

**REDUCED MODELS OF NETWORKS OF COUPLED
ENZYMATIC REACTIONS AND
LINEARIZATION OF MICHAELIS-MENTEN
DIFFERENTIAL EQUATIONS**

A Dissertation
Presented to
the Faculty of the Department of Mathematics
University of Houston

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

By
Ajit Kumar
May 2011

**REDUCED MODELS OF NETWORKS OF COUPLED
ENZYMATIC REACTIONS AND
LINEARIZATION OF MICHAELIS-MENTEN
DIFFERENTIAL EQUATIONS**

Ajit Kumar

APPROVED:

Dr. Krešimir Josić,
Chairman

Dr. Andrew Török

Dr. Gabor Balazsi

Dr. Robert Azencott

Dean, College of Natural Sciences and Mathematics

Acknowledgements

I have been blessed with the help and support of a lot of people during the completion of my thesis. My adviser, Dr. Krešimir Josić, has played the most significant role. All the work in this thesis is in collaboration with him. I am deeply indebted to him for accepting me as his student, and for guiding me all these years.

Next major thanks goes to Drs. Andrew Török, Gabor Balazsi, and Robert Azencott, who very generously agreed to serve on my thesis committee. Many thanks to them for taking their time out to read my thesis and for suggesting new directions to work on.

I would also like to thank the Department of Mathematics at UH, for letting me study in this world-class research environment. All the faculty and staff of this place are extremely friendly and cooperative. Another special thanks goes to the faculty of Mathematical Sciences Foundation, New Delhi, who encouraged me to pursue advanced studies in Mathematics.

On personal note, I am extremely thankful to my lovely girlfriend, Sneha, and to all my fellow graduate students, Harbir, Meghna, Pankaj, Aanchal, Ankita, Anando, Negi, Aarti, Ali, Vasudha, Paps, Justin, and many more, for making my grad school years really memorable.

Finally, I would like to express my deepest gratitude to my family back home in India, especially my parents Shri. Rakesh Kr. Sinha and Smt. Pramila Devi. The work in this thesis is as much theirs as is mine and my adviser's.

**REDUCED MODELS OF NETWORKS OF COUPLED
ENZYMATIC REACTIONS AND
LINEARIZATION OF MICHAELIS-MENTEN
DIFFERENTIAL EQUATIONS**

An Abstract of a Dissertation
Presented to
the Faculty of the Department of Mathematics
University of Houston

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

By
Ajit Kumar
May 2011

Abstract

The Michaelis-Menten scheme has played a central role in our understanding of biochemical processes. It has long been understood how this scheme can be used to approximate the dynamics of irreversible enzymatic reactions. However, a similar approximation in the case of networks, where the product of one reaction can act as an enzyme in another, has not been fully developed. In this thesis such an approximation is developed for a class of coupled enzymatic networks where the individual interactions are of Michaelis-Menten type. In addition, sufficient conditions for the validity of the *total Quasi-Steady State Assumption (tQSSA)*, obtained in a single protein case by Borghans, de Boer, and Segel are extended to sufficient conditions that guarantee the validity of the tQSSA for a large class of enzymatic networks. The resulting reduced equations approximate a network's dynamics and involve only protein concentrations. This significantly reduces the number of equations necessary to model such systems. The validity of this approximation is proved using geometric singular perturbation theory and results about matrix differentiation. The ideas used in deriving the approximating equations are quite general, and can be used to systematize other model reductions.

This thesis also examines a particular class of non-linear dynamical systems that appear frequently as models of biological networks. The non-linearity in these systems model the interaction of network elements and take the form of sigmoidal functions. It is shown that, in certain limits, these equations can be approximated by piecewise linear dynamical systems. These approximations are naturally determined by the structure of the original system and can again be justified using geometric singular perturbation theory. The validity of both reductions is demonstrated in a number of biologically inspired example networks.

Contents

0	Introduction and motivation	1
1	Geometric Singular Perturbation Theory (GSPT)	9
1.1	Three main theorems of the GSPT	10
1.2	Example application	14
2	Introduction of the total Quasi-Steady States Assumption (tQSSA)	20
2.1	Meaning and extension of the tQSSA	22
2.1.1	Extension of the tQSSA	24
2.2	Sufficient conditions for the validity of tQSSA	26
2.2.1	Results of Borghans’s et al. [6]	26
2.2.2	Results on tQSSA obtained by Tzafriri [65]	27
2.2.3	Khoo and Hegland’s proof for tQSSA using GSPT [37]	29
2.2.4	Validity of Tzafriri’s claim using the GSPT	30
3	General description of the network in this study	32
3.1	Analysis of a two-protein network	32
3.1.1	New coordinates and reduction under the tQSSA	35
3.1.2	Comparison of our reduction with MM differential equations	37
3.2	The general problem	38

3.2.1	Description of the network	39
3.2.2	Inherent constraints on this network	42
3.2.3	ODE associated with this network	43
3.2.4	The total substrate coordinates	43
3.2.5	The tQSSA and the resulting reduced equations	45
3.2.6	Proof of Lemma 3.2.1	47
3.2.7	An example network where the above reduction scheme fails	56
4	Validity of tQSSA in a network	58
4.1	The stability of the slow manifold	59
4.1.1	Stability of slow manifold in the absence of some connections	71
4.2	Validity of the tQSSA in the general setup	72
4.2.1	Another approach for validity of tQSSA in isolated MM reaction	72
4.2.2	Validity of the tQSSA for two interacting proteins	74
4.2.3	Extension of scaling from two dimension to several dimension	77
5	Linearization of Michaelis-Menten differential equations	83
5.1	Introduction	83
5.2	Example problems	86
5.3	General setup	91
5.4	Discussion	98
A	The Law of Mass Action	99
B	Differentiation with respect to a matrix	102
C	Some Linear Algebra results	104
	Bibliography	110

List of Figures

- 1.1 The solid line represents the exact solution of the system $\epsilon \frac{dx}{dt} = -(x - \epsilon)$, $\frac{dy}{dt} = -y$, with $\epsilon = 0.03$ and the initial value (the little circle at top-right corner) $x(0) = 1, y(0) = 0.8$. Here $\mathcal{M}_0 := \{(x, y) | x = 0\}$ and $\mathcal{M}_\epsilon := \{(x, y) | x = \epsilon\}$ (the vertical dotted-dashed line). The horizontal dashed line represents the solution of $\frac{dx}{ds} = -x$, $\frac{dy}{ds} = 0$, with initial value $x(0) = 1, y(0) = 0.8$. The vertical dotted line on y axis beginning at the solid dot and ending at the origin represents the solution of the system $0 = -x$, $\frac{dy}{dt} = -y$, initial value $y(0) = 0.8$. The triple arrow denotes *fast* dynamics because those are on the time scale $s = t/\epsilon$ 13
- 1.2 Projected solutions of Eq. (1.12) on the u - v_1 plane, for two different values of ϵ . For the dashed (black) line, $\epsilon = 0.1$; and for the thick solid (blue) line, $\epsilon = 0.05$ was used. The thin solid (red) line is the slow manifold, $\mathcal{M}_0$, described by Eq. (1.15). We observe that for small ϵ , solutions first get quickly attracted to $\mathcal{M}_0$ then move on it slowly. The attraction gets sharper as ϵ becomes smaller. Other parameters used: $\lambda_1 = \lambda_2 = \lambda_3 = \lambda_4 = \alpha = 1$; $B = 1$ (dashed (black) line), $B = 2$ (thick solid (blue) line). Initial value: $v_1 = 0, v_2 = 0, x = 0.9$. 19

- 2.1 Proper choice of the initial values of the reduced system. The empty circle at $\bar{X} = 1$, $C = 0$, represents the initial value for the full system. The solid dot is the initial value of the reduced system. The dash-dotted (red) line represents the attracting *slow manifold*. (a) The solid curve represents the numerical solution of Eq. (2.4). The solution rapidly converges to the manifold, and evolves slowly along the manifold after this transient. The dashed line satisfies $\bar{X} = X_T$. The solid dot at the intersection of the dashed line and the slow manifold represents the projection of the initial condition onto the slow manifold given by Eq. (2.5b). Thus $\bar{X}(0) = X_T$ is the proper initial condition for the reduced system (2.6). (b) The solid line represents the numerical solution of Eq. (2.3). After a quick transient, the solution again converges to the slow manifold. However, since the initial transient is not orthogonal to the X axis, the initial conditions do not project vertically onto the slow manifold. Instead, the initial transient follows the line $X + C = X_T$ (dashed), and the intersection of this line and the slow manifold represents the proper choice of the initial value for Eq. (2.8a). (c) Comparison of solutions of Eq. (2.3) and the reduced system (2.8a). The graph in the inset offers a magnified view of the boxed region, showing the quick transient to the slow manifold. We used: $X_T = E_T = k_1 = k_2 = 1$, $k_{-1} = 3$, which, using Eq. (2.8b), gives the initial condition for the reduced system, $\hat{X}(0) = 0.83$. 25
- 3.1 A simplified description of interactions between two regulators of the G2-to-mitosis phase (G2/M) transition in the eukaryotic cell cycle [49] (See text). (a) X and Y phosphorylate and deactivate each other. For instance, the protein X exists in a *phosphorylated* X_p and *unphosphorylated* X state, and the conversion X to X_p is catalyzed by Y_p . The conversion of X_p to X is catalyzed by the phosphatase E_1 . (b) Comparison of the numerical solution of Eq. (3.1) and Eq. (3.8). Here $k_1 = 5$, $k_{-1} = 1$, $k_2 = 1$, $E_1^T = 10$, $E_2^T = 2$, $X_T = 10$, $Y_T = 10.1$ as in [11]. The initial values for Eq. (3.1) are $X(0) = 10$, $Y(0) = 1.1$, $X_p(0) = 0$, $Y_p(0) = 9$, $C_x(0) = 0$, $C_y(0) = 0$, $C_x^e(0) = 0$, $C_y^e(0) = 0$, $E_1(0) = 10$, $E_2(0) = 2$. The initial values of the reduced system, $\hat{X}_p(0) = 0.12$, $\hat{Y}_p(0) = 0.83$ are obtained by the projection onto the slow manifold defined by Eq. (3.7). 33
- 3.2 (a),(b): Plot of the the first coordinate of the right hand side of Eq. (3.8) from two different viewing angles. (c) Plot of the right hand side of Eq. (3.9a). The viewing angle for subfigures (b) and (c) are same. All the parameters are same as in Fig. 3.1. The difference in shape of (b) and (c) suggest that Eq. (3.9) is not consistent with the Law of Mass Action. 38
- 3.3 A section of the network illustrating the terminology used in describing protein interaction networks. Each shaded region represents a node and encompasses either an enzyme or a single protein that is part of an MM type reaction. Each dotted arrow represents an edge in the network. The solid arrows represent transitions within the nodes, and do not define an edge in the network. 39

3.4	A hypothetical network for which the reduction described in sections 3.2.4–3.2.5 leads to a differential–algebraic system of equations. The concentrations of the intermediate complexes appear in a nonlinear way in the resulting algebraic equations. A further reduction to a form involving only the protein concentrations is therefore not apparent.	56
5.1	Comparison of the numerical solution of Eq. (5.1) (dashed black) and the solution of the approximate system as listed in Table 5.1 (solid colored) for two different values of J (We used $J = 10^{-2}$ in (a); and $J = 10^{-4}$ in (b).). The different colors denote the switching behavior of the solution from one region to next. We used $\delta = 0.01$. Solution of the linear approximation started in the region $\mathcal{R}_0^0$ (Initial value: $u_1 = 0.6, u_2 = 0.4$), and as soon as u_2 became smaller than δ , the region switched to $\mathcal{R}_2^0$ and driving linear differential equation also switched accordingly. It should be noted that the approximate solution is discontinuous. The reason is that as soon as the solution crossed the horizontal line, $u_2 = \delta$, the solution jumped (see inset) to the manifold, described by the algebraic part of the linear differential algebraic system prevalent in the region $\mathcal{R}_2^0$. The solution finally stopped in the region $\mathcal{R}_2^1$.	90
5.2	Comparison of the numerical solution of Eq. (5.7) (dashed black) and the solution of the approximate linear system (not explicitly provided) for two different sets of J and δ . For (a)–(c) $J = 10^{-2}, \delta = 0.06$; for (c)–(f) $J = 10^{-4}, \delta = 0.01$. The approximate solution changes color when switching between different subdomains. Note that the approximate solution is discontinuous in general. The reason is that as soon as the solution enters a new region, the solution jumps (see inset) to the manifold defined by the algebraic part of the linear differential algebraic system corresponding to the new region.	91

Introduction and motivation

Biology is increasingly being viewed by mathematicians as a new playground, a new field to explore. Advances in experimental technologies allow for the acquisition of large amounts of data describing numerous aspects of different organisms. These data suggest that to describe even a tiny living cell, we need enormous amounts of information. Mathematics has helped us understand the structure and function of the interacting processes within a cell. Mathematicians have also gained significantly from their interactions with biologists. Many biological phenomena could not be described neatly by existing mathematical theories. This lead to the development of new branches of mathematics. Biology therefore, has taken the role played by physics a century ago [1].

The broad aim for mathematicians would be to build a connection between the known laws of physics and biological observations. We are far away from this goal presently, and it may not be achievable if taken in a literal sense. However, a lot of work has been done to allow us to ask questions relevant to biology so that they can be addressed using mathematical techniques. In the words of Uri Alon “observing the activities in a living

cell is like watching a dance. Proteins, enzymes, genes, etc, assemble together when they are needed; they perform their job with utmost efficiency in presence of cellular noise, and when their work is done they so effortlessly disassemble or inactivate or get busy in some other job” [2]. Nontrivial theories are required to explain these phenomena. How do cells function in the way they do? Is their action efficient? How do they manage to overcome the noise that accompany all the processes within a cell? How does information get passed between cells and generations? The list of questions goes on.

This thesis is a modest attempt to further develop the mathematical theories used for understanding cell mechanism. One such theory investigates so called Molecular Interaction Networks. This theory assumes that cells can be described as containers full of fluid. Biomolecules like proteins, enzymes, etc., are tiny particles floating in this liquid body. The behavior of the cell can then be studied by modeling the outcome of collisions between these molecules. If one wants to understand the function of proteins and enzymes in the life span of the cell then one needs to study their interactions, and hence the importance of Protein Interaction Networks (PIN).

Depending on the question, one uses different types of mathematical tools to analyze models of PINs. For example, an ideal aim of a study of PINs (or in fact any Molecular Interaction Network) would be to come up with a description of the position of each and every molecule at any given time. Considering the fact that even a single gram of any substance contains on the order of 10^{23} molecules, this goal is clearly not achievable.

A simpler question is to describe the behavior of certain averages for the different types of molecules in the system. Assuming that for all the collisions between two types of molecules, a given percentage will lead to a new product, assuming that temperature, volume and pressure remain constant, the chemical species are well mixed, then one can

write an ordinary differential equation whose solution will be the probability distribution describing the populations of the different molecules involved. This differential equation is known as Chemical Master Equation (CME) [19, 29]. However, the CME is very cumbersome to use because for most practical purposes it turns out to be infinite dimensional. Its analytical solutions are known only for very few simple reactions [12, 34, 39]. In general, the CME can only be solved through simulation algorithms like the Stochastic Simulation Algorithm (SSA) [20, 29] and its variants the *slow scale* Stochastic Simulation Algorithm (ssSSA) [4, 8, 56]; and the *nested* Stochastic Simulation Algorithm (nSSA) [70]. Approximating the CME by lower dimensional systems is a subject of active research [27, 41, 45, 53].

Another common method to study chemical reactions is through mass action kinetics. If sufficiently large numbers of molecules are present, it is possible to obtain much simpler differential equation to approximate the population of various kinds of molecules with high accuracy. This set of differential equations are related to the set of chemical reactions through the Law of Mass Action [68, 69]. This law can be applied to obtain an ODE model directly from the list of reactants and products involved, and a list of possible reactions. Theoretically, this differential equation can be derived, under the assumption of the existence of the “macroscopic infinitesimal”, as the mean behavior of the probability distribution guaranteed by the CME [21, 29]. However, the history of the Law of Mass Action is much older than the CME, as chemists have been using it for more than a hundred years [30, 68, 69]

Although the assumptions that allow for a theoretical justification of the Law of Mass Action are quite stringent, experimental data suggest that even in the noisy, nonhomogeneous environment of living cells the Law of Mass Action may do a decent job in describing the population of chemical species [16, 44, 63]. Encouraged by the experimental

results and tempted by the relative simplicity of the resulting equations, extensive research has been done on the analysis of the models of PINs obtained from the Law of Mass Action [11, 25, 51, 64].

We will focus on one class of such PINs. It is believed that proteins regulate the activity of each other through activation or inhibition. Such interactions are believed to be much cheaper than producing a protein from scratch when it is needed and destroying it when its job is done [2]. The chief feature of the PIN we consider is that proteins and enzymes activate and deactivate each other through a scheme which is popularly known as the Michaelis-Menten (MM) reaction, named after Leonor Michaelis and Maud Menten (1913) [7, 43]. In this reaction an enzyme, E , reacts with a protein, X , resulting in an intermediate complex, C . In turn, this complex dissociates into an “active” product, X^* , and the enzyme E . It is frequently assumed that the formation of C is reversible while its dissociation is not [7, 43, 46].



The MM reaction became popular because of its simplicity and effective explanation of the activation and inhibition relations among proteins. In a paper by Goldbeter and Koshland in 1982, it was shown that, when the total enzyme concentration is much smaller than the total substrate concentration in reaction (1), the substrate concentration can be modeled by a particular type of differential equation which is commonly known as the MM differential equation [11, 24, 52]. The MM differential equation can be obtained in the following way: If we apply the Law of Mass Action on reaction (1) then, using some natural constraints, one gets a system of two dimensional autonomous differential equation, say

$$\frac{dX}{dt} = f(X, C) \text{ and } \frac{dC}{dt} = g(X, C).$$

The MM differential equation is obtained by replacing the time derivative of C with zero,

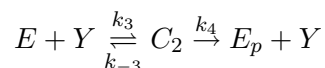
leading to an algebraic equation for C , *i.e.* $0 = g(X, C)$. Solving this equation for C as a function of X , say $C = h(X)$, and plugging this solution into the differential equation for X we get

$$\frac{dX}{dt} = f(X, h(x)).$$

The assumption that some of the variables are in steady state, or, equivalently, the replacement of the derivative of some of variables with zero, is known as the Quasi-Steady State Assumption (QSSA). In the preceding example, the variable C is in quasi-steady state with respect to the variable X . The intuitive idea is that before X changes appreciably, C changes fast and equilibrates. The QSSA therefore posits that, if in the dynamical system, variables change in very different time scales, then the system can be simplified by assuming that the fast variables will reach a “local” steady state instantaneously.

Extensive research has been done to study the mathematical logic behind the QSSA. Heuristic self consistent arguments [6], as well as rigorous theories involving monotone dynamical systems have been used in the analysis [48]. The most common and intuitive approach to the problem is through the use of the *Geometric Singular Perturbation Theory (GSPT)* [15]. Several application of the GSPT to explain the QSSA exist in the literature [28, 59, 62]. As the GSPT plays a central role behind the mathematics of this thesis, it has been reviewed in the first chapter.

The simplicity of the MM reactions and the MM differential equations prompted their use in systems where MM reactions are not isolated, but embedded in a larger set of reactions. Such a system would be obtained if, for example, a reaction like



is coupled with reaction (1). However, the mathematical basis for the QSSA, which was needed to derive the MM differential equation, only existed for an isolated MM reaction.

To the best of our knowledge, no theory exists to justify the use of the MM differential equations in a general system of coupled MM reactions. A large part of this thesis consists of a theoretical justification of something similar to QSSA, which is valid for systems of coupled Michaelis-Menten reactions.

The asymptotic limits, under which QSSA can be assumed on a MM reaction, does not make sense if we have several simultaneous MM reactions in the system, and the protein of one MM reaction is behaving like the enzyme for some other reaction. To justify the QSSA for such a system we will need the proteins to be present in both large and small amount at the same time, which is obviously absurd [11]. To remedy this situation, other versions of the QSSA were proposed. (Recall that in the original derivation of MM differential equation the intermediate complex C was assumed to be in steady state.) Segel and Slemrod proposed reverse QSSA (rQSSA) where the substrate X was assumed to be in steady state [59]. However, we are not aware of any study which attempts to extend the rQSSA to a system of MM reactions.

Another version of QSSA, which is of particular interest to us, is the total QSSA (tQSSA), proposed by Borghans, et al. in 1996 [6] and later refined by Tzafriri in 2003 [65]. The tQSSA posits that the sum of substrate and the intermediate complex, $X + C$, is in steady state. In a recent paper by Ciliberto, et al. an extension of tQSSA was defined for a system of MM reactions [11]. They also presented simulation results which suggest that tQSSA might be a very good tool to simplify the dynamical systems in a system of coupled MM reactions.

This brings us to our main contribution in the field of PINs. In this thesis we have proved that, the asymptotic limit which implies tQSSA, as obtained by Borghans, et al. in [6] for the isolated MM reaction (1), can be extended to a system of such reactions.

Thus we provide theoretical explanation of the simulation result obtained by Ciliberto, et al. [11]; also extending significantly the results of Pedersen, et al. [51], who have obtained results regarding the validity of the tQSSA for a system of two MM reactions. In addition to that, we have noted that direct use tQSSA leads to an algebraic system of coupled quadratic equation which can be difficult to solve. We discuss a method to circumvent this problem.

Closely connected to the PINs are gene interaction networks. As the name suggests, these network model the expression of gene and their interactions within the cells. Typically one does not use the Law of Mass Action to model this network. The main reason could be that the list of reactions are not known in enough detail. Another reason could be that the copies of gene are not believed to be present in large enough number. The differential equation models one sees in literature are typically custom made to fit the data. It is widely believed that gene activity can be approximated by Boolean variables, they are either ON or OFF. To model this switch like behavior, Hill functions *i.e.*, functions of type $f(x) := x^n/(x^n + J^n)$, where n is the Hill coefficient and J is the threshold, appear more than often in the equations [2, 13, 23, 40, 49, 50, 64]. The problem with the Hill functions are that they produce highly non-linear differential equations. Several theories exist to approximate these non-linear differential equations with piecewise-linear differential equations [9, 22, 33, 54, 55]. The general argument in these studies is that, in the limit of large Hill coefficient, near the threshold, the non-linear differential equation can be decomposed into *fast* and *slow* variables. Then, using the GSPT one gets a reduced system which turns out to be linear.

In the last chapter of this thesis we will re-investigate the non-linearity induced by the Hill functions and will introduce a class of differential equations, which can be approximated

by a piecewise-linear system. Furthermore, we will see that this reduction is independent of the Hill coefficient but rather depends on the threshold size. In particular, our linear approximation will be derived in the asymptotic limit of small threshold.

Outline of the thesis:

This thesis is organized as follows: In Chapter 1 we review the basics of the GSPT. This theory is the mathematical backbone of the results discussed in this report. In this chapter we also discuss MM reactions, the meaning of QSSA and its justification using the GSPT, in detail.

Chapters 2–4 contains our work on PINs. In Chapter 2, the notion of tQSSA for a single MM reaction is introduced. Various different arguments in support of the tQSSA have been reviewed. Merits of these arguments have also been discussed. This chapter also includes a novel justification of the tQSSA for a single MM reaction. In Chapter 3, a general class of coupled MM reaction is described. The notion of the tQSSA for this reaction system has been introduced. The problem of coupled quadratic equation by direct application of the tQSSA and a method to get out of that problem has been discussed. In Chapter 4, we describe how we can extend the justification of the tQSSA from one MM reaction to the system of coupled MM reactions.

Chapter 5 discusses our results on linear approximations of the differential equations involving Hill functions. This chapter is essentially independent from Chapter 2–4.

Brief notes on necessary technicalities like the Law of Mass Action, “differentiation with respect to a matrix variable” have been provided in the appendix. The appendix also includes some of our other purely mathematical results, which we discovered in the course of our investigation. These results, however, do not necessarily contribute to the topics in the main chapters.

Chapter 1

Geometric Singular Perturbation Theory (GSPT)

In this chapter I will review the basics of Geometric Singular Perturbation Theory (GSPT) and present the main theorems along with their interpretations. For the proofs of these theorems and more detailed expositions on the GSPT, the reader may consult [15, 28, 35, 36, 71] and references therein. In the last section of this chapter I will provide an application of this theory to obtain a rigorous justification of the Quasi-Steady State Assumption (QSSA) in a system of two coupled Michaelis–Menten reactions.

1.1 Three main theorems of the GSPT

Consider a system of ordinary differential equation of the form

$$\begin{aligned} \frac{dv}{dt} &= g(u, v, \epsilon), & v(0) &= v_0, \\ \epsilon \frac{du}{dt} &= f(u, v, \epsilon), & u(0) &= u_0, \end{aligned} \quad (1.1)$$

where $u \in \mathbb{R}^k$ and $v \in \mathbb{R}^l$ with $k, l \geq 1$; and $u_0 \in \mathbb{R}^k$ and $v_0 \in \mathbb{R}^l$ are the initial values. The parameter ϵ is small and positive ($0 < \epsilon \ll 1$). The right hand side functions f and g are smooth functions such that $f(u, v, 0) \not\equiv 0$, $g(u, v, 0) \not\equiv 0$, and $\lim_{\epsilon \rightarrow 0} \epsilon g(u, v, \epsilon) \equiv 0$.

The following three theorems, due to Fenichel, say that we can say much about Eq. (1.1) just by studying it in the limiting case of $\epsilon = 0$.

Theorem 1.1 (Fenichel [15]) *Suppose $\mathcal{M}_0 \subset \{(u, v) \in \mathbb{R}^{k+l} \mid f(u, v, 0) = 0\}$ is compact, possibly with boundary, and normally hyperbolic, that is, the eigenvalues λ_i of the Jacobian $\frac{\partial f}{\partial u}(u, v, 0)|_{\mathcal{M}_0}$ all satisfy $\operatorname{Re}(\lambda_i) \neq 0$. Suppose f and g are smooth. Then for sufficiently small $\epsilon > 0$, there exist a manifold $\mathcal{M}_\epsilon$, $\mathcal{O}(\epsilon)$ close and diffeomorphic to $\mathcal{M}_0$ that is locally invariant under the flow of the system (1.1).*

For system (1.1), for $\epsilon = 0$, suppose that the normally hyperbolic manifold $\mathcal{M}_0 \subset \{(u, v) \in \mathbb{R}^{k+l} \mid f(u, v, 0) = 0\}$ has an $l + m$ dimensional stable manifold $W^s(\mathcal{M}_0)$ and an $l + n$ dimensional unstable manifold $W^u(\mathcal{M}_0)$, with $m + n = k$. In other words, suppose that the Jacobian $\frac{\partial f}{\partial u}(u, v, 0)|_{\mathcal{M}_0}$ has m eigenvalues λ_i with $\operatorname{Re}(\lambda_i) < 0$ and n eigenvalues λ_j with $\operatorname{Re}(\lambda_j) > 0$. Then we have the Fenichel's second theorem.

Theorem 1.2 (Fenichel [15]) *Suppose $\mathcal{M}_0 \subset \{(u, v) \in \mathbb{R}^{k+l} \mid f(u, v, 0) = 0\}$ is compact, possibly with boundary, and normally hyperbolic, that is, the eigenvalues λ_i of the Jacobian*

$\frac{\partial f}{\partial u}(u, v, 0)|_{\mathcal{M}_0}$ all satisfy $\operatorname{Re}(\lambda_i) \neq 0$. Suppose f and g are smooth. Then for sufficiently small $\epsilon > 0$, there exist manifolds $W^s(\mathcal{M}_\epsilon)$ and $W^u(\mathcal{M}_\epsilon)$ that are $\mathcal{O}(\epsilon)$ close and diffeomorphic to $W^s(\mathcal{M}_0)$ and $W^u(\mathcal{M}_0)$, respectively, and that are locally invariant under the flow of the system (1.1).

Let $x \cdot t$ denote the new point under the flow, described by Eq. (1.1), after time t , starting from the initial value x . Also, let Δ be a neighborhood of $\mathcal{M}_\epsilon$, and for all $V \subset \Delta$ define

$$V \cdot_\Delta t := \{x \cdot t \mid x \in V \text{ and } x \cdot [0, t] \subset \Delta\}.$$

The third theorem is the following.

Theorem 1.3 (Fenichel [15]) *Suppose $\mathcal{M}_0 \subset \{(u, v) \in \mathbb{R}^{k+l} \mid f(u, v, 0) = 0\}$ is compact, possibly with boundary, and normally hyperbolic and suppose f and g are smooth. Then for every $v_\epsilon \in \mathcal{M}_\epsilon$, $0 < \epsilon \ll 1$, there exist manifolds $W^s(v_\epsilon) \subset W^s(\mathcal{M}_\epsilon)$ and $W^u(v_\epsilon) \subset W^u(\mathcal{M}_\epsilon)$ that are $\mathcal{O}(\epsilon)$ close and diffeomorphic to $W^s(v_0)$ and $W^u(v_0)$, respectively. The families $\{\mathcal{W}^{u,s}(v_\epsilon) \mid v_\epsilon \in \mathcal{M}_\epsilon\}$ are invariant in the sense that*

$$\mathcal{W}^s(v_\epsilon) \cdot_\Delta t \subset \mathcal{W}^s(v_\epsilon \cdot_\Delta t) \text{ if } v_\epsilon \cdot r \in \Delta \text{ for all } r \in [0, t], \text{ and}$$

$$\mathcal{W}^u(v_\epsilon) \cdot_\Delta t \subset \mathcal{W}^u(v_\epsilon \cdot_\Delta t) \text{ if } v_\epsilon \cdot r \in \Delta \text{ for all } r \in [t, 0].$$

Interpretation of the above theorems

The basic ideas behind these theorems can be illustrated by assuming, for simplicity, that

$$\mathcal{M}_0 := \left\{ (u, v) \in \mathbb{R}^{k \times l} \mid f(u, v, 0) = 0 \right\},$$

and $\mathcal{M}_0$ is *normally hyperbolic* and *stable*, that is, the eigenvalues λ_i of the Jacobian $\frac{\partial f}{\partial u}(u, v, 0)|_{\mathcal{M}_0}$ all satisfy $Re(\lambda_i) < 0$. Then, for ϵ sufficiently small, the solutions of Eq. (1.1) follow an initial transient, which can be approximated by

$$\begin{aligned} \frac{dv}{ds} &= 0, & v(0) &= v_0, \\ \frac{du}{ds} &= f(u, v, 0), & u(0) &= u_0, \end{aligned} \tag{1.2a}$$

where $t = \epsilon s$. After this transient, the solutions are $\mathcal{O}(\epsilon)$ close to the solutions of the reduced differential-algebraic system

$$\begin{aligned} \frac{dv}{dt} &= g(u, v, 0), & v(0) &= v_0, \\ 0 &= f(u, v, 0). \end{aligned} \tag{1.2b}$$

More precisely there is an invariant, slow manifold $\mathcal{M}_\epsilon$, $\mathcal{O}(\epsilon)$ close to $\mathcal{M}_0$. Solutions of Eq. (1.1) are attracted to $\mathcal{M}_0$ exponentially fast, and can be approximated by concatenating the fast transient described by Eq. (1.2a), and the solution of the reduced Eq. (1.2b) (see Fig (1.1) for an illustrative example).

The slow manifold, $\mathcal{M}_0$, consists of the fixed points of Eq. (1.2a). The condition that the eigenvalues, λ_i , of the Jacobian $\frac{\partial f}{\partial u}(u, v, 0)|_{\mathcal{M}_0}$ all satisfy $Re(\lambda_i) < 0$ implies that these fixed points are stable .

