Leading order asymptotics in the Goldbeter–Koshland switch

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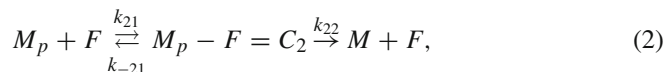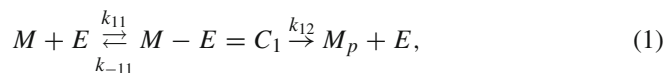
Abstract An asymptotic analysis at leading order of the Goldbeter–Koshland switch, the simplest futile cycle, is carried out in detail. After a nondimensionalization of the problem, we find the leading order uniform expansions of the reactants, identifying the proper time scales in a total setting. Comparison with numerical integration confirms the goodness of our analysis.

Keywords Asymptotic expansions · Systems biology · Enzyme kinetics

1 Introduction

One of the main phenomena characterizing living organisms is the capability of responding to internal and external stimuli, such as depletion of nutrients, variation of hormone levels, reception of sensory signals. In general, this activity is carried out with a cascade of events, whose building block is the covalent modification of proteins. This process can be summarized in the scheme below

$$M + E \underset{k_{-11}}{\overset{k_{11}}{\rightleftarrows}} M - E = C_1 \overset{k_{12}}{\rightarrow} M_p + E, \qquad (1)$$

$$M_p + F \underset{k_{-21}}{\overset{k_{21}}{\rightleftarrows}} M_p - F = C_2 \overset{k_{22}}{\rightarrow} M + F, \qquad (2)$$

which describes, for example, the cycle of phosphorylation and dephosphorylation of an unmodified substrate M into a modified form M_p, where the interconversion

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of the forms is catalyzed by two converter enzymes, E and F. This system, called the Goldbeter–Koshland (GK) switch, was considered in the pioneering work [6] and modelled in terms of Michaelis–Menten (MM) kinetics; assuming Michaelis–Menten conditions (i.e., excess of substrates with respect to enzymes and negligible intermediate complexes) the authors derived reduced equations for the time evolution of the reactants and a closed form for the steady states. But when MM conditions are not valid, their analysis obviously ceases to be feasible.

At the end of last century Borghans et al. [1] introduced, with a simple change of variable, the total quasi-steady-state approximation (tQSSA) for a single MM reaction, that has shown to be the more adequate do describe regimes where MM kinetics (that is, mass action kinetics) do not occur under MM conditions. In [3] the authors showed that the tQSSA is the leading order outer solution of a particular asymptotic expansion. Recently the tQSSA has been applied to networks of coupled enzymatic reactions [4] and also specifically to the GK module [2,9]. Anyway, none of these works use singular perturbation theory to catch the relevant time scales and correct approximations. This is exactly the aim of this paper. A similar approach, in a geometric framework, can be found in [7], even if the authors make an oversimplification of the kinetic parameters.

In this paper the tQSSA has been exploited as a tool for a proper nondimensionalization and, more importantly, we have found, in our leading order outer expansions, the tQSSA results of [9]. The paper is organized as follows. In Sect. 2 we write the equations governing the dynamics of the system, then we rewrite them in a total setting and we find the proper nondimensionalization. In Sect. 3 we find an appropriate small parameter, expand the inner and the outer solution in function of that parameter and we write the leading order uniform expansion. Finally, in Sect. 4 we compare our approximations with the numerical integration of the full system and we summarize our results and the future work.

