DYNAMICS AND ASYMPTOTIC BEHAVIORS OF BIOCHEMICAL NETWORKS

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ABSTRACT OF THE DISSERTATION

Dynamics and Asymptotic Behaviors of Biochemical Networks

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The purpose of this dissertation is to study the dynamics and asymptotic behaviors of biochemical networks using a "modular" approach. New mathematics is motivated and developed to analyze modules in terms of the number of steady states and the stability of the steady states.

One of the main contributions of the thesis is to extend Hirsch's generic convergence result from monotone systems to systems "close" to monotone using geometric singular perturbation theory. A monotone system is a dynamical system for which the comparison principle holds, that is, "bigger" initial states lead to "bigger" future states. In monotone systems, every net feedback loop is positive. On the other hand, negative feedback loops are important features of many systems, since they are required for adaptation and precision. We show that, provided that these negative loops act at a comparatively fast time scale, the generic convergence property still holds. This is an appealing result, which suggests that monotonicity has broader implications than previously thought. One particular application of great interest is that of double phosphorylation dephosphorylation cycles. Other recurring modules in biochemical networks are also analyzed in detail.

For systems without time scale separation, we study the global stability of one

special class of systems, called monotone tridiagonal systems with negative feedback. The key technique is to rule out periodic orbits using the theory of second compound matrices.

We also investigate the effect of diffusion on the stability of a constant steady state for systems with more general structures represented by graphs. This work extends the passivity-based stability criterion developed by Arcak and Sontag to reaction diffusion equations.

Upon assembling different modules, the dynamics of individual modules might be affected. One particular effect is called "retroactivity" in the systems biology literature. We propose designs and conditions under which the retroactivity can be attenuated.

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Dedication

To my parents Xianying Ma and Wansen Wang.

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Chapter 1

Introduction

A promising approach to handling the complexity of biochemical networks is to decompose networks into small modules. In the influential papers [34] by Hartwell *et al* and [50] by Lauffenburger, the authors point out the critical role of modules in biological organization. Subsequently, a wave of interesting work emerged regarding modularity in biochemical networks, see for instance, [2, 5, 69, 72, 77].

In a "bottom-up modularity" approach, one first identifies basic modules (motifs) in the network, then uses mathematical modeling to analyze these relatively simple motifs. Finally, the whole network can be set up by integrating these basic building blocks and studying their interconnections.

Following this approach, the present work relies upon a "graphical" way of analyzing individual modules as well as the interconnections among them.

In Chapter 2, we introduced such a module, which in biochemistry is sometimes called a "futile cycle". It involves two or more inter-convertible forms of one protein. The protein, denoted here by S_0, is ultimately converted into a product, denoted here by S_n, through a cascade of "activation" reactions triggered or facilitated by an enzyme E; conversely, S_n is transformed back (or "deactivated") into the original S_0, helped on by the action of a second enzyme F, see Figure 1.1.

Figure 1.1: A futile cycle of size n.

Futile cycles constitute a ubiquitous motif in cellular signaling pathways. Typically,

the enzymatic activation and de-activation are given by phosphorylation dephosphorylation reactions. One very important instance is that of Mitogen-Activated Protein Kinase (MAPK) cascades, which regulate primary cellular activities such as proliferation, differentiation, and apoptosis ([9, 14, 42, 100]) in eukaryotes from yeast to humans.

We set up a mathematical model based on mass action kinetics, and study the number of positive steady states of this system. We show analytically that

1. for some parameter ranges, there are at least $n+1$ (if n is even) or n (if n is odd) steady states;

2. there never are more than $2n-1$ steady states (in particular, this implies that for $n=2$, including single levels of MAPK cascades, there are at most three steady states);

3. for parameters near the standard Michaelis-Menten quasi-steady state conditions, there are at most $n+1$ steady states; and

4. for parameters far from the standard Michaelis-Menten quasi-steady state conditions, there is at most one steady state.