Note that v is constant in Eq. (1.2a) and only changes in Eq. (1.2b). Another way of saying the same thing would be that v only changes on the time scale t and is essentially constant on the time scale s . Furthermore, u is an independent variable on the time scale s but in the time scale t , it plays an indirect role (because, in principle, $0 = f(u, v, 0)$ can be solved for u and plugged into $g(u, v, 0)$ to make Eq. (1.2b) a closed form equation in v only). This difference between the time scales in which u and v change is also loosely referred as *existence of separation of time scales*. In the beginning v stays approximately

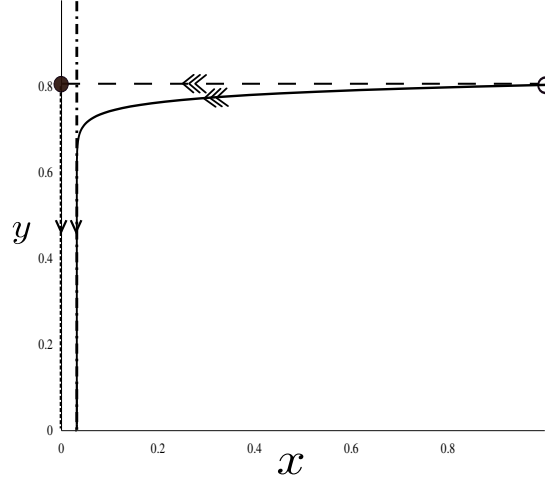


Figure 1.1: The solid line represents the exact solution of the system $\epsilon \frac{dx}{dt} = -(x - \epsilon)$, $\frac{dy}{dt} = -y$, with $\epsilon = 0.03$ and the initial value (the little circle at top-right corner) $x(0) = 1, y(0) = 0.8$. Here $\mathcal{M}_0 := \{(x, y) | x = 0\}$ and $\mathcal{M}_\epsilon := \{(x, y) | x = \epsilon\}$ (the vertical dotted-dashed line). The horizontal dashed line represents the solution of $\frac{dx}{ds} = -x$, $\frac{dy}{ds} = 0$, with initial value $x(0) = 1, y(0) = 0.8$. The vertical dotted line on y axis beginning at the solid dot and ending at the origin represents the solution of the system $0 = -x$, $\frac{dy}{dt} = -y$, initial value $y(0) = 0.8$. The triple arrow denotes *fast* dynamics because those are on the time scale $s = t/\epsilon$.

constant while u is changing, and later u stops playing an explicit role in the dynamics, and v starts changing on the slower time scale t . Because of this separation, I will refer to u as the *fast* variable and v as the *slow* variable.

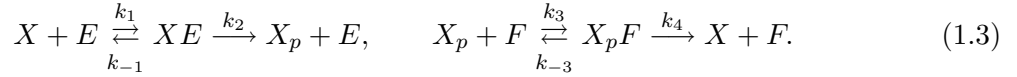
In applications, dynamical system do not in general have the form of Eq. (1.1). Significant effort can go into the search for a proper change or scaling of coordinates so that the given equation take the form of Eq. (1.1). There are frequently no exact rules to obtain such scalings, and the work may involve calculated guesses. However, finding such a scaling is half the job. The other half of the effort goes into verifying that the manifold $\mathcal{M}_0$ is normally hyperbolic and stable.

In the next section we will see how the GSPT can be used to get a rigorous justification of the QSSA in a system of two coupled Michaelis Menten reactions.

1.2 Example application

Derivation of Michaelis Menten (MM) type differential equation

The Michaelis-Menten (MM) scheme is a fundamental building block of many models of protein interactions [7, 43]. The following diagram represents two MM reactions working in opposite directions. One contributes to the activation of a protein while the other works towards deactivation.



The symbols X and X_p represent unphosphorylated and phosphorylated proteins; and E and F represent the enzymes participating in the phosphorylation and dephosphorylation processes, respectively. In the first reaction, the substrate, X , binds with the enzyme, E , to produce an intermediate complex, XE . This intermediate complex later dissociates into the phosphorylated form of the substrate, X_p , and the enzyme molecule. We also assume that the formation of XE is reversible while its dissociation is not (It is possible to build a theory even when the breaking down of XE is reversible [26, 66], but we will not cover that direction here.) Similarly, in the second reaction the enzyme, F , works to reverse the effect of the first reaction. It catalyzes the conversion of X_p back to X through the formation of another intermediate complex, X_pF . The symbols $k_1, k_{-1}, k_2, k_3, k_{-3}$ and k_4 are the reaction rate constants. This phosphorylation/dephosphorylation process is also known as a *Goldbeter-Koshland (GK) switch* [11, 24, 52] or a *futile cycle* [3].

The MM differential equation is a simplified model for tracking the time evolution of the concentration of the protein in the reactions (1.3). Let

$$x := [X], \quad c_1 := [XE], \quad e := [E], \quad x_p := [X_p], \quad c_2 := [X_pF], \quad f := [F],$$

1.2. EXAMPLE APPLICATION

where any symbol enclosed in square brackets denotes its molar concentration. The following differential equation is known as the MM differential equation [11, 13, 46].

$$\frac{dx}{dt} = k_2 f_0 \frac{x_0 - x}{k_{m_1} + x_0 - x} - k_4 e_0 \frac{x}{k_{m_2} + x}, \quad (1.4)$$

where $k_{m_1} := (k_2 + k_{-1})/k_1$ and $k_{m_2} := (k_4 + k_{-3})/k_3$ are known as *Michaelis Menten constants*; and x_0, e_0 and f_0 are total concentrations of substrate and respective enzymes in the MM reaction scheme (1.3).

In this section, using the GSPT, we will show that, if we assume the validity of the Law of Mass Action for reactions (1.3), and if the total concentrations of both the enzymes are very small compared to the total concentration of the substrate, *i.e.*, if $e_0 \ll x_0$ and $f_0 \ll x_0$; and if the total concentrations of the two enzymes are of the same order, *i.e.* if $e_0 \approx f_0$, then Eq. (1.4) is a valid model of reactions (1.3). More precisely, we will show that Eq. (1.4) is a valid reduction of the equations that describe reactions (1.3) assuming the Law of Mass Action.

The reader may refer to the Appendix A for a quick tutorial on the Law of Mass Action or may consult references like [30]. The application of the Law of Mass Action to reaction (1.3) yields the following set of differential equations:

$$\begin{aligned} \frac{dx}{dt} &= -k_1 x e + k_{-1} c_1 + k_4 c_2, & \frac{dx_p}{dt} &= -k_3 x_p f + k_{-3} c_2 + k_2 c_1, \\ \frac{dc_1}{dt} &= k_1 x e - (k_{-1} + k_2) c_1, & \frac{dc_2}{dt} &= k_3 x_p f - (k_{-3} + k_4) c_2, \\ \frac{de}{dt} &= -k_1 x e + (k_{-1} + k_2) c_1, & \frac{df}{dt} &= -k_3 x_p f + (k_{-3} + k_4) c_2. \end{aligned} \quad (1.5)$$

Eq. (1.5) is generally studied with the following initial conditions,

$$x(0) = x_0, \quad e(0) = e_0, \quad f(0) = f_0, \quad x_p(0) = c_1(0) = c_2(0) = 0.$$

This choice means that there are no intermediate complexes at the beginning of reactions.

1.2. EXAMPLE APPLICATION

By the very nature of reaction scheme, this dynamical system has the following constraints:

$$e(t) + c_1(t) \equiv e_0, \quad f(t) + c_2(t) \equiv f_0, \quad x(t) + x_p(t) + c_1(t) + c_2(t) \equiv x_0, \quad t \geq 0. \quad (1.6)$$

It is easy to check that these constraints are consistent with the initial conditions and with Eq. (1.5). Using Eq (1.6), we can eliminate a number of variables in Eq. (1.5) to obtain

$$\begin{aligned} \frac{dx}{dt} &= -k_1 e x_0 + (k_1 x + k_{-1}) c_1 + k_4 c_2, \\ \frac{dc_1}{dt} &= k_1 e_0 x - (k_1 x + k_{-1} + k_2) c_1, \\ \frac{dc_2}{dt} &= k_3 (x_0 - x - c_1 - c_2) (f_0 - c_2) - (k_{-3} + k_4) c_2. \end{aligned} \quad (1.7)$$

Suppose Eq. (1.7) evolves so that before x changes considerably, c_1 and c_2 change fast and equilibrate. Then Eq. (1.7) can be replaced by the simpler equation

$$\frac{dx}{dt} = -k_1 e x_0 + (k_1 x + k_{-1}) c_1 + k_4 c_2, \quad (1.8a)$$

$$0 = k_1 e_0 x - (k_1 x + k_{-1} + k_2) c_1, \quad (1.8b)$$

$$0 = k_3 (x_0 - x - c_1 - c_2) (f_0 - c_2) - (k_{-3} + k_4) c_2. \quad (1.8c)$$

This replacement of the derivative of c_1 and c_2 with 0, *i.e.*, the reduction from Eq. (1.7) to Eq. (1.8), holds under what is known as Quasi-Steady State Assumption(QSSA) [59]. Then, Eq. (1.8b) and Eq. (1.8c) can be solved for c_1 and c_2 , in terms of x ; and the result can be plugged into Eq. (1.8a). The resulting equation is exactly the MM differential equation (1.4).

We next use GSPT to get a rigorous reduction of Eq. (1.7), and hence a rigorous justification of the QSSA. As discussed earlier, to apply the results of GSPT, proper scaling of variables is necessary to recast the Eq. (1.7) into the form of Eq. (1.1). In general there is more than one way to scale the variables. Different scalings yield different sets of rescaled equations, and expressions for ϵ . Not surprisingly, different reduced equations will

1.2. EXAMPLE APPLICATION

therefore be obtained in different parameter ranges. The scaling we use here is motivated by an example in [46] and is one of the earliest and simplest found in the literature. Define

$$\tau := k_1 e_0 t, \quad u(\tau) := \frac{x(t)}{x_0}, \quad v_1(\tau) := \frac{c_1(t)}{e_0}, \quad v_2(\tau) := \frac{c_2(t)}{f_0}, \quad (1.9)$$

and

$$\epsilon_1 := \frac{e_0}{x_0}, \quad \epsilon_2 := \frac{f_0}{x_0}.$$

With the above change of variables, Eq. (1.7) takes the form

$$\begin{aligned} \epsilon_1 \frac{dv_1}{d\tau} &= u - (u + \lambda_1 + \lambda_3)v_1, \\ \epsilon_1 \frac{dv_2}{d\tau} &= -(\alpha(1 - u) + \lambda_4 + \lambda_2)v_2 + \alpha(1 - u) - \epsilon_1 \alpha v_1(1 - v_2) - \epsilon_2 \alpha v_2(1 - v_2), \\ \epsilon_1 \frac{du}{d\tau} &= -\epsilon_1(u + (u + \lambda_1)v_1) + \epsilon_2 \lambda_2 v_2, \end{aligned} \quad (1.10)$$

where,

$$\lambda_1 = \frac{k_{-1}}{k_1 x_0}, \quad \lambda_2 = \frac{k_4}{k_1 x_0}, \quad \lambda_3 = \frac{k_2}{k_1 x_0}, \quad \lambda_4 = \frac{k_{-3}}{k_1 x_0}, \quad \alpha = \frac{k_3}{k_1}. \quad (1.11)$$

Using the notation $B := \frac{\epsilon_2}{\epsilon_1} = \frac{f_0}{e_0}$ and $\epsilon = \epsilon_1$ in Eq. (1.10) we get

$$\begin{aligned} \epsilon \frac{dv_1}{d\tau} &= u - (u + \lambda_1 + \lambda_3)v_1, \\ \epsilon \frac{dv_2}{d\tau} &= -(\alpha(1 - u) + \lambda_4 + \lambda_2)v_2 + \alpha(1 - u) - \epsilon \alpha v_1(1 - v_2) - \epsilon B \alpha v_2(1 - v_2), \\ \frac{du}{d\tau} &= -u + (u + \lambda_1)v_1 + B \lambda_2 v_2. \end{aligned} \quad (1.12)$$

Eq. (1.12) clearly has the form of Eq. (1.1). Hence by the results in the GSPT, after a fast transient, Eq. (1.12) will follow the dynamics of a simpler system and that simpler system can be obtained by plugging in $\epsilon = 0$ in Eq. (1.12). And in the limit $\epsilon \rightarrow 0$, Eq. (1.12) takes the form

$$0 = u - (u + \lambda_1 + \lambda_3)v_1, \quad (1.13a)$$

$$0 = -(\alpha(1 - u) + \lambda_4 + \lambda_2)v_2 + \alpha(1 - u), \quad (1.13b)$$

$$\frac{du}{d\tau} = -u + (u + \lambda_1)v_1 + B \lambda_2 v_2. \quad (1.13c)$$

Solving for v_1 and v_2 from the Eqs. (1.13a) and (1.13b), we get

$$v_1 = \frac{u}{u + \lambda_1 + \lambda_3}, \quad v_2 = \frac{1 - u}{(1 - u) + \frac{\lambda_4 + \lambda_2}{\alpha}}. \quad (1.14)$$

So, after the fast initial transient the solution of Eq. (1.12) tends to stay close to the *slow manifold*

$$\mathcal{M}_0 = \left\{ (u, v_1, v_2) : u \in [0, 1], \quad v_1 = \frac{u}{u + \lambda_1 + \lambda_3}, \quad v_2 = \frac{1 - u}{(1 - u) + \frac{\lambda_4 + \lambda_2}{\alpha}} \right\}. \quad (1.15)$$

Using Eq. (1.14) in Eq. (1.13c), we get the following “governing” differential equation near the manifold $\mathcal{M}_0$.

$$\frac{du}{d\tau} = B\lambda_2 \frac{1 - u}{\frac{\lambda_4 + \lambda_2}{\alpha} + 1 - u} - \lambda_3 \frac{u}{\lambda_1 + \lambda_3 + u}. \quad (1.16)$$

The above equation is essentially a Michaelis Menten type equation in dimensionless form.

Using the change of variables, as defined in Eq. (1.9), and Eq. (1.11) we see that Eq. (1.16) becomes Eq. (1.4).

The verification that the *slow manifold* defined by the first two equations of Eq. (1.13) is normally hyperbolic and stable is nearly trivial. Let the right side of the first and second equation of Eq. (1.13) be $F(u, v_1, v_2)$ and $G(u, v_1, v_2)$, respectively. *i.e.* $F(u, v_1, v_2) := u - (u + \lambda_1 + \lambda_3)v_1$, and $G(u, v_1, v_2) := -(\alpha(1 - u) + \lambda_4 + \lambda_2)v_2 + \alpha(1 - u)$. Then, clearly we have

$$\frac{\partial[F, G]}{\partial[v_1, v_2]} = \begin{bmatrix} -(u + \lambda_1 + \lambda_3) & 0 \\ 0 & -(\alpha(1 - u) + \lambda_4 + \lambda_2) \end{bmatrix}.$$

The Jacobian is therefore diagonal with negative eigenvalues.

Fig. 1.2 shows the solutions of Eq. (1.12) for two small values of ϵ . We do indeed observe that solutions first get quickly attracted to $\mathcal{M}_0$ then move on it slowly. The attraction gets sharper as ϵ becomes smaller.

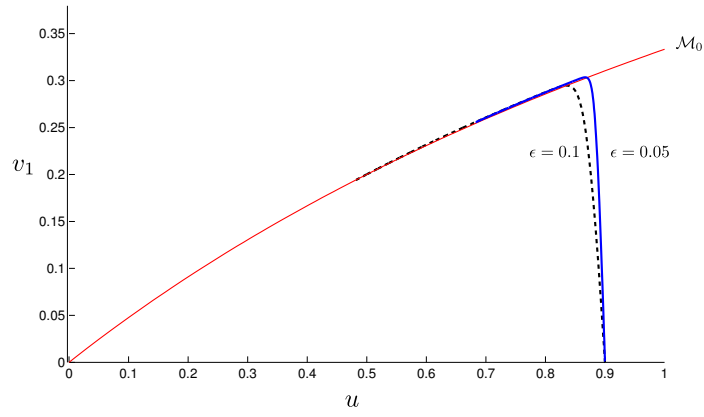


Figure 1.2: Projected solutions of Eq. (1.12) on the u - v_1 plane, for two different values of ϵ . For the dashed (black) line, $\epsilon = 0.1$; and for the thick solid (blue) line, $\epsilon = 0.05$ was used. The thin solid (red) line is the slow manifold, $\mathcal{M}_0$, described by Eq. (1.15). We observe that for small ϵ , solutions first get quickly attracted to $\mathcal{M}_0$ then move on it slowly. The attraction gets sharper as ϵ becomes smaller. Other parameters used: $\lambda_1 = \lambda_2 = \lambda_3 = \lambda_4 = \alpha = 1$; $B = 1$ (dashed (black) line), $B = 2$ (thick solid (blue) line). Initial value: $v_1 = 0, v_2 = 0, x = 0.9$.

Chapter 2

Introduction of the total Quasi-Steady States Assumption (tQSSA)

In the last chapter we saw that a classic assumption in the derivation of the MM type differential equation is that the enzyme concentration is much lower than the substrate concentration. A weaker requirement was derived in [59], where it was shown that even if the enzyme concentration is small compared to the *sum* of substrate concentration and the Michaelis-Menten constant then the QSSA holds, and the MM type reduction is justified. Although these conditions hold for a single MM type reaction, *i.e.* when there is only one substrate, theoretical justification for the use of MM type differential equations, in a general network of coupled MM reactions, is yet to be found [6, 11, 65]. Unless we answer this question, any prediction, made by models created by using of MM differential equation in a network of enzymatic reactions, will be unreliable. For examples of such model, see [23, 49, 50, 64].

As mentioned before, the QSSA plays a major role in the derivation of the MM differential equations. Hence, to prove the validity of MM type differential equations, for a system of coupled MM reactions, one must justify a generalized version of the QSSA that holds for such a system. Although the QSSA has not been extended directly to a system of coupled MM reactions, other versions of the QSSA which may be more suitable for the network have been developed. In this thesis we will extend one such version, namely the tQSSA, to a system of coupled MM reactions.

The tQSSA was proposed by Borghans, et al. (1996) [6]. The original result was applicable only to an isolated MM reaction. In this chapter we will discuss the meaning of the tQSSA, in the way it was first introduced; and we will discuss some other explanations of tQSSA in terms of GSPT. In Section 2.1, we will describe what we mean by the tQSSA. In addition, in Section 2.1.1, we will show how we can get around a technical issue of solving quadratic equations that appear in the reduced equations obtained under the tQSSA. In Section 2.2, we will study several different justifications of the tQSSA. Section 2.2.1 discusses the results of Borghans, et al. [59]; Section 2.2.2 discusses the justification given by Tzafriri [65]. We will also discuss some potential issues in these arguments. In Section 2.2.3, we will study a more precise proof of the validity of tQSSA, based on GSPT. Here we follow Khoo and Hegland [37], who gave a rigorous proof of Borghans et al.'s result [6]. In the last section, Section 2.2.4, we will show that we can also use GSPT to prove the validity of Tzafriri's condition as a sufficient condition for the tQSSA [65].

2.1 Meaning and extension of the tQSSA

The Michaelis-Menten (MM) scheme [7, 43] is a fundamental building block of many models of protein interactions: An enzyme, E , reacts with a protein, X , resulting in an intermediate complex, C . In turn, this complex dissociates into a product, X_p , and the enzyme E . It is frequently assumed that formation of C is reversible while its dissociation is not. The process is represented by the following sequence of reactions [7, 43, 46]



It should be noted that the reaction (2.1) is exactly the first of the two Michaelis-Menten reaction in Eq. (1.3). The reader may notice the term “isolated” in the name of this section. We add this term because subsequently we will consider examples where the substrate of one reaction can act as an enzyme in another. Here the term “isolated” simply means that there is only one enzyme species and only one substrate species.

For notational convenience we will use variable names to denote both the chemical species and its concentration. For instance, E denotes both an enzyme and its concentration. Reaction (2.1) reaction obeys two natural constraints: The total amount of protein and enzyme remain constant. *i.e.*

$$X + C + X_p = X_T, \quad \text{and} \quad E + C = E_T, \quad (2.2)$$

for positive constants X_T and E_T . In conjunction with the constraints (2.2), the Law of Mass Action (see Appendix A) yields the following system of ordinary differential equations that models reaction (2.1)

$$\begin{aligned} \frac{dX}{dt} &= -k_1 X(E_T - C) + k_{-1} C, & X(0) &= X_T, \\ \frac{dC}{dt} &= k_1 X(E_T - C) - (k_{-1} + k_2) C, & C(0) &= 0. \end{aligned} \quad (2.3)$$

In Chapter 1, we studied the derivation of the MM equation. The conclusion was that when the concentration of the substrate-bound enzyme, C , equilibrates quickly, then system (2.3) can be reduced by one dimension and the resulting one dimensional differential equation was called the MM equation. We say that the QSSA holds if C is quickly attracted to quasi-steady state value, while X changes little during this transient. Sufficient conditions under which the QSSA is valid have been studied extensively [17, 24, 60]. However, it has also been observed that the QSSA is too restrictive [6, 65], as mentioned in the introduction of this chapter. Hence, other less restrictive versions of the QSSA were proposed.

In particular, the tQSSA was proposed to obtain a reduction that is valid for a wider range of parameters than the QSSA. The trick used in the tQSSA is to introduce a new variable $\bar{X} := X + C$. Eq. (2.3) can then be rewritten as

$$\frac{d\bar{X}}{dt} = -k_2 C, \quad \bar{X}(0) = X_T, \quad (2.4a)$$

$$\frac{dC}{dt} = k_1[\bar{X}E_T - (\bar{X} + E_T + k_m)C + C^2], \quad C(0) = 0, \quad (2.4b)$$

where $k_m = (k_{-1} + k_2)/k_1$ is the *Michaelis-Menten constant*.

The tQSSA posits that C equilibrates quickly compared to $\bar{X}$ [6, 65]. Under this assumption we obtain the following differential-algebraic system

$$\frac{d\bar{X}}{dt} = -k_2 C, \quad \bar{X}(0) = X_T, \quad (2.5a)$$

$$0 = k_1[\bar{X}E_T - (\bar{X} + E_T + k_m)C + C^2]. \quad (2.5b)$$

Solving Eq. (2.5b) and noting that only the negative branch of solutions is stable, we can express C in terms of $\bar{X}$ to obtain a closed, first order differential equation for $\bar{X}$,

$$\frac{d\bar{X}}{dt} = -k_2 \frac{(\bar{X} + E_T + k_m) - \sqrt{(\bar{X} + E_T + k_m)^2 - 4\bar{X}E_T}}{2}, \quad \bar{X}(0) = X_T. \quad (2.6)$$

Although the reduced equation is given in the $\bar{X}, C$ coordinates, it is easy to revert to the original variables X, C . Therefore, from Eq. (2.6) one can recover an approximation to the solution of Eq. (2.3).

2.1.1 Extension of the tQSSA

An essential step in the tQSSA reduction is the solution of the quadratic equation (2.5b). A direct extension of this approach to networks of chemical reactions typically leads to a coupled system of quadratic equations (see Eq. (3.18)) [11, 51, 52]. The solution of this system may not be unique, and generally may need to be obtained numerically. However, an approach introduced by Bennett, et al. [5], can be used to obtain the desired solution from a system of linear equations.

In particular, we keep the tQSSA, but look for a reduced equation in the original coordinates, X, C . Using $\bar{X} = X + C$ to eliminate $\bar{X}$ from Eq. (2.5b), we obtain

$$0 = k_1 (X(E_T - C) - k_m C). \quad (2.7)$$

Eq. (2.7) and Eq. (2.5b) are equivalent, but Eq. (2.7) is linear in C , and leads to

$$C = \frac{XE_T}{k_m + X}, \text{ and } \bar{X} = X + \frac{XE_T}{k_m + X}.$$

Using these formulas in Eq. (2.5a), and applying the chain rule gives

$$\frac{\partial}{\partial \bar{X}} \left(X + \frac{XE_T}{k_m + X} \right) \frac{dX}{dt} = -k_2 \frac{XE_T}{k_m + X} \implies \frac{dX}{dt} = -k_2 \left(1 + \frac{k_m E_T}{(k_m + X)^2} \right)^{-1} \frac{XE_T}{k_m + X}. \quad (2.8a)$$

The reduced Eq. (2.8a) was obtained under the assumption that there is no significant change in $\bar{X} = X + C$ during the rapid equilibration. After equilibration, $C = XE_T/(k_m + X)$ (See Fig. 2.1). Therefore, the initial value for Eq. (2.8a), denote by $\hat{X}(0)$, can be

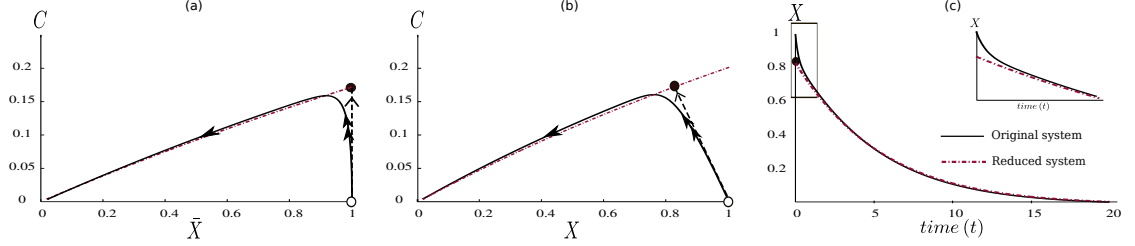


Figure 2.1: Proper choice of the initial values of the reduced system. The empty circle at $\bar{X} = 1$, $C = 0$, represents the initial value for the full system. The solid dot is the initial value of the reduced system. The dash-dotted (red) line represents the attracting *slow manifold*. (a) The solid curve represents the numerical solution of Eq. (2.4). The solution rapidly converges to the manifold, and evolves slowly along the manifold after this transient. The dashed line satisfies $\bar{X} = X_T$. The solid dot at the intersection of the dashed line and the slow manifold represents the projection of the initial condition onto the slow manifold given by Eq. (2.5b). Thus $\bar{X}(0) = X_T$ is the proper initial condition for the reduced system (2.6). (b) The solid line represents the numerical solution of Eq. (2.3). After a quick transient, the solution again converges to the slow manifold. However, since the initial transient is not orthogonal to the X axis, the initial conditions do not project vertically onto the slow manifold. Instead, the initial transient follows the line $X + C = X_T$ (dashed), and the intersection of this line and the slow manifold represents the proper choice of the initial value for Eq. (2.8a). (c) Comparison of solutions of Eq. (2.3) and the reduced system (2.8a). The graph in the inset offers a magnified view of the boxed region, showing the quick transient to the slow manifold. We used: $X_T = E_T = k_1 = k_2 = 1$, $k_{-1} = 3$, which, using Eq. (2.8b), gives the initial condition for the reduced system, $\hat{X}(0) = 0.83$.

obtained from the initial values $X(0), C(0)$ using

$$\hat{X}(0) + \frac{E_T \hat{X}(0)}{\hat{X}(0) + k_m} = X(0) + C(0) = X_T. \quad (2.8b)$$

Fig. 2.1c) shows that the solutions of the full system (2.3) and the reduced system (2.8a) are close when initial conditions are mapped correctly.

The tQSSA implies that Eq. (2.4) can be approximated by Eq. (2.5). Therefore, to explore the conditions under which Eq. (2.8a) is a valid reduction of Eq. (2.3) we need to investigate the parameter regime in which the transition from Eq. (2.4) to Eq. (2.5) is justified. In the next section we will review some of the well known results guaranteeing the tQSSA.

2.2 Sufficient conditions for the validity of tQSSA

2.2.1 Results of Borghans's et al. [6]

In this subsection we will outline the arguments used by Borghans's et al. in [6] to justify the tQSSA on an isolated MM reaction. Recall that tQSSA in this case simply means an approximation of Eq. (2.4) with Eq. (2.5).

To estimate the range of validity of the tQSSA one estimates the time scale of the fast transient and the slow transient. With fast(slow) time scale we mean, the time scale on which the fast(slow) variables change.

The first step is to estimate the fast time scale in which C changes, say t_C . Underlying assumptions are: (1) $\bar{X}$ does not change significantly till C gets sucked in near the slow manifold. In the calculation of t_C we will replace the right hand side of Eq. (2.4a) with 0; (2) the second assumption is that C begins from an initial value of zero and remains relatively small. The implication of this assumption is that we can ignore any terms like C^2 (Ignoring the C^2 term is also sometime referred as Padè approximation). With this assumption one can approximate the Eq. (2.4) in the fast time scale with

$$\begin{aligned}\frac{d\bar{X}}{dt} &= 0, \\ \frac{dC}{dt} &= k_1[X_T E_T - (X_T + E_T + k_m)C].\end{aligned}\tag{2.9}$$

The right hand side of Eq. (2.9) is linear in C and the coefficient of C is $k_1(X_T + E_T + k_m)$. Thus, in some sense, one can say that in Eq. (2.9), C changes in the time scale of $1/(k_1(X_T + E_T + k_m))$ and this is what one calls the fast time scale, *i.e.*

$$t_C = \frac{1}{k_1(X_T + E_T + k_m)}.$$

To estimate the slow time scale $t_{\bar{X}}$, we calculate the maximum change of $\bar{X}$ divided by the

maximum rate of change of total substrate after the fast transient. This can roughly set to be $X_T/|d\bar{X}/dt|_{\max}$ and with the quasi equilibrium value of C from Eq. (2.9), one obtains

$$t_{\bar{X}} = \frac{X_T}{|d\bar{X}/dt|_{\max}} = \frac{X_T + E_T + k_m}{k_2 E_T}$$

After obtaining t_C and $t_{\bar{X}}$, Borghans's et al. argue that a necessary and sufficient condition for the validity of tQSSA is

$$\epsilon = \frac{t_C}{t_{\bar{X}}} = \frac{k_2}{k_1} \frac{E_T}{(X_T + E_T + k_m)^2} \ll 1. \quad (2.10)$$

In later sections (see Sections 2.2.3 and 4.2.1) we will see that one can arrive at the same conclusion by using the GSPT.

2.2.2 Results on tQSSA obtained by Tzafriri [65]

In [65], the author produced another argument to go from Eq. (2.4) to Eq. (2.5). Tzafriri's argument is similar to Borghans's argument, except that no assumption of Padé approximation is required. By using

$$C_{\pm}(\bar{X}) := \frac{(\bar{X} + E_T + k_m) \pm \sqrt{(\bar{X} + E_T + k_m)^2 - 4E_T\bar{X}}}{2} \quad (2.11)$$

as the roots of the quadratic equation: $k_1[\bar{X}E_T - (\bar{X} + E_T + k_m)C + C^2] = 0$, we can rewrite the Eq. (2.4) as

$$\frac{d\bar{X}}{dt} = -k_2 C, \quad \bar{X}(0) = X_T, \quad (2.12a)$$

$$\frac{dC}{dt} = k_1(C - C_+(\bar{X}))(C - C_-(\bar{X})), \quad C(0) = 0. \quad (2.12b)$$

Under the assumptions that C is the fast variable and $\bar{X}$ is the slow variable, one can assume that in the fast initial transient there has not been any significant change in $\bar{X}$. Hence, we can replace $\bar{X}$ with X_T in Eq. (2.12b) to get

$$\frac{dC}{dt} = k_1(C - C_+(X_T))(C - C_-(X_T)).$$

This is a Riccati equation and it can be solved analytically [57] to get

$$C_i(t) := C_-(X_T) \frac{1 - e^{-t/t_C}}{1 - \frac{C_-(X_T)}{C_+(X_T)} e^{-t/t_C}}$$

where t_C is defined as

$$t_C := \frac{1}{k_1 [C_+(X_T) - C_-(X_T)]} = \frac{1}{k_1 \sqrt{(X_T + E_T + k_m)^2 - 4E_T X_T}}. \quad (2.13)$$

Then, the author of [65] argued that a sufficient condition for the validity of tQSSA can be obtained by requiring that the fractional decrease of $\bar{X}(t)$ during the initial transient should be small, *i.e.*

$$\frac{X_T - \bar{X}(t)}{X_T} \leq \frac{k_2 C_i(t)}{X_T} t \ll 1.$$

And because the duration of the initial transient is of the order t_C , one gets a new sufficient condition of the validity of tQSSA by plugging in $t = t_C$ in the above equation

$$\epsilon := \frac{k_2 C_-(X_T)}{X_T} t_C \ll 1 \quad (2.14)$$

where $C_-(.)$ and t_C are defined in Eqs. (2.11) and (2.13), respectively. One can also write Eq.(2.14) as

$$\epsilon := \frac{t_C}{t_{\bar{X}}} \ll 1, \quad \text{where} \quad t_{\bar{X}} := \frac{X_T}{k_2 C_-(X_T)} \quad (2.15)$$

can be interpreted as the time scale for the slow variable.