2 Model equations and nondimensionalization

Using the law of mass action (1) can be translated into the following system of (dimensional) differential equations (here we use the same symbol to denote both the reactant and its concentration):

$$
\begin{aligned}
\frac{dM}{dt} &= -k_{11}ME + k_{-11}C_1 + k_{22}C_2 \\
\frac{dM_p}{dt} &= -k_{21}M_pF + k_{-21}C_2 + k_{12}C_1 \\
\frac{dC_1}{dt} &= k_{11}ME - (k_{-11} + k_{12})C_1 \\
\frac{dC_2}{dt} &= k_{21}M_pF - (k_{-21} + k_{22})C_2 \\
\frac{dE}{dt} &= -k_{11}ME + (k_{-11} + k_{12})C_1 \\
\frac{dF}{dt} &= -k_{21}M_pF + (k_{-21} + k_{22})C_2
\end{aligned}
\tag{3}
$$

with initial conditions

$$M(0) = M_T, \quad M_p(0) = 0, \quad C_1(0) = 0, \quad C_2(0) = 0. \tag{4}$$

Summing up equations, it is easily seen that the system admits three conservation laws:

$$M + M_p + C_1 + C_2 = M_T, \tag{5}$$
$$E + C_1 = E_T, \quad F + C_2 = F_T. \tag{6}$$

Introducing as in [9] the total substrates $\overline{M} = M + C_1$ and $\overline{M}_p = M_p + C_2$ and using (6), we can write

$$
\begin{aligned}
\frac{d\overline{M}}{dt} &= -k_{12}C_1 + k_{22}C_2 = -\frac{d\overline{M}_p}{dt} \\
\frac{dC_1}{dt} &= k_{11}\left[C_1^2 - (E_T + \overline{M} + K_M^1)C_1 + E_T\overline{M} \right] \\
\frac{dC_2}{dt} &= k_{21}\left[C_2^2 - (F_T + \overline{M}_p + K_M^2)C_2 + F_T\overline{M}_p \right]
\end{aligned}
\tag{7}
$$

where $K_M^i = \frac{k_{-i1}+k_{i2}}{k_{i1}}$, $i = 1, 2$ are the *Michaelis constants* of reactions (1–2). It follows that (5) is converted in

$$\overline{M} + \overline{M}_p = M_T. \tag{8}$$

Proceeding as in [3], if we adopt the change of variables

$$\overline{M} = \alpha_1\overline{m}, \quad \overline{M}_p = \alpha_2\overline{m}_p, \quad C_1 = \beta_1 c_1, \quad C_2 = \beta_2 c_2, \quad t = \gamma\tau \tag{9}$$

we find that Eqs. (7) become:

$$
\begin{aligned}
\frac{\alpha_1}{\gamma}\frac{d\overline{m}}{d\tau} &= -k_{12}\beta_1 c_1 + k_{22}\beta_2 c_2 = -\frac{\alpha_2}{\gamma}\frac{d\overline{m}_p}{d\tau} \\
\frac{\beta_1}{\gamma}\frac{dc_1}{d\tau} &= k_{11}\left[\beta_1^2 c_1^2 - (E_T + K_M^1 + \alpha_1\overline{m})\beta_1 c_1 + E_T\alpha_1\overline{m} \right] \\
\frac{\beta_2}{\gamma}\frac{dc_2}{d\tau} &= k_{21}\left[\beta_2^2 c_2^2 - (F_T + K_M^2 + \alpha_2\overline{m}_p)\beta_2 c_2 + F_T\alpha_2\overline{m}_p \right].
\end{aligned}
\tag{10}
$$

Let us first scale the inner variables, since they are supplemented by the initial conditions. It follows immediately that $\alpha_1 = \alpha_2 = M_T$, while to ensure that all terms on the right hand side of the second and third equations of (10) are of the same magnitude, neglecting the quadratic terms and setting for scaling purposes $\overline{m} = \overline{m}_p = c_1 = c_2 = 1$, we find that

$$\beta_1 = \frac{M_T E_T}{M_T + E_T + K_M^1}$$

and

$$\beta_2 = \frac{M_T F_T}{M_T + F_T + K_M^2}.$$

To find γ, the usual procedure would be to require that the left side of the second (or third) equation of (10) have same magnitude of the right side. However, this would in general lead to two different values of γ; we choose therefore to take the arithmetic mean of such values, i.e.

$$\gamma = \frac{1}{2}\left(\frac{1}{k_{11}(M_T + E_T + K_M^1)} + \frac{1}{k_{21}(M_T + F_T + K_M^2)}\right) \tag{11}$$

The value of $t_c = \gamma$ is, for the Golbether–Koshland switch, the fast time scale of complexes formation, corresponding to the analogous one found in [1,3,5] for the single reaction case.

