1 \left[E_0 S_0 s - (E_0 + S_0 s + \kappa_m) C_0 c + (C_0 c)^2 \right] \times t_{\bar{S}} \\ &= \frac{1}{\epsilon} \left(s - \frac{E_0 + S_0 s + \kappa_m}{E_0 + S_0 + \kappa_m} c + \gamma c^2 \right), \end{aligned} \tag{5}$$

with

$$\gamma = \frac{E_0 S_0}{(S_0 + \kappa_m + E_0)^2},$$

and initial condition $c(0) = 0$. As $\epsilon \to 0$, equation (5) becomes the algebraic equation

$$s - \frac{E_0 + S_0 s + \kappa_m}{E_0 + S_0 + \kappa_m} c + \gamma c^2 = 0 .$$

By applying the approach discussed by Cha and Cha [5] or Padé approximation [1], an approximation c_O (subscript 'O' here denotes the outer solution) for c is obtained:

$$c_O(T) = \frac{E_0 + S_0 + \kappa_m}{E_0 + S_0 s + \kappa_m} s . \tag{6}$$

It turns out that the same approximation can also be derived from the tQSSA. Note that this solution does not satisfy the initial condition $c(0) = 0$, and is only valid in the quasi-steady state. We call this approximation an outer solution. Substituting the dimensionless variables and (6) into equation (2) gives

$$\frac{ds}{dT} = -\frac{E_0 + S_0 + \kappa_m}{E_0 + S_0 s + \kappa_m} s , \quad s(0) = 1 . \tag{7}$$

Solving equation (7) gives the outer solution of s, s_O, in the form

$$(E_0 + \kappa_m) \ln s_O(T) + S_0(s_O(T) - 1) + (E_0 + S_0 + \kappa_m) T = 0 \tag{8}$$

which is also the same as the solution obtained via tQSSA.

3.3 Inner solution

Since the outer solutions do not satisfy the initial conditions, we presume there is another set of early time solutions in an initial or pre-steady state layer. Here the solutions are called inner solutions.

Consequently, a new rate equation is derived for the substrate concentration upon substitution of the dimensionless variables s and τ into equa-

tion (2):

$$\frac{ds}{d\tau} = -\frac{\kappa_2 E_0}{\kappa_1 (E_0 + S_0 + \kappa_m)^2} c\,, \quad s(0) = 1\,. \tag{9}$$

One sees that as $\epsilon \to 0$, equation (9) becomes $\frac{ds}{d\tau} = 0$ and so s is approximately constant throughout the pre-steady state. After imposing the initial condition, the inner solution (represented by subscript 'I') in this region is

$$s_I(\tau) = s(0) = 1\,. \tag{10}$$

On the other hand, insert the scaled variables into equation (3) and replace $s = 1$, the rate equation of complex is reformulated as

$$\frac{dc}{d\tau} = 1 - c - \gamma c^2\,, \quad c(0) = 0\,. \tag{11}$$

Solving equation (11) gives the inner solution of complex concentration

$$c_I(\tau) = \frac{2\left[\exp\left(\sqrt{4\gamma - 1}\tau\right) - 1\right]}{\left(1 - \sqrt{1 - 4\gamma}\right)\exp\left(\sqrt{4\gamma - 1}\tau\right) - \left(1 + \sqrt{1 - 4\gamma}\right)}$$

which satisfies the actual biochemical phenomena, that is, no complex concentration appears at the beginning; in other words, the initial condition $c(0) = 0$ holds by the inner solution.

3.4 Matching and uniform approximation

The inner solution, valid in the transient period, together with the outer solution, valid in the post-transient period, comprise a total solution for the system. These solutions have a common limit or overlap term, that is, where the outer solution begins to take over from inner solution.

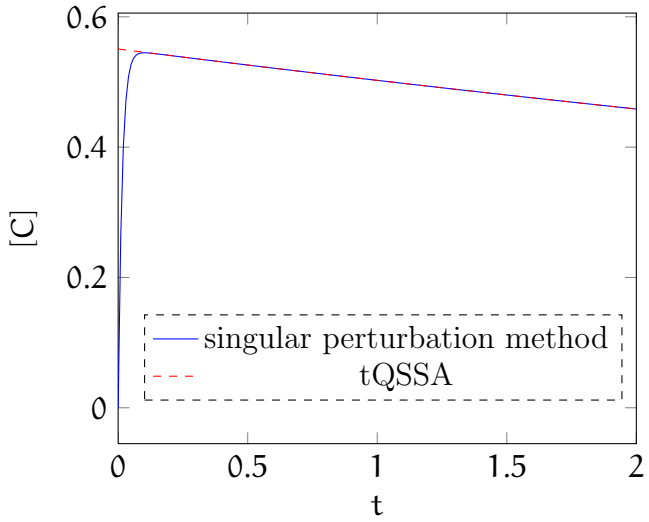

FIGURE 1: The computed complex concentrations with $E_0 = 200$, $S_0 = 180$, $\kappa_1 = 0.001$, $\kappa_2 = 30$ and $\kappa_{-1} = 35$.