Remark: It should be noted that the self-consistent approach used by Borghans, et al. and Tzafriri, though intuitive, is somewhat heuristic. In the next two subsections we will see the use of GSPT to obtain rigorous proofs of the above claims. The first one proves Borgans' result and the second one proves Tzafriri's result.

Another limitation of the above self-consistency approaches is that they only seem to work for an isolated Michaelis-Menten reaction and not for a system of simultaneous MM reactions. In other words it is not obvious how to use a self-consistency approach when dealing with more than two independent variables.

2.2.3 Khoo and Hegland's proof for tQSSA using GSPT [37]

As we discussed in Chapter 1, to apply the results of the GSPT on any dynamical system, one frequently needs to rescale the variables, so that the given differential equation takes the form as in Eq. (1.1). In [37], the authors worked with the following scaling. Using

$$t_C = \frac{1}{k_1(X_T + E_T + k_m)}, \quad t_{\bar{X}} = \frac{X_T + E_T + k_m}{k_2 E_T}, \quad \epsilon = \frac{t_C}{T_{\bar{X}}} = \frac{k_2}{k_1} \frac{E_T}{(X_T + E_T + k_m)^2}, \quad (2.16)$$

the scaled variables are defined as

$$\tau = \frac{t}{t_C}, \quad \bar{x}(\tau) = \frac{\bar{X}(t)}{X_T}, \quad c(\tau) = \frac{C(t)}{\gamma}, \quad \text{where} \quad \gamma = \frac{X_T E_T}{X_T + E_T + k_m}.$$

With these new variables Eq. (2.4) takes the form

$$\begin{aligned} \frac{d\bar{x}}{d\tau} &= -\epsilon c, \\ \frac{dc}{d\tau} &= \bar{x} - \frac{X_T \bar{x} + E_T + k_m}{X_T + E_T + k_m} c + \frac{X_T E_T}{(X_T + E_T + k_m)^2} c^2. \end{aligned}$$

This equation can be rescaled further, so that it will fit directly into the form of Eq. (1.1), by a new time $T = \tau/\epsilon$.

$$\begin{aligned} \frac{d\bar{x}}{dT} &= -c, \\ \epsilon \frac{dc}{dT} &= \bar{x} - \frac{X_T \bar{x} + E_T + k_m}{X_T + E_T + k_m} c + \frac{X_T E_T}{(X_T + E_T + k_m)^2} c^2. \end{aligned}$$

Therefore the results of the GSPT implies that $\epsilon \ll 1$, where ϵ is defined in Eq. (2.16), is a sufficient condition for the validity of tQSSA. As we mentioned earlier, this is exactly the same condition claimed by Borghans, et al.

2.2.4 Validity of Tzafriri's claim using the GSPT

As discussed in Section 2.2.2, Tzafriri argued in [65] that the condition

$$\epsilon := \frac{t_C}{t_{\bar{X}}} \ll 1 \quad (2.17)$$

is a valid condition for reduction from Eq. (2.4) to Eq. (2.5), where t_C and $t_{\bar{X}}$ were defined as:

$$t_{\bar{X}} := \frac{X_T}{k_2 C_-(X_T)}, \quad t_C := \frac{1}{k_1 [C_+(X_T) - C_-(X_T)]} = \frac{1}{k_1 \sqrt{(X_T + E_T + k_m)^2 - 4E_T X_T}}, \quad (2.18)$$

where

$$C_{\pm}(X_T) := \frac{(X_T + E_T + k_m) \pm \sqrt{(X_T + E_T + k_m)^2 - 4E_T X_T}}{2}.$$

However, the self consistent argument used by Tzafriri can be made rigorous by using the techniques of the GSPT. And that is what we will do in this section. Once again, variables will be scaled appropriately to recast the Eq. (2.4) into a form of Eq. (1.1). Using C_- as short for $C_-(X_T)$ we define the new variables as

$$\tau := \frac{t}{t_C}, \quad \bar{x}(\tau) := \frac{\bar{X}(t)}{X_T}, \quad c(\tau) := \frac{C(t)}{C_-}. \quad (2.19)$$

Let

$$r := \frac{4E_T X_T}{(E_T + k_m + X_T)^2}.$$

Then, by the definition of $\epsilon, t_C, t_{\bar{X}}$ and r we note that ϵ and r can be reformulated into the form

$$\epsilon = \frac{t_C}{t_{\bar{X}}} = \frac{k_2}{k_1 X_T} \frac{1 - \sqrt{1 - r}}{\sqrt{1 - r}}, \quad \text{and } r = 1 - \frac{1}{\left(1 + \epsilon \frac{k_1 X_T}{k_2}\right)^2}. \quad (2.20)$$

Recasting Eq. (2.4) in term of the new variables, as defined in Eq. (2.19), and using Eq. (2.20) we get

$$\begin{aligned}\frac{d\bar{x}}{d\tau} &= -c, \\ \epsilon \frac{dc}{d\tau} &= \epsilon \frac{k_1 X_T}{k_2} c^2 - \left[\frac{(E_T + k_m + X_T \bar{x})}{(E_T + k_m + X_T) \sqrt{1-r}} \right] c + \frac{r}{4(\sqrt{1-r} - (1-r))} \bar{x}. \quad (2.21)\end{aligned}$$

We see that Eq. (2.21) has the structure of Eq. (1.1). Hence the results of the GSPT can be applied for small ϵ . For two cases it will be meaningful to use the limit $\epsilon = 0$.

Case 1: Suppose $\epsilon \frac{k_1 X_T}{k_2} \rightarrow 0$ in the limit $\epsilon \rightarrow 0$. Eq. (2.20) then implies that $r \rightarrow 0$. Also, $r/(\sqrt{1-r} - (1-r))$ does not vanish because

$$\lim_{r \rightarrow 0} \frac{r}{(\sqrt{1-r} - (1-r))} = \lim_{r \rightarrow 0} \frac{1}{\frac{-1}{2\sqrt{1-r}} + 1} = 2.$$

Therefore, in this case, in the limit of small ϵ we can eliminate the derivative of c as well as the c^2 term from Eq. (2.21).

Case 2: Second case is that $\epsilon \frac{k_1 X_T}{k_2} \not\rightarrow 0$ but $\epsilon \frac{k_1 X_T}{k_2}$ stays bounded in the limit $\epsilon \rightarrow 0$. Eq. (2.20) then implies that r stays bounded away from one. Hence, in this case too the right hand side is well defined in the limit of $\epsilon \rightarrow 0$. Though in this case we can not ignore the c^2 term.

Note that the above argument will break down if $\epsilon \frac{k_1 X_T}{k_2} \rightarrow \infty$ in the limit $\epsilon \rightarrow 0$ possible because that would imply that $r \rightarrow 1$. In this limit the right hand side of Eq. (2.21) will be undefined.

Chapter 3

General description of the network in this study

We start with an example application of our theory on a real network.

3.1 Analysis of a two-protein network

To illustrate the main ideas used in reducing the corresponding equations, we start with a concrete example of two interacting proteins.

Fig. 3.1a) is a simplified depiction of the interactions between two regulators of the G2-to-mitosis phase (G2/M) transition in the eukaryotic cell cycle [49]. Here, Y represents *MPF* (M-phase promoting factor, a dimer of *Cdc2* and cyclin B) and X represents *Wee1* (a kinase that phosphorylates and deactivates *Cdc2*). The proteins exist in a *phosphorylated* state, X_p, Y_p , and an *unphosphorylated* state, X, Y , with the phosphorylated state being less active. The proteins X and Y deactivate each other, and hence act as antagonists. In

3.1. ANALYSIS OF A TWO-PROTEIN NETWORK

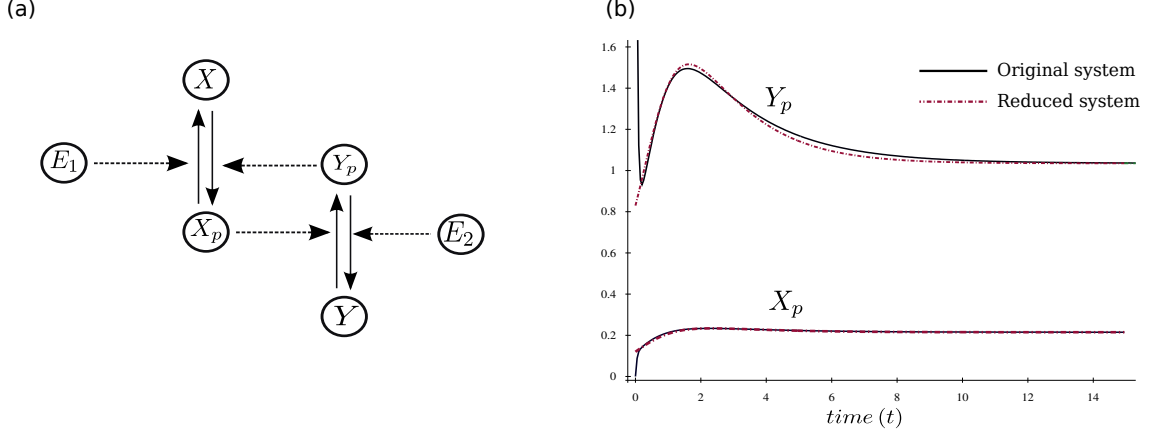
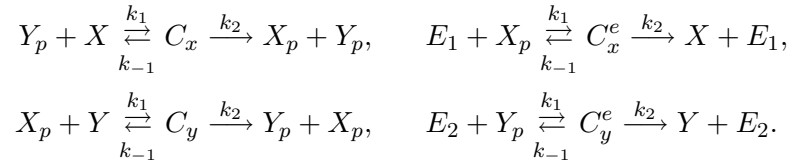


Figure 3.1: A simplified description of interactions between two regulators of the G2-to-mitosis phase (G2/M) transition in the eukaryotic cell cycle [49] (See text). (a) X and Y phosphorylate and deactivate each other. For instance, the protein X exists in a *phosphorylated* X_p and *unphosphorylated* X state, and the conversion X to X_p is catalyzed by Y_p . The conversion of X_p to X is catalyzed by the phosphatase E_1 . (b) Comparison of the numerical solution of Eq. (3.1) and Eq. (3.8). Here $k_1 = 5, k_{-1} = 1, k_2 = 1, E_1^T = 10, E_2^T = 2, X_T = 10, Y_T = 10.1$ as in [11]. The initial values for Eq. (3.1) are $X(0) = 10, Y(0) = 1.1, X_p(0) = 0, Y_p(0) = 9, C_x(0) = 0, C_y(0) = 0, C_x^e(0) = 0, C_y^e(0) = 0, E_1(0) = 10, E_2(0) = 2$. The initial values of the reduced system, $\hat{X}_p(0) = 0.12, \hat{Y}_p(0) = 0.83$ are obtained by the projection onto the slow manifold defined by Eq. (3.7).

this network E_1 and E_2 represent phosphatases that catalyze the conversion of X_p and Y_p to X and Y , respectively. Each dotted arrow in Fig. 3.1a) is associated with exactly one MM type reaction in the list of reactions given below. The sources of the arrows act as enzymes. Therefore, Fig. 3.1a) represents the following network of reactions



To simplify the exposition, we have assumed some homogeneity in the rates. Since the total concentration of proteins and enzymes is assumed fixed, the system obeys the following set

of constraints

$$\begin{aligned}
 X_T &= X(t) + X_p(t) + C_x(t) + C_y(t) + C_x^e(t), \\
 E_1^T &= C_x^e(t) + E_1(t), \\
 Y_T &= Y(t) + Y_p(t) + C_x(t) + C_y(t) + C_y^e(t), \\
 E_2^T &= C_y^e(t) + E_2(t)
 \end{aligned}$$

for $t \geq 0$, where X_T, Y_T, E_1^T, E_2^T are constant and represent the total concentrations of the respective proteins and enzymes. Along with these constraints the concentrations of the ten species in the reaction evolve according to

$$\begin{aligned}
 \frac{dX_p}{dt} &= -k_1 \underbrace{(Y_T - Y_p - C_x - C_y - C_y^e)}_{=Y} X_p - k_1 X_p \underbrace{(E_1^T - C_x^e)}_{=E_1} \\
 &\quad + k_{-1} C_x^e + (k_{-1} + k_2) C_y + k_2 C_x, \\
 \frac{dY_p}{dt} &= -k_1 \underbrace{(X_T - X_p - C_x - C_y - C_x^e)}_{=X} Y_p - k_1 Y_p \underbrace{(E_2^T - C_y^e)}_{=E_2} \\
 &\quad + k_{-1} C_y^e + (k_{-1} + k_2) C_x + k_2 C_y, \\
 \frac{dC_x}{dt} &= k_1 \underbrace{(X_T - X_p - C_x - C_y - C_x^e)}_{=X} Y_p - (k_{-1} + k_2) C_x, \\
 \frac{dC_y}{dt} &= k_1 \underbrace{(Y_T - Y_p - C_x - C_y - C_y^e)}_{=Y} X_p - (k_{-1} + k_2) C_y, \\
 \frac{dC_x^e}{dt} &= k_1 X_p \underbrace{(E_1^T - C_x^e)}_{=E_1} - (k_{-1} + k_2) C_x^e, \\
 \frac{dC_y^e}{dt} &= k_1 Y_p \underbrace{(E_2^T - C_y^e)}_{=E_2} - (k_{-1} + k_2) C_y^e,
 \end{aligned} \tag{3.1}$$

with initial values

$$C_x(0) = 0, \quad C_y(0) = 0, \quad C_x^e(0) = 0, \quad C_y^e(0) = 0. \tag{3.2}$$

The initial values of X_p and Y_p are some arbitrary positive numbers.

Following the approach in the previous chapter, we reduce Eq. (3.1) to a two-dimensional system. Assuming the validity of the tQSSA, we obtain an approximating differential–algebraic system. Solving the algebraic equations, which are linear in the original coordinates, leads to a closed, reduced system of ODEs. The parameter regime for the validity of the tQSSA will be discussed in the next chapter.

3.1.1 New coordinates and reduction under the tQSSA

To extend the tQSSA we define a new set of variables by adding the concentration of the free state of a species to the concentrations of all intermediate complexes formed by that particular species as reactant [11],

$$\begin{aligned}\bar{X}_p &:= X_p + C_y + C_x^e, \\ \bar{Y}_p &:= Y_p + C_x + C_y^e.\end{aligned}\tag{3.3}$$

Under the tQSSA, the intermediate complexes equilibrate quickly compared to the variables $\bar{X}_p$ and $\bar{Y}_p$. In the coordinates defined by Eq. (3.3), Eq. (3.1) takes the form

$$\frac{d\bar{X}_p}{dt} = k_2 C_x - k_2 C_x^e, \tag{3.4a}$$

$$\frac{d\bar{Y}_p}{dt} = k_2 C_y - k_2 C_y^e, \tag{3.4b}$$

$$0 = k_1(X_T - \bar{X}_p - C_x)(\bar{Y}_p - C_x - C_y^e) - (k_{-1} + k_2)C_x, \tag{3.4c}$$

$$0 = k_1(Y_T - \bar{Y}_p - C_y)(\bar{X}_p - C_y - C_x^e) - (k_{-1} + k_2)C_y, \tag{3.4d}$$

$$0 = k_1(\bar{X}_p - C_y - C_x^e)(E_1^T - C_x^e) - (k_{-1} + k_2)C_x^e, \tag{3.4e}$$

$$0 = k_1(\bar{Y}_p - C_x - C_y^e)(E_2^T - C_y^e) - (k_{-1} + k_2)C_y^e. \tag{3.4f}$$

Solving the coupled system of quadratic equations (3.4c-3.4f) in terms of $\bar{X}_p, \bar{Y}_p$ appears to be possible only numerically, as it is equivalent to finding the roots of a degree 16

polynomial [11]. However, since we are interested in the dynamics of X_p and Y_p , we can proceed as in the previous chapter (see Section 2.1.1): Using Eq. (3.3) in (3.4c-3.4f) gives a linear system in C_x, C_y, C_x^e, C_y^e . Defining $k_m := (k_{-1} + k_2)/k_1$, this system can be written in matrix form as

$$\begin{bmatrix} Y_p + k_m & Y_p & Y_p & 0 \\ X_p & X_p + k_m & 0 & X_p \\ 0 & 0 & X_p + k_m & 0 \\ 0 & 0 & 0 & Y_p + k_m \end{bmatrix} \begin{bmatrix} C_x \\ C_y \\ C_x^e \\ C_y^e \end{bmatrix} = \begin{bmatrix} Y_p(X_T - X_p) \\ X_p(Y_T - Y_p) \\ X_p E_1^T \\ Y_p E_2^T \end{bmatrix}. \quad (3.5)$$

The coefficient matrix above is invertible and Eq. (3.5) can be solved to obtain C_x, C_y, C_x^e, C_y^e as functions of X_p, Y_p . Denoting the resulting solutions as $C_x(X_p, Y_p), C_y(X_p, Y_p), C_x^e(X_p, Y_p), C_y^e(X_p, Y_p)$ and using them in Eqs.(3.4a-3.4b) we obtain the following

$$\frac{d}{dt} \begin{bmatrix} \bar{X}_p \\ \bar{Y}_p \end{bmatrix} = k_2 \begin{bmatrix} C_x(X_p, Y_p) - C_x^e(X_p, Y_p) \\ C_y(X_p, Y_p) - C_y^e(X_p, Y_p) \end{bmatrix}.$$

Reverting to the original coordinates, X_p and Y_p , and using the chain rule gives a closed system

$$\begin{aligned} \frac{d}{dt} \begin{bmatrix} X_p + C_y(X_p, Y_p) + C_x^e(X_p, Y_p) \\ Y_p + C_x(X_p, Y_p) + C_y^e(X_p, Y_p) \end{bmatrix} &= k_2 \begin{bmatrix} C_x(X_p, Y_p) - C_x^e(X_p, Y_p) \\ C_y(X_p, Y_p) - C_y^e(X_p, Y_p) \end{bmatrix} \implies \\ \begin{bmatrix} 1 + \frac{\partial C_y}{\partial X_p} + \frac{\partial C_x^e}{\partial X_p} & \frac{\partial C_y}{\partial Y_p} + \frac{\partial C_x^e}{\partial Y_p} \\ \frac{\partial C_x}{\partial X_p} + \frac{\partial C_y^e}{\partial X_p} & 1 + \frac{\partial C_x}{\partial Y_p} + \frac{\partial C_y^e}{\partial Y_p} \end{bmatrix} \frac{d}{dt} \begin{bmatrix} X_p \\ Y_p \end{bmatrix} &= k_2 \begin{bmatrix} C_x(X_p, Y_p) - C_x^e(X_p, Y_p) \\ C_y(X_p, Y_p) - C_y^e(X_p, Y_p) \end{bmatrix}. \end{aligned} \quad (3.6)$$

The initial values of Eq. (3.6) are determined by projecting the initial values, given by Eq. (3.2), onto the slow manifold. Unfortunately, they can be expressed only implicitly. The reduction from Eq. (3.1) to Eq. (3.6) was obtained under the assumption that $\bar{X}_p = X_p + C_y + C_x^e$ and $\bar{Y}_p = Y_p + C_x + C_y^e$ are slow variables, and hence constant during the

transient to the slow manifold. Therefore the projections of the initial conditions onto the slow manifold, $\hat{X}_p(0)$ and $\hat{Y}_p(0)$, are related to the original initial conditions as

$$\begin{aligned} \hat{X}_p(0) + C_y(\hat{X}_p(0), \hat{Y}_p(0)) + C_x^e(\hat{X}_p(0), \hat{Y}_p(0)) &= X_p(0) + C_y(0) + C_x^e(0) = X_T, \\ \hat{Y}_p(0) + C_x(\hat{X}_p(0), \hat{Y}_p(0)) + C_y^e(\hat{X}_p(0), \hat{Y}_p(0)) &= Y_p(0) + C_x(0) + C_y^e(0) = Y_T. \end{aligned} \quad (3.7)$$

We have therefore shown that, if the tQSSA holds, and if the coefficient matrix on the left hand side of Eq. (3.6) is invertible, then

$$\frac{d}{dt} \begin{bmatrix} X_p \\ Y_p \end{bmatrix} = k_2 \begin{bmatrix} 1 + \frac{\partial C_y}{\partial X_p} + \frac{\partial C_x^e}{\partial X_p} & \frac{\partial C_y}{\partial Y_p} + \frac{\partial C_x^e}{\partial Y_p} \\ \frac{\partial C_x}{\partial X_p} + \frac{\partial C_y^e}{\partial X_p} & 1 + \frac{\partial C_x}{\partial Y_p} + \frac{\partial C_y^e}{\partial Y_p} \end{bmatrix}^{-1} \begin{bmatrix} C_x(X_p, Y_p) - C_x^e(X_p, Y_p) \\ C_y(X_p, Y_p) - C_y^e(X_p, Y_p) \end{bmatrix}, \quad (3.8)$$

with initial value obtained by solving Eq. (3.7), is a valid approximation of Eq. (3.1). Fig. 3.1b) shows that the solutions of the two systems are indeed close, after an initial transient.

3.1.2 Comparison of our reduction with MM differential equations

Lets compare Eq. (3.8) with the equation one generally sees in the literature to model the network discussed in this section. For example, in [64] the following system of MM differential equations has been used.

$$\frac{dX_p}{dt} = k_2 Y_p \frac{X_T - X_p}{k_m + X_T - X_p} - k_2 E_1^T \frac{X_p}{k_m + X_p}, \quad (3.9a)$$

$$\frac{dY_p}{dt} = k_2 X_p \frac{Y_T - Y_p}{k_m + Y_T - Y_p} - k_2 E_2^T \frac{Y_p}{k_m + Y_p}. \quad (3.9b)$$

In Fig. 3.2 we compare the right hand side of Eq. (3.9a) and the first coordinate of the right hand side of Eq. (3.8). The difference in two functions strongly suggests that the use of MM Menten differentials equation on a system of coupled MM reactions is not consistent with

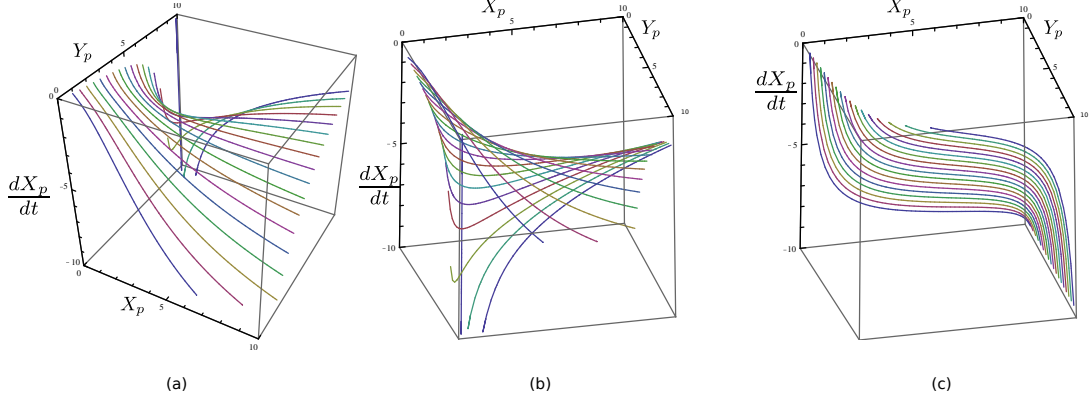


Figure 3.2: (a),(b): Plot of the the first coordinate of the right hand side of Eq. (3.8) from two different viewing angles. (c) Plot of the right hand side of Eq. (3.9a). The viewing angle for subfigures (b) and (c) are same. All the parameters are same as in Fig. 3.1. The difference in shape of (b) and (c) suggest that Eq. (3.9) is not consistent with the Law of Mass Action.

the Law of Mass Action. In particular, Eq. (3.9a) has sigmoidal shape where as Eq. (3.8) do not.

3.2 The general problem

In this section we describe a general class of protein interaction networks to which the reduction schemes described in the previous section can be applied. We again assume that the proteins interact via MM type reactions, and obtain a reduction under the generalized tQSSA [11]. We will follow the steps that lead to the reduced systems in the previous section: After describing the model and the conserved quantities, we recast the equations in terms of the “total” protein concentrations (*cf.* Section 3.1.1). Under a generalized tQSSA, these equations can be reduced to an differential-algebraic system. We show that the algebraic part of the system is linear in the original coordinates (*cf.* sections 2.1.1 and 3.1.1), so that the reduced system can be described by a differential equation with

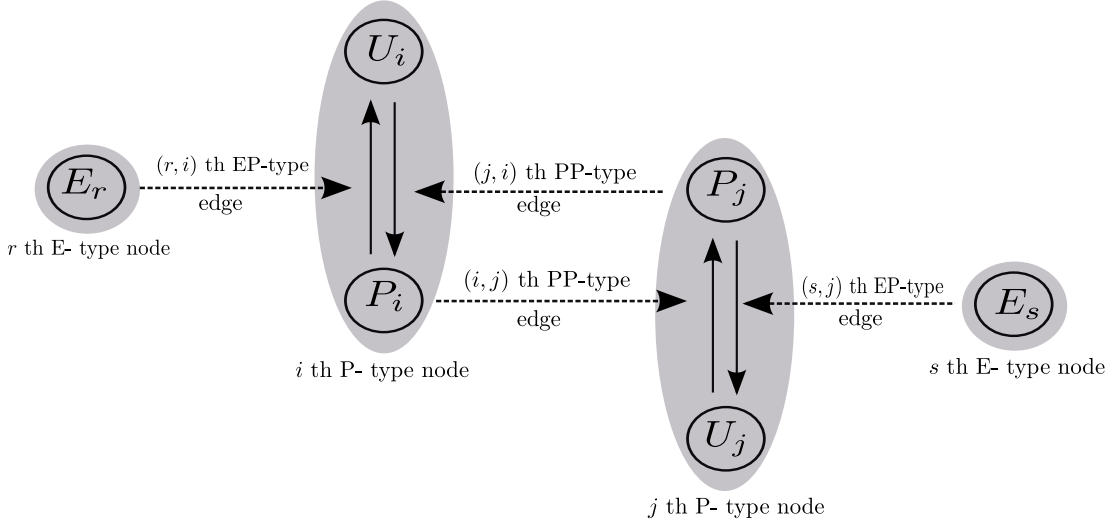


Figure 3.3: A section of the network illustrating the terminology used in describing protein interaction networks. Each shaded region represents a node and encompasses either an enzyme or a single protein that is part of an MM type reaction. Each dotted arrow represents an edge in the network. The solid arrows represent transitions within the nodes, and do not define an edge in the network.

dimension equal to the number of interacting proteins.

Other examples of biologically motivated network that to which this scheme can be applied can be found in [10, 13, 23, 32, 49, 50, 61, 64].

3.2.1 Description of the network

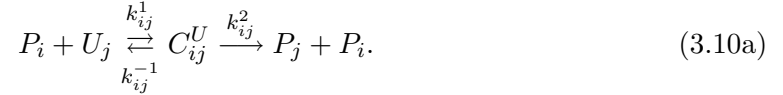
The network of reaction we consider can be described in terms of nodes and edges. The nodes represent enzymes as well as proteins, while the edges represent the catalytic effect one species has on another. Proteins are assumed to come in two states, phosphorylated and unphosphorylated. Both states are represented by a single node in this network. Fig. 3.3 and the following description make these definitions precise.

3.2. THE GENERAL PROBLEM

In a network of n interacting proteins, and n associated enzymes, we define the following:

Nodes: The two types of nodes in this network represent proteins (P-type nodes) and enzymes (E-type nodes). Each protein can exist in either an *active* or *inactive* form. The *inactive* form of the i th protein is denoted by U_i , and the *active* form by P_i . The i th P-type node is formed by grouping together U_i and P_i . In addition there are n species of enzymes, E_i , which exist in only one state.

Edges: All edges in the network are *directed*, and represent the catalytic effect of a species in a MM type reaction. There are two types of edges: *PP-type* edges connect two P-type nodes, while *EP-type* edges connect E-type nodes to P-type nodes. In particular, a PP-type edge from node i to node j represents the following MM type reaction in which P_i catalyzes the conversion of U_j to the *active* form P_j ,

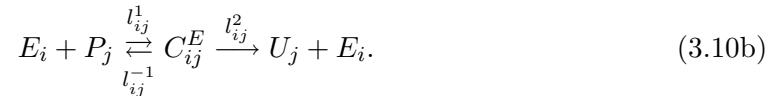


Note that autocatalysis is possible. The rate constants $k_{i,j}^1, k_{i,j}^{-1}, k_{i,j}^2$, associated to each edge, can be grouped into weighted “connectivity matrices”

$$K_1 = [k_{ij}^1]_{n \times n}, \quad K_{-1} = [k_{ij}^{-1}]_{n \times n}, \quad K_2 = [k_{ij}^2]_{n \times n}.$$

In the absence of an edge, that is, when P_i does not catalyze the phosphorylation of U_j , the corresponding (i, j) -th entry in K_1, K_{-1} , and K_2 is set to zero.

EP-type edges are similar to PP-type edges, with enzymes acting as catalysts. To each pair of enzyme, E_i , and protein, P_j , we associate three rate constants $l_{i,j}^1, l_{i,j}^{-1}, l_{i,j}^2$ of the corresponding reaction in which E_i is a catalyst in the conversion of P_j into U_j ,



3.2. THE GENERAL PROBLEM

The rate constants can again be arranged into matrices

$$L_1 = \begin{bmatrix} l_{ij}^1 \end{bmatrix}_{n \times n}, \quad L_{-1} = \begin{bmatrix} l_{ij}^{-1} \end{bmatrix}_{n \times n}, \quad L_2 = \begin{bmatrix} l_{ij}^2 \end{bmatrix}_{n \times n},$$

with zero entries again denoting the absence of interactions.

These definitions imply that the active form of one protein always catalyzes the production of the active form of another protein. This assumption excludes certain interactions (see Section 3.2.7 for an example). However, the reduction is easiest to describe under these assumptions, and this setting does include a lot, if not all, of the enzymatic network considered in literature.

For notational convenience we define $U = [U_1, U_2, \dots, U_n]^t$, $P = [P_1, P_2, \dots, P_n]^t$, and $E = [E_1, E_2, \dots, E_n]^t$, and arrange intermediate complexes into matrices,

$$C_U = \begin{bmatrix} C_{11}^U & C_{12}^U & \dots & C_{1n}^U \\ C_{21}^U & C_{22}^U & \dots & C_{2n}^U \\ \vdots & \vdots & \ddots & \vdots \\ C_{n1}^U & C_{n2}^U & \dots & C_{nn}^U \end{bmatrix}, \quad C_E = \begin{bmatrix} C_{11}^E & C_{12}^E & \dots & C_{1n}^E \\ C_{21}^E & C_{22}^E & \dots & C_{2n}^E \\ \vdots & \vdots & \ddots & \vdots \\ C_{n1}^E & C_{n2}^E & \dots & C_{nn}^E \end{bmatrix}.$$

Initially all intermediate complexes are assumed to start at zero concentration. Therefore, any intermediate complex corresponding to a reaction that has zero rates, will remain at zero concentration for all time.