3 Asymptotic expansions

Substituting in (10) the values of $\alpha_1, \alpha_2, \beta_1, \beta_2$ and γ we have the inner equations:

$$\frac{d\overline{m}}{d\tau} = \varepsilon\left(\eta_1 c_2 - \eta_2 c_1\right) = -\frac{d\overline{m}_p}{d\tau}$$

$$\frac{dc_1}{d\tau} = A\left[\sigma_1 c_1^2 - \frac{M_T \overline{m} + E_T + K_M^1}{M_T + E_T + K_M^1}c_1 + \overline{m}\right] \tag{12}$$

$$\frac{dc_2}{d\tau} = B\left[\sigma_2 c_2^2 - \frac{M_T(1-\overline{m}) + F_T + K_M^2}{M_T + F_T + K_M^2}c_2 + \overline{m}_p\right].$$

with initial conditions $\overline{m}(0) = 1, \overline{m}_p(0) = c_1(0) = c_2(0) = 0$ and where all the constants appearing in (12) are defined as follows:

$$\varepsilon = \frac{E_T F_T\left[k_{11}(M_T + E_T + K_M^1) + k_{21}(M_T + F_T + K_M^2)\right]}{2\left[(M_T + E_T + K_M^1)(M_T + F_T + K_M^2)\right]^2},$$

$$\eta_1 = K_2 \frac{M_T + E_T + K_M^1}{v_{max}^1}, \quad \eta_2 = K_1 \frac{M_T + F_T + K_M^2}{v_{max}^2},$$

$$A = \frac{1}{2}\left(1 + \frac{k_{11}}{k_{21}} \cdot \frac{M_T + E_T + K_M^1}{M_T + F_T + K_M^2}\right), \quad B = \frac{1}{2}\left(1 + \frac{k_{21}}{k_{11}} \cdot \frac{M_T + F_T + K_M^2}{M_T + E_T + K_M^1}\right),$$

$$\sigma_1 = \frac{M_T E_T}{(M_T + E_T + K_M^1)^2}, \quad \sigma_2 = \frac{M_T F_T}{(M_T + F_T + K_M^2)^2}$$

where $K_1 = \frac{k_{12}}{k_{11}}, K_2 = \frac{k_{22}}{k_{21}}, v_{max}^1$ and v_{max}^2 are the Van Slyke–Cullen costants and the maximal velocities of reactions (1–2).

Let us expand the solutions of (12) in the form

$$\overline{m} = \mu_0 + \varepsilon\mu_1 + o(\varepsilon), \quad \overline{m}_p = \mu_{p0} + \varepsilon\mu_{p1} + o(\varepsilon), \quad c_1 = \Gamma_{1,0} + \varepsilon\Gamma_{1,1} + o(\varepsilon),$$
$$c_2 = \Gamma_{2,0} + \varepsilon\Gamma_{2,1} + o(\varepsilon),$$

Substituting in (12) we find at leading order that $\mu_0 = const. = 1$ and $\mu_{p0}(0) = const. = 0$, while $\Gamma_{1,0}$ and $\Gamma_{2,0}$ are given by

$$\frac{d\Gamma_{1,0}}{d\tau} = A\left(\sigma_1\Gamma_{1,0}^2 - \Gamma_{1,0} + 1\right)$$
$$\frac{d\Gamma_{2,0}}{d\tau} = B\left(\sigma_2\Gamma_{2,0}^2 - \frac{F_T + K_M^2}{M_T + F_T + K_M^2}\Gamma_{2,0}\right) \tag{13}$$

whose solutions, complying with (4), is easily found as $\Gamma_{2,0} \equiv 0$ and

$$\Gamma_{1,0} = \frac{\Phi^+\Phi^-(\exp(A\sqrt{1-4\sigma_1}\tau) - 1)}{\Phi^+\exp(A\sqrt{1-4\sigma_1}\tau) - \Phi^-}, \tag{14}$$