Consider now $\epsilon \to 0$, $\tau \to \infty$ and $T \to 0$ respectively. The common limit of the inner and outer solutions is defined as

$$\lim_{\epsilon\to 0}[y_O(T)|_{T=0}] = \lim_{\epsilon\to 0}[y_I(\tau)|_{\tau=\infty}].$$

In other words, the inner and outer solutions are matched if in the limit of $\epsilon \to 0$, the inner solution as $\tau \to \infty$ is equal to the outer solution as $T \to 0$.

Lastly, the final solution, the so-called uniform approximation is obtained by adding the inner and outer solutions and subtracting their common part.

Now, let us apply the matching condition to the outer and inner solutions of the substrate concentration, that is, to equations (8) and (10). We have

$$\lim_{\epsilon\to 0}[s_O(T)|_{T=0}] = \lim_{\epsilon\to 0}[s_I(\tau)|_{\tau=\infty}] = 1.$$

On the other hand, checking the limit of the outer and inner solutions of the complex gives

$$\lim_{\epsilon\to 0}[c_O(T)|_{T=0}] = \lim_{\epsilon\to 0}[c_I(\tau)|_{\tau=\infty}] \approx 2\left(1+\sqrt{1-4\gamma}\right)^{-1}.$$

The uniform approximations for s and c are eventually derived as

$$s_u = s_O + s_I - 1 = s_O, \tag{12}$$

$$c_u = c_O + c_I - 2\left(1+\sqrt{1-4\gamma}\right)^{-1}. \tag{13}$$

Through equation (12), we find that the approximations obtained via tQSSA and its singular perturbation procedures are exactly the same, thus tQSSA is considered as a reasonable approximation to the substrate concentration. On the other hand, the singular perturbation analysis has successfully corrected the defect of tQSSA; that is, the initial condition is satisfied by introducing the inner solution, c_I (refer Figure 1).

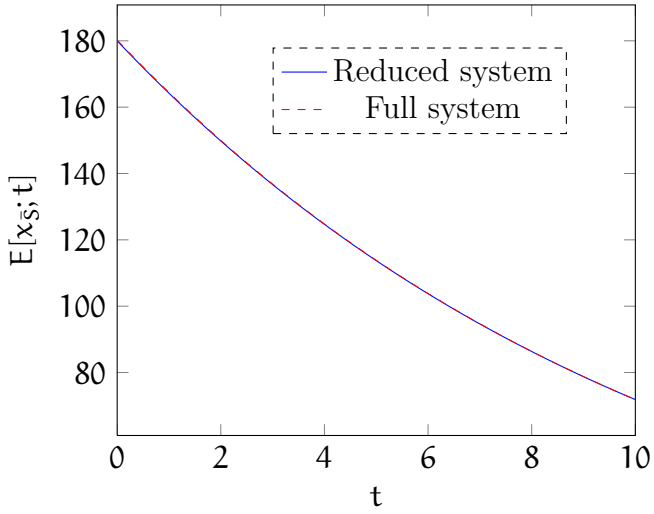

FIGURE 2: Expected value of total substrate computed with $x_{e_0} = 200$, $x_{s_0} = 180$, $\kappa_1 = 0.001$, $\kappa_2 = 30$ and $\kappa_{-1} = 35$, via two approaches: full system of CME and the reduced system, namely the CME in conjunction with tQSSA.

4 Applying the tQSSA to the chemical master equation

In molecular biology, stochasticity is an important driver. Here, we consider the Michaelis–Menten mechanism in reaction (1) where we initially have x_{e_0} copies of enzyme, x_{s_0} copies of the total substrate and zero copies of complex and product. The probability of a state $X = (x_c, x_{\bar{S}})$ at time t is denoted by $\Pr(x_c, x_{\bar{S}}; t)$ and is governed by the chemical master equation (CME):

$$\frac{d\Pr(X;t)}{dt} = \sum_{i=1}^{3} \alpha_i(X - z_i)\Pr(X - z_i; t) - \alpha_i(X, t)\Pr(X; t). \qquad (14)$$