For instance, in the case of the two protein example discussed in Section 3.1, we have

$$C_U = \begin{bmatrix} 0 & C_y \\ C_y & 0 \end{bmatrix}, \quad C_E = \begin{bmatrix} C_x^e & 0 \\ 0 & C_y^e \end{bmatrix}, \quad U = \begin{bmatrix} X \\ Y \end{bmatrix}, \quad P = \begin{bmatrix} X_p \\ Y_p \end{bmatrix}, \quad E = \begin{bmatrix} E_1 \\ E_2 \end{bmatrix},$$

$$K_1 = \begin{bmatrix} & k_1 \\ k_1 & \end{bmatrix}, \quad K_{-1} = \begin{bmatrix} & k_{-1} \\ k_{-1} & \end{bmatrix}, \quad K_2 = \begin{bmatrix} & k_2 \\ k_2 & \end{bmatrix},$$

$$L_1 = \begin{bmatrix} l_1 & \\ & l_1 \end{bmatrix}, \quad L_{-1} = \begin{bmatrix} l_{-1} & \\ & l_{-1} \end{bmatrix}, \quad L_2 = \begin{bmatrix} l_2 & \\ & l_2 \end{bmatrix}.$$

3.2.2 Inherent constraints on this network

Assuming that the system is isolated from the environment implies that the total concentration of each enzyme, E_i^T , remains constant. Therefore,

$$E_i + \sum_{s=1}^n C_{is}^E = E_i^T, \quad i \in \{1, 2, \dots, n\}. \quad (3.11a)$$

Similarly, for each protein the total concentration, U_i^T , of its *inactive* and *active* form, and the intermediate complexes is constant,

$$U_i + P_i + \left(\sum_{s=1}^n C_{is}^U + \sum_{r=1}^n C_{ri}^U - C_{ii}^U \right) + \sum_{r=1}^n C_{ri}^E = U_i^T, \quad i \in \{1, 2, \dots, n\}. \quad (3.11b)$$

Let

$$V_n = \underbrace{[\ 1 \quad 1 \quad \dots \quad 1 \]^t}_{n \text{ times}}, \quad E_T = [\ E_1^T \ E_2^T \ \dots \ E_n^T \]^t, \text{ and } U_T = [\ U_1^T \ U_2^T \ \dots \ U_n^T \]^t,$$

and denote the $n \times n$ identity matrix by I_n . In addition, we use the *Hadamard product* of matrices, denoted by $*$, to simplify notation¹. Constraints (3.11) can now be written concisely in matrix form

$$E_T = E + C_E V_n,$$

$$U_T = U + P + C_U V_n + C_U^t V_n - (I_n * C_U) V_n + C_E^t V_n.$$

¹For instance, the Hadamard product of matrices $A = \begin{bmatrix} a & b \\ c & d \end{bmatrix}$, and $B = \begin{bmatrix} e & f \\ g & h \end{bmatrix}$, is $A * B = \begin{bmatrix} ae & bf \\ cg & dh \end{bmatrix}$.

3.2.3 ODE associated with this network

Applying the Law of Mass Action to the system of reactions described by (3.10a-3.10b) yields a $(2n^2 + n)$ dimensional dynamical system,

$$\begin{aligned} \frac{dP_i}{dt} &= \sum_{s=1}^n \left(-k_{is}^1 P_i U_s + (k_{is}^{-1} + k_{is}^2) C_{is}^U \right) \\ &\quad + \sum_{r=1}^n \left(k_{ri}^2 C_{ri}^U - l_{ri}^1 E_r P_i + l_{ri}^{-1} C_{ri}^E \right), \quad P_i(0) = p_i^0, \\ \frac{dC_{ij}^U}{dt} &= k_{ij}^1 P_i U_j - (k_{ij}^{-1} + k_{ij}^2) C_{ij}^U, \quad C_{ij}^U(0) = 0, \\ \frac{dC_{ij}^E}{dt} &= l_{ij}^1 E_i P_j - (l_{ij}^{-1} + l_{ij}^2) C_{ij}^E, \quad C_{ij}^E(0) = 0, \end{aligned} \quad (3.12)$$

Due to the constraints (3.11a,3.11b), U_i, E_i , are *affine linear* function of P_i, C_{ij}^U, C_{ij}^E and can be used to close Eq. (3.12). Our aim is to reduce this $2n^2 + n$ dimensional system to an n dimensional system involving only P_i .

3.2.4 The total substrate coordinates

In this section we generalize the change of variables to the “total” protein concentrations. These variables will act as the slow variables in our differential equations. Let

$$\bar{P}_i := P_i + \sum_{s=1}^n C_{is}^U + \sum_{r=1}^n C_{ri}^E, \quad i \in \{1, 2, \dots, n\}, \quad (3.13)$$

so that Eq. (3.12) takes the form

$$\frac{d\bar{P}_i}{dt} = \sum_{r=1}^n k_{ri}^2 C_{ri}^U - \sum_{r=1}^n l_{ri}^2 C_{ri}^E, \quad (3.14a)$$

$$\frac{dC_{ij}^U}{dt} = k_{ij}^1 P_i U_j - (k_{ij}^{-1} + k_{ij}^2) C_{ij}^U, \quad (3.14b)$$

$$\frac{dC_{ij}^E}{dt} = l_{ij}^1 E_i P_j - (l_{ij}^{-1} + l_{ij}^2) C_{ij}^E. \quad (3.14c)$$

3.2. THE GENERAL PROBLEM

To close this system we use Eqs. (3.11a,3.11b) with Eq. (3.13), to obtain

$$\begin{aligned}
U_i &= U_i^T - P_i - \sum_{s=1}^n C_{is}^U - \sum_{r=1}^n (C_{ri}^U + C_{ri}^E) + C_{ii}^U \\
&= U_i^T - \bar{P}_i - \sum_{r=1}^n C_{ri}^U + C_{ii}^U, \\
E_i &= E_i^T - \sum_{s=1}^n C_{is}^E, \\
P_i &= \bar{P}_i - \sum_{s=1}^n C_{is}^U - \sum_{r=1}^n C_{ri}^E.
\end{aligned} \tag{3.15}$$

Defining $\bar{P} := (\bar{P}_1, \bar{P}_2, \dots, \bar{P}_n)^t$, Eq. (3.13) can be written in vector form as $\bar{P} = P + C_U V_n + C_E^t V_n$, and Eqs. (3.14) and (3.15) can be written in matrix form as

$$\frac{d\bar{P}}{dt} = (K_2 * C_U)^t V_n - (L_2 * C_E)^t V_n, \tag{3.16a}$$

$$\frac{dC_U}{dt} = K_1 * (P U^t) - (K_{-1} + K_2) * C_U, \tag{3.16b}$$

$$\frac{dC_E}{dt} = L_1 * (E P^t) - (L_{-1} + L_2) * C_E, \tag{3.16c}$$

where

$$\begin{aligned}
U &= U_T - P - C_U V_n - C_U^t V_n - C_E^t V_n + (I_n * C_U) V_n \\
&= U_T - \bar{P} - C_U^t V_n + (I_n * C_U) V_n,
\end{aligned} \tag{3.17a}$$

$$E = E_T - C_E V_n, \tag{3.17b}$$

$$P = \bar{P} - C_U V_n - C_E^t V_n. \tag{3.17c}$$

Eqs. (3.14–3.15) and (3.16–3.17) are different representations of the same set of reactions. Both forms are helpful in understanding different properties of the dynamics: Eq. (3.14–3.15) will be used to examine the conditions under which separation of time scales holds, while Eq. (3.16–3.17) will be used to prove the attractivity of the slow manifold and obtain the reduced equations.

3.2.5 The tQSSA and the resulting reduced equations

The general form of the tQSSA states that the intermediate complexes, C_U and C_E , equilibrate faster than $\bar{P}$. This assumption implies that, after a fast transient, Eq. (3.16) can be approximated by the differential-algebraic system

$$\frac{d\bar{P}}{dt} = (K_2 * C_U)^t V_n - (L_2 * C_E)^t V_n, \quad (3.18a)$$

$$0 = K_1 * (PU^t) - (K_{-1} + K_2) * C_U, \quad (3.18b)$$

$$0 = L_1 * (EP^t) - (L_{-1} + L_2) * C_E. \quad (3.18c)$$

In particular, according to GSPT (see Chapter 1), if the *slow manifold*

$$\mathcal{M}_0 = \left\{ (\bar{P}, C_U, C_E) \left| \begin{array}{l} 0 = K_1 * (PU^t) - (K_{-1} + K_2) * C_U; \\ 0 = L_1 * (EP^t) - (L_{-1} + L_2) * C_E \end{array} \right. \right\} \quad (3.19)$$

is normally hyperbolic and stable, then the solutions of Eq. (3.16) are attracted to and shadow solutions on $\mathcal{M}_0$. Later on we will show that the above slow manifold is always normally hyperbolic and stable (see Section 4.1).

If we consider the system (3.18b,c) entry-wise then it consists of $2n^2$ coupled quadratic equations in $2n^2 + n$ variables, namely the entries of $\bar{P}, C_U, C_E$ (note that U, E are functions of $\bar{P}, C_U, C_E$). As discussed in Section 3.1.1, we can avoid solving coupled quadratic equations by seeking a solution in terms of P instead of $\bar{P}$. Using Eq. (3.17a,b) we eliminate E, U from Eqs. (3.18b,c) to obtain

$$\begin{aligned} K_1 * [P (V_n^t C_U^t + V_n^t C_U - V_n^t (I_n * C_U)) + P V_n^t C_E] + (K_{-1} + K_2) * C_U \\ = K_1 * [P (U_T^t - P^t)], \end{aligned} \quad (3.20a)$$

$$L_1 * (C_E (V_n P^t)) + (L_{-1} + L_2) * C_E = L_1 * (E_T P^t). \quad (3.20b)$$

Although complicated, Eq. (3.20) is linear in the entries of C_U and C_E . The following Lemma, proved in Section 3.2.6, shows that the equations are also solvable.

Lemma 3.2.1 *Suppose $K_1 = [k_{ij}^1]$, $K_{-1} = [k_{ij}^{-1}]$, $K_2 = [k_{ij}^2]$, $L_1 = [l_{ij}^1]$, $L_{-1} = [l_{ij}^{-1}]$, $L_2 = [l_{ij}^2] \in \mathbb{R}^{n \times n}$ are real matrices with non-negative entries. Furthermore, assume that for any pair $i, j \in \{1, 2, \dots, n\}$ either $k_{ij}^1 = k_{ij}^{-1} = k_{ij}^2 = 0$, or all these coefficients are positive, and similarly for the coefficients l_{ij}^1, l_{ij}^{-1} , and l_{ij}^2 . If $U_T, E_T, P \in \mathbb{R}_+^{n \times 1}$ are real vectors with positive entries, and $V_n = [1 \ 1 \ \dots \ 1]^t$ is a vector of size n , then Eq. (3.20) has a unique solution for $C_U, C_E \in \mathbb{R}^{n \times n}$ in terms of P .*

We denote the solution of Eq. (3.20), guaranteed through Lemma 3.2.1, by $\tilde{C}_U(P), \tilde{C}_E(P)$. This solution can be used to close Eq. (3.18a), by using Eq. (3.17c) to obtain

$$\begin{aligned} \frac{d\bar{P}}{dt} &= \frac{dP}{dt} + \frac{d}{dt} \left(\tilde{C}_U(P) V_n \right) + \frac{d}{dt} \left(\tilde{C}_E(P)^t V_n \right) \\ &= \left[I + \frac{\partial}{\partial P} \left(\tilde{C}_U(P) V_n \right) + \frac{\partial}{\partial P} \left(\tilde{C}_E(P)^t V_n \right) \right] \frac{dP}{dt} \end{aligned} \quad (3.21)$$

With Eq. (3.18a), this leads to a closed system in P ,

$$\left[I + \frac{\partial}{\partial P} \left(\tilde{C}_U(P) V_n \right) + \frac{\partial}{\partial P} \left(\tilde{C}_E(P)^t V_n \right) \right] \frac{dP}{dt} = (K_2 * \tilde{C}_U(P))^t V_n - (L_2 * \tilde{C}_E(P))^t V_n. \quad (3.22)$$

The initial value of Eq. (3.22), denoted by $\hat{P}(0)$, must be chosen as the projection of the initial value $P(0)$ of Eq. (3.12), onto the manifold $\mathcal{M}_0$. The reduction is obtained under the assumption that during the initial transient there has not been any significant change in $\bar{P} = P + C_U V_n + C_E^t V_n$. Therefore the projection, $\hat{P}(0)$, of the initial conditions onto the slow manifold is related to the original initial conditions, $U(0), P(0), C_U(0), C_E(0)$, by

$$\hat{P}(0) + \tilde{C}_U(\hat{P}(0)) V_n + \tilde{C}_E^t(\hat{P}(0)) V_n = P(0) + C_U(0) V_n + C_E^t(0) V_n = P(0).$$

In summary, if tQSSA is valid, then Eq. (3.22) is a reduction of Eq. (3.12).

3.2.6 Proof of Lemma 3.2.1

Note that the unknowns in Eq. (3.20) are matrices and the structure of the equation is somewhat similar to a Lyapunov equation, $AX + XB = C$, where the matrices A, B, C are known and X is unknown. A standard approach to solving Lyapunov equations is to vectorize the matrices (see [31]), resulting in an equation of the type $[(I_m \otimes A) + (B^t \otimes I_n)] \text{vec}(X) = \text{vec}(C)$ (see Appendix B for the definition of term *vectorize* and the vec operator and the $\hat{\cdot}$ operator). Proving solvability then essentially reduces to proving the non-singularity of the coefficient matrix $[(I_m \otimes A) + (B^t \otimes I_n)]$. We will use this approach to show the solvability of Eq. (3.20).

In the proof of this Lemma we first assume that all possible reactions occur at nonzero rates so that all entries in the matrices $K_1, K_2, K_{-1}, L_1, L_2$, and L_{-1} are strictly positive. The result is then generalized to the case when some reaction rates are zero, so that not all reactions occur.

Note that Eq. (3.20b) is uncoupled from Eq. (3.20a). (The notations and terms used below are described in Appendix B.) Using Theorems B.1 and B.2, we vectorize Eq. (3.20b) to obtain

$$\begin{aligned}
 \text{vec} [L_1 * (C_E (V_n P^t)) + (L_{-1} + L_2) * C_E] &= \text{vec} [L_1 * (C_E (V_n P^t))] + \text{vec} [(L_{-1} + L_2) * C_E] \\
 &= \hat{L}_1 \text{vec} [C_E (V_n P^t)] + (\hat{L}_{-1} + \hat{L}_2) \text{vec} (C_E) \\
 &= \hat{L}_1 (P V_n^t \otimes I_n) \text{vec} (C_E) + (\hat{L}_{-1} + \hat{L}_2) \text{vec} (C_E) \\
 &= [\hat{L}_1 (P V_n^t \otimes I_n) + (\hat{L}_{-1} + \hat{L}_2)] \text{vec} (C_E). \tag{3.23}
 \end{aligned}$$

The following lemma shows that the matrix multiplying $\text{vec}(C_E)$ in this equation is invertible.

Lemma 3.2.2 *If $A, B \in \mathbb{R}_+^{n^2 \times n^2}$ are diagonal matrices with positive entries on the diagonal, $Y \in \mathbb{R}_+^{n \times 1}$ is a column vector with positive entries, $V_n = [1 \ 1 \ \cdots \ 1]^t$ is a column vector of size n , and I_n is the $n \times n$ identity matrix, then the $n^2 \times n^2$ matrix*

$$D = A(YV_n^t \otimes I_n) + B$$

is invertible.

Proof: Invertibility of D is equivalent to invertibility of $B^{-1}D$. Therefore, it is sufficient to prove the result with $B = I_{n^2 \times n^2} =: I$, so that $D = A(YV_n^t \otimes I_n) + I$. We will show that $A(YV_n^t \otimes I_n)$ can not have -1 as an eigenvalue. Since I commute with every matrix, it will follow that D cannot have 0 as an eigenvalue and we will be done. Let

$$A = \begin{bmatrix} A_1 & & & \\ & A_2 & & \\ & & \ddots & \\ & & & A_n \end{bmatrix}, \quad Y = \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{bmatrix},$$

where $A_i \in \mathbb{R}_+^{n \times n}$, $i \in \{1, 2, \dots, n\}$ are diagonal matrices, and $y_i \in \mathbb{R}_+$. Now

$$YV_n^t \otimes I_n = \begin{bmatrix} y_1 & y_1 & y_1 \\ y_2 & y_2 & y_2 \\ \vdots & \vdots & \vdots \\ y_n & y_n & y_n \end{bmatrix} \otimes I_n = \begin{bmatrix} y_1 I_n & y_1 I_n & y_1 I_n \\ y_2 I_n & y_2 I_n & y_2 I_n \\ \vdots & \vdots & \vdots \\ y_n I_n & y_n I_n & y_n I_n \end{bmatrix}.$$

This implies that

$$A(YV_n^t \otimes I_n) = \begin{bmatrix} y_1 A_1 & y_1 A_1 & y_1 A_1 \\ y_2 A_2 & y_2 A_2 & y_2 A_2 \\ \vdots & \vdots & \vdots \\ y_n A_n & y_n A_n & y_n A_n \end{bmatrix}. \quad (3.24)$$

3.2. THE GENERAL PROBLEM

Suppose λ is an eigenvalue of $A(YV_n^t \otimes I_n)$, and

$$\bar{X} = \begin{bmatrix} X_1 \\ X_2 \\ \vdots \\ X_n \end{bmatrix},$$

$X_i \in \mathbb{C}^{n \times 1}$, $i \in \{1, 2, \dots, n\}$ is one corresponding eigenvector. Using Eq. (3.24) we have

$$\begin{bmatrix} y_1 A_1 & y_1 A_1 & y_1 A_1 \\ y_2 A_2 & y_2 A_2 & y_2 A_2 \\ \vdots & \vdots & \vdots \\ y_n A_n & y_n A_n & y_n A_n \end{bmatrix} \begin{bmatrix} X_1 \\ X_2 \\ \vdots \\ X_n \end{bmatrix} = \lambda \begin{bmatrix} X_1 \\ X_2 \\ \vdots \\ X_n \end{bmatrix}.$$

This implies that for all $k \in \{1, 2, \dots, n\}$,

$$\begin{bmatrix} y_1 A_{1_k} & y_1 A_{1_k} & y_1 A_{1_k} \\ y_2 A_{2_k} & y_2 A_{2_k} & y_2 A_{2_k} \\ \vdots & \vdots & \vdots \\ y_n A_{n_k} & y_n A_{n_k} & y_n A_{n_k} \end{bmatrix} \begin{bmatrix} X_{1_k} \\ X_{2_k} \\ \vdots \\ X_{n_k} \end{bmatrix} = \lambda \begin{bmatrix} X_{1_k} \\ X_{2_k} \\ \vdots \\ X_{n_k} \end{bmatrix}, \quad (3.25)$$

where A_{i_k} is (k, k) -th entry in the matrix A_i , and X_{i_k} is the k th entry in the vector X_i .

Therefore, if λ is an eigenvalue of $A(YV_n^t \otimes I_n)$ then it must be an eigenvalue of one of its $n \times n$ principal submatrices which have the form of the coefficient matrix in Eq. (3.25) and whose eigenvalues we know are either zero or $\sum_{i=1}^n y_i A_{i_k}$ (see reason in the footnote²). Hence λ can not be -1 , and hence D cannot have a zero eigenvalue. $\square$

²We have

$$\begin{bmatrix} y_1 A_{1_k} & y_1 A_{1_k} & y_1 A_{1_k} \\ y_2 A_{2_k} & y_2 A_{2_k} & y_2 A_{2_k} \\ \vdots & \vdots & \vdots \\ y_n A_{n_k} & y_n A_{n_k} & y_n A_{n_k} \end{bmatrix}^t \begin{bmatrix} 1 \\ 1 \\ \vdots \\ 1 \end{bmatrix} = \sum_{i=1}^n y_i A_{i_k} \begin{bmatrix} 1 \\ 1 \\ \vdots \\ 1 \end{bmatrix}.$$

Since the coefficient matrix in the above equation is rank one, $\sum_{i=1}^n y_i A_{i_k}$ is the only non-zero eigenvalue.

This settles the problem of solvability of C_E in Eq. (3.20b). We can use this solution to eliminate C_E from Eq. (3.20a). Rewriting Eq. (3.20a) with all the known terms on the right hand side we obtain

$$\begin{aligned} K_1 * [P(V_n^t C_U^t + V_n^t C_U - V_n^t(I_n * C_U))] + (K_{-1} + K_2) * C_U \\ = K_1 * [P(U_T^t - P^t)] - K_1 * [PV_n^t C_E]. \end{aligned} \quad (3.26)$$

We can write

$$\text{vec} [P(V_n^t C_U^t + V_n^t C_U - V_n^t(I_n * C_U))] = (I_n \otimes P) \text{vec} [V_n^t C_U^t + V_n^t C_U - V_n^t(I_n * C_U)]. \quad (3.27)$$

Since $(C_U V_n)^t$ is a row vector, we have $\text{vec}[(C_U V_n)^t] = \text{vec}(C_U V_n)$. Therefore, using Theorems B.1 and B.2 we get

$$\begin{aligned} \text{vec}(V_n^t C_U^t) &= \text{vec}(C_U V_n) = (V_n^t \otimes I_n) \text{vec}(C_U), \\ \text{vec}(V_n^t C_U) &= (I_n \otimes V_n^t) \text{vec}(C_U), \\ \text{vec}(V_n^t(I_n * C_U)) &= (I_n \otimes V_n^t) \text{vec}(I_n * C_U) = (I_n \otimes V_n^t) \hat{I}_n \text{vec}(C_U). \end{aligned}$$

Plugging these in Eq. (3.27) we get

$$\begin{aligned} \text{vec} [P(V_n^t C_U^t + V_n^t C_U - V_n^t(I_n * C_U))] \\ = (I_n \otimes P) \left[(V_n^t \otimes I_n) + (I_n \otimes V_n^t) - (I_n \otimes V_n^t) \hat{I}_n \right] \text{vec}(C_U) \\ = \left[(I_n \otimes P)(V_n^t \otimes I_n) + (I_n \otimes PV_n^t) - (I_n \otimes PV_n^t) \hat{I}_n \right] \text{vec}(C_U). \end{aligned}$$

The vectorized form of the left hand side of Eq. (3.26) is

$$\left[\hat{K}_1 \left\{ (I_n \otimes P)(V_n^t \otimes I_n) + (I_n \otimes PV_n^t) - (I_n \otimes PV_n^t) \hat{I}_n \right\} + (\hat{K}_{-1} + \hat{K}_{-1}) \right] \text{vec}(C_U).$$

The following Lemma shows that the matrix multiplying $\text{vec}(C_U)$ in this expression is invertible.

3.2. THE GENERAL PROBLEM

Lemma 3.2.3 *If $A, B \in \mathbb{R}_+^{n^2 \times n^2}$ are diagonal matrices with positive entries on the diagonal, $Y \in \mathbb{R}_+^{n \times 1}$ is a column vector with positive entries, $V_n = [1 \ 1 \ \cdots \ 1]^t$ is a column vector of size n , then the $n^2 \times n^2$ matrix*

$$D = A \left((I_n \otimes Y) (V_n^t \otimes I_n) + (I_n \otimes Y V_n^t) - (I_n \otimes Y V_n^t) \hat{I}_n \right) + B$$

is invertible.

Proof: The invertibility of D is equivalent to invertibility of $A^{-1}D$. We can therefore assume that $A = I_{n^2}$. Now

$$\begin{aligned} & (I_n \otimes Y) (V_n^t \otimes I_n) \\ = & \left[\begin{array}{ccc|ccc|c|ccc} y_1 & 0 & 0 & y_1 & 0 & 0 & & y_1 & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \ddots & \vdots \\ y_n & 0 & 0 & y_n & 0 & 0 & \dots & y_n & 0 & 0 & 0 \end{array} \right. \\ & \left. \begin{array}{ccc|ccc|c|ccc} 0 & y_1 & 0 & 0 & y_1 & 0 & & 0 & y_1 & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \ddots & \vdots \\ 0 & y_n & 0 & 0 & y_n & 0 & \dots & 0 & y_n & 0 & 0 \end{array} \right. \\ & \left. \begin{array}{ccc|ccc|c|ccc} & & & & & & \vdots & & & & \\ & & & & & & \vdots & & & & \end{array} \right. \\ & \left. \begin{array}{ccc|ccc|c|ccc} 0 & 0 & y_1 & 0 & 0 & y_1 & & 0 & 0 & y_1 & \\ \vdots & \vdots & \ddots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & y_n & 0 & 0 & y_n & \dots & 0 & 0 & y_n & \end{array} \right] , \end{aligned}$$

3.2. THE GENERAL PROBLEM

and

$$\begin{aligned}
 & (I_n \otimes YV_n^t) \\
 = & \begin{bmatrix}
 y_1 & y_1 & & y_1 & & & & \\
 \vdots & \vdots & \dots & \vdots & & & & \\
 y_n & y_n & & y_n & & & & \\
 & & & & y_1 & y_1 & & y_1 \\
 & & & & \vdots & \vdots & \dots & \vdots \\
 & & & & y_n & y_n & & y_n \\
 & & & & & & \ddots & \\
 & & & & & & & y_1 & y_1 & & y_1 \\
 & & & & & & & \vdots & \vdots & \dots & \vdots \\
 & & & & & & & y_n & y_n & & y_n
 \end{bmatrix}.
 \end{aligned}$$

So,

$$\begin{aligned}
 & (I_n \otimes YV_n^t) (I_{n^2} - \widehat{I}_n) \\
 = & \begin{bmatrix}
 0 & y_1 & & y_1 & & & & \\
 \vdots & \vdots & \dots & \vdots & & & & \\
 0 & y_n & & y_n & & & & \\
 & & & & y_1 & 0 & & y_1 \\
 & & & & \vdots & \vdots & \dots & \vdots \\
 & & & & y_n & 0 & & y_n \\
 & & & & & & \ddots & \\
 & & & & & & & y_1 & y_1 & & 0 \\
 & & & & & & & \vdots & \vdots & \dots & \vdots \\
 & & & & & & & y_n & y_n & & 0
 \end{bmatrix},
 \end{aligned}$$

3.2. THE GENERAL PROBLEM

and

$$\begin{aligned}
 & (I_n \otimes Y) (V_n^t \otimes I_n) + (I_n \otimes YV_n^t) - (I_n \otimes YV_n^t) \hat{I}_n \\
 = & \left[\begin{array}{ccc|ccc|c|ccc}
 y_1 & y_1 & y_1 & y_1 & 0 & 0 & & y_1 & 0 & 0 \\
 \vdots & \vdots & \dots & \vdots & \vdots & \dots & \vdots & \vdots & \vdots & \vdots \\
 y_n & y_n & y_n & y_n & 0 & 0 & & y_n & 0 & 0 \\
 \hline
 0 & y_1 & 0 & y_1 & y_1 & y_1 & & 0 & y_1 & 0 \\
 \vdots & \vdots & \dots & \vdots & \vdots & \dots & \vdots & \vdots & \vdots & \vdots \\
 0 & y_n & 0 & y_n & y_n & y_n & & 0 & y_n & 0 \\
 \hline
 & & & & & & \ddots & & & \\
 \hline
 0 & 0 & y_1 & 0 & 0 & y_1 & & y_1 & y_1 & y_1 \\
 \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\
 0 & 0 & y_n & 0 & 0 & y_n & & y_n & y_n & y_n
 \end{array} \right].
 \end{aligned}$$

Clearly, its sufficient to show the invertibility of D with $y_1 = y_2 = \dots = y_n = 1$. We

3.2. THE GENERAL PROBLEM

examine

$$D - B = \left[\begin{array}{ccc|ccc|c|ccc} 1 & 1 & & 1 & & & 1 & 0 & & 0 \\ \vdots & \vdots & \dots & \vdots & & & \vdots & \vdots & \dots & \vdots \\ 1 & 1 & & 1 & 0 & & 0 & & & 0 \\ \hline 0 & 1 & & 0 & 1 & 1 & & 1 & & 0 \\ \vdots & \vdots & \dots & \vdots & \vdots & \vdots & \dots & \vdots & & \vdots \\ 0 & 1 & & 0 & 1 & 1 & & 1 & & 0 \\ \hline & & & & & & & & \ddots & \\ & & & & & & & & & \\ \hline 0 & 0 & & 1 & 0 & 0 & & 1 & & 1 \\ \vdots & \vdots & \dots & \vdots & \vdots & \vdots & \dots & \vdots & & \vdots \\ 0 & 0 & & 1 & 0 & 0 & & 1 & & 1 \end{array} \right].$$

Now let

$$V = \left[\begin{array}{cccccccc} v_{11} & \dots & v_{1n} & v_{21} & \dots & v_{2n} & \dots & v_{n1} & \dots & v_{nn} \end{array} \right]^t$$

be an eigenvector of D corresponding to a zero eigenvalue. We aim to show that $V = 0$.

Let

$$B = \text{diag} \left[\begin{array}{cccccccc} b_{11} & \dots & b_{1n} & b_{21} & \dots & b_{2n} & \dots & b_{n1} & \dots & b_{nn} \end{array} \right].$$

Then for each $i, j \in \{1, 2, \dots, n\}$,

$$\underbrace{\sum_{s=1}^n v_{is} + \sum_{\substack{r=1 \\ r \neq i}}^n v_{ri}}_{:= -\lambda_i} = -b_{ij}v_{ij}. \quad (3.28)$$

Note that the left hand side of this equation, which we denote by $-\lambda_i$, is independent of

j . Hence, for all $i, j \in \{1, 2, \dots, n\}$ we obtain $v_{ij} = \frac{\lambda_i}{b_{ij}}$. Using this observation in Eq. (3.28) we get

$$\sum_{s=1}^n \frac{\lambda_i}{b_{is}} + \sum_{\substack{r=1 \\ r \neq i}}^n \frac{\lambda_r}{b_{ri}} = -\lambda_i, \quad \forall i \in \{1, 2, \dots, n\}.$$

This equality can be written in matrix form as

$$\begin{bmatrix} 1 + \sum_{s=1}^n \frac{1}{b_{1s}} & \frac{1}{b_{21}} & \cdots & \frac{1}{b_{n1}} \\ \frac{1}{b_{12}} & 1 + \sum_{s=1}^n \frac{1}{b_{2s}} & \cdots & \frac{1}{b_{n2}} \\ & & \ddots & \\ \frac{1}{b_{1n}} & \frac{1}{b_{2n}} & \cdots & 1 + \sum_{s=1}^n \frac{1}{b_{ns}} \end{bmatrix} \begin{bmatrix} \lambda_1 \\ \lambda_2 \\ \vdots \\ \lambda_n \end{bmatrix} = 0$$

The coefficient matrix is diagonally dominant along the columns, and hence invertible.

This implies that $\lambda_i = 0$, and so $v_{ij} = 0$. $\square$

Lemmas 3.2.2 and 3.2.3 together complete the proof of Lemma 3.2.1 for the case when all the entries in the connectivity matrices are strictly positive. This proof can be extended to general connectivity matrices, as stated in the Lemma 3.2.1 in the following way.