where

$$\Phi^\pm = \frac{1 \pm \sqrt{1-4\sigma_1}}{2\sigma_1}$$

Note that

$$\lim_{\tau\to\infty}\Gamma_{1,0}(\tau) = \Phi^-. \tag{15}$$

Summarizing, we have that the (dimensional) leading order inner expansions of the reactants are:

$$M^{inn} = M_T\left\{1 - \frac{E_T\Phi^+\Phi^-[\exp(A\sqrt{1-4\sigma_1}\frac{t}{t_c}) - 1]}{(M_T + E_T + K_M^1)[\Phi^+\exp(A\sqrt{1-4\sigma_1}\frac{t}{t_c}) - \Phi^-]}\right\},$$
$$M_p^{inn} \equiv 0, \tag{16}$$
$$C_1^{inn} = \left\{\frac{M_T E_T\Phi^+\Phi^-[\exp(A\sqrt{1-4\sigma_1}\frac{t}{t_c}) - 1]}{(M_T + E_T + K_M^1)[\Phi^+\exp(A\sqrt{1-4\sigma_1}\frac{t}{t_c}) - \Phi^-]}\right\},$$
$$C_2^{inn} \equiv 0.$$

Let us look, now, for the outer solutions of (10). Only the timescale needs to be changed, therefore we choose as new timescale the value $t_s = \frac{t_c}{\varepsilon}$. Note that proceeding in this way the slow time scale becomes:

$$t_s = \frac{\gamma}{\varepsilon} = \frac{(M_T + E_T + K_M^1)(M_T + F_T + K_M^2)}{v_{max}^1 v_{max}^2}$$

In other words, the slow time scale of the Goldbeter–Koshland switch is the product of the slow time scales of every single reaction, found in [3].

With this choice (10) become

$$\frac{d\overline{m}}{d\tau} = (\eta_1 c_2 - \eta_2 c_1) = -\frac{d\overline{m}_p}{d\tau}$$

$$\varepsilon\frac{dc_1}{d\tau} = A\left[\sigma_1 c_1^2 - \frac{M_T\overline{m} + E_T + K_M^1}{M_T + E_T + K_M^1}c_1 + \overline{m}\right] \qquad (17)$$

$$\varepsilon\frac{dc_2}{d\tau} = B\left[\sigma_2 c_2^2 - \frac{M_T\overline{m}_p + F_T + K_M^2}{M_T + F_T + K_M^2}c_2 + \overline{m}_p\right].$$

Let us expand the solutions of (17) in the form

$$\overline{m} = \overline{m}_0 + \varepsilon\overline{m}_1 + o(\varepsilon), \quad \overline{m}_p = \overline{m}_{p0} + \varepsilon\overline{m}_{p1} + o(\varepsilon), \quad c_1 = c_{1,0} + \varepsilon c_{1,1} + o(\varepsilon),$$
$$c_2 = c_{2,0} + \varepsilon c_{2,1} + o(\varepsilon)$$

Substituting in (17) we find at leading order that

$$\frac{d\overline{m}_0}{d\tau} = \left(\eta_1 c_{2,0} - \eta_2 c_{1,0}\right) = \frac{d\overline{m}_{p0}}{d\tau},$$

$$\sigma_1 c_{1,0}^2 - \frac{M_T\overline{m}_0 + E_T + K_M^1}{M_T + E_T + K_M^1}c_{1,0} + \overline{m}_0 = 0, \qquad (18)$$

$$\sigma_2 c_{2,0}^2 - \frac{M_T\overline{m}_{p0} + F_T + K_M^2}{M_T + F_T + K_M^2}c_{2,0} + \overline{m}_{p0} = 0.$$

which correspond to the equations obtained in the tQSSA for the Goldbeter–Koshland switch [9].