Chapter 3 is motivated by studying the dynamics and stability of a double phosphorylation dephosphorylation futile cycle. When the amount of enzymes E and F is negligible compared to the amount of the total substrate S, the system is strongly monotone. In a strongly monotone system, every net feedback loop is positive. The resulting dynamics is relatively simple. Hirsch's Generic Convergence Theorem for monotone system ([36, 37, 38, 39, 82]) says that almost every bounded solution of a strongly monotone system converges to a set of equilibria.

The futile cycle example as well as many systems in biology are not monotone, at least with respect to any standard orthant order. Negative feedback is required for adaptation and precision. However, we show in Chapter 3 that if the negative feedback acts on a sufficiently fast time scale, the most important dynamical property of strongly monotone systems – convergence to steady states – is preserved. Geometric singular

perturbation is the major tool used in the proofs. Several applications are worked out at the end of this chapter.

Chapter 4 deals with negative feedback without the assumption of time scale separation. We focus on global asymptotic stability of monotone tridiagonal systems with negative feedback. Monotone tridiagonal systems possess the Poincaré-Bendixson property, which implies that, if orbits are bounded, if there is a unique steady state and this unique steady state is asymptotically stable, and if one can rule out periodic orbits, then the steady state is globally asymptotically stable.

To rule out periodic orbits, we use the theory of second additive compound matrices. Sanchez in [75] studied the special case of cyclic systems. Our results treat a more general class of systems which includes cyclic systems. A specific and classical instance of tridiagonal systems with negative feedback is the Goldbeter model for circadian oscillations in the *Drosophila* PER ("period") protein ([30]). We apply our main theoretical results to this model and provide conditions under which the unique equilibrium is globally asymptotically stable.

Chapter 5 studies global asymptotic stability for more general interconnected systems with positive and negative feedback. The connections in such systems are represented by graphs, which are not restricted to the tridiagonal case. We present a stability test for a class of such systems inspired by the work of Arcak and Sontag in [8] and Jovanović, Arcak, and Sontag in [46].

In their novel work [8], Arcak and Sontag developed a passivity-based stability criterion for general interconnected systems in a "well-mixed" environment (no diffusion), under the assumption that a unique equilibrium exists. Jovanović, Arcak, and Sontag studied the effect of diffusion in [46] but only for the special case of cyclic graphs. Chapter 5 extends the result in [46] so as to encompass the broader class of interconnected systems. By constructing a composite convex Lyapunov function, we prove that the unique equilibrium of the ordinary differential equations is globally asymptotically stable when diffusion is added to the system. The interconnection structure of the network and the signs of the interconnection terms are incorporated into a *dissipativity matrix*, which is crucial in constructing the Lyapunov function.

Chapter 6 focuses on the interconnections among different modules. In general, when two modules are connected, the dynamics of the individual module might be affected. If we define the upstream system and downstream system in the direction of which the signal is traveling, the effect from downstream to upstream will be called "retroactivity". We discuss in Chapter 6 conditions under which the retroactivity can be attenuated. The results here extend those by Del Vecchio, Ninfa, and Sontag in [94] to a more general class of interconnected systems and fill in mathematical details using singular perturbation arguments.

The present thesis is based on the author's papers listed below:

- The material in Section 2.2 was published in the Journal of Mathematical Biology [98] in joint with Eduardo Sontag.
- A preliminary version of Chapter 3 is published in Proceedings of the second Multidisciplinary International Symposium on Positive Systems: Theory and Applications [97]. More general results are published in the Journal of Nonlinear Science [99]. Both of them are collaborated with Eduardo Sontag.
- The material in Chapter 4 is part of a joint paper [96] coauthored with Patrick de Leenheer and Eduardo Sontag accepted to the 2008 IEEE conference on Decision and Control.
- Chapter 5 is a paper in preparation for submission joint with Eduardo Sontag and Murat Arcak.
- Chapter 6 is also a paper that is being prepared for submission coauthored with Domitilla Del Vecchio and Eduardo Sontag.

Chapter 2

Multistationarity in Biochemical Networks

2.1 Introduction

One of the ideas to understand the complexity in biochemical networks is to decompose them into subunits that describe processes arising frequently in the networks. The smaller subunits are often simpler to analyze, and provide insights to properties of the whole network.