Suppose that some of the entries in the connectivity matrix are zero. Let,

$$I_K = [I_K(i, j)]_{i,j=1}^n \quad \text{such that} \quad I_K(i, j) = \begin{cases} 1 & \text{if } k_{ij}^1, k_{ij}^{-1}, k_{ij}^2 \text{ are nonzero,} \\ 0 & \text{if } k_{ij}^1 = k_{ij}^{-1} = k_{ij}^2 = 0, \end{cases} \quad (3.29a)$$

$$I_L = [I_L(i, j)]_{i,j=1}^n \quad \text{such that} \quad I_L(i, j) = \begin{cases} 1 & \text{if } l_{ij}^1, l_{ij}^{-1}, l_{ij}^2 \text{ are nonzero,} \\ 0 & \text{if } l_{ij}^1 = l_{ij}^{-1} = l_{ij}^2 = 0. \end{cases} \quad (3.29b)$$

Hence I_K and I_L are the unweighted connectivity matrices of the reaction network. The matrices of intermediate variables, corresponding to existing connections, now have the

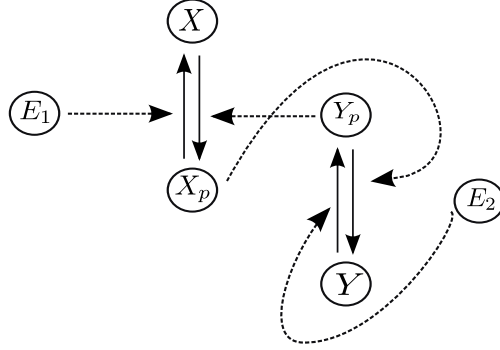


Figure 3.4: A hypothetical network for which the reduction described in sections 3.2.4–3.2.5 leads to a differential–algebraic system of equations. The concentrations of the intermediate complexes appear in a nonlinear way in the resulting algebraic equations. A further reduction to a form involving only the protein concentrations is therefore not apparent.

form

$$C_U^{I_K} = I_K * C_U \quad C_E^{I_L} = I_L * C_L. \quad (3.30)$$

Replacing C_U with $C_U^{I_K}$ and C_E with $C_E^{I_L}$ in Eq. (3.20), one can easily check that the solution of the non-zero entries of $C_U^{I_K}$ and $C_E^{I_L}$ does not depend on the zero entries of K_1 , K_2 , K_{-1} , L_1 , L_2 , L_{-1} . This observation completes the proof of Lemma 3.2.1.

3.2.7 An example network where the above reduction scheme fails

We end this chapter by pointing out that not all enzymatic networks belong to the class we described. For example, our full reduction scheme does not work for the network depicted in Fig. 3.4.

This network is a slight modification of the network in Fig. 3.1a). Although the tQSSA can still be justified (we will see that in the next chapter), the algebraic part of the reduced

3.2. THE GENERAL PROBLEM

equations cannot be solved using our approach. These equations have the form

$$\begin{aligned}
0 &= \underbrace{(X_T - X_p - C_x^e - C_x - C_y)}_{=X} \underbrace{(Y_T - Y - C_y - C_x - C_y^e)}_{=Y_p} - k_m C_x, \\
0 &= X_p \underbrace{(Y_T - Y - C_y - C_x - C_y^e)}_{=Y_p} - k_m C_y, \\
0 &= (E_1^T - C_x^e) X_p - k_m C_x^e, \\
0 &= (E_2^T - C_y^e) Y - k_m C_y^e,
\end{aligned}$$

which has to be solved for C_x, C_y, C_x^e, C_y^e in terms of X_p, Y . Immediately we run into problems because the first equation in the above algebraic system is quadratic in the unknown variables.

To determine whether the reduction scheme described in this chapter is applicable to a given network one can follow these guidelines:

For each protein exactly one of its form, either phosphorylated or unphosphorylated, can act as enzyme. Lets label this form of the proteins to be active and the form that is not acting as enzyme to be inactive. Then, if for each protein, the active form is acting as enzymes to convert only the inactive form of other proteins into their active form, then the present reduction scheme is applicable.

Chapter 4

Validity of tQSSA in a network

In the last chapter we built a hypothetical network and described the corresponding meaning of tQSSA. In addition to that we showed what simplification one can get if tQSSA is assumed. To make the story more complete the natural question is when can one assume the tQSSA. This is the question that we address in this chapter.

The method we use is very much the same as the one used in the derivation of Michaelis-Menten equation for an isolated MM reaction in Section 1.2. The original equations will be scaled in such a way that the theorems of GSPT can be applied. Another technical issue that we will deal here is to show that the manifold, on which the dynamics is being reduced to, is asymptotically stable. One should note that it is necessary to show this if one wishes to apply the GSPT.

We have divided this chapter into two main part. In the first part we show that the slow manifold is stable. In the second part we obtain the necessary scaling to frame the original equation to the form where the theorems of GSPT can be applied.

4.1 The stability of the slow manifold

In this section we will show that the slow manifold $\mathcal{M}_0$ defined by Eq. (3.19) is always normally hyperbolic and stable. As we said earlier, this is a necessary requirement because we intend to use GSPT to justify the validity of the reduction obtained under the generalized tQSSA. The results of this sections also apply to the slow manifolds that arise in isolated Michaelis-Menten reaction and the two protein example we discussed in Section 3.1, as those are particular examples of the general setup.

First we will need some new notations and definitions to simplify the computations. Suppose that A and B are matrices of dimensions $n \times k$ and $n \times l$, respectively. We denote by $[A : B]$ the $n \times (k + l)$ matrix obtained by adjoining B to A . We use this definition to combine the different coefficient matrices, and let

$$C := [C_U : C_E^t], \quad Q_1 := [K_1 : L_1^t], \quad Q_2 := [K_{-1} + K_2 : L_{-1}^t + L_2^t].$$

(Note the t in the subscript of C_E^t in $[C_U : C_E^t]$ and similarly in other definitions above. This t stands for matrix transpose.) We also define

$$Z := \begin{bmatrix} U \\ E \end{bmatrix}, \quad \bar{Z} := \begin{bmatrix} U_T - \bar{P} \\ E_T \end{bmatrix}, \quad I_{2n}^n := \begin{bmatrix} I_n \\ 0 \end{bmatrix}, \quad \text{and} \quad V_{2n} = \underbrace{\begin{bmatrix} 1 & 1 & \dots & 1 \end{bmatrix}}_{2n \text{ times}}^t.$$

Using this notation the right hand side of Eqs. (3.17a-3.17b) can be written as

$$\begin{aligned} Z &= \begin{bmatrix} U \\ E \end{bmatrix} = \begin{bmatrix} U_T - \bar{P} \\ E_T \end{bmatrix} - \begin{bmatrix} C_U^t V_n \\ C_E V_n \end{bmatrix} + \begin{bmatrix} (I_n * C_U^t) V_n \\ 0 \end{bmatrix} \\ &= \bar{Z} - C^t V_n + \left(\begin{bmatrix} I_n \\ 0 \end{bmatrix} * C^t \right) V_n \\ &= \bar{Z} - (C^t - I_{2n}^n * C^t) V_n, \end{aligned}$$

and Eq. (3.17c) can be written as $P = \bar{P} - CV_{2n}$. Therefore, Eqs. (3.16b-3.16c) can be merged to obtain

$$\frac{dC}{dt} = \underbrace{Q_1 * (PZ^t) - Q_2 * C}_{:=F(C)}. \quad (4.1)$$

The manifold $\mathcal{M}_0$, as defined in Eq. (3.19), can now be redefined as

$$\mathcal{M}_0 = \{C \in \mathbb{R}^{n \times 2n} \mid Q_1 * (PZ^t) - Q_2 * C = F(C) = 0\}.$$

To show that $\mathcal{M}_0$ is normally hyperbolic and stable we need to show that the Jacobian, $\frac{\partial F}{\partial C}$, evaluated at $\mathcal{M}_0$ has eigenvalues with only negative real parts. We will show that $\frac{\partial F}{\partial C}$ has eigenvalues with negative real parts everywhere, and hence at all points of $\mathcal{M}_0$, *a fortiori*.

The mapping $F : \mathbb{R}^{n \times 2n} \rightarrow \mathbb{R}^{n \times 2n}$ is a matrix valued function of the matrix variables C . Therefore $\frac{\partial F}{\partial C}$ represents differentiation with respect to a matrix. This operation is defined by “flattening” a $m \times n$ matrix to a $mn \times 1$ vector and taking the gradient. More precisely, suppose $M = [M_{.1} : M_{.2} : \dots : M_{.n}]$ is a $m \times n$ matrix, where $M_{.j}$ is the j th column of M . Then define

$$\text{vec}(M) := \begin{bmatrix} M_{.1} \\ M_{.2} \\ \vdots \\ M_{.n} \end{bmatrix} \in \mathbb{C}^{mn \times 1}, \quad \text{and} \quad \widehat{M} := \text{diag}(\text{vec}(M)) \in \mathbb{C}^{mn \times mn}. \quad (4.2)$$

Therefore, $\text{vec}(M)$ is obtained by stacking the columns of M on top of each other, and $\widehat{M}$ is the $mn \times mn$ diagonal matrix whose diagonal entries are given by $\text{vec}(M)$.

Suppose $G : \mathbb{C}^{p \times q} \rightarrow \mathbb{C}^{m \times n}$ is a matrix valued function with $X \in \mathbb{C}^{p \times q} \mapsto G(X) \in$

$\mathbb{C}^{m \times n}$. Then the derivative of G with respect to X is defined as

$$\frac{\partial G}{\partial X} := \frac{\partial \text{vec}(G)}{\partial \text{vec}(X)}, \quad (4.3)$$

where the right hand side is the Jacobian [42]. In the appendix we list some important properties of these operators which will be used subsequently (see Appendix B).

A direct application of Theorem B.4 yields

$$\frac{\partial F}{\partial C} = \frac{\partial \text{vec}(F)}{\partial \text{vec}(C)} = \hat{Q}_1 \frac{\partial \text{vec}(PZ^t)}{\partial \text{vec}(C)} - \hat{Q}_2 \frac{\partial \text{vec}(C)}{\partial \text{vec}(C)}.$$

We first assume that all the entries in the connectivity matrices are positive, so that all entries in the matrix C are *actual variables*. At the end of this section we show how to remove this assumption.

Replacing $\partial \text{vec}(C)/\partial \text{vec}(C)$ with the identity matrix, I_{2n^2} , adding $\hat{Q}_2$ to both side, using Theorems B.1, B.2, B.3, B.4, and treating $\bar{P}$ and $\bar{Z}$ as independent of C we obtain

$$\begin{aligned} \hat{Q}_2 + \frac{\partial \text{vec}(F)}{\partial \text{vec}(C)} &= \hat{Q}_1 \left[(Z \otimes I_n) \frac{\partial \text{vec}(P)}{\partial \text{vec}(C)} + (I_{2n} \otimes P) \frac{\partial \text{vec}(Z^t)}{\partial \text{vec}(C)} \right] \\ &= \hat{Q}_1 \left[- (Z \otimes I_n) \frac{\partial \text{vec}(CV_{2n})}{\partial \text{vec}(C)} - (I_{2n} \otimes P) \frac{\partial \text{vec} \left(((C^t - I_{2n}^n * C^t) V_n)^t \right)}{\partial \text{vec}(C)} \right] \\ &= \hat{Q}_1 \left[- (Z \otimes I_n) \frac{\partial \text{vec}(CV_{2n})}{\partial \text{vec}(C)} - (I_{2n} \otimes P) \frac{\partial \text{vec} (V_n^t C - V_n^t ((I_{2n}^n)^t * C))}{\partial \text{vec}(C)} \right] \\ &= \hat{Q}_1 \left[- (Z \otimes I_n) (V_{2n}^t \otimes I_n) \frac{\partial \text{vec}(C)}{\partial \text{vec}(C)} \right. \\ &\quad \left. - (I_{2n} \otimes P) \left\{ \frac{\partial \text{vec}(V_n^t C)}{\partial \text{vec}(C)} - \frac{\partial \text{vec}(V_n^t ((I_{2n}^n)^t * C))}{\partial \text{vec}(C)} \right\} \right] \\ &= \hat{Q}_1 \left[- (ZV_{2n}^t \otimes I_n) - (I_{2n} \otimes P) \left\{ (I_{2n} \otimes V_n^t) - (I_{2n} \otimes V_n^t) \widehat{(I_{2n}^n)^t} \right\} \right] \\ &= \hat{Q}_1 \left[- (ZV_{2n}^t \otimes I_n) - (I_{2n} \otimes PV_n^t) + (I_{2n} \otimes PV_n^t) \widehat{(I_{2n}^n)^t} \right] \\ &= -\hat{Q}_1 \left[(ZV_{2n}^t \otimes I_n) + (I_{2n} \otimes PV_n^t) \left(I_{2n^2} - \widehat{(I_{2n}^n)^t} \right) \right]. \end{aligned}$$

Here $\widehat{(I_{2n}^n)^t}$ is the matrix obtained by applying the *hat* operator, defined in Eq. (4.2), to the transpose of I_{2n}^n .

This computation shows that the Jacobian matrix of interest has the form

$$J := \frac{\partial F}{\partial C} = -\widehat{Q}_1 \left[(ZV_{2n}^t \otimes I_n) + (I_{2n} \otimes PV_n^t) \left(I_{2n^2} - \widehat{(I_{2n}^n)^t} \right) \right] - \widehat{Q}_2. \quad (4.4)$$

The following Lemma shows that this Jacobian matrix always has eigenvalues with negative real part.

Lemma 4.1.1 *Suppose $Z \in \mathbb{R}_+^{2n \times 1}$ is a $2n$ dimensional vector with positive entries, $Y \in \mathbb{R}_+^{n \times 1}$ is an n dimensional vector with positive entries, $\Lambda, \Gamma \in \mathbb{R}^{2n^2 \times 2n^2}$ are diagonal matrices with positive entries on the diagonal. Further assume that R_n and R_{2n} are row vectors of size n and $2n$ respectively with all entries equal to 1. Then the $2n^2 \times 2n^2$ matrix*

$$J = \Lambda \left[(ZR_{2n} \otimes I_n) + (I_{2n} \otimes YR_n) \left(I_{2n^2} - \widehat{(I_{2n}^n)^t} \right) \right] + \Gamma \quad (4.5)$$

has eigenvalues with strictly positive real parts.

This Lemma applies to connectivity matrices with strictly positive entries. In Section 4.1.1 we show how to generalize the Lemma to the case when the connectivity matrices contain zero entries. In this case only the principal submatrix of the Jacobian, J , corresponding to the positive entries of the connectivity matrices needs to be examined. Since this principal submatrix contains all the non-zero element of J , the result follows. We therefore obtain the following corollary.

Corollary 4.1.2 *The manifold $\mathcal{M}_0$ defined in Eq. (3.19) is normally hyperbolic and stable.*

We first prove the above Lemma under the assumption that $K_1, K_2, K_{-1}, L_1, L_2$, and L_{-1} are strictly positive. At the end of this section we show how to generalize the proof to the case when some of the reactions do not occur.

First we start with a preliminary lemma.

Lemma 4.1.3 *Suppose $Z \in \mathbb{R}_+^{2n \times 1}$ is a $2n$ dimensional vector with positive entries, $Y \in \mathbb{R}_+^{n \times 1}$ is an n -dimensional vector with positive entries, and $\widehat{\Psi} = [\widehat{\psi}_{ij}]$, $\widehat{\Gamma} = [\widehat{\gamma}_{ij}] \in \mathbb{R}_+^{n \times 2n}$ real matrices with positive entries. Let $\lambda \in \mathbb{C}$ be a complex number with nonpositive real part. If $V = [v_{ij}] \in \mathbb{C}^{n \times 2n}$ is a complex matrix that satisfies the following system of linear homogeneous equations,*

$$\frac{1}{y_i} \sum_{s=1}^{2n} v_{is} + \frac{1}{z_j} \sum_{\substack{r=1 \\ r \neq j}}^n v_{rj} = \frac{\widehat{\psi}_{ij}}{y_i z_j} (\lambda - \widehat{\gamma}_{ij}) v_{ij}, \quad \begin{array}{l} 1 \leq i \leq n, \\ 1 \leq j \leq n, \end{array} \quad (4.6a)$$

$$\frac{1}{y_i} \sum_{s=1}^{2n} v_{is} + \frac{1}{z_j} \sum_{r=1}^n v_{rj} = \frac{\widehat{\psi}_{ij}}{y_i z_j} (\lambda - \widehat{\gamma}_{ij}) v_{ij}, \quad \begin{array}{l} 1 \leq i \leq n, \\ n+1 \leq j \leq 2n, \end{array} \quad (4.6b)$$

then V is the zero matrix.

Proof: Let $V = [v_{ij}] \in \mathbb{C}^{n \times 2n}$ satisfy Eq. (4.6). We will show that $v_{ij} = 0$ for all i, j .

Let

$$R_i := \sum_{j=1}^{2n} v_{ij}, \quad 1 \leq i \leq n, \quad C_j := \begin{cases} \sum_{\substack{i=1 \\ i \neq j}}^n v_{ij}, & 1 \leq j \leq n, \\ \sum_{i=1}^n v_{ij}, & n+1 \leq j \leq 2n. \end{cases}$$

Then Eq. (4.6) can be written as

$$\frac{1}{y_i} R_i + \frac{1}{z_j} C_j = \frac{\widehat{\psi}_{ij}}{y_i z_j} (\lambda - \widehat{\gamma}_{ij}) v_{ij}, \quad \begin{array}{l} 1 \leq i \leq n, \\ 1 \leq j \leq 2n, \end{array}$$

Setting $a_{ij} = \frac{\widehat{\psi}_{ij}}{y_i z_j} (\lambda - \widehat{\gamma}_{ij})$, we have

$$\frac{1}{a_{ij} y_i} R_i + \frac{1}{a_{ij} z_j} C_j = v_{ij}, \quad 1 \leq i \leq n, \quad 1 \leq j \leq 2n. \quad (4.7)$$

4.1. THE STABILITY OF THE SLOW MANIFOLD

By summing Eq. (4.7) over i and j separately we obtain the following system of linear equations in the unknowns $\{R_1, R_2, \dots, R_n, C_1, C_2, \dots, C_{2n}\}$

$$R_i \frac{1}{y_i} \sum_{j=1}^{2n} \frac{1}{a_{ij}} + \sum_{j=1}^{2n} \frac{1}{z_j a_{ij}} C_j = R_i, \quad 1 \leq i \leq n, \quad (4.8a)$$

$$\sum_{i=1}^n \frac{1}{y_i a_{ij}} R_i + C_j \frac{1}{z_j} \sum_{i=1}^n \frac{1}{a_{ij}} = C_j, \quad 1 \leq j \leq 2n \quad (4.8b)$$

Eq. (4.8) can be written in matrix form as

$$\underbrace{\left[\begin{array}{ccc|ccc} -1 + \frac{1}{y_1} \sum_{j=1}^{2n} \frac{1}{a_{1j}} & & & \frac{1}{z_1 a_{11}} & \cdots & \frac{1}{z_{2n} a_{1,2n}} \\ & \ddots & & \vdots & & \vdots \\ & & -1 + \frac{1}{y_n} \sum_{j=1}^{2n} \frac{1}{a_{nj}} & \frac{1}{z_1 a_{n1}} & \cdots & \frac{1}{z_{2n} a_{n,2n}} \\ \hline \frac{1}{y_1 a_{11}} & \cdots & \frac{1}{y_n a_{n1}} & -1 + \frac{1}{z_1} \sum_{i=1}^n \frac{1}{a_{i1}} & & \\ \vdots & & \vdots & & \ddots & \\ \frac{1}{y_1 a_{1,2n}} & \cdots & \frac{1}{y_n a_{n,2n}} & & & -1 + \frac{1}{z_{2n}} \sum_{i=1}^n \frac{1}{a_{i,2n}} \end{array} \right]}_{:=A} \begin{bmatrix} R_1 \\ R_2 \\ \vdots \\ R_n \\ C_1 \\ C_2 \\ \vdots \\ C_{2n} \end{bmatrix} = 0. \quad (4.9)$$

We next show that the coefficient matrix, A , is invertible. This will imply that $R_i = C_j = 0, \forall i, j$. This, together with (4.7), will force v_{ij} to be zero and we will be done.

To show the non-singularity of A it is sufficient to show the non singularity of the product of A with a non-singular diagonal matrix

$$A \begin{bmatrix} y_1 & & & & & \\ & \ddots & & & & \\ & & y_n & & & \\ & & & z_1 & & \\ & & & & \ddots & \\ & & & & & z_{2n} \end{bmatrix}$$

$$= \underbrace{\begin{bmatrix} -y_1 + \sum_{j=1}^{2n} \frac{1}{a_{1j}} & & & \frac{1}{a_{1,2n}} \\ & \ddots & & \vdots \\ & & -y_n + \sum_{j=1}^{2n} \frac{1}{a_{nj}} & \frac{1}{a_{n,2n}} \\ \hline \frac{1}{a_{11}} & \cdots & \frac{1}{a_{n1}} & -z_1 + \sum_{i=1}^n \frac{1}{a_{i1}} \\ \vdots & & \vdots & \ddots \\ \frac{1}{a_{1,2n}} & \cdots & \frac{1}{a_{n,2n}} & -z_{2n} + \sum_{i=1}^n \frac{1}{a_{i,2n}} \end{bmatrix}}_{=X}.$$

Note that X is a complex symmetric matrix (i.e. $X = X^t$). To show the non singularity of X , it is sufficient to show that X has no zero eigenvalue. Assume that α is an eigenvalue of X and $u \in \mathbb{R}^{3n}$ a corresponding eigenvector. Break X into two Hermitian matrices,

$$X = \underbrace{\frac{X + X^*}{2}}_{:=S} + i \underbrace{\frac{X - X^*}{2i}}_{:=T} = S + iT,$$

where X^* is the conjugate transpose of X). Then,

$$\alpha \langle u, u \rangle = \langle Xu, u \rangle = \langle Su, u \rangle + i \langle Tu, u \rangle.$$

To show that α is not zero, it is sufficient to show that $\langle Su, u \rangle$ is not zero for any $0 \neq u \in \mathbb{R}^{3n}$. Note that, since S , and T are Hermitian, the terms $\langle Su, u \rangle$ and $\langle Tu, u \rangle$ are always real.

But since X is a complex symmetric matrix, $S_{ij} = \frac{X_{ij} + \bar{X}_{ji}}{2} = \frac{X_{ij} + \bar{X}_{ij}}{2} = \text{Re}(X_{ij})$, where S_{ij} , and X_{ij} are the (i, j) -th entries of the matrices S and X respectively, and $\bar{X}_{ij}$ is the complex conjugate of the complex number X_{ij} , and $\text{Re}(X_{ij})$ is the real part of X_{ij} .

Therefore,

$$S = \left[\begin{array}{ccc|ccc} -y_1 + \sum_{j=1}^{2n} \operatorname{Re} \frac{1}{a_{1j}} & & & \operatorname{Re} \frac{1}{a_{11}} & \dots & \operatorname{Re} \frac{1}{a_{1,2n}} \\ & \ddots & & \vdots & & \vdots \\ & & -y_n + \sum_{j=1}^{2n} \operatorname{Re} \frac{1}{a_{nj}} & \operatorname{Re} \frac{1}{a_{n1}} & \dots & \operatorname{Re} \frac{1}{a_{n,2n}} \\ \hline & \operatorname{Re} \frac{1}{a_{11}} & \dots & \operatorname{Re} \frac{1}{a_{n1}} & & \\ & \vdots & & \vdots & & \\ \operatorname{Re} \frac{1}{a_{1,2n}} & \dots & \operatorname{Re} \frac{1}{a_{n,2n}} & & -z_1 + \sum_{i=1}^n \operatorname{Re} \frac{1}{a_{i1}} & \\ & & & & \ddots & \\ & & & & & -z_{2n} + \sum_{i=1}^n \operatorname{Re} \frac{1}{a_{i,2n}} \end{array} \right].$$

Recall that $a_{ij} = \frac{\widehat{\psi}_{ij}}{y_i z_j} (\lambda - \widehat{\gamma}_{ij})$. If the real part of λ is nonpositive then the real parts of a_{ij} are negative. This implies that $\operatorname{Re} \frac{1}{a_{ij}} < 0$ for all i, j . In turn, this implies that S is diagonally dominant, and all the eigenvalues of S are negative and real, since S is a real symmetric matrix.

Therefore $\langle Su, u \rangle < 0$ for all $u \in \mathbb{R}^{3n}$, and α cannot be zero. This implies that X is invertible, which further implies that A is invertible. So, $R_i = C_j = 0$ for i, j . Eq. (4.7) therefore implies that $V = 0$. $\square$

Now we are ready to prove the claim that the slow manifold, arising in the general setup, is always normally hyperbolic and stable.

Proof of Lemma 4.1.1:

Proof: We will prove the lemma by contradiction. Let

$$Z = \begin{bmatrix} z_1 \\ z_2 \\ \vdots \\ z_{2n} \end{bmatrix}, \quad Y = \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{bmatrix}.$$

Then

$$ZR_{2n} \otimes I_n = \begin{bmatrix} z_1 & z_1 & & z_1 \\ z_2 & z_2 & \dots & z_2 \\ \vdots & \vdots & & \vdots \\ z_{2n} & z_{2n} & & z_{2n} \end{bmatrix} \otimes I_n = \underbrace{\begin{bmatrix} z_1 I_n & z_1 I_n & & z_1 I_n \\ z_2 I_n & z_2 I_n & \dots & z_2 I_n \\ \vdots & \vdots & & \vdots \\ z_{2n} I_n & z_{2n} I_n & & z_{2n} I_n \end{bmatrix}}_{2n \text{ block columns}},$$

$$I_{2n} \otimes YR_n = I_{2n} \otimes \underbrace{\begin{bmatrix} y_1 & y_1 & & y_1 \\ y_2 & y_2 & \dots & y_2 \\ \vdots & \vdots & & \vdots \\ y_n & y_n & & y_n \end{bmatrix}}_{=YR_n} = \underbrace{\begin{bmatrix} YR_n & & & \\ & YR_n & & \\ & & \ddots & \\ & & & YR_n \end{bmatrix}}_{2n \text{ block columns}}.$$

Let $R_n^{(i)} = \begin{bmatrix} 1 & \dots & 1 & 0 & 1 & \dots & 1 \end{bmatrix}$ be a row vector with a zero in the i -th place and 1s everywhere else. Then,

$$(I_{2n} \otimes YR_n) \left(I_{2n^2} - \widehat{I_{2n}^n}^t \right) = \underbrace{\begin{bmatrix} YR_n^{(1)} & & & \\ & \ddots & & \\ & & YR_n^{(n)} & \\ & & & YR_n \\ & & & & \ddots \\ & & & & & YR_n \end{bmatrix}}_{2n \text{ block columns}}.$$

Therefore,

$$ZR_{2n} \otimes I_n + (I_{2n} \otimes YR_n) \left(I_{2n^2} - \widehat{I_{2n}^n}^t \right)$$

$$= \left[\begin{array}{ccc|ccc} z_1 I_n + Y R_n^{(1)} & \dots & z_1 I_n & z_1 I_n & \dots & z_1 I_n \\ \vdots & \ddots & \vdots & \vdots & \ddots & \vdots \\ z_n I_n & \dots & z_n I_n + Y R_n^{(n)} & z_n I_n & \dots & z_n I_n \\ \hline z_{n+1} I_n & \dots & z_{n+1} I_n & z_{n+1} I_n + Y R_n & \dots & z_{n+1} I_n \\ \vdots & \ddots & \vdots & \vdots & \ddots & \vdots \\ z_{2n} I_n & \dots & z_{2n} I_n & z_{2n} I_n & \dots & z_{2n} I_n + Y R_n \end{array} \right].$$

Let

$$\Lambda = \begin{bmatrix} \Lambda^{(1)} & & & \\ & \Lambda^{(2)} & & \\ & & \ddots & \\ & & & \Lambda^{(2n)} \end{bmatrix}, \quad \Gamma = \begin{bmatrix} \Gamma^{(1)} & & & \\ & \Gamma^{(2)} & & \\ & & \ddots & \\ & & & \Gamma^{(2n)} \end{bmatrix},$$

where $\Lambda^{(k)}, \Gamma^{(k)}, k \in \{1, 2, \dots, 2n\}$ are $n \times n$ diagonal blocks of Λ, Γ respectively. Hence

$$J = \left[\begin{array}{c|c} A_{11} & A_{12} \\ \hline A_{21} & A_{22} \end{array} \right],$$

where

$$\begin{aligned}
 A_{11} &= \begin{bmatrix} z_1 \Lambda^{(1)} + \Lambda^{(1)} Y R_n^{(1)} + \Gamma^{(1)} & \dots & z_1 \Lambda^{(1)} \\ \vdots & \ddots & \vdots \\ z_n \Lambda^{(n)} & \dots & z_n \Lambda^{(n)} + \Lambda^{(n)} Y R_n^{(n)} + \Gamma^{(n)} \end{bmatrix}, \\
 A_{12} &= \begin{bmatrix} z_1 \Lambda^{(1)} & \dots & z_1 \Lambda^{(1)} \\ \vdots & \ddots & \vdots \\ z_n \Lambda^{(n)} & \dots & z_n \Lambda^{(n)} \end{bmatrix}, \\
 A_{21} &= \begin{bmatrix} z_{n+1} \Lambda^{(n+1)} & \dots & z_{n+1} \Lambda^{(n+1)} \\ \vdots & \ddots & \vdots \\ z_{2n} \Lambda^{(2n)} & \dots & z_{2n} \Lambda^{(2n)} \end{bmatrix}, \\
 A_{22} &= \begin{bmatrix} z_{n+1} \Lambda^{(n+1)} + \Lambda^{(n+1)} Y R_n + \Gamma^{(n+1)} & \dots & z_{n+1} \Lambda^{(n+1)} \\ \vdots & \ddots & \vdots \\ z_{2n} \Lambda^{(2n)} & \dots & z_{2n} \Lambda^{(2n)} + \Lambda^{(2n)} Y R_n + \Gamma^{(n+1)} \end{bmatrix}.
 \end{aligned}$$

Let λ be an eigenvalue of J , with a corresponding eigenvector

$$V = \begin{bmatrix} V^{(1)} \\ V^{(2)} \\ \vdots \\ V^{(2n)} \end{bmatrix} \in \mathbb{C}^{2n^2}, \quad \text{where } V^{(k)} = \begin{bmatrix} v_1^{(k)} \\ v_2^{(k)} \\ \vdots \\ v_n^{(k)} \end{bmatrix} \in \mathbb{C}^n, k \in \{1, 2, \dots, 2n\}.$$

We will show that $v_l^{(k)} = 0$ for all l, k . By definition of eigenvalues and using the block

structure of J we get

$$\begin{bmatrix} z_1 \Lambda^{(1)} \sum_{j=1}^{2n} V^{(j)} + \Lambda^{(1)} Y R_n^{(1)} V^{(1)} + \Gamma^{(1)} V^{(1)} \\ \vdots \\ z_n \Lambda^{(n)} \sum_{j=1}^{2n} V^{(j)} + \Lambda^{(n)} Y R_n^{(n)} V^{(n)} + \Gamma^{(n)} V^{(n)} \\ z_{n+1} \Lambda^{(n+1)} \sum_{j=1}^{2n} V^{(j)} + \Lambda^{(n+1)} Y R_n V^{(n+1)} + \Gamma^{(n+1)} V^{(n+1)} \\ \vdots \\ z_{2n} \Lambda^{(2n)} \sum_{j=1}^{2n} V^{(j)} + \Lambda^{(2n)} Y R_n V^{(2n)} + \Gamma^{(2n)} V^{(2n)} \end{bmatrix} = \begin{bmatrix} \lambda V^{(1)} \\ \vdots \\ \lambda V^{(n)} \\ \lambda V^{(n+1)} \\ \vdots \\ \lambda V^{(2n)} \end{bmatrix}.$$

Looking at the above equation row by row we get

$$z_k \Lambda^{(k)} \sum_{j=1}^{2n} V^{(j)} + \Lambda^{(k)} Y R_n^{(k)} V^{(k)} + \Gamma^{(k)} V^{(k)} = \lambda V^{(k)}, \quad k \in \{1, 2, \dots, n\} \quad (4.10a)$$

$$z_k \Lambda^{(k)} \sum_{j=1}^{2n} V^{(j)} + \Lambda^{(k)} Y R_n V^{(k)} + \Gamma^{(k)} V^{(k)} = \lambda V^{(k)}, \quad k \in \{n+1, \dots, 2n\} \quad (4.10b)$$

Note that Eq. (4.10) is still in matrix multiplication form. Writing it further in terms of each of its rows, for each $k \in \{1, 2, \dots, 2n\}$ and $l \in \{1, 2, \dots, n\}$, we have (For notational simplicity let $(\Lambda^{(k)})^{-1} := \Psi^{(k)}$)

$$\frac{1}{y_l} \sum_{j=1}^{2n} v_l^{(j)} + \frac{1}{z_k} \sum_{\substack{h=1 \\ h \neq l}}^n v_h^{(k)} = \frac{\psi_l^{(k)}}{y_l z_k} (\lambda - \gamma_l^{(k)}) v_l^{(k)}, \quad k \in \{1, \dots, n\}, \quad (4.11a)$$

$$\frac{1}{y_l} \sum_{j=1}^{2n} v_l^{(j)} + \frac{1}{z_k} \sum_{h=1}^n v_h^{(k)} = \frac{\psi_l^{(k)}}{y_l z_k} (\lambda - \gamma_l^{(k)}) v_l^{(k)}, \quad k \in \{n+1, \dots, 2n\}. \quad (4.11b)$$

Now, Lemma 4.1.3 applied to Eq. (4.11) immediately yields that $v_l^{(k)} = 0$ for all l, k . This implies that the real part of λ cannot be nonpositive. This completes the proof of stability of J . $\square$

4.1.1 Stability of slow manifold in the absence of some connections

Lemma 4.1.1 show that the slow manifold defined by Eq. (3.19) is normally hyperbolic and stable when all entries in the connectivity matrices are positive. We next show how to extend the result to the case when some reactions are absent.