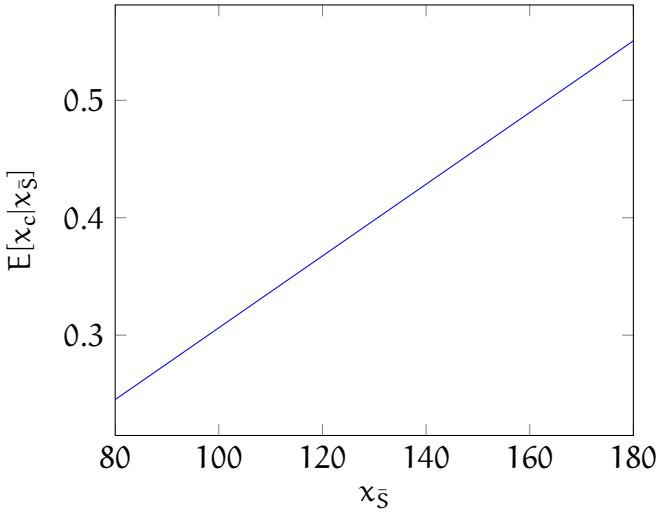

FIGURE 3: The conditional expectation of the complex level, $E[x_c \mid x_{\bar{S}}]$, obtained through equations (15) and (16).

Here, α_i is the ith reaction's propensity: $\alpha_1 = \kappa_1(x_{e_0} - x_c)(x_{\bar{S}} - x_c)$, $\alpha_2 = \kappa_2 x_c$ and $\alpha_3 = \kappa_{-1} x_c$. Furthermore, z_i is the ith reaction's stoichiometric vector: $z_1 = [1, 0]^T$, $z_2 = [-1, -1]^T$ and $z_3 = [-1, 0]^T$.

According to Goutsias [6], the conditional probability $\Pr(x_c \mid x_{\bar{S}}; t)$ approximately solves

$$\begin{aligned}\frac{d \Pr(x_c \mid x_{\bar{S}}; t)}{dt} = &- (\kappa_1(x_{e_0} - x_c)(x_{\bar{S}} - x_c) + (\kappa_{-1} + \kappa_2)x_c) \Pr(x_c \mid x_{\bar{S}}; t) \\ &+ \kappa_1(x_{e_0} - x_c + 1)(x_{\bar{S}} - x_c + 1) \Pr(x_c - 1 \mid x_{\bar{S}}; t) \\ &+ (\kappa_{-1} + \kappa_2)(x_c + 1) \Pr(x_c + 1 \mid x_{\bar{S}}; t).\end{aligned} \tag{15}$$

Recall that the tQSSA leads to the DAE system (4), and, in particular, a constant c_O for any given s_O. Following an idea of Cao et al. [4], this suggests that for a given $x_{\bar{S}}$ the conditional probability $\Pr(x_c \mid x_{\bar{S}})$ is stationary, that

TABLE 1: Comparison of the computations of the full and reduced systems.

Limiting factor, x_{s_0}	90	120	150	180
Computation time for full system (s)	392	609	1261	2430
Computation time for reduced system (s)	5.0	5.9	7.3	8.4
Ratio	78	103	173	289
Percent relative error of $E[x_{\bar{S}}]$ (%)	0.14	0.14	0.14	0.14

is

$$\frac{d\Pr(x_c \mid x_{\bar{S}}; t)}{dt} = 0\,. \tag{16}$$

It follows that $\Pr(x_c \mid x_{\bar{S}}; t)$ can be found by solving the homogeneous system obtained by replacing the left hand side of equation (15) by zero.

On the other hand, summing the CME (14) over x_c and replacing $\Pr(x_c, x_{\bar{S}}; t)$ by $\Pr(x_c \mid x_{\bar{S}})\Pr(x_{\bar{S}}; t)$ in the CME gives

$$\frac{d\Pr(x_{\bar{S}}; t)}{dt} = \kappa_2 E[x_c \mid x_{\bar{S}} + 1]\Pr(x_{\bar{S}} + 1; t) - \kappa_2 E[x_c \mid x_{\bar{S}}]\Pr(x_{\bar{S}}; t). \tag{17}$$

Note that once $\Pr(x_c \mid x_{\bar{S}}; t)$ and, hence, $E[x_c \mid x_{\bar{S}}]$ have been determined, the CME (17) is of lower dimension than the original CME (14).

5 Discussion

We revisited the tQSSA for simple enzymatic reactions and derived the reduced equations using a perturbation ansatz. While we mainly do this for the deterministic case we also derive a model for the stochastic case.

The application of the reduced system obtained using this approach, equations (16) and (17), leads to substantial computational savings, and the results obtained match favorably with the results of the full system, by equation (14), see Figures 2 and 3 and Table 1. Since x_{s_0} is the limiting

factor [3] of the system, the sizes of the propensity matrices of full and reduced systems are $\chi_{s_0}^2 \times \chi_{s_0}^2$ and $\chi_{s_0} \times \chi_{s_0}$ respectively. Our computations (see Table 1), shown that the computation time grows exponentially for the full system but linearly for the reduced system. The relative error computed for the expected value of the total substrate turns out to be around 10^{-3}. The justification of the application of tQSSA to the CME will be discussed in future work.

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