One aspect of studying the asymptotic behavior of subunits is to examine their numbers of steady states. A system with more than one steady state is said to be multistationary, and different levels of multistationarity can be regarded as the system's capacity for information storage.

Multistationarity and multistability have attracted much attention in recent years, see, for instance, [4, 5, 28, 67, 78, 79, 97, 99]. In this chapter, we use different approaches to study several ubiquitous motifs in biochemical networks. The first motif is called "futile cycle". We not only show the existence of multistationarity in this motif, but also provide lower and upper bounds for the number of positive steady states. The second motif is also a "futile cycle" but with different mechanism. We show that instead of multistationarity this motif can only support a unique positive steady state regardless of the values of the kinetic parameters involved. The last example is a system which admits a unique steady state in the deterministic model, while exhibits bistability in the stochastic model.

2.2 "Futile Cycle" Motifs

2.2.1 Introduction

One particular motif that has attracted much attention in recent years is the cycle formed by two or more inter-convertible forms of one protein. The protein, denoted here by S_0, is ultimately converted into a product, denoted here by S_n, through a cascade of "activation" reactions triggered or facilitated by an enzyme E; conversely, S_n is transformed back (or "deactivated") into the original S_0, helped on by the action of a second enzyme F. See Figure 1.1.

Such structures, often called "futile cycles" (also called substrate cycles, enzymatic cycles, or enzymatic inter-conversions, see [74]), serve as basic blocks in cellular signaling pathways and have pivotal impact on the signaling dynamics. Futile cycles underlie signaling processes such as guanosine triphosphate cycles [20], bacterial two-component systems and phosphorelays [11, 32], actin treadmilling [15], and glucose mobilization [47], as well as metabolic control [86] and cell division and apoptosis [87] and cell-cycle checkpoint control [52]. One very important instance is that of Mitogen-Activated Protein Kinase (MAPK) cascades, which regulate primary cellular activities such as proliferation, differentiation, and apoptosis [9, 14, 42, 100] in eukaryotes from yeast to humans.

MAPK cascades usually consist of three tiers of similar structures with multiple feedbacks [13, 25, 102], see Figure 2.1. Each individual level of the MAPK cascades is a futile cycle as depicted in Figure 1.1 with $n = 2$, see also Figure 2.2.

The paper [58] by Markevich, Hoek, and Kholodenko is the first to demonstrate the possibility of multistationarity at a single cascade level, and motivated the need for analytical studies of the number of steady states. Conradi *et al.* studied the existence of multistationarity in their paper [18], employing algorithms based on Feinberg's chemical reaction network theory (CRNT). (For details on CRNT, see [22, 23].) The CRNT algorithm confirms multistationarity in a single level of MAPK cascades, and provides a set of kinetic constants which can give rise to multistationarity. However, the CRNT algorithm does not provide information regarding the precise number of steady states

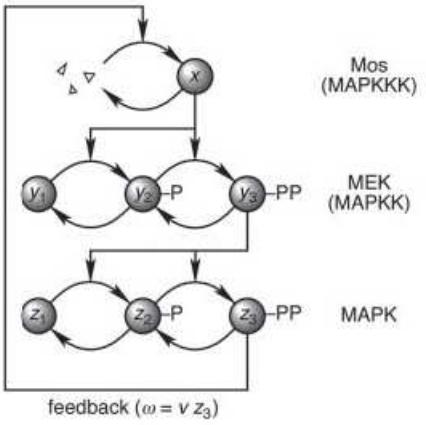

Figure 2.1: This is a schematic view of the MAPK cascades from the paper [5] by Angeli, Ferrell, and Sontag. In this picture, z_1, z_2, and z_3 (y_1, y_2, and y_3) stand for the unphosphorylated, monophosphorylated, and double phosphorylated forms of MAPK (MAPKK), respectively; x represents the protein MAPKKK.

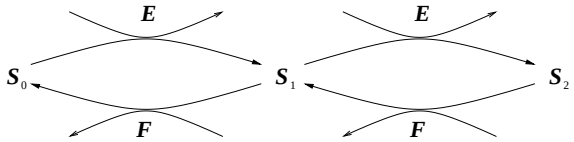

Figure 2.2: A futile cycle of size two.

and how the number varies with the change of parameters.