Recall the definitions of the unweighted connectivity matrices, I_K, I_L , and the associated matrices $C_U^{I_K}$ and $C_E^{I_L}$ given in Eqs. (3.29) and (3.30). Let I_{L^t} be a $n \times n$ matrix with ones at the places where L_1^t, L_2^t, L_{-1}^t are non zero and zero where L_1^t, L_2^t, L_{-1}^t are zeros. Now, recall the definition of C in Section 4.1 and define

$$C_0 = \begin{bmatrix} I_U & I_{L^t} \end{bmatrix} * C$$

Then, in the sense that we only need to differentiate along the coordinates corresponding to positive connections, one can formally write

$$I_0 := \frac{\partial \text{vec}(C_0)}{\partial \text{vec}(C_0)} = \begin{bmatrix} \widehat{I_K} & 0 \\ 0 & \widehat{I_{L^t}} \end{bmatrix}. \quad (4.12)$$

Replacing C with C_0 in the definition of F and repeating the whole process of finding the Jacobian of F , now with respect to C_0 , and using Eq. (4.12) we obtain the new Jacobian

$$J_0 := \frac{\partial \text{vec}(F(C_0))}{\partial \text{vec}(C_0)} = I_0 J I_0,$$

where the matrix J is the Jacobian matrix given in Eq. (4.4). If the connectivity matrices have zero entries, then I_0 will have zero entries in the diagonal. Therefore, some eigenvalues of J_0 will be zero. But, this does not affect the stability of slow manifold because we only need to look for the stability along the directions of intermediate complexes that occur in the reactions. That is, we only need to look at the principal submatrix of J_0 corresponding to the positive entries in the diagonal of I_0 . Let this principal submatrix be J_0^+ . But, since

$I_0 J_0 I_0 = I_0 J I_0$, we see that J_0^+ is also a principal submatrix of J . And J_0^+ is independent of zero entries in the connectivity matrices. Since Lemma 4.1.1 implies that, when all the entries in connectivity matrices are positive, J has eigenvalues with only negative real parts, we get that J_0^+ will have eigenvalues with only negative real parts. We conclude that the results hold even if some entries in the connectivity matrices are zero.

4.2 Validity of the tQSSA in the general setup

In this section we will describe the scaling we use to cast the original equations in to a form where GSPT can be applied. First we will revisit the isolated MM reaction case and describe the scaling in that. Then we will discuss how this scaling extends to the network we are studying.

4.2.1 Another approach for validity of tQSSA in isolated MM reaction

It should be noted that we have a certain degree of freedom in scaling of variables. We found that an informal method called *pairwise balance*, developed in [17, 59] can be extended to the network we have in mind. Let us first describe this method for simplest case of isolated MM reaction.

We define a new set of dimensionless variables as

$$\tau = \frac{t}{T_{\bar{X}}}, \quad \bar{x}(\tau) = \frac{\bar{X}(t)}{X_T}, \quad c(\tau) = \frac{C(t)}{\beta}. \quad (4.13)$$

Eq. (2.4) has the following form in the new variables

$$\frac{d\bar{x}}{d\tau} = -T_{\bar{X}} \frac{k_2 \beta}{X_T} c, \quad (4.14a)$$

$$\frac{\beta}{k_1 T_{\bar{X}} X_T E_T} \frac{dc}{d\tau} = \bar{x} - \frac{X_T \bar{x} + E_T + k_m}{X_T E_T} \beta c + \frac{\beta^2}{X_T E_T} c^2. \quad (4.14b)$$

We have some freedom in defining β and $T_{\bar{X}}$, and we follow [17, 59] in obtaining a particular choice. We choose β and $T_{\bar{X}}$ so that the coefficients of the right hand side of Eq. (4.14a-4.14b) are of order one in magnitude. Since $\bar{x}$ is order one, the coefficient of c in the right hand side of Eq. (4.14b) is of order $\frac{X_T + E_T + k_m}{X_T E_T} \beta$. Also, because all the variables being scaled in such a way so that they are restricted to order one region, the linear terms will dominate. It follows that a proper choice of β is

$$\beta = \frac{X_T E_T}{X_T + E_T + k_m}. \quad (4.15a)$$

Once we know the value for β we can prescribe the value for $T_{\bar{X}}$ by requiring that the coefficient of c in the right hand side of (4.14a) is of order one. This implies that

$$T_{\bar{X}} = \frac{X_T}{k_2 \beta}. \quad (4.15b)$$

The definition of $T_{\bar{X}}$ implies that $T_{\bar{X}} \frac{k_2 \beta}{X_T} = 1$. Also, the way β was defined implies that all the coefficients on the right hand side of (4.14b) are bounded above by 1.

Hence, if the parameters are chosen such that the right hand side of (4.14) is always of order one magnitude then tQSSA should hold in the limit

$$\epsilon := \frac{\beta}{k_1 T_{\bar{X}} X_T E_T} = \frac{k_2}{k_1} \frac{E_T}{(E_T + X_T + k_m)^2} \rightarrow 0$$

A simple lemma below shows that an elegant way to control the ϵ can be to make the ratio of E_T and Van Slyke-Cullen constant [67], k_2/k_1 too large or too small. That is $\epsilon \rightarrow 0$ in either of the following two limit

$$\frac{E_T}{k_2/k_1} \rightarrow 0, \quad \text{or} \quad \frac{E_T}{k_2/k_1} \rightarrow \infty.$$

Lemma 4.2.1 (*Bound on ϵ*): *If $k_1, k_2, k_{-1}, e, x \in \mathbb{R}_+$, then*

$$\epsilon := \frac{k_2}{k_1} \frac{e}{(e + x + \frac{k_{-1} + k_2}{k_1})^2} \leq \frac{k_1 e k_2}{(k_1 e + k_2)^2} \leq \frac{1}{4}.$$

Proof: Since k_1, k_2, k_{-1}, e, x are all positive,

$$\frac{k_2}{k_1} \frac{e}{(e + x + \frac{k_{-1} + k_2}{k_1})^2} \leq \frac{k_2}{k_1} \frac{e}{(e + \frac{k_2}{k_1})^2} = \frac{k_1 e k_2}{(k_1 e + k_2)^2}.$$

Since for any positive number s , $s + 1/s \geq 2$, we obtain

$$\frac{k_1 e k_2}{(k_1 e + k_2)^2} \leq \frac{1}{\left(\sqrt{\frac{k_1 e}{k_2}} + \sqrt{\frac{k_2}{k_1 e}}\right)^2} \leq \frac{1}{4}.$$

□

This bound is sharp because for $k_1 = 1$, $k_2 = 1$, $k_{-1} \rightarrow 0$, $e = 1$, $x \rightarrow 0$ we obtain $\epsilon \rightarrow 1/4$.

Note that the above expression for ϵ is same as the one obtained by Borghans, et al. in [6] (see section 2.2.1). But the pairwise balance method we have used does not suffer from self consistency and we will see that this method can be extended from isolated MM reaction to the network setup we have in hand.

4.2.2 Validity of the tQSSA for two interacting proteins

In this section we will see how the change of variables discussed in the previous section extends to the two protein example we discussed in Section 3.1. Recall that using Eq. (3.3)

in Eq. (3.1) yields

$$\begin{aligned}
 \frac{d\bar{X}_p}{dt} &= k_2 C_x - k_2 C_x^e, \\
 \frac{d\bar{Y}_p}{dt} &= k_2 C_y - k_2 C_y^e, \\
 \frac{dC_x}{dt} &= k_1 (X_T - \bar{X}_p - C_x)(\bar{Y}_p - C_x - C_y^e) - (k_{-1} + k_2) C_x, \\
 \frac{dC_y}{dt} &= k_1 (Y_T - \bar{Y}_p - C_y)(\bar{X}_p - C_y - C_x^e) - (k_{-1} + k_2) C_y, \\
 \frac{dC_x^e}{dt} &= k_1 (\bar{X}_p - C_y - C_x^e)(E_1^T - C_x^e) - (k_{-1} + k_2) C_x^e, \\
 \frac{dC_y^e}{dt} &= k_1 (\bar{Y}_p - C_x - C_y^e)(E_2^T - C_y^e) - (k_{-1} + k_2) C_y^e.
 \end{aligned} \tag{4.16}$$

And recall that the assumption that C_x, C_y, C_x^e, C_y^e , equilibrate quickly in above system is what we call the tQSSA in this example. To reveal the asymptotic limits for which the tQSSA holds, we again rescale the Eq. (4.16). In particular, $\bar{X}_p$ and $\bar{Y}_p$ are scaled by the total concentration of the respective proteins. To scale the intermediate complexes, each MM reaction in this network is treated as isolated. The scaling factors are then obtained analogously to β in Eq. (4.15a). Let

$$\begin{aligned}
 \alpha_x &:= \frac{X_T Y_T}{X_T + Y_T + k_m}, & \alpha_y &:= \frac{X_T Y_T}{X_T + Y_T + k_m}, \\
 \beta_x^e &:= \frac{X_T E_1^T}{X_T + E_1^T + k_m}, & \beta_y^e &:= \frac{Y_T E_2^T}{Y_T + E_2^T + k_m},
 \end{aligned}$$

and

$$T_s := \max \left\{ \frac{X_T}{k_2 \alpha_x}, \frac{X_T}{k_2 \beta_x^e}, \frac{Y_T}{k_2 \alpha_y}, \frac{Y_T}{k_2 \beta_y^e} \right\}.$$

Therefore, T_s is obtained analogously to $T_{\bar{X}}$ in Eq. (4.15b). The reason for choosing the maximum will become evident shortly. The rescaled variables are now defined as

$$\begin{aligned}
 \tau &:= \frac{t}{T_s}, & \bar{x}_p(\tau) &:= \frac{\bar{X}_p(t)}{X_T}, & \bar{y}_p(\tau) &:= \frac{\bar{Y}_p(t)}{Y_T}, \\
 c_x(\tau) &:= \frac{C_x(t)}{\alpha_x}, & c_y(\tau) &:= \frac{C_y(t)}{\alpha_y}, & c_x^e(\tau) &:= \frac{C_x^e(t)}{\beta_x^e}, & c_y^e(\tau) &:= \frac{C_y^e(t)}{\beta_y^e}.
 \end{aligned} \tag{4.17}$$

4.2. VALIDITY OF THE TQSSA IN THE GENERAL SETUP

Using Eq. (4.17) in Eq. (4.16) we obtain

$$\frac{d\bar{x}_p}{d\tau} = \frac{k_2\alpha_x T_s}{X_T} c_x - \frac{k_2\beta_x^e T_s}{X_T} c_x^e, \quad (4.18a)$$

$$\frac{d\bar{y}_p}{d\tau} = \frac{k_2\alpha_y T_s}{Y_T} c_y - \frac{k_2\beta_y^e T_s}{Y_T} c_y^e, \quad (4.18b)$$

$$\underbrace{\frac{\alpha_x}{k_1 X_T Y_T T_s}}_{\leq \epsilon_x} \frac{dc_x}{d\tau} = \left[\bar{y}_p - \bar{x}_p \bar{y}_p - \frac{\alpha_x}{X_T} c_x \bar{y}_p - \frac{\alpha_x}{Y_T} c_x - \frac{\beta_y^e}{Y_T} c_y^e + \frac{\alpha_x}{Y_T} c_x \bar{x}_p + \frac{\beta_y^e}{Y_T} c_y^e \bar{x}_p + \frac{\alpha_x^2}{X_T Y_T} c_x^2 + \frac{\alpha_x \beta_y^e}{X_T Y_T} c_x c_y^e - \frac{\alpha_x k_m}{X_T Y_T} c_x \right], \quad (4.18c)$$

$$\underbrace{\frac{\alpha_y}{k_1 X_T Y_T T_s}}_{\leq \epsilon_y} \frac{dc_y}{d\tau} = \left[\bar{x}_p - \frac{\beta_x^e}{X_T} c_x^e - \frac{\alpha_y}{X_T} c_y - \bar{x}_p \bar{y}_p + \frac{\beta_x^e}{X_T} c_x^e \bar{y}_p + \frac{\alpha_y}{X_T} c_y \bar{y}_p - \frac{\alpha_y}{Y_T} c_y \bar{x}_p + \frac{\alpha_y \beta_x^e}{X_T Y_T} c_x^e c_y + \frac{\alpha_y^2}{X_T Y_T} c_y^2 - \frac{\alpha_y k_m}{X_T Y_T} c_y \right], \quad (4.18d)$$

$$\underbrace{\frac{\beta_x^e}{k_1 X_T E_1^T T_s}}_{\leq \epsilon_x^e} \frac{dc_x^e}{d\tau} = \bar{x}_p - \frac{\beta_x^e}{E_1^T} c_x^e \bar{x}_p - \frac{\beta_x^e}{X_T} c_x^e - \frac{\alpha_y}{X_T} c_y + \frac{(\beta_x^e)^2}{E_1^T X_T} (c_x^e)^2 + \frac{\alpha_y \beta_x^e}{E_1^T X_T} c_x^e c_y - \frac{\beta_x^e k_m}{E_1^T X_T} c_x^e, \quad (4.18e)$$

$$\underbrace{\frac{\beta_y^e}{k_1 E_2^T Y_T T_s}}_{\leq \epsilon_y^e} \frac{dc_y^e}{d\tau} = \bar{y}_p - \frac{\beta_y^e}{E_2^T} c_y^e \bar{y}_p - \frac{\alpha_x}{Y_T} c_x - \frac{\beta_y^e}{Y_T} c_y^e + \frac{\alpha_x \beta_y^e}{E_2^T Y_T} c_x c_y^e + \frac{(\beta_y^e)^2}{E_2^T Y_T} (c_y^e)^2 - \frac{\beta_y^e k_m}{E_2^T Y_T} c_y^e, \quad (4.18f)$$

where

$$\begin{aligned} \epsilon_x &:= \frac{k_2}{k_1} \frac{Y_T}{(X_T + Y_T + k_m)^2}, & \epsilon_y &:= \frac{k_2}{k_1} \frac{X_T}{(Y_T + X_T + k_m)^2}, \\ \epsilon_x^e &:= \frac{k_2}{k_1} \frac{E_1^T}{(X_T + E_1^T + k_m)^2}, & \epsilon_y^e &:= \frac{k_2}{k_1} \frac{E_2^T}{(Y_T + E_2^T + k_m)^2}. \end{aligned}$$

The bounds on these coefficients follow from the definition of T_s . Since $(1/T_s) \leq (k_2\alpha_x/X_T)$,

$$\frac{\alpha_x}{k_1 X_T Y_T T_s} \leq \frac{k_2}{k_1} \frac{\alpha_x^2}{X_T^2 Y_T} = \frac{k_2}{k_1} \frac{1}{X_T^2 Y_T} \left(\frac{X_T Y_T}{X_T + Y_T + k_m} \right)^2 = \epsilon_x.$$

Similarly,

$$\frac{\alpha_y}{k_1 X_T Y_T T_s} \leq \epsilon_y, \quad \frac{\beta_x^e}{k_1 X_T E_1^T T_s} \leq \epsilon_x^e, \quad \text{and} \quad \frac{\beta_x^e}{k_1 E_2^T Y_T T_s} \leq \epsilon_y^e.$$

Finally, we define

$$\epsilon := \max \{ \epsilon_x, \epsilon_y, \epsilon_x^e, \epsilon_y^e \}. \quad (4.19)$$

The definitions of scaling factors in (4.17) imply that all the coefficients on the right hand side of (4.18c–4.18f) are $\mathcal{O}(1)$. Therefore, in the asymptotic limit $\epsilon \rightarrow 0$, Eq. (4.18) defines a singularly perturbed system. Since the two equations are related by the scaling given in Eq. (4.17), we can conclude that in the limit $\epsilon \rightarrow 0$, the tQSSA is valid.

We have already proved in Section 4.1 that the slow manifold arising from these networks are in general normally hyperbolic and stable. Since the two protein of this subsection is a particular class of the general network, the stability and normal hyperbolicity of its slow manifold follows from the general discussion in Section 4.1.

4.2.3 Extension of scaling from two dimension to several dimension

We next investigate the asymptotic limits under which the tQSSA is valid in the general setting. We follow the approach given in the previous sections to obtain a suitable rescaling of the variables. While this rescaling does not change the stability of the slow manifold, $\mathcal{M}_0$, it allows us to more easily describe the asymptotic limits in which the timescales are separated, and the system is singularly perturbed.

Recall that Eq. (3.16) and Eq. (3.14) are equivalent. The concise form given in Eq. (3.16) was useful in obtaining a reduction and checking the stability of the slow manifold. However, to obtain sufficient conditions for the validity of the tQSSA, we will work with Eqs. (3.14) and (3.15).

Let $l_{ij}^m := (l_{ij}^{-1} + l_{ij}^2)/l_{ij}^1$, $k_{ij}^m := (k_{ij}^{-1} + k_{ij}^2)/k_{ij}^1$ denote the MM constants. Then the following scaling factors are natural generalizations of those introduced in Section 4.2.2,

$$\beta_{ij} := \frac{E_i^T U_j^T}{E_i^T + U_j^T + l_{ij}^m}, \quad \alpha_{ij} := \frac{U_i^T U_j^T}{U_i^T + U_j^T + k_{ij}^m}, \quad i, j \in \{1, 2, \dots, n\}.$$

Note that for each pair (i, j) either all of $k_{ij}^1, k_{ij}^{-1}, k_{ij}^2$ are all zero or all nonzero. In the case that $k_{ij}^1 = k_{ij}^{-1} = k_{ij}^2 = 0$ we define $k_{ij}^m := 0$. Similarly, if $l_{ij}^1 = l_{ij}^{-1} = l_{ij}^2 = 0$ then $l_{ij}^m := 0$.

Let

$$T_{\bar{U}} := \max \left\{ \max_{i,j} \left\{ \frac{U_j^T}{l_{ij}^2 \beta_{ij}} \right\}, \max_{i,j} \left\{ \frac{U_j^T}{k_{ij}^2 \alpha_{ij}} \right\} \right\} = \frac{U_{j_0}^T}{l_{i_0 j_0}^2 \beta_{i_0 j_0}}, \quad \text{for some } i_0, j_0 \in \{1, 2, \dots, n\}.$$

We next define the following dimensionless rescaling of the variables in Eq. (3.14)

$$\tau = \frac{t}{T_{\bar{U}}}, \quad \text{and} \quad \bar{p}_i(\tau) = \frac{\bar{P}_i(t)}{U_i^T}, \quad c_{ij}^u(\tau) = \frac{C_{ij}^U(t)}{\alpha_{ij}}, \quad c_{ij}^e(\tau) = \frac{C_{ij}^E(t)}{\beta_{ij}}, \quad i, j \in \{1, 2, \dots, n\}. \quad (4.20)$$

After rescaling, Eqs. (3.14) take the form

$$\frac{d\bar{p}_i}{d\tau} = \sum_{r=1}^n \left(\frac{k_{ri}^2 \alpha_{ri} U_{j_0}^T}{l_{i_0 j_0}^2 \beta_{i_0 j_0} U_i^T} c_{rj}^u - \frac{l_{ri}^2 \beta_{ri} U_{j_0}^T}{l_{i_0 j_0}^2 \beta_{i_0 j_0} U_i^T} c_{rj}^e \right), \quad (4.21a)$$

$$\begin{aligned} \left(\frac{\beta_{ij}}{l_{ij}^1 E_i^T U_j^T T_{\bar{U}}} \right) \frac{dc_{ij}^e}{d\tau} &= 1 - c_{ij}^e \\ &- \left[\sum_{\substack{s=1 \\ s \neq j}}^n \frac{\beta_{is}}{E_i^T} c_{is}^e \right] \left[1 - \bar{x}_j - \frac{1}{U_j^T} \sum_{\substack{r=1 \\ r \neq i}}^n \left(\beta_{rj} c_{rj}^e + \sum_{\substack{s=1 \\ s \neq j}}^n \alpha_{js} c_{js}^u \right) \right] \\ &- \frac{1}{U_j^T} \left[U_j^T \bar{x}_j + \sum_{\substack{r=1 \\ r \neq i}}^n \beta_{rj} c_{rj}^e + \sum_{\substack{s=1 \\ s \neq j}}^n \alpha_{js} c_{js}^u \right] \\ &- \frac{1}{U_j^T} \left[U_j^T \bar{x}_j + \sum_{\substack{r=1 \\ r \neq i}}^n \beta_{rj} c_{rj}^e + \sum_{\substack{s=1 \\ s \neq j}}^n \alpha_{js} c_{js}^u + \sum_{\substack{s=1 \\ s \neq i}}^n \beta_{is} c_{is}^e \right] \frac{\beta_{ij} c_{ij}^e}{E_i^T} \\ &+ \frac{(\beta_{ij} c_{ij}^e)^2}{E_i^T U_j^T}. \end{aligned} \quad (4.21c)$$

The rescaled form of Eq. (3.14b) is similar to the rescaled form of Eq. (3.14c), and we therefore omit it. If we define

$$\epsilon_{ij} := \frac{k_{ij}^2}{k_{ij}^1} \frac{U_i^T}{(U_i^T + U_j^T + k_{ij}^m)^2}, \quad \epsilon_{ij}^e := \frac{l_{ij}^2}{l_{ij}^1} \frac{E_i^T}{(E_i^T + U_j^T + l_{ij}^m)^2},$$

and let

$$\epsilon := \max \left\{ \max_{i,j} \{ \epsilon_{ij} \}, \max_{i,j} \{ \epsilon_{ij}^e \} \right\}, \quad (4.22)$$

then the following theorem defines the conditions under which Eq. (4.21) defines a singularly perturbed system and, hence, conditions under which GSPT is applicable.

Theorem 4.2.2 *If for all non-zero $k_{ij}^1, k_{ij}^2, k_{ij}^{-1}$ and for all non zero $l_{ij}^1, l_{ij}^2, l_{ij}^{-1}$ and for all U_i^T, E_i^T*

$$\begin{aligned} \mathcal{O} \left(\frac{k_{ij}^1}{k_{rs}^1} \right) &= \mathcal{O} \left(\frac{l_{ij}^1}{k_{rs}^1} \right) = \mathcal{O} \left(\frac{l_{ij}^1}{l_{rs}^1} \right) = \mathcal{O}(1), \\ \mathcal{O} \left(\frac{k_{ij}^2}{k_{rs}^2} \right) &= \mathcal{O} \left(\frac{l_{ij}^2}{k_{rs}^2} \right) = \mathcal{O} \left(\frac{l_{ij}^2}{l_{rs}^2} \right) = \mathcal{O}(1), \\ \mathcal{O} \left(\frac{k_{ij}^{-1}}{k_{rs}^{-1}} \right) &= \mathcal{O} \left(\frac{l_{ij}^{-1}}{k_{rs}^{-1}} \right) = \mathcal{O} \left(\frac{l_{ij}^{-1}}{l_{rs}^{-1}} \right) = \mathcal{O}(1), \\ \mathcal{O} \left(\frac{X_i^T}{X_j^T} \right) &= \mathcal{O} \left(\frac{X_i^T}{E_j^T} \right) = \mathcal{O} \left(\frac{E_i^T}{E_j^T} \right) = \mathcal{O}(1), \end{aligned} \quad 1 \leq i, j, r, s \leq n,$$

in the limit $\epsilon \rightarrow 0$, then Eq. (4.21) is a singularly perturbed system with the structure of Eq. (1.1). In particular, the $\bar{p}_i$ are the slow variables, and the c_{ij} and c_{ij}^e are the fast variables.

Proof: For each i there always exist indices r, s such that $k_{ri}^2 \neq 0 \neq k_{si}^2$. Hence, the the right hand side of Eq. (4.21a) is not identically zero for any $i \in \{1, 2, \dots, n\}$. Furthermore, by assumption all coefficients on the right hand side of Eq. (4.21a) are $\mathcal{O}(1)$ as $\epsilon \rightarrow 0$. This implies that ϵ times the right hand side of Eq. (4.21a) is identically zero, in the limit $\epsilon \rightarrow 0$.

Secondly, the definition of β_{ij} implies that all coefficients on the right hand side of Eq. (4.21c) are less than or equal to 1. Also, by definition, at least one coefficient has value

exactly equal to 1. Hence, the right hand side of Eq. (4.21c) is not identically zero in the limit $\epsilon \rightarrow 0$.

The definitions of $\epsilon, \alpha_{ij}, \beta_{ij}, T_{\bar{U}}$ imply that coefficients of $\frac{dc_{ij}^e}{d\tau}$ in Eq. (4.21c) are less than or equal to ϵ . For example

$$\frac{\beta_{ij}}{l_{ij}^1 E_i^T U_j^T} \frac{1}{T_{\bar{U}}} \leq \frac{\beta_{ij}}{l_{ij}^1 E_i^T U_j^T} \frac{l_{ij}^2 \beta_{ij}}{U_j^T} = \epsilon_{ij}^e \leq \epsilon.$$

Hence, in the limit $\epsilon \rightarrow 0$, the left hand side of Eq. (4.21c) vanishes while the right hand side does not. To conclude the proof we only need to show the stability of the slow manifold in rescaled coordinates. But we have already shown that for unscaled coordinates in Section 4.1 and a non-singular scaling of variable, as in Eq. (4.20), will not affect the eigenvalues of the Jacobian. $\square$ Hence, under the assumptions of the above theorem,

Eq. (4.21) has the form of Eq. 1.1. Hence, switching back to unscaled variables we conclude that in the limit $\epsilon \rightarrow 0$, tQSSA is valid, *i.e.* the reduction from Eq. (3.16) to Eq. (3.18) is valid.

4.2.3.1 The assumption of zero initial concentrations of intermediate complexes and the choice of scaling

Before concluding, we discuss the significance of zero initial concentrations of intermediate complexes and the benefit of the choice of scaling we used to verify the asymptotic limits in which the system is singularly perturbed. Proposition 4.2.3 below proves that if the reaction starts with zero initial concentration of intermediate complexes then the solution of both Eqs. (3.16) and (4.21) are trapped in an $\mathcal{O}(1)$ neighborhood of the origin. Hence, separation of time scale in Eq.(4.21), implied by Theorem 4.2.2 can be used to obtain the reduction of Eq. (3.16) given by Eq. (3.18). This is important, since GSPT would not be

applicable if the rescaling were to send $\mathcal{O}(1)$ solutions of Eq. (3.16) to solutions of Eq. (4.21) that are unbounded as $\epsilon \rightarrow 0$.

Proposition 4.2.3 *The $(2n^2 + n)$ -dimensional hypercube Ω defined by*

$$\Omega := \left\{ \{\bar{p}_i\}, \{c_{ij}^u\}, \{c_{ij}^e\} \mid 0 \leq \bar{p}_i \leq 1, 0 \leq c_{ij}^u \leq 2, 0 \leq c_{ij}^e \leq 2, \forall i, j \in \{1, 2, \dots, n\} \right\},$$

is invariant under the flow of Eq. (4.21).

Proof: By the construction of the differential equations from the Law of Mass Action, all the species concentration variables can take only non-negative values. This together with the conservation constraints (3.11b) force the $\bar{P}_i$ to take values between 0 and U_i^T . Therefore $0 \leq \bar{p}_i(\tau) \leq 1, \forall \tau > 0$, provided the initial conditions are chosen in Ω .

Positivity of variables also implies that $c_{ij}^u(\tau) \geq 0, c_{ij}^e(\tau) \geq 0$ if the flow starts inside Ω . So we only need to show that $c_{ij}^u(\tau) \leq 2$ and $c_{ij}^e(\tau) \leq 2$. It is sufficient to show that $\left. \frac{dc_{ij}^u}{d\tau} \right|_{c_{ij}^u=2} \leq 0$, and $\left. \frac{dc_{ij}^e}{d\tau} \right|_{c_{ij}^e=2} \leq 0$, or equivalently that $\left. \frac{dC_{ij}^U}{dt} \right|_{C_{ij}^U=2\alpha_{ij}} \leq 0$, and $\left. \frac{dC_{ij}^E}{dt} \right|_{C_{ij}^E=2\beta_{ij}} \leq 0$. But

$$\begin{aligned} \left. \frac{dC_{ij}^U}{dt} \right|_{C_{ij}^U=2\alpha_{ij}} &= k_{ij}^1 \left[P_i U_j - (k_{ij}^{-1} + k_{ij}^2) C_{ij}^U \right] \Big|_{C_{ij}^U=2\alpha_{ij}} \\ &= k_{ij}^1 \left[\left(\bar{P}_i - \sum_{s=1}^n C_{is}^U - \sum_{r=1}^n C_{ri}^E \right) \left(U_i^T - \bar{P}_i - \sum_{r=1}^n C_{ri}^U \right) \right. \\ &\quad \left. - (k_{ij}^{-1} + k_{ij}^2) C_{ij}^U \right] \Big|_{C_{ij}^U=2\alpha_{ij}} \\ &\leq k_{ij}^1 (P_i^T - 2\alpha_{ij}) (U_j - 2\alpha_{ij}) - (k_{ij}^{-1} + k_{ij}^2) 2\alpha_{ij} \\ &= k_{ij}^1 [(P_i^T - 2\alpha_{ij}) (U_j^T - 2\alpha_{ij}) - k_{ij}^m 2\alpha_{ij}] \\ &\leq 0. \end{aligned}$$

Similarly we can show that C_{ij}^E is decreasing when $C_{ij}^E = \beta_{ij}$. This concludes the proof.

□

From this we conclude that the assumptions of Theorem 4.2.2 and the zero initial values of intermediate complexes together imply the tQSSA.

Finally, we combine the results of Section 4.1 with Theorem 4.2.2 and Proposition 4.2.3 to obtain the main result of this study.

Theorem 4.2.4 *If the parameters of Eq. (3.12) are such that assumptions of Theorem 4.2.2 are satisfied and the initial values of intermediate complexes are zero, then the tQSSA holds. For ϵ defined by Eq. (4.22), there exists an ϵ_0 such that for all $0 < \epsilon < \epsilon_0$, the solutions of Eq. (3.16) are $\mathcal{O}(\epsilon)$ close to the solutions of Eq. (3.18) after an exponentially fast transient. Eq. (3.12) can therefore be reduced to the n -dimensional Eq. (3.22) involving only the protein concentrations, P_i .*

Chapter 5

Linearization of Michaelis-Menten differential equations

In this chapter we show that a particular class of non-linear dynamical system, with nonlinearities represented by Hill-functions, can be approximated by a piecewise-linear dynamical system. This linear system is naturally determined from the structure of the original system. The approximation can again be explained on the basis of the GSPT. Successful application of this theory on two simple examples are also presented.

5.1 Introduction

Ordinary differential equations are commonly used to model interactions between enzymes, proteins, and genes. Such interactions are frequently described using sigmoidal functions. A particularly common choice is the Hill function, $f(x) = x^n/(x^n + J^n)$, and its variants [2]. The resulting systems of ODEs are generally not tractable analytically. This is particularly

important for large networks, where the potential complexity of the dynamics, and large number of parameters may make the system difficult to analyze using only numerical methods.