The second and third equations are algebraic in $c_{1,0}$ and $c_{2,0}$ with solutions

$$c_{1,0}^{\pm} = \frac{M_T\overline{m}_0 + E_T + K_M^1 \pm \sqrt{(M_T\overline{m}_0 + E_T + K_M^1)^2 - 4M_T E_T\overline{m}_0}}{2\beta_1}$$

$$c_{2,0}^{\pm} = \frac{M_T\overline{m}_{p0} + F_T + K_M^2 \pm \sqrt{[M_T\overline{m}_{p0} + F_T + K_M^2]^2 - 4M_T F_T\overline{m}_{p0}}}{2\beta_2} \qquad (19)$$

It is easy to see that only $c_{1,0}^-$ and $c_{2,0}^-$ are biologically significant; then the first equation of (18) becomes

$$\frac{d\overline{m}_0}{d\tau} = \left(\eta_1 c_{2,0}^- - \eta_2 c_{1,0}^-\right) = -\frac{d\overline{m}_{p0}}{d\tau}. \qquad (20)$$

with initial conditions given by the matching conditions $\overline{m}_0(0) = \lim_{\tau\to\infty}\mu_0(\tau) \equiv 1$ (and $\overline{m}_{p0}(0) = 0$) for the leading order terms in the inner and outer expansions

of $\overline{m}$ (and of $\overline{m}_p$); thus we have automatically that $c_{1,0}(0) = c^-_{1,0}(0) = \Phi^-$ and $c_{2,0}(0) = c^-_{2,0}(0) = 0$. Therefore, we have that the (dimensional) leading order outer expansions of the reactants are:

$$M^{out} = M_T \left(\overline{m}_0 \left(\frac{t}{t_s} \right) - \frac{E_T}{M_T + E_T + K^1_M} c^-_{1,0} \left(\frac{t}{t_s} \right) \right),$$

$$M^{out}_p = M_T \left(\overline{m}_{p0} \left(\frac{t}{t_s} \right) - \frac{F_T}{M_T + F_T + K^2_M} c^-_{2,0} \left(\frac{t}{t_s} \right) \right), \quad (21)$$

$$C^{out}_1 = \frac{M_T E_T}{M_T + E_T + K^1_M} c^-_{1,0} \left(\frac{t}{t_s} \right),$$

$$C^{out}_2 = \frac{M_T F_T}{M_T + F_T + K^2_M} c^-_{2,0} \left(\frac{t}{t_s} \right),$$

Following [8] we are now in position to write the (dimensional) leading order uniform expansions, adding the inner and the outer solutions and subtracting their common part, i. e.,

$$M^{un} = M_T \left[\overline{m}_0 \left(\frac{t}{t_s} \right) - \frac{E_T}{M_T + E_T + K^1_M} \left(\Gamma_{1,0} \left(\frac{t}{t_c} \right) + c^-_{1,0} \left(\frac{t}{t_s} \right) - \Phi^- \right) \right],$$

$$M^{un}_p = M_T \left[\overline{m}_{p0} \left(\frac{t}{t_s} \right) - \frac{F_T}{M_T + F_T + K^2_M} c^-_{2,0} \left(\frac{t}{t_s} \right) \right], \quad (22)$$

$$C^{un}_1 = \frac{M_T E_T}{M_T + E_T + K^1_M} \left(\Gamma_{1,0} \left(\frac{t}{t_c} \right) + c^-_{1,0} \left(\frac{t}{t_s} \right) - \Phi^- \right),$$

$$C^{un}_2 = \frac{M_T F_T}{M_T + F_T + K^2_M} c^-_{2,0} \left(\frac{t}{t_s} \right).$$