In [33], Gunawardena proposed a novel approach to the study of steady states of futile cycles. His approach, which was focused in the question of determining the proportion of maximally phosphorylated substrate, was developed under the simplifying quasi-steady state assumption that substrate is in excess. Nonetheless, our study of multistationarity uses in a key manner the basic formalism in [33], even for the case when substrate is not in excess.

In Section 2.2.2, we state our basic assumptions regarding the model. The basic formalism and background for the approach are given in Section 2.2.3. The main focus is on Section 2.2.4, where we derive various bounds on the number of steady states of a futile cycle of size n. The first result is a lower bound for the number of steady states. Currently available results on lower bounds, as in [90], can only handle the case when quasi-steady state assumptions are valid; we substantially extend these results to the

fully general case by means of a perturbation argument which allows one to get around these restricted assumptions. Another novel feature of our results is the derivation of an upper bound of $2n-1$, valid for all kinetic constants. Models in molecular cell biology are characterized by a high degree of uncertainly in parameters, hence such results valid over the entire parameter space are of special significance. However, when more information on the parameters is available, we have sharper upper bounds, see Theorems 2.7 and 2.8. A summary of our results can be found in Section 2.2.5.

We remark that the results here do not address the stability of the steady states. However, we see from simulations that the stable and unstable steady states tend to alternate if ranked by the ratio of their steady state concentrations of the kinase and the phosphatase. Complementary work dealing with the *dynamical* behavior of futile cycles of size two is studied in [4] and Section 3.5.2. In Section 3.5.2, we will show that the system exhibits generic convergence to steady states but no more complicated behavior, at least within restricted parameter ranges, while [4] showed a persistence property (no species tends to be eliminated) for any possible parameter values. See [7] for a global convergence result in the single-phosphorylation case.

2.2.2 Model assumptions

Before presenting mathematical details, let us first discuss the basic biochemical assumptions that go into the model. In general, phosphorylation and dephosphorylation can follow either a distributive or a processive mechanism. In the processive mechanism, the kinase (phosphatase) facilitates two or more phosphorylations (dephosphorylations) before the final product is released, whereas in the distributive mechanism, the kinase (phosphatase) facilitates at most one phosphorylation (dephosphorylation) in each molecular encounter. In the case of $n=2$, a futile cycle that follows the processive mechanism can be represented by reactions as follows:

$$S_0 + E \longleftrightarrow ES_0 \longleftrightarrow ES_1 \longrightarrow S_2 + E$$

$$S_2 + F \longleftrightarrow FS_2 \longleftrightarrow FS_1 \longrightarrow S_0 + F;$$

and the distributive mechanism can be represented by reactions:

$$S_0 + E \longleftrightarrow ES_0 \longrightarrow S_1 + E \longleftrightarrow ES_1 \longrightarrow S_2 + E$$

$$S_2 + F \longleftrightarrow FS_2 \longrightarrow S_1 + F \longleftrightarrow FS_1 \longrightarrow S_0 + F.$$

Biological experiments have demonstrated that both dual phosphorylation and dephosphorylation in MAPK are distributive, see [13, 25, 102]. In their paper [18], Conradi *et al.* showed mathematically that if either phosphorylation or dephosphorylation follows a processive mechanism, then the steady state will be unique, which, it is argued in [18], contradicts experimental observations. We therefore assume that both phosphorylations and dephosphorylations in the futile cycles follow the distributive mechanism.

Our structure of futile cycles in Figure 1.1 also implicitly assumes a sequential instead of a random mechanism. By a sequential mechanism, we mean that the kinase phosphorylates the substrates in a specific order, and the phosphatase works in the reversed order.

A few kinases are known to be sequential, for example, the auto-phosphorylation of FGF-receptor-1 kinase [27]. This assumption dramatically reduces the number of different phospho-forms and simplifies our analysis. In a special case when the kinetic constants of each phosphorylation are the same and the kinetic constants of each dephosphorylation are the same, the random mechanism can be easily included in the sequential case.

To model the reactions, we assume mass action kinetics, which is standard in mathematical modeling of molecular events in biology.