While the ODEs describing such networks are inherently non-linear, they can be treated analytically in certain limits. In particular, the approaches that have been proposed to analyze models of gene interaction networks can be broadly classified into three categories: *Quasi-Steady State Approximations* (QSSA), *Piecewise-Linear Approximations* (PLA) and *discretization of continuous time ODEs* [55].

In the previous chapters we have described how to extend the QSSA approximation to a network level [38]. Here we will be primarily concerned with PLAs. In particular, we show that in certain limits interactions between network elements become switch-like, and more tractable [2, 13].

We introduce a class of network models that are well-approximated by piecewise-linear differential equations in certain limits. Similar approaches have been used in different contexts. In a recent study piecewise-linear functions of the form developed in [22] have been shown to be well suited for the modeling of genetic regulatory networks [9]. More results regarding generic properties of this simplification can be found in [33, 54]. The basic idea behind such reductions is to approximate a Hill function by a Heaviside function. As the Hill coefficient, n , increases, the regions within which the Hill function changes value from close to 1 to close to 0 become thinner. These regions partition the domain in such a way that for each block a fixed set of coordinates are away from the thresholds of Hill functions and the remaining coordinates are near the thresholds. Then the Hill functions corresponding to the coordinates which are away from the thresholds are treated as known parameters, either 0 or 1. This leaves the Hill functions corresponding to the coordinates

which are near thresholds. But in this region it is reasonable to assume that the values of the Hill functions change very rapidly. With this assumption the Hill functions are then introduced as the new variables. This induces a differential equation with *fast* variables as Hill functions and the slow variables as the the coordinates which are away from the thresholds. Then one proceeds like one would in any standard QSSA like argument. In the limit of large Hill coefficient, the fast variables will be assumed to reach their steady states instantaneously. These steady states will be some function of the the coordinates which are away from the thresholds. Hence, for each block one gets a closed form differential equation only in terms of the coordinates which are away from thresholds.

Here we take a similar approach, but work in a different limit. We again start with the Hill function, $x^n/(x^n + J^n)$, but consider the limit of small J , rather than large n . The subsequent results hold for any fixed n . For the sake of simplicity we therefore assume $n = 1$. Thus the network interactions are modeled by functions of the form $x/(x + J)$ (activation) or $(1 - x)/(1 - x + J)$ (repression). Equations involving this special class of Hill functions are known as Michaelis-Menten equations, where J is the Michaelis-Menten constant [11, 13, 24, 43]. There are many examples of such models of which we only list a representative sample [13, 23, 40, 49, 50, 64] The results in this paper are obtained in the asymptotic limit of small J , $0 < J \ll 1$. Recently the asymptotic limit $J \rightarrow 0$ was considered in a reduction of a protein interaction network to a Boolean network [13]. However, we are here concerned with a rigorous justification underlying such reductions, as well as examples with dynamically rich behavior.

Remark: The theoretical justification of the use of Hill functions, like the Michaelis-Menten differential equations, is debatable. But the subject of this chapter is not the biological phenomena modeled by the differential equations, but the differential equations

themselves.

The main idea behind the reduction we propose is simple. For example, suppose we want to get rid of the non-linear term, say $f(x) = x/(x + J)$. When $x \gg J \approx 0$ then $f(x) \approx 1$ and when $x \approx 0$ then we use $x = 0$ to eliminate the non-linear terms. This induces a natural decomposition of the domain into a nested sequence of hypercubes such that for each level of nesting we get a separate linear equation. The examples in the next section will make this idea more clear.

We proceed as follows: In Section 5.2 we illustrate our approach in some simple examples and provide numerical evidence for the validity of our claim. In Section 5.3 we describe a general class of differential equations which subsumes these examples. Furthermore, in this section we justify our approach mathematically using GSPT. At the end of the chapter we discuss some limitations of these reductions.

5.2 Example problems

In this section we demonstrate the reduction methods heuristically with two simple examples. We provide a mathematical justification for the different steps in the subsequent section. First, consider two mutually repressing elements within a biological network. Such biological *toggle switches* have been discussed widely [13, 18, 64]. Let u_1 and u_2 represent the activity of each element, respectively. Suppose these variables can take values only in the interval $[0, 1]$, where $u_i = 1$ means the maximum activity of the i -th element and $u_i = 0$ means no activity of the same element. The mutually repressing activity of these

two elements can be modeled by the following system of differential equations

$$\begin{aligned}\frac{du_1}{dt} &= 0.5 \frac{1 - u_1}{J + 1 - u_1} - u_2 \frac{u_1}{J + u_1}, \\ \frac{du_2}{dt} &= 0.5 \frac{1 - u_2}{J + 1 - u_2} - u_1 \frac{u_2}{J + u_2},\end{aligned}\tag{5.1}$$

where J is some positive constant. From the very structure of Eq. (5.1) it is clear that its solution will remain in the cube $[0, 1]^2 = \{(u_1, u_2) \mid 0 \leq u_1, u_2 \leq 1\}$ (see Proposition 5.3.1).

We show that, in the limit of small J ($0 < J \ll 1$), this non-linear differential equation can be approximated by a piecewise-linear differential equation defined as follows: When J is small, and x is not too close to zero the expression $x/(J + x)$ is approximately unity. More precisely, we choose a small positive number $\delta > 0$, which will be dependent on J . This δ will measure the closeness of any coordinate to the boundary. When $x < \delta$ we replace $x/(J + x)$ by 1, and when $x > 1 - \delta$ then we replace $(1 - x)/(J + 1 - x)$ by 1.

With this convention in mind we will break the cube $[0, 1]^2$ into several subregions where one or more rational expressions from the right hand side can be eliminated. For example let

$$\mathcal{R}_0^0 := \{(u_1, u_2) \in [0, 1]^2 \mid \delta \leq u_1 \leq 1 - \delta \text{ and } \delta \leq u_2 \leq 1 - \delta\}.\tag{5.2}$$

Then, Eq. (5.1), restricted to $\mathcal{R}_0^0$ can be approximated by the linear differential equation.

$$\frac{du_1}{dt} = 0.5 - u_2, \quad \frac{du_2}{dt} = 0.5 - u_1.\tag{5.3}$$

If one of the coordinate is near the boundary, while the other is in the interior, the approximation is different. For instance, let

$$\mathcal{R}_1^0 := \{(u_1, u_2) \in [0, 1]^2 \mid u_1 < \delta \text{ and } \delta \leq u_2 \leq 1 - \delta\}.\tag{5.4}$$

In this case we are forced to keep the nonlinear term $u_1/(J + u_1)$, and the approximation

in this region has the form

$$\frac{du_1}{dt} = 0.5 - u_2 \frac{u_1}{J + u_1}, \quad (5.5a)$$

$$\frac{du_2}{dt} = 0.5 - u_1. \quad (5.5b)$$

This equation can be simplified further. Since u_1 is near the boundary, and the boundaries are invariant, it's derivative must be small. We therefore set $\frac{du_1}{dt} = 0$ in Eq. (5.5a) and $u_1 = 0$ in Eq. (5.5b) to obtain

$$0 = 0.5 - u_2 \frac{u_1}{J + u_1}, \quad (5.6a)$$

$$\frac{du_2}{dt} = 0.5. \quad (5.6b)$$

Note that Eq. (5.6b) is linear and decoupled from Eq. (5.6a), while Eq. (5.6a) is an algebraic system which can be solved for u_1 .

A similar reduction can be obtained in all other parts of the domain $[0, 1]^2$. In each subregion we obtain a linear equation for the variable(s) which is away from the boundary. Variable(s) near the boundary are assumed to be approximately in steady state, and that lead to algebraic equations which can be solved in terms of the interior variables.

In Table 5.1 we list all the subregion of $[0, 1]^2$ and the differential–algebraic system that approximates Eq. (5.1) within the respective subregion. Each approximate solution has the potential of exiting the region within which it is defined, and entering another region. The global approximate solution of Eq. (5.1) is obtained by concatenating entries from Table 5.1. Fig. 5.1 shows that the approximation can be very good when J is small and δ is chosen appropriately. In general the global solution will be discontinuous. The reason is that as soon as the solution enters a new region, the solution jumps to the manifold defined by the algebraic part of the linear differential algebraic system corresponding to the new region.

Region's name	u_1	u_2	Approximating linear system
$\mathcal{R}_0^0$	$\delta \leq u_1 \leq 1 - \delta$	$\delta \leq u_2 \leq 1 - \delta$	$\frac{du_1}{dt} = 0.5 - u_2,$ $\frac{du_2}{dt} = 0.5 - u_1$
$\mathcal{R}_0^1$	$u_1 > 1 - \delta$	$\delta \leq u_2 \leq 1 - \delta$	$0 = 0.5 \frac{1-u_1}{J+1-u_1} - u_2,$ $\frac{du_2}{dt} = -0.5$
$\mathcal{R}_0^2$	$\delta \leq u_1 \leq 1 - \delta$	$u_2 > 1 - \delta$	$\frac{du_1}{dt} = -0.5,$ $0 = 0.5 \frac{1-u_2}{J+1-u_2} - u_1$
$\mathcal{R}_1^0$	$u_1 < \delta$	$\delta \leq u_2 \leq 1 - \delta$	$0 = 0.5 - u_2 \frac{u_1}{J+u_1},$ $\frac{du_2}{dt} = 0.5$
$\mathcal{R}_2^0$	$\delta \leq u_1 \leq 1 - \delta$	$u_2 < \delta$	$\frac{du_1}{dt} = 0.5,$ $0 = 0.5 - u_1 \frac{u_2}{J+u_2}$
$\mathcal{R}_0^{12}$	$u_1 > 1 - \delta$	$u_2 > 1 - \delta$	$0 = 0.5 \frac{1-u_1}{J+1-u_1} - 1,$ $0 = 0.5 \frac{1-u_2}{J+1-u_2} - 1$
$\mathcal{R}_{12}^0$	$u_1 < \delta$	$u_2 < \delta$	$0 = 0.5 - J \frac{u_1}{J+u_1},$ $0 = 0.5 - J \frac{u_2}{J+u_2}$
$\mathcal{R}_2^1$	$u_1 > 1 - \delta$	$u_2 < \delta$	$0 = 0.5 \frac{1-u_1}{J+1-u_1},$ $0 = 0.5 - \frac{u_2}{J+u_2}$
$\mathcal{R}_1^2$	$u_1 < \delta$	$u_2 > 1 - \delta$	$0 = 0.5 - \frac{u_1}{J+u_1},$ $0 = 0.5 \frac{1-u_2}{J+1-u_2}$

Table 5.1: List of differential-algebraic systems that approximate Eq. (5.1) in different parts of the domain. The column gives the names of the regions. The superscript lists the coordinates which are near 1, with 0 denoting the empty set. The subscript lists coordinates which are near 0. For example, $\mathcal{R}_1^2$ denotes that subregion with $u_1 \approx 1$ and $u_2 \approx 0$, and $\mathcal{R}_0^2$ the subregion where u_2 is near 1, but u_1 is away from the boundary. The middle column define the subregion explicitly. The right column gives the approximating differential-algebraic system valid in that region

The same reduction can be applied to systems of arbitrary dimension. We next consider the *repressilator* [14, 64] which can be described by

$$\begin{aligned}
 \frac{du_1}{dt} &= 0.6 \frac{1-u_1}{J+1-u_1} - u_3 \frac{u_1}{J+u_1}, \\
 \frac{du_2}{dt} &= 0.4 \frac{1-u_2}{J+1-u_2} - u_1 \frac{u_2}{J+u_2}, \\
 \frac{du_3}{dt} &= 0.3 \frac{1-u_3}{J+1-u_3} - u_2 \frac{u_3}{J+u_3}.
 \end{aligned} \tag{5.7}$$

The cyclic repression of the three elements in this network can lead to oscillations. Indeed,

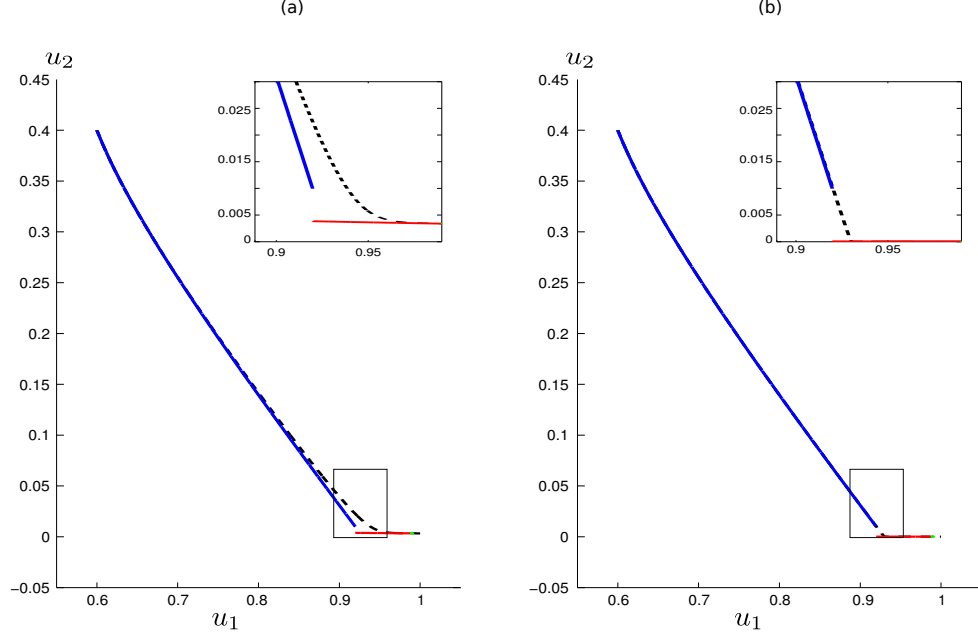


Figure 5.1: Comparison of the numerical solution of Eq. (5.1) (dashed black) and the solution of the approximate system as listed in Table 5.1 (solid colored) for two different values of J (We used $J = 10^{-2}$ in (a); and $J = 10^{-4}$ in (b).). The different colors denote the switching behavior of the solution from one region to next. We used $\delta = 0.01$. Solution of the linear approximation started in the region $\mathcal{R}_0^0$ (Initial value: $u_1 = 0.6, u_2 = 0.4$), and as soon as u_2 became smaller than δ , the region switched to $\mathcal{R}_2^0$ and driving linear differential equation also switched accordingly. It should be noted that the approximate solution is discontinuous. The reason is that as soon as the solution crossed the horizontal line, $u_2 = \delta$, the solution jumped (see inset) to the manifold, described by the algebraic part of the linear differential algebraic system prevalent in the region $\mathcal{R}_2^0$. The solution finally stopped in the region $\mathcal{R}_2^1$.

system (5.7) supports oscillatory solutions over a large range of values of J . The domain of this system, $[0, 1]^3$, can be divided into 27 subdomains: 1 interior, 6 faces, 12 edges, and 8 vertices, with an approximating equation within each subdomain. The global approximation is obtained by concatenating these approximations across the different regions. We demonstrate the validity of the approximation in Fig. 5.2. In particular, the numerically obtained solutions to the full system given by Eq. (5.7), and the approximate solution both exhibit oscillations. As mentioned before, solutions tend to be discontinuous at the intersection of two subdomains.

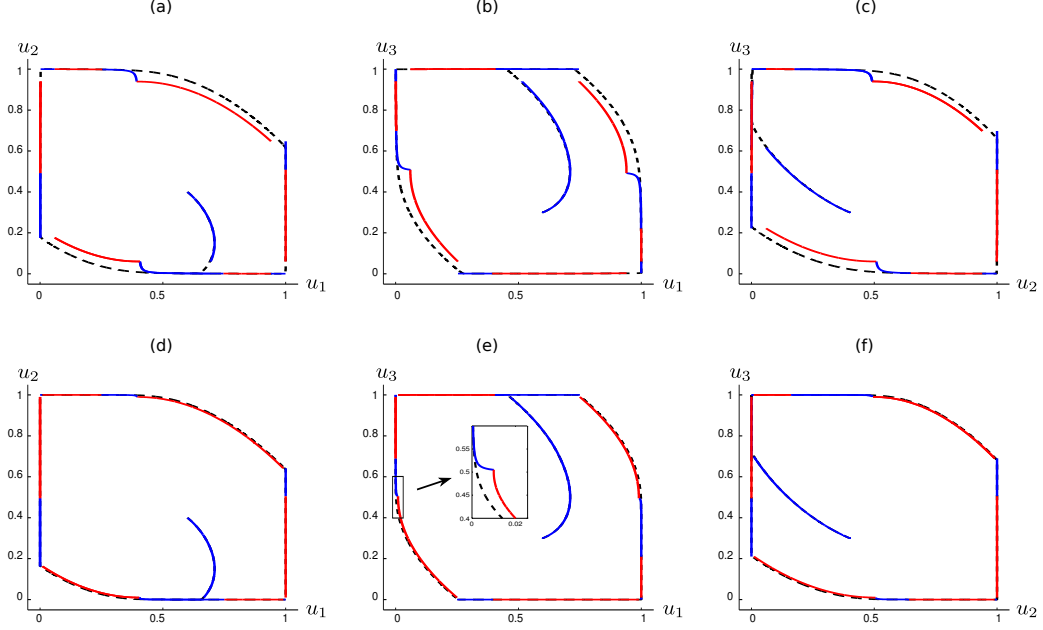


Figure 5.2: Comparison of the numerical solution of Eq. (5.7) (dashed black) and the solution of the approximate linear system (not explicitly provided) for two different sets of J and δ . For (a)-(c) $J = 10^{-2}, \delta = 0.06$; for (c)-(f) $J = 10^{-4}, \delta = 0.01$. The approximate solution changes color when switching between different subdomains. Note that the approximate solution is discontinuous in general. The reason is that as soon as the solution enters a new region, the solution jumps (see inset) to the manifold defined by the algebraic part of the linear differential algebraic system corresponding to the new region.

5.3 General setup

The approximations described in the previous section can be extended to more general differential equations. Suppose we have n variable $u_1, u_2, \dots, u_n$, ($n \geq 2$) whose evolution is described by

$$\frac{du_i}{dt} = (\text{activation function}) \frac{1 - u_i}{J_i^A + 1 - u_i} - (\text{inhibition function}) \frac{u_i}{J_i^I + u_i}, \quad (5.8)$$

where J_i^A, J_i^I are some positive constants. The initial conditions are assumed to satisfy $u_i(0) \in [0, 1]$ for all i . The activation/inhibition functions that appear in Eq. (5.8) capture the interaction of u_i with other variables. We will define them to be non-negative. This

will force the trajectories to stay inside the unit cube $[0, 1]^n$ (see Proposition 5.3.1).

Activation/Inhibition functions: Let $W = [w_{ij}]$ be an $n \times n$ real matrix. The entry w_{ij} of W gives the contribution of the j -th variable on the growth rate of i -th variable. If $w_{ij} > 0$, then w_{ij} it will appear in the activation function for u_i ; and if $w_{ij} < 0$ then $-w_{ij}$ will appear in the inhibition function for u_i . Therefore W represents a *connectivity matrix*.

Let $b = [b_1 \ b_2 \ \dots \ b_n]^t$ be an $n \times 1$ real vector. The entries b_i represent the threshold between the activation function and the inhibition function for u_i . The value $|b_i|$ will appear in the activation or the inhibition function for u_i , depending on whether $b_i > 0$ or $b_i < 0$, respectively. To be more precise, we use the convention $x^+ = \max\{x, 0\}$ and $x^- = \max\{-x, 0\}$ for all $x \in \mathbb{R}$, and define the activation functions A_i and the inhibition functions I_i as

$$A_i := \sum_{j=1}^n w_{ij}^+ u_j + b_i^+, \quad I_i := \sum_{j=1}^n w_{ij}^- u_j + b_i^-. \quad (5.9)$$

Using this notation, Eqs. (5.8) take the form

$$\frac{du_i}{dt} = A_i \frac{1 - u_i}{J_i^A + 1 - u_i} - I_i \frac{u_i}{J_i^I + u_i}. \quad (5.10)$$

The next result illustrates that the cube $[0, 1]^n$ is invariant under the flow of dynamical system (5.10).

Proposition 5.3.1 *The cube $[0, 1]^n$ is invariant for the dynamical system (5.10).*

Proof: It will be enough to show that the vector field at any point on the boundary is directed inward. Since, A_i s and I_i s will always take nonnegative values, we observe that for any i ,

$$\left. \frac{du_i}{dt} \right|_{u_i=0} = A_i \frac{1}{J_i^A + 1} \geq 0, \quad \text{and} \quad \left. \frac{du_i}{dt} \right|_{u_i=1} = -I_i \frac{1}{J_i^I + 1} \leq 0.$$

This means that as soon as the solution reaches the boundary, it will be forced to move inward. $\square$

We can express Eq. (5.10) in matrix notation in following way: Let,

$$u := \begin{bmatrix} u_1 & u_2 & \cdots & u_n \end{bmatrix}^t, \quad W^+ := [w_{ij}^+], \quad W^- := [w_{ij}^-], \quad b^+ = \begin{bmatrix} b_1^+ & b_2^+ & \cdots & b_n^+ \end{bmatrix}^t,$$

$$b^- = \begin{bmatrix} b_1^- & b_2^- & \cdots & b_n^- \end{bmatrix}^t, \text{ and}$$

$$F(u) := \text{diag} \left(\frac{1 - u_1}{J_1^A + 1 - u_1}, \frac{1 - u_2}{J_2^A + 1 - u_2}, \dots, \frac{1 - u_n}{J_n^A + 1 - u_n} \right),$$

$$G(u) := \text{diag} \left(\frac{u_1}{J_1^I + u_1}, \frac{u_2}{J_2^I + u_2}, \dots, \frac{u_n}{J_n^I + u_n} \right).$$

Then, we can write Eq. (5.10) in matrix form as

$$\frac{du}{dt} = F(u)(W^+u + b^+) - G(u)(W^-u + b^-). \quad (5.11)$$

For example, with the *repressilator* we have

$$W = \begin{bmatrix} 0 & 0 & -1 \\ -1 & 0 & 0 \\ 0 & -1 & 0 \end{bmatrix} \quad \text{and} \quad b = \begin{bmatrix} 0.6 \\ 0.4 \\ 0.3 \end{bmatrix},$$

and $J_1^A = J_2^A = J_3^A = J_1^I = J_2^I = J_3^I = J$.

Linear approximation of Eq. (5.10)

We follow the reduction procedure outlined in Section 5.2. For notational convenience we consider the case $J_i^A = J_i^I = J$, with J small and positive. The general case is equivalent. Let δ be some positive number which will be used to define the thickness of the boundary layers, and which will depend on J in general. The division of the square $[0, 1]^2$, listed in Table 5.1, can be generalized to the n -dimensional cube $[0, 1]^n$ as follows.

Let T and S be two disjoint subsets of the set $\{1, 2, \dots, n\}$ and define

$$\mathcal{R}_S^T := \left\{ (u_1, u_2, \dots, u_n) \in [0, 1]^n \mid \begin{array}{l} u_s < \delta \text{ for all } s \in S; \quad u_t > 1 - \delta \text{ for all } t \in T; \\ \text{and } \delta \leq u_k \leq 1 - \delta \text{ for all } k \notin S \cup T \end{array} \right\}.$$

Therefore, $\mathcal{R}_S^T$ corresponds to the part of the cube $[0, 1]^n$ where the coordinates u_s , $s \in S$ are close to zero; and the coordinates u_t , $t \in T$ are close to one; and all the other coordinates are away from the extreme values zero and one. In Table 5.1, and in Eqs. (5.2) and (5.4) we used the above notation together with following conventions: $\mathcal{R}_0^T := \mathcal{R}_S^T$ when S is empty; $\mathcal{R}_S^0 := \mathcal{R}_S^T$ when T is empty; and $\mathcal{R}_0^0 := \mathcal{R}_S^T$ when T , S both are empty.

The system of equations (5.10) is reduced to a different linear algebraic–differential system within each region $\mathcal{R}_S^T$. We follow the reduction from Eq. (5.1) to Eq. (5.5). For $i \notin S \cup T$ we obtain the linear system

$$\frac{du_i}{dt} = \sum_{j=1}^n a_{ij} u_j + b_i; \quad (5.12a)$$

for $s \in S$ some of the non linear terms will stay and we get

$$\frac{du_s}{dt} = \left(\sum_{j=1}^n a_{sj}^+ u_j + b_s^+ \right) - \left(\sum_{j=1}^n a_{sj}^- u_j + b_s^- \right) \frac{u_s}{J + u_s}; \quad (5.12b)$$

and for $t \in T$ we will have

$$\frac{du_t}{dt} = \left(\sum_{j=1}^n a_{tj}^+ u_j + b_t^+ \right) \frac{1 - u_t}{J + 1 - u_t} - \left(\sum_{j=1}^n a_{tj}^- u_j + b_t^- \right). \quad (5.12c)$$

Eq. (5.12) is simpler than Eq. (5.10), but it is not solvable yet. Following the reduction from Eq. (5.5) to Eq. (5.6), we note that in the region $\mathcal{R}_S^T$, $u_s, s \in S$ are close to zero and $u_t, t \in T$ are close to one. The first simplification we propose is to plug in these approximate values of u_s and u_t , *i.e.* $u_s = 0$ and $u_t = 1$ in the activation and inhibition functions appearing in Eq. (5.12). The second simplification comes from the fact that

boundary of $[0, 1]^n$ is inflowing and the $\mathcal{R}_S^T$ is thin. Therefore, when u_s, u_t are near the boundary, their derivative must be small (this is justified below). Thus it makes sense to assume that coordinates u_s for $s \in S$ and u_t for $t \in T$ are in steady state. With these two simplifications Eq. (5.12) takes the form

$$\frac{du_i}{dt} = \sum_{j \notin S \cup T} a_{ij} u_j + \sum_{j \in T} a_{ij} + b_i \quad i \notin S \cup T; \quad (5.13a)$$

$$0 = \sum_{j \notin S \cup T} a_{sj}^+ u_j + \sum_{t \in T} a_{st}^+ + b_s^+ - \left(\sum_{j \notin S \cup T} a_{sj}^- u_j + \sum_{t \in T} a_{st}^- + b_s^- \right) \frac{u_s}{J + u_s}; \quad s \in S, \quad (5.13b)$$

$$0 = - \left(\sum_{j \notin S \cup T} a_{tj}^+ u_j + \sum_{j \in T} a_{tj}^+ + b_t^+ \right) \frac{1 - u_t}{J + 1 - u_t} + \sum_{j \notin S \cup T} a_{tj}^- u_j + \sum_{j \in T} a_{tj}^- + b_t^-, \quad t \in T. \quad (5.13c)$$

Eq. (5.13) is completely solvable since Eq. (5.13a) is linear and decoupled from boundary variables. Furthermore, Eqs.(5.13b) and (5.13c) are solvable for u_s and u_t , respectively, as functions of the solution of Eq. (5.13).

Finally we justify our claim that the variables that are close to the boundary can be assumed to be in steady state. We define the following new variables to “magnify” the boundary region.

$$\begin{aligned} \tilde{u}_s &:= \frac{u_s}{J} & \text{for } s \in S, \\ \tilde{u}_t &:= \frac{1 - u_t}{J} & \text{for } t \in T. \end{aligned} \quad (5.14)$$

Using Eq. (5.14) in Eq. (5.12) we get:

For $i \notin S \cup T$

$$\frac{du_i}{dt} = \sum_{j \notin S \cup T} a_{ij} u_j + \sum_{j \in T} a_{ij} + J \left(\sum_{s \in S} a_{is} \tilde{u}_s - \sum_{t \in T} a_{it} \tilde{u}_t \right) + b_i; \quad (5.15a)$$

and for $s \in S$, we have

$$\begin{aligned}
 J \frac{d\tilde{u}_s}{dt} = & \sum_{j \notin S \cup T} a_{sj}^+ u_j + \sum_{t \in T} a_{st}^+ + J \left(\sum_{j \in S} a_{sj}^+ \tilde{u}_j - \sum_{t \in T} a_{st}^+ \tilde{u}_t \right) + b_s^+ \\
 & - \left(\sum_{j \notin S \cup T} a_{sj}^- u_j + \sum_{t \in T} a_{st}^- + b_s^- \right) \frac{\tilde{u}_s}{1 + \tilde{u}_s} - J \left(\sum_{j \in S} a_{sj}^+ \tilde{u}_j - \sum_{t \in T} a_{st}^+ \tilde{u}_t \right) \frac{\tilde{u}_s}{1 + \tilde{u}_s},
 \end{aligned} \tag{5.15b}$$

and similarly, for $t \in T$, we have

$$\begin{aligned}
 J \frac{d\tilde{u}_t}{dt} = & - \left(\sum_{j \notin S \cup T} a_{tj}^+ u_j + \sum_{j \in T} a_{tj}^+ + b_t^+ \right) \frac{\tilde{u}_t}{1 + \tilde{u}_t} - J \left(\sum_{s \in S} a_{ts}^+ \tilde{u}_s - \sum_{j \in T} a_{tj}^+ \tilde{u}_j \right) \frac{\tilde{u}_t}{1 + \tilde{u}_t} \\
 & + \sum_{j \notin S \cup T} a_{tj}^- u_j + \sum_{j \in T} a_{tj}^- + b_t^- + J \left(\sum_{s \in S} a_{ts}^+ \tilde{u}_s - \sum_{j \in T} a_{tj}^+ \tilde{u}_j \right).
 \end{aligned} \tag{5.15c}$$

Note that Eq. (5.15) exactly has the form of Eq. (1.1), with $u_i, i \notin S \cup T$ as the *slow variable*; and $\tilde{u}_s, s \in S$ and $\tilde{u}_t, t \in T$ as the *fast variables*. Therefore, following the discussion on the GSPT (see Chapter 1), for small J we can approximate the Eq. (5.15) by plugging in $J = 0$ in Eq. (5.15). Doing that yields the following differential-algebraic system.

$$\frac{du_i}{dt} = \sum_{j \notin S \cup T} a_{ij} u_j + \sum_{j \in T} a_{ij} + b_i, \quad i \notin S \cup T; \tag{5.16a}$$

$$0 = \sum_{j \notin S \cup T} a_{sj}^+ u_j + \sum_{t \in T} a_{st}^+ + b_s^+ - \left(\sum_{j \notin S \cup T} a_{sj}^- u_j + \sum_{t \in T} a_{st}^- + b_s^- \right) \frac{\tilde{u}_s}{1 + \tilde{u}_s}, \quad s \in S; \tag{5.16b}$$

$$0 = - \left(\sum_{j \notin S \cup T} a_{tj}^+ u_j + \sum_{j \in T} a_{tj}^+ + b_t^+ \right) \frac{\tilde{u}_t}{1 + \tilde{u}_t} + \sum_{j \notin S \cup T} a_{tj}^- u_j + \sum_{j \in T} a_{tj}^- + b_t^-, \quad t \in T. \tag{5.16c}$$

Normal hyperbolicity of the *slow manifold* defined by Eqs. (5.16b) and (5.16c):

Before we can safely conclude that Eq. (5.16) is a valid reduction of Eq. (5.15), we still need to verify one more requirement of the theorems of the GSPT. That is, the manifold to which the dynamics is being reduced must be asymptotically stable. In the present scenario this means that the manifold defined by Eqs. (5.16b) and (5.16c) has to be normally hyperbolic and stable. We show next that this additional requirement is easy to verify.