4 Discussion

We have integrated numerically (3) and we have compared the results with our uniform expansions. This is shown in Figs. 1, 2. In Fig. 1 our leading order approximations describe very well the dynamics of the unmodified substrate M and its nearby complex C_1: in these cases, our expansions are made, as usual, of an inner part coupled with an outer one. For what regards the modified complex M_p and its nearby complex C_2 (see Fig. 2), our approximations are very good in catching the asymptotic values of the reactants, while they are quite unsatisfactory in describing the initial dynamics, even if the value of ε is extremely small, with our choice of kinetic parameters. This is clearly due to the fact that in this latter case the inner expansions, at leading order, are identically zero and therefore to have a better description of the very first dynamics of these reactants we should better use first order asymptotics. Note also that the time needed to reach a quasi-steady state, in this latter case, is much longer (about one order of magnitude), but this probably depends from the fact that the Michaelis
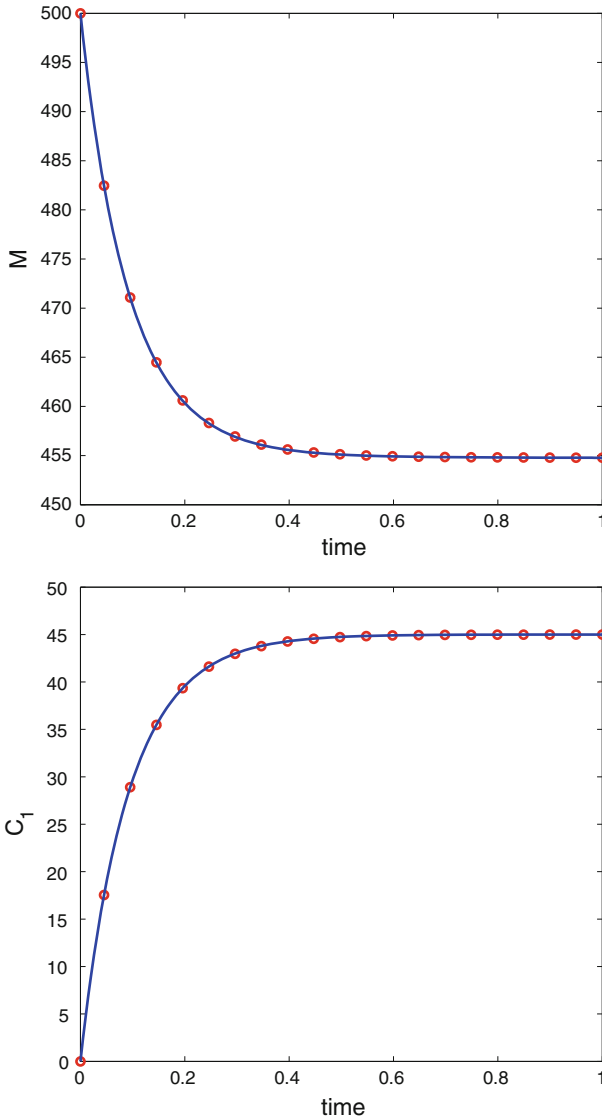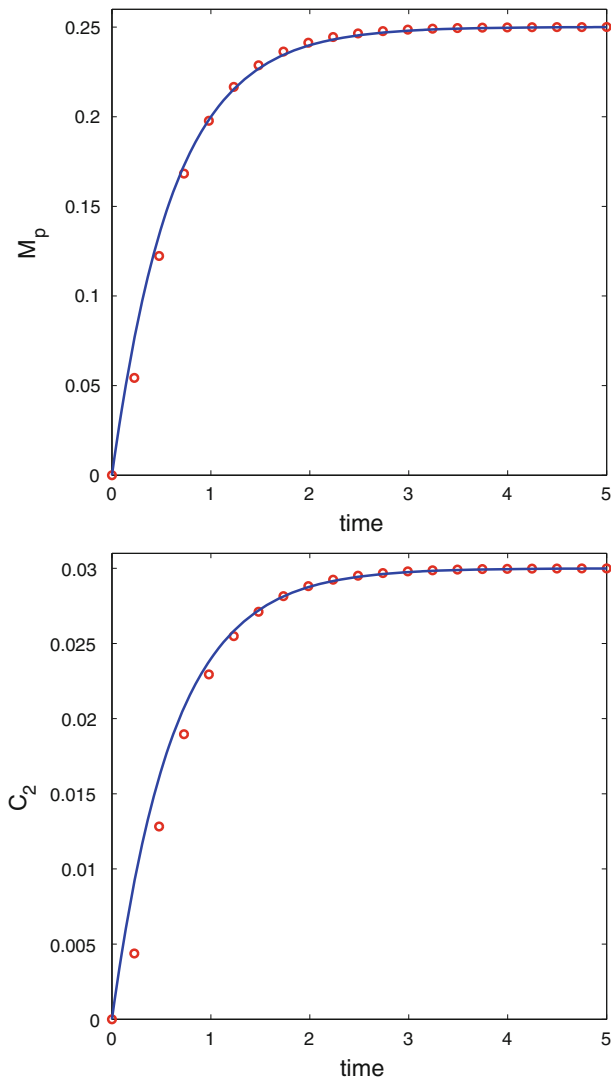
Fig. 1 Dynamics of M (*left* panel) and of C_1 (*right* panel): full system (*circles*) and leading order approximation (*solid*). Kinetic parameters as in Table 1, $M_T = 500$, $E_T = 50$, $F_T = 60$