2.2.3 Mathematical formalism

Let us set up a mathematical framework for studying the steady states of a futile cycle of size n. We first write down all the elementary chemical reactions in Figure 1.1:

$$\begin{aligned}
S_0 + E &\underset{k_{\mathrm{off}_0}}{\overset{k_{\mathrm{on}_0}}{\rightleftarrows}} ES_0 \overset{k_{\mathrm{cat}_0}}{\rightarrow} S_1 + E \\
&\vdots \\
S_{n-1} + E &\underset{k_{\mathrm{off}_{n-1}}}{\overset{k_{\mathrm{on}_{n-1}}}{\rightleftarrows}} ES_{n-1} \overset{k_{\mathrm{cat}_{n-1}}}{\rightarrow} S_n + E \\
S_1 + F &\underset{l_{\mathrm{off}_0}}{\overset{l_{\mathrm{on}_0}}{\rightleftarrows}} FS_1 \overset{l_{\mathrm{cat}_0}}{\rightarrow} S_0 + F \\
&\vdots \\
S_n + F &\underset{l_{\mathrm{off}_{n-1}}}{\overset{l_{\mathrm{on}_{n-1}}}{\rightleftarrows}} FS_n \overset{l_{\mathrm{cat}_{n-1}}}{\rightarrow} S_{n-1} + F
\end{aligned}$$

where k_{on_0}, etc., are kinetic parameters for binding and unbinding, ES_0 denotes the complex consisting of the enzyme E and the substrate S_0, and so forth. These reactions can be modeled by $3n + 3$ differential-algebraic equations according to mass action kinetics:

$$\begin{aligned}
\frac{ds_0}{dt} &= -k_{\mathrm{on}_0} s_0 e + k_{\mathrm{off}_0} c_0 + l_{\mathrm{cat}_0} d_1, \\
\frac{ds_i}{dt} &= -k_{\mathrm{on}_i} s_i e + k_{\mathrm{off}_i} c_i + k_{\mathrm{cat}_{i-1}} c_{i-1} - l_{\mathrm{on}_{i-1}} s_i f + l_{\mathrm{off}_{i-1}} d_i + l_{\mathrm{cat}_i} d_{i+1}, \qquad (2.1) \\
&\qquad\qquad i = 1, \ldots, n-1, \\
\frac{dc_j}{dt} &= k_{\mathrm{on}_j} s_j e - (k_{\mathrm{off}_j} + k_{\mathrm{cat}_j}) c_j, \quad j = 0, \ldots, n-1, \\
\frac{dd_k}{dt} &= l_{\mathrm{on}_{k-1}} s_k f - (l_{\mathrm{off}_{k-1}} + l_{\mathrm{cat}_{k-1}}) d_k, \quad k = 1, \ldots, n,
\end{aligned}$$

together with the algebraic "conservation equations":

$$\begin{aligned} E_{\text{tot}} &= e + \sum_0^{n-1} c_i, \\ F_{\text{tot}} &= f + \sum_1^{n} d_i, \\ S_{\text{tot}} &= \sum_0^{n} s_i + \sum_0^{n-1} c_i + \sum_1^{n} d_i. \end{aligned} \tag{2.2}$$

The variables $s_0, \ldots, s_n, c_0, \ldots, c_{n-1}, d_1, \ldots, d_n, e, f$ stand for the concentrations of

$$S_0, \ldots, S_n, ES_0, \ldots, ES_{n-1}, FS_1, \ldots, FS_n, E, F$$

respectively. For each positive vector

$$\begin{aligned} \kappa =&(k_{\text{on}_0}, \ldots, k_{\text{on}_{n-1}}, k_{\text{off}_0}, \ldots, k_{\text{off}_{n-1}}, k_{\text{cat}_0}, \ldots, k_{\text{cat}_{n-1}}, \\ & l_{\text{on}_0}, \ldots, l_{\text{on}_{n-1}}, l_{\text{off}_0}, \ldots, l_{\text{off}_{n-1}}, l_{\text{cat}_0}, \ldots, l_{\text{cat}_{n-1}}) \in \mathbb{R}_+^{6n} \end{aligned}$$

(of "kinetic constants") and each positive triple $\mathcal{C} = (E_{\text{tot}}, F_{\text{tot}}, S_{\text{tot}})$, we have a different system $\Sigma(\kappa, \mathcal{C})$.