Let $\hat{u} = \{u_{i_1}, \dots, u_{i_m}\}$ where $\{i_1, \dots, i_m\} = \{1, 2, \dots, n\} \setminus (S \cup T)$, be the coordinates of u which are away from the boundary, and denote the right hand side of Eq. (5.16b) by $F_s(\hat{u}, \tilde{u}_{i_s})$, for all $s \in S$. *i.e.*

$$F_s(\hat{u}, \tilde{u}_{i_s}) := \sum_{j \notin S \cup T} a_{sj}^+ u_j + \sum_{t \in T} a_{st}^+ + b_s^+ - \left(\sum_{j \notin S \cup T} a_{sj}^- u_j + \sum_{t \in T} a_{st}^- + b_s^- \right) \frac{\tilde{u}_s}{1 + \tilde{u}_s}.$$

Then, clearly we have

$$\frac{\partial F_s}{\partial \tilde{u}_{i_s}} = - \left(\sum_{j \notin S \cup T} a_{sj}^- u_j + \sum_{t \in T} a_{st}^- + b_s^- \right) \left(\frac{1}{1 + \tilde{u}_s} \right)^2 < 0$$

for all $s \in S$. Similarly, by denoting the right hand side of Eq. (5.16c) by $G_t(\hat{u}, \tilde{u}_{i_t})$, for all $t \in T$. *i.e.*

$$G_t(\hat{u}, \tilde{u}_{i_t}) := - \left(\sum_{j \notin S \cup T} a_{tj}^+ u_j + \sum_{j \in T} a_{tj}^+ + b_t^+ \right) \frac{\tilde{u}_t}{1 + \tilde{u}_t} + \sum_{j \notin S \cup T} a_{tj}^- u_j + \sum_{j \in T} a_{tj}^- + b_t^-,$$

we see that

$$\frac{\partial G_t}{\partial \tilde{u}_{i_t}} = - \left(\sum_{j \notin S \cup T} a_{tj}^+ u_j + \sum_{j \in T} a_{tj}^+ + b_t^+ \right) \left(\frac{\tilde{u}_t}{1 + \tilde{u}_t} \right)^2 < 0.$$

Hence, the manifold defined by Eqs. (5.16b) and (5.16c) is normally hyperbolic and stable.

This completes the proof of the claim that, for small J , the variables $u_s, s \in S$ and $u_t, t \in T$ can be safely assumed to be in steady state in Eq. (5.12). Hence the reduction of the non-linear system (5.10) to a solvable system (5.13) is justified if J is very small.

5.4 Discussion

A special class of non-linear differential equation was studied. The non-linear terms involved were like Hill functions. We showed that if the Michaelis-Menten constants are small enough then the non-linearity of the system can be circumvented by appropriate linear systems. This induces a natural decomposition of the domain into a nested sequence of hypercubes. Example problems were worked out and mathematical justification through the geometrical singular perturbation theory was discussed.

A potential limitation in our arguments is that we have an approximation which is valid only in an asymptotic limit. Another major limitation of our analysis is that we have not provided a systematic relationship between the thickness of the boundary, δ , and the Michaelis-Menten constant, J . Intuitively we believe that δ should go to zero as J goes to zero, but more research is required to make this relation more concrete. Also, more study needs to be done to approximate the error induced in the system by the replacement of non-linear terms with unity.

Appendix A

The Law of Mass Action

Consider an elementary chemical reaction



where a, b_1, b_2, p are positive integers and A, B, P are molar concentration of some chemical species, and k_1, k_{-1} are reaction rate coefficients. For this reaction, the **the Law of Mass Action** states that the rate of change of each of the reactant or product is governed by the following rules:

The contribution from the Forward reaction:

$$k_1 A^a B^{b_1} = -\frac{1}{a} \frac{dA}{dt} = \frac{1}{b_2 - b_1} \frac{dB}{dt} = \frac{1}{p} \frac{dP}{dt} \quad (\text{A.2})$$

The contribution from the Backward reaction:

$$k_{-1} B^{b_2} P^p = \frac{1}{a} \frac{dA}{dt} = \frac{1}{b_1 - b_2} \frac{dB}{dt} = -\frac{1}{p} \frac{dP}{dt}. \quad (\text{A.3})$$

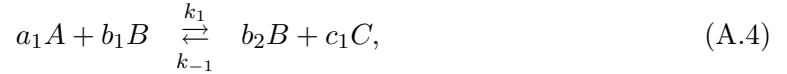
The final differential equation to model the concentration of different chemical involved in reaction (A.1) is obtained by adding the contribution from Eq. (A.2) and Eq. (A.3)

$$\begin{aligned}\frac{dA}{dt} &= -k_1 a A^a B^{b_1} + k_{-1} a B^{b_2} P^p, \\ \frac{dB}{dt} &= -k_1 (b_1 - b_2) A^a B^{b_1} + k_{-1} (b_1 - b_2) B^{b_2} P^p, \\ \frac{dP}{dt} &= k_1 p A_a B^{b_1} - k_{-1} p B^{b_2} P^p.\end{aligned}$$

Similarly, if the reaction is broken into more than one elementary reaction, rate of change of each reactant/product can be calculated from individual elementary reaction and finally added. Here is an example.

Complex reaction

Complex reactions are chain of elementary reactions. So, the Law of Mass Action can be applied on individual steps and added. Consider the following two stage reaction.



Then, by applying the Law of Mass Action in each of the above step and in each direction, we get:

From forward direction of (A.4),

$$k_1 A^{a_1} B^{b_1} = -\frac{1}{a_1} \frac{dA}{dt} = \frac{1}{b_2 - b_1} \frac{dB}{dt} = \frac{1}{c_1} \frac{dC}{dt},$$

from backward direction of (A.4),

$$k_{-1} B^{b_2} C^{c_1} = \frac{1}{a_1} \frac{dA}{dt} = -\frac{1}{b_2 - b_1} \frac{dB}{dt} = -\frac{1}{c_1} \frac{dC}{dt},$$

from forward direction of (A.5),

$$k_2 A^{a_2} C^{c_2} = -\frac{1}{a_2} \frac{dA}{dt} = -\frac{1}{c_2} \frac{dC}{dt} = \frac{1}{e} \frac{dE}{dt} = \frac{1}{f} \frac{dF}{dt},$$

from backward direction of (A.5),

$$k_{-2}E^eF^f = -\frac{1}{e}\frac{dE}{dt} = -\frac{1}{f}\frac{dF}{dt} = \frac{1}{a_2}\frac{dA}{dt} = \frac{1}{c_2}\frac{dC}{dt}.$$

From these we can write down the dynamics of the whole reaction as

$$\begin{aligned}\frac{dA}{dt} &= -k_1a_1A^{a_1}B^{b_1} + k_{-1}a_1B^{b_2}C^{c_1} - k_2a_2A^{a_2}C^{c_2} + k_{-2}a_2E^eF^f, \\ \frac{dB}{dt} &= k_1(b_2 - b_1)A^{a_1}B^{b_1} - k_{-1}(b_2 - b_1)B^{b_2}C^{c_1}, \\ \frac{dC}{dt} &= k_1c_1A^{a_1}B^{b_1} - k_{-1}c_1B^{b_2}C^{c_1} - k_2c_2A^{a_2}C^{c_2} + k_{-2}c_2E^eF^f, \\ \frac{dE}{dt} &= k_2eA^{a_2}C^{c_2} - k_{-2}eE^eF^f, \\ \frac{dF}{dt} &= k_2fA^{a_2}C^{c_2} - k_{-2}fE^eF^f.\end{aligned}$$

Appendix B

Differentiation with respect to a matrix

The theory of differentiation with respect to a matrix is described in [42]. Suppose $M = [M_{.1} : M_{.2} : \dots : M_{.n}]$ is a $m \times n$ matrix, where $M_{.j}$ is the j th column of M . Then define

$$\text{vec}(M) := \begin{bmatrix} M_{.1} \\ M_{.2} \\ \vdots \\ M_{.n} \end{bmatrix} \in \mathbb{C}^{mn \times 1}, \quad \text{and} \quad \widehat{M} := \text{diag}(\text{vec}(M)) \in \mathbb{C}^{mn \times mn}.$$

Therefore, $\text{vec}(M)$ is obtained by stacking the columns of M on top of each other, and $\widehat{M}$ is the $mn \times mn$ diagonal matrix whose diagonal entries are given by $\text{vec}(M)$.

Suppose $G : \mathbb{C}^{p \times q} \rightarrow \mathbb{C}^{m \times n}$ is a matrix valued function with $X \in \mathbb{C}^{p \times q} \mapsto G(X) \in \mathbb{C}^{m \times n}$. Then the derivative of G with respect to X is defined as

$$\frac{\partial G}{\partial X} := \frac{\partial \text{vec}(G)}{\partial \text{vec}(X)},$$

where the right hand side is the Jacobian [42]. Below we list some important properties of these operators as they relate to differentiation with respect to a matrix. Proofs can be

found in [42].

Theorem B.1 ([47, 58]) *For any three matrices A, B and C such that the matrix product ABC is defined,*

$$\text{vec}(ABC) = (C^t \otimes A) \text{vec}(B).$$

Theorem B.2 ([42]) *For any two matrices A and B of equal size*

$$\text{vec}(A * B) = \hat{A} \text{vec}(B) = \hat{B} \text{vec}(A).$$

Theorem B.3 (Product rule [42]) *Let $G : \mathbb{C}^{p \times q} \rightarrow \mathbb{C}^{m \times r}$ and $H : \mathbb{C}^{p \times q} \rightarrow \mathbb{C}^{r \times n}$ be two differentiable function then*

$$\frac{\partial \text{vec}(GH)}{\partial \text{vec}(X)} = (H^t \otimes I_m) \frac{\partial \text{vec}(G)}{\partial \text{vec}(X)} + (I_n \otimes G) \frac{\partial \text{vec}(H)}{\partial \text{vec}(X)}.$$

Theorem B.4 (Hadamard product rule [42]) *Let $G : \mathbb{C}^{p \times q} \rightarrow \mathbb{C}^{m \times n}$ and $H : \mathbb{C}^{p \times q} \rightarrow \mathbb{C}^{m \times n}$ be two differentiable functions then*

$$\frac{\partial \text{vec}(G * H)}{\partial \text{vec}(X)} = \hat{H} \frac{\partial \text{vec}(G)}{\partial \text{vec}(X)} + \hat{G} \frac{\partial \text{vec}(H)}{\partial \text{vec}(X)}.$$

Appendix C

Some Linear Algebra results

These results were discovered while we were trying to prove the stability of Jacobian matrix that came up in the system of coupled Michaelis-Menten reaction in Chapter 4 (see Eq. (4.4)). We have not used these in the main part of the thesis, but these might still interest the reader.

Lemma C.1 *If $Y \in \mathbb{R}_+^{n \times 1}$ is a column vector with positive entries, $V = [1 \ 1 \ \cdots \ 1]^t$ is a column vector of size n with all entries as ones and $\Lambda \in \mathbb{R}_+^{n \times n}$ diagonal matrix with all positive entries in the diagonal then the $n \times n$ matrix*

$$D = YV^t + \Lambda$$

has only real eigenvalues.

Proof: Let,

$$Y = \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{bmatrix}, \quad \Lambda = \begin{bmatrix} \lambda_1 & & & \\ & \lambda_2 & & \\ & & \ddots & \\ & & & \lambda_n \end{bmatrix}.$$

And let $\lambda = a + ib$ be an eigenvalue with $b \neq 0$ and choose corresponding eigenvector

$$\bar{V} = \begin{bmatrix} v_1 \\ v_2 \\ \vdots \\ v_n \end{bmatrix} = \begin{bmatrix} r_1 + is_1 \\ r_2 + is_2 \\ \vdots \\ r_n + is_n \end{bmatrix}$$

such that

$$\sum_{k=1}^n v_k = 1. \quad (\text{C.1})$$

By the definition of eigenvector and using (C.1) $\forall k \in \{1, 2, \dots, n\}$ we have

$$y_k + \lambda_k v_k = \lambda v_k.$$

So

$$\begin{aligned} y_k &= (\lambda - \lambda_k) v_k \\ &= ((a - \lambda_k) + ib)(r_k + is_k) \\ &= ((a - \lambda_k)r_k - bs_k) + i((a - \lambda_k)s_k + br_k). \end{aligned} \quad (\text{C.2})$$

Since y_k is real and $b \neq 0$ we can set the imaginary part to zero and solve for r_k

$$(a - \lambda_k)s_k + br_k = 0, \quad (\text{C.3})$$

$$r_k = -(a - \lambda_k) \frac{s_k}{b}. \quad (\text{C.4})$$

Pluggin this in (C.2) we get

$$y_k = -((a - \lambda_k)^2 + b^2) \frac{s_k}{b}. \quad (\text{C.5})$$

Since $y_k > 0$, equations (C.3),(C.5) together implies that all the entries of eigenvector have nonzero real and imaginary parts.

Now by (C.1) we have $\sum_{k=1}^n s_k = 0$. Which implies that for some k_0 , $\frac{s_{k_0}}{b} > 0$. Which will imply that $y_{k_0} < 0$ which is a contradiction on the assumption that Y has positive entries.

Hence λ must be real. $\square$

Lemma C.2 *If $Y \in \mathbb{R}_+^{n \times 1}$ is a column vector with positive entire , $V = [1 \ 1 \ \cdots \ 1]^t$ is a column vector of size n with all entries as ones and $\Lambda \in \mathbb{R}_+^{n \times n}$ diagonal matrix with all positive entries in the diagonal then the $n \times n$ matrix*

$$D = YV^t + \Lambda$$

has only positive eigenvalues.

Proof: Let,

$$Y = \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{bmatrix}, \quad \Lambda = \begin{bmatrix} \lambda_1 & & & \\ & \lambda_2 & & \\ & & \ddots & \\ & & & \lambda_n \end{bmatrix}.$$

By lemma (C.1) we know that D can have only real eigenvalues. Let, if possible, $\lambda < 0$ be a negative eigenvalue and choose corresponding eigenvector

$$\bar{V} = \begin{bmatrix} v_1 \\ v_2 \\ \vdots \\ v_n \end{bmatrix}$$

such that

$$\sum_{k=1}^n v_k = 1. \quad (\text{C.6})$$

and v_k , $k \in \{1, 2, \dots, n\}$ are real (we can do this separating the real and imaginary part of any eigenvector as long as the eigenvalue is real).

Now, by the definition of eigenvector and using (C.6) $\forall k \in \{1, 2, \dots, n\}$ we have

$$\begin{aligned} y_k + \lambda_k v_k &= \lambda v_k, \\ y_k &= (\lambda - \lambda_k) v_k. \end{aligned} \quad (\text{C.7})$$

But since $\lambda < 0$ and $\lambda_k > 0$, (C.7) would imply that $v_k < 0 \forall k \in \{1, 2, \dots, n\}$ which will be a contradiction to (C.6). So λ can not be negative. $\square$

Lemma C.3 Suppose $Z \in \mathbb{R}_+^{2n \times 1}$ is a $2n$ dimensional vector with positive entries; $Y \in \mathbb{R}_+^{n \times 1}$ is an n dimensional vector with positive entries; I_n, I_{2n}, I_{2n^2} is identity matrices of order $n, 2n, 2n^2$, respectively. Assume further that R_n and R_{2n} are row vectors of size n and $2n$ respectively with all entries equal to 1. Then the $2n^2 \times 2n^2$ matrix

$$J := (ZR_{2n} \otimes I_n) + (I_{2n} \otimes YR_n) + I_{2n^2} \quad (\text{C.8})$$

has eigenvalues with strictly positive real parts.

Proof: The first thing to note is that the terms on the right hand side of Eq. (C.8) commute:

$$\begin{aligned}
(ZR_{2n} \otimes I_n)(I_{2n} \otimes YR_n) &= (ZR_{2n}I_{2n}) \otimes (I_n YR_n) \\
&= (I_{2n}ZR_{2n}I_{2n}) \otimes (YR_nI_n) \\
&= (I_{2n} \otimes YR_n)(ZR_{2n} \otimes I_n),
\end{aligned}$$

and obviously

$$\begin{aligned}
(ZR_{2n} \otimes I_n)I_{2n^2} &= I_{2n^2}(ZR_{2n} \otimes I_n), \\
(I_{2n} \otimes YR_n)I_{2n^2} &= I_{2n^2}(I_{2n} \otimes YR_n) \otimes I_n.
\end{aligned}$$

We next show that each term of the right hand side of Eq. (C.8) has non negative eigenvalue.

Lets focus on $(ZR_{2n} \otimes I_n)$ first. By a property of tensor products, we have

$$\sigma(ZR_{2n} \otimes I_n) = \sigma(ZR_{2n}), \quad (\text{C.9})$$

where with $\sigma(A)$ we mean the spectrum of the matrix A . Let

$$Z = \begin{bmatrix} z_1 \\ z_2 \\ \vdots \\ z_{2n} \end{bmatrix} \quad \text{and} \quad Y = \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{bmatrix}.$$

We see that ZR_{2n} is a rank one matrix and has one positive eigenvalue $\sum_{i=1}^{2n} z_i$, because

$$\begin{bmatrix} z_1 & z_1 & \dots & z_1 \\ z_2 & z_2 & \dots & z_2 \\ \vdots & & & \vdots \\ z_{2n} & z_{2n} & \dots & z_{2n} \end{bmatrix} \begin{bmatrix} z_1 \\ z_2 \\ \vdots \\ z_{2n} \end{bmatrix} = \sum_{i=1}^{2n} z_i \begin{bmatrix} z_1 \\ z_2 \\ \vdots \\ z_{2n} \end{bmatrix}.$$

Hence, all the other eigenvalues of ZR_{2n} will be zero. Hence Eq. (C.9) implies that

$$\sigma(ZR_{2n} \otimes I_n) = \left\{ \sum_{i=1}^{2n} z_i, 0 \right\}.$$

Similarly,

$$\sigma(I_{2n} \otimes YR_n) = \left\{ \sum_{i=1}^n y_i, 0 \right\}.$$

And obviously

$$\sigma(I_{2n^2}) = \{1\}.$$

And now we invoke a linear algebra property that, since the terms in the right hand side of Eq. (C.8) commute, we get (with a slight abuse of notations on addition of sets)

$$\begin{aligned} \sigma(J) &= \sigma[(ZR_{2n} \otimes I_n) + (I_{2n} \otimes YR_n) + I_{2n^2}] \\ &\subseteq \sigma(ZR_{2n} \otimes I_n) + \sigma(I_{2n} \otimes YR_n) + \sigma(I_{2n^2}) \\ &= \left\{ \sum_{i=1}^{2n} z_i, 0 \right\} + \left\{ \sum_{i=1}^n y_i, 0 \right\} + \{1\} \\ &= \left\{ 1, 1 + \sum_{i=1}^{2n} z_i, 1 + \sum_{i=1}^n y_i, \sum_{i=1}^{2n} z_i + \sum_{i=1}^n y_i \right\}. \end{aligned}$$

This implies that J has all positive eigenvalues. This concludes the proof. $\square$

Bibliography

- [1] Mathematics is biology’s next microscope, only better; biology is mathematics’ next physics, only better. *PLoS Biol*, 2(12):e439+, December 2004.
- [2] Uri Alon. *An Introduction to Systems Biology: Design Principles of Biological Circuits*. Chapman and Hall/CRC, 1st edition, July 2006.
- [3] David Angeli, Patrick De Leenheer, and Eduardo Sontag. Graph-theoretic characterizations of monotonicity of chemical networks in reaction coordinates. *Journal of Mathematical Biology*, 61(4):581–616, October 2010.
- [4] Debashis Barik, Mark R. Paul, William T. Baumann, Yang Cao, and John J. Tyson. Stochastic simulation of Enzyme-Catalyzed reactions with disparate timescales. *Biophysical Journal*, 95(8):3563–3574, October 2008.
- [5] M. Bennett, D. Volfson, L. Tsimring, and J. Hasty. Transient dynamics of genetic regulatory networks. *Biophysical Journal*, 92(10):3501–3512, May 2007.
- [6] José Borghans, Rob de Boer, and Lee Segel. Extending the quasi-steady state approximation by changing variables. *Bulletin of Mathematical Biology*, 58(1):43–63, January 1996.
- [7] G. E. Briggs and J. B. Haldane. A note on the kinetics of enzyme action. *The Biochemical Journal*, 19(2):338–339, 1925.
- [8] Yang Cao, Daniel T. Gillespie, and Linda R. Petzold. The slow-scale stochastic simulation algorithm. *The Journal of Chemical Physics*, 122(1):014116+, January 2005.
- [9] Richard Casey, Hidde Jong, and Jean-Luc Gouzé. Piecewise-linear models of genetic regulatory networks: Equilibria and their stability. *Journal of Mathematical Biology*, 52(1):27–56, January 2006.

- [10] P. B. Chock and E. R. Stadtman. Superiority of interconvertible enzyme cascades in metabolite regulation: analysis of multicyclic systems. *Proceedings of the National Academy of Sciences of the United States of America*, 74(7):2766–2770, July 1977.
- [11] Andrea Ciliberto, Fabrizio Capuani, and John J. Tyson. Modeling networks of coupled enzymatic reactions using the total quasi-steady state approximation. *PLoS Computational Biology*, 3(3)(3):e45, March 2007.
- [12] I. G. Darvey, B. W. Ninham, and P. J. Staff. Stochastic models for Second-Order chemical reaction kinetics. the equilibrium state. *The Journal of Chemical Physics*, 45(6):2145–2155, 1966.
- [13] M. Davidich and S. Bornholdt. The transition from differential equations to boolean networks: A case study in simplifying a regulatory network model. *Journal of Theoretical Biology*, 255(3):269–277, December 2008.
- [14] Michael B. Elowitz and Stanislas Leibler. A synthetic oscillatory network of transcriptional regulators. *Nature*, 403(6767):335–338, January 2000.
- [15] N. Fenichel. Geometric singular perturbation theory for ordinary differential equations. *Journal of Differential Equations*, 31(1):53–98, January 1979.
- [16] M. Fernandez and G. Carta. Characterization of protein adsorption by composite silica-polyacrylamide gel anion exchangers i. equilibrium and mass transfer in agitated contactors. *Journal of Chromatography A*, 746(2):169–183, October 1996.
- [17] C. L. Frenzen and P. K. Maini. Enzyme kinetics for a two-step enzymic reaction with comparable initial enzyme-substrate ratios. *Journal of Mathematical Biology*, 26:689–703, 1988.
- [18] Timothy S. Gardner, Charles R. Cantor, and James J. Collins. Construction of a genetic toggle switch in escherichia coli. *Nature*, 403(6767):339–342, January 2000.
- [19] D. T. Gillespie. A rigorous derivation of the chemical master equation. *Physica A Statistical Mechanics and its Applications*, 188(1-3):404–425, September 1992.
- [20] Daniel T. Gillespie. Exact stochastic simulation of coupled chemical reactions. *The Journal of Physical Chemistry*, 81(25):2340–2361, December 1977.
- [21] Daniel T. Gillespie. The chemical langevin equation. *The Journal of Chemical Physics*, 113(1):297–306, 2000.
- [22] L. Glass and S. Kauffman. The logical analysis of continuous, non-linear biochemical control networks. *Journal of Theoretical Biology*, 39(1):103–129, April 1973.
- [23] A. Goldbeter. A minimal cascade model for the mitotic oscillator involving cyclin and cdc2 kinase. *Proceedings of the National Academy of Sciences of the United States of America*, 88(20):9107–9111, October 1991.

- [24] Albert Goldbeter and Daniel E. Koshland. An amplified sensitivity arising from covalent modification in biological systems. *Proceedings of the National Academy of Sciences of the United States of America*, 78(11):6840–6844, 1981.
- [25] R. Grima and S. Schnell. A systematic investigation of the rate laws valid in intracellular environments. *Biophysical Chemistry*, 124(1):1–10, October 2006.
- [26] H. M. Hardin, A. Zagaris, K. Krab, and H. V. Westerhoff. Simplified yet highly accurate enzyme kinetics for cases of low substrate concentrations. *FEBS Journal*, 276(19):5491–5506, October 2009.
- [27] M. Hegland, C. Burden, L. Santoso, S. MacNamara, and H. Booth. A solver for the stochastic master equation applied to gene regulatory networks. *Journal of Computational and Applied Mathematics*, 205(2):708–724, August 2007.
- [28] Geertje Hek. Geometric singular perturbation theory in biological practice. *Journal of Mathematical Biology*, 60(3):347–386, March 2010.
- [29] Desmond J. Higham. Modeling and simulating chemical reactions. *SIAM Rev.*, 50:347–368, May 2008.
- [30] F. Horn and R. Jackson. General mass action kinetics. *Archive for Rational Mechanics and Analysis*, 47(2):81–116, January 1972.
- [31] R. A. Horn and C. R. Johnson. *Topics in Matrix Analysis*, chapter 4, pages 268–269. Cambridge University Press, 1991.
- [32] C. Y. Huang and J. E. Ferrell. Ultrasensitivity in the mitogen-activated protein kinase cascade. *Proceedings of the National Academy of Sciences of the United States of America*, 93(19):10078–10083, September 1996.
- [33] Liliana Ironi, Luigi Panzeri, Erik Plahte, and Valeria Simoncini. Dynamics of actively regulated gene networks. *Physica D*, 240:779–794, 2011.
- [34] Tobias Jahnke and Wilhelm Huisinga. Solving the chemical master equation for monomolecular reaction systems analytically. *Journal of Mathematical Biology*, 54(1):1–26, January 2007.
- [35] Christopher Jones. Geometric singular perturbation theory. In *Dynamical Systems*, volume 1609 of *Lecture Notes in Mathematics*, chapter 2, pages 44–118. Springer Berlin Heidelberg, 1995.
- [36] Tasso J. Kaper. An introduction to geometrical methods and dynamical systems for singular perturbation problems. In *Analyzing Multiscale Phenomena Using Singular Perturbation Methods: American Mathematical Society Short Course, January 5-6, 1998, Baltimore, Maryland (Proc. Sym. Ap.)*, pages 85–132, 1998.

- [37] C. F. Khoo and M. Hegland. The total quasi-steady state assumption: its justification by singular perturbation theory and its application to the chemical master equation. *ANZIAM Journal*, 50:C429 – C443, 2008.
- [38] Ajit Kumar and Krešimir Josić. Reduced models of networks of coupled enzymatic reactions. *Journal of Theoretical Biology*, March 2011.
- [39] Ian J. Laurenzi. An analytical solution of the stochastic master equation for reversible bimolecular reaction kinetics. *The Journal of Chemical Physics*, 113(8):3315–3322, 2000.
- [40] Wenzhe Ma, Ala Trusina, Hana El-Samad, Wendell A. Lim, and Chao Tang. Defining network topologies that can achieve biochemical adaptation. *Cell*, 138(4):760–773, August 2009.
- [41] Shev MacNamara and Kevin Burrage. Krylov and steady-state techniques for the solution of the chemical master equation for the mitogen-activated protein kinase cascade. *Numerical Algorithms*, 51(3):281–307, July 2009.
- [42] R. J. Magnus and H. Neudecker. Matrix differential calculus with applications to simple, hadamard, and kronecker products. *Journal of Mathematical Psychology*, 29(4):474–492, December 1985.
- [43] Leonor Michaelis and Maud Menten. Die kinetik der inwertin wirkung. *Biochemische Zeitschrift*, (49):333–369, 1913.
- [44] H. J. Motulsky and L. C. Mahan. The kinetics of competitive radioligand binding predicted by the law of mass action. *Molecular Pharmacology*, 25(1):1–9, January 1984.
- [45] Brian Munsky and Mustafa Khammash. The finite state projection algorithm for the solution of the chemical master equation. *The Journal of Chemical Physics*, 124(4):044104+, January 2006.
- [46] J. D. Murray. *Mathematical Biology II*. Springer, 3rd edition, January 2003.
- [47] H. Neudecker. Some theorems on matrix differentiation with special reference to kronecker matrix products. *Journal of the American Statistical Association*, 64(327):953–963, 1969.
- [48] L. Noethen and S. Walcher. Quasi-steady state in the Michaelis-Menten system. *Non-linear Analysis: Real World Applications*, 8(5):1512–1535, December 2007.
- [49] B. Novak and J. J. Tyson. Numerical analysis of a comprehensive model of m-phase control in xenopus oocyte extracts and intact embryos. *Journal of cell science*, 106(4):1153–1168, December 1993.

- [50] Bela Novak, Zsuzsa Pataki, Andrea Ciliberto, and John J. Tyson. Mathematical model of the cell division cycle of fission yeast. *Chaos (Woodbury, N.Y.)*, 11(1):277–286, March 2001.
- [51] M. Pedersen, A. Bersani, E. Bersani, and G. Cortese. The total quasi-steady-state approximation for complex enzyme reactions. *Mathematics and Computers in Simulation*, 79(4):1010–1019, December 2008.
- [52] Morten Pedersen, Alberto Bersani, and Enrico Bersani. Quasi steady-state approximations in complex intracellular signal transduction networks - a word of caution. *Journal of Mathematical Chemistry*, 43(4):1318–1344, May 2008.
- [53] Slaven Peleš, Brian Munsky, and Mustafa Khammash. Reduction and solution of the chemical master equation using time scale separation and finite state projection. *The Journal of Chemical Physics*, 125(20):204104+, 2006.
- [54] Erik Plahte and Sissel Kjoglum. Analysis and generic properties of gene regulatory networks with graded response functions. *Physica D: Nonlinear Phenomena*, 201(1-2):150 – 176, 2005.
- [55] A. Polynikis, S. J. Hogan, and M. di Bernardo. Comparing different ODE modelling approaches for gene regulatory networks. *Journal of theoretical biology*, 261(4):511–530, December 2009.
- [56] Christopher V. Rao and Adam P. Arkin. Stochastic chemical kinetics and the quasi-steady-state assumption: Application to the gillespie algorithm. *The Journal of Chemical Physics*, 118(11):4999–5010, 2003.
- [57] William T. Reid. *Riccati Differential Equations*, volume 86 of *Mathematics in Science and Engineering*. Academic Press, New York-London, 1972.
- [58] W. E. Roth. On direct product matrices. *Bulletin of American Mathematical Society*, 40:461–468, 1934.
- [59] L. A. Segel and M. Slemrod. The quasi-steady state assumption: a case study in perturbation. *SIAM Review*, 31(3):446–477, 1989.
- [60] Lee Segel. On the validity of the steady state assumption of enzyme kinetics. *Bulletin of Mathematical Biology*, 50(6):579–593, November 1988.
- [61] Guy Shinar, Ron Milo, Maria R. Martinez, and Uri Alon. Input output robustness in simple bacterial signaling systems. *Proceedings of the National Academy of Sciences of the United States of America*, 104(50):19931–19935, December 2007.
- [62] Matthias Stiefenhofer. Quasi-steady-state approximation for chemical reaction networks. *Journal of Mathematical Biology*, 36(6):593–609, July 1998.

- [63] Y. Sykulev, R. J. Cohen, and H. N. Eisen. The law of mass action governs antigen-stimulated cytolytic activity of CD8+ cytotoxic t lymphocytes. *Proceedings of the National Academy of Sciences of the United States of America*, 92(26):11990–11992, December 1995.
- [64] J. J. Tyson, K. C. Chen, and B. Novak. Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell. *Current Opinion in Cell Biology*, 15(2):221–231, 2003.
- [65] A. R. Tzafriri. Michaelis menten kinetics at high enzyme concentrations. *Bulletin of Mathematical Biology*, 65(6):1111–1129, 2003.
- [66] A. R. Tzafriri and E. R. Edelman. The total quasi-steady-state approximation is valid for reversible enzyme kinetics. *Journal of theoretical biology*, 226(3):303–313, February 2004.
- [67] Donald D. Van Slyke and Glenn E. Cullen. The mode of action of urease and of enzymes in general. *Journal of Biological Chemistry*, 19(2):141–180, October 1914.
- [68] P. Waage and C. M. Gulberg. Studies concerning affinity. *C. M. Forhandling: Videnskabs-Selskabet i Christiana*, 35, 1864.
- [69] P. Waage and C. M. Gulberg. Studies concerning affinity (English translation). *Journal of Chemical Education*, 63(12):1044+, December 1986.
- [70] E. Weinan, Di Liu, and Eric V. Eijnden. Nested stochastic simulation algorithm for chemical kinetic systems with disparate rates. *The Journal of Chemical Physics*, 123(19):194107+, 2005.
- [71] Stephen Wiggins. *Normally Hyperbolic Invariant Manifolds in Dynamical Systems*. Springer-Verlag, 1994.