constant of reaction (2) is about one order of magnitude bigger than the one of (1). Let us remark once again, by the way, that our expansions of M_p and C_2 reproduce, at leading order, the total quasi-steady state approximation, introduced in [1] and applied to the Goldbeter–Koshland switch in [9].

Summarizing, in this work we have presented, for the very first time without any assumption on the kinetic parameters, an asymptotic expansion for the covalent modification cycle, also known as Goldbeter–Koshland switch, in a total setting. We do

Fig. 2 Dynamics of M_p (*left* panel) and of C_2 (*right* panel): full system (*circles*) and leading order approximation (*solid*). Kinetic parameters as in Table 1, $M_T = 500$, $E_T = 50$, $F_T = 60$

Table 1 Kinetic parameters, resulting Michaelis constants and perturbation parameter of reaction (1–2)

k_{11}	k_{-11}	k_{12}	k_{21}	K_M^1	K_M^2	ε
0.02	1	0.01	0.032	50.5	500	1.7×10^{-7}

think that this method can be generalized to biochemical networks of increasing complexity. Another point to work on is the possibility to use first order asymptotic to have better descriptions of the very first dynamics of all the reactants.

References

1. J. Borghans, R. de Boer, L.A. Segel, Extending the quasi-steady state approximation by changing variables. Bull. Math. Biol. **58**, 43–63 (1996)
2. A. Ciliberto, F. Capuani, J.J. Tyson, Modeling networks of coupled anzymatic reactions using the total quasi-steady state approximation. PLoS Comput. Biol. **3**, 463–472 (2007)
3. G. Dell'Acqua, A.M. Bersani, A perturbation solution of Michaelis-Menten kinetics in a 'total' framework. J. Math. Chem. **50**, 1136–1148 (2012)
4. G. Dell'Acqua, A.M. Bersani, Quasi steady-state approximations and multistability in the double phosphorylation-dephosphorylation cycle. Comm. Com. Inf. Sc. **273**, 155–172 (2013)
5. J.W. Dingee, A.B. Anton, A new perturbation solution to the Michaelis-Menten problem. AIChE J. **54**, 1344–1357 (2008)
6. A. Goldbeter, D.E. Koshland, An amplified sensitivity arising from covalent modification in biological system. Proc. Natl. Acad. Sci. **78**, 6840–6844 (1981)
7. A. Kumar, K. Josić, Reduced models of networks of coupled enzymatic reactions. J. Theor. Biol. **278**, 87–106 (2011)
8. C.C. Lin, L.A. Segel, *Mathematics Applied to Deterministic Problems in the Natural Sciences* (Society for Industrial and Applied Mathematics (SIAM), Philadelphia, 1988)
9. M.G. Pedersen, A.M. Bersani, E. Bersani, G. Cortese, The total quasi-steady-state approximation for complex enzyme reactions. Math. Comput. Simulat. **79**, 1010–1019 (2008)