Let us write the coordinates of a vector $x \in \mathbb{R}_+^{3n+3}$ as:

$$x = (s_0, \ldots, s_n, c_0, \ldots, c_{n-1}, d_1, \ldots, d_n, e, f),$$

and define a mapping

$$\Phi : \mathbb{R}_+^{3n+3} \times \mathbb{R}_+^{6n} \times \mathbb{R}_+^{3} \longrightarrow \mathbb{R}^{3n+3}$$

with components $\Phi_1, \ldots, \Phi_{3n+3}$ where the first $3n$ components are

$$\Phi_1(x, \kappa, \mathcal{C}) = -k_{\text{on}_0} s_0 e + k_{\text{off}_0} c_0 + l_{\text{cat}_0} d_1,$$

and so forth, listing the right hand sides of the equations (2.1), Φ_{3n+1} is

$$e + \sum_0^{n-1} c_i - E_{\text{tot}},$$

and similarly for Φ_{3n+2} and Φ_{3n+3}, we use the remaining equations in (2.2).

For each $\kappa, \mathcal{C}$, let us define a set

$$\mathcal{Z}(\kappa, \mathcal{C}) = \{x \,|\, \Phi(x, \kappa, \mathcal{C}) = 0\}.$$

Observe that, by definition, given $x \in \mathbb{R}_+^{3n+3}$, x is a positive steady state of $\Sigma(\kappa, \mathcal{C})$ if and only if $x \in \mathcal{Z}(\kappa, \mathcal{C})$. So, the mathematical statement of the central problem is to count the number of elements in $\mathcal{Z}(\kappa, \mathcal{C})$. Our analysis will be greatly simplified by the following preprocessing. Let us introduce a function

$$\Psi : \mathbb{R}_+^{3n+3} \times \mathbb{R}_+^{6n} \times \mathbb{R}_+^3 \longrightarrow \mathbb{R}^{3n+3}$$

with components $\Psi_1, \ldots, \Psi_{3n+3}$ defined as

$$\begin{aligned}
\Psi_1 &= \Phi_1 + \Phi_{n+1}, \\
\Psi_i &= \Phi_i + \Phi_{n+i} + \Phi_{2n+i-1} + \Psi_{i-1}, \quad i = 2, \ldots, n, \\
\Psi_j &= \Phi_j, \quad j = n+1, \ldots, 3n+3.
\end{aligned}$$

It is easy to see that

$$\mathcal{Z}(\kappa, \mathcal{C}) = \{x \,|\, \Psi(x, \kappa, \mathcal{C}) = 0\},$$

but now the first $3n$ equations are

$$\begin{aligned}
\Psi_i &= l_{\mathrm{cat}_{i-1}} d_i - k_{\mathrm{cat}_{i-1}} c_{i-1} = 0, \quad i = 1, \ldots, n, \\
\Psi_{n+1+j} &= k_{\mathrm{on}_j} s_j e - (k_{\mathrm{off}_j} + k_{\mathrm{cat}_j}) c_j = 0, \quad j = 0, \ldots, n-1, \\
\Psi_{2n+k} &= l_{\mathrm{on}_{k-1}} s_k f - (l_{\mathrm{off}_{k-1}} + l_{\mathrm{cat}_{k-1}}) d_k = 0, \quad k = 1, \ldots, n,
\end{aligned}$$

and can be easily solved as

$$s_{i+1} = \lambda_i (e/f) s_i, \tag{2.3}$$

$$c_i = \frac{e s_i}{K_{M_i}}, \tag{2.4}$$

$$d_{i+1} = \frac{f s_{i+1}}{L_{M_i}}, \tag{2.5}$$

where

$$\lambda_i = \frac{k_{\mathrm{cat}_i} L_{M_i}}{K_{M_i} l_{\mathrm{cat}_i}}, \quad K_{M_i} = \frac{k_{\mathrm{cat}_i} + k_{\mathrm{off}_i}}{k_{\mathrm{on}_i}}, \quad L_{M_i} = \frac{l_{\mathrm{cat}_i} + l_{\mathrm{off}_i}}{l_{\mathrm{on}_i}}, \quad i = 0, \ldots, n-1. \tag{2.6}$$

For each κ, we introduce three functions $\varphi_0^\kappa, \varphi_1^\kappa, \varphi_2^\kappa : \mathbb{R}_+ \longrightarrow \mathbb{R}_+$ as follows,

$$\begin{aligned}
\varphi_0^\kappa(u) &= 1 + \lambda_0 u + \lambda_0 \lambda_1 u^2 + \cdots + \lambda_0 \cdots \lambda_{n-1} u^n, \\
\varphi_1^\kappa(u) &= \frac{1}{K_{M_0}} + \frac{\lambda_0}{K_{M_1}} u + \cdots + \frac{\lambda_0 \cdots \lambda_{n-2}}{K_{M_{n-1}}} u^{n-1}, \\
\varphi_2^\kappa(u) &= \frac{\lambda_0}{L_{M_0}} u + \frac{\lambda_0 \lambda_1}{L_{M_1}} u^2 + \cdots + \frac{\lambda_0 \cdots \lambda_{n-1}}{L_{M_{n-1}}} u^n.
\end{aligned}$$

We may now write

$$\sum_0^n s_i = s_0\left(1+\lambda_0\left(\frac{e}{f}\right)+\lambda_0\lambda_1\left(\frac{e}{f}\right)^2+\cdots+\lambda_0\cdots\lambda_{n-1}\left(\frac{e}{f}\right)^n\right) = s_0\varphi_0^\kappa\left(\frac{e}{f}\right),$$
$$\sum_0^{n-1} c_i = es_0\left(\frac{1}{K_{M_0}}+\frac{\lambda_0}{K_{M_1}}\left(\frac{e}{f}\right)+\cdots+\frac{\lambda_0\cdots\lambda_{n-2}}{K_{M_{n-1}}}\left(\frac{e}{f}\right)^{n-1}\right) = es_0\varphi_1^\kappa\left(\frac{e}{f}\right), \quad (2.7)$$
$$\sum_1^n d_i = fs_0\left(\frac{\lambda_0}{L_{M_0}}\left(\frac{e}{f}\right)+\frac{\lambda_0\lambda_1}{L_{M_1}}\left(\frac{e}{f}\right)^2+\cdots+\frac{\lambda_0\cdots\lambda_{n-1}}{L_{M_{n-1}}}\left(\frac{e}{f}\right)^n\right) = fs_0\varphi_2^\kappa\left(\frac{e}{f}\right).$$

Although the equation $\Psi = 0$ represents $3n+3$ equations with $3n+3$ unknowns, next we will show that it can be reduced to two equations with two unknowns, which have the same number of positive solutions as $\Psi = 0$. Let us first define a set

$$\mathcal{S}(\kappa,\mathcal{C}) = \{(u,v)\in\mathbb{R}_+\times\mathbb{R}_+ \,|\, G_1^{\kappa,\mathcal{C}}(u,v)=0, G_2^{\kappa,\mathcal{C}}(u,v)=0\},$$

where $G_1^{\kappa,\mathcal{C}}, G_2^{\kappa,\mathcal{C}}:\mathbb{R}_+^2\longrightarrow\mathbb{R}$ are given by

$$G_1^{\kappa,\mathcal{C}}(u,v) = v\left(u\varphi_1^\kappa(u)-\varphi_2^\kappa(u)E_{\rm tot}/F_{\rm tot}\right)-E_{\rm tot}/F_{\rm tot}+u,$$
$$G_2^{\kappa,\mathcal{C}}(u,v) = \varphi_0^\kappa(u)\varphi_2^\kappa(u)v^2+\left(\varphi_0^\kappa(u)-S_{\rm tot}\varphi_2^\kappa(u)+F_{\rm tot}u\varphi_1^\kappa(u)+F_{\rm tot}\varphi_2^\kappa(u)\right)v-S_{\rm tot}.$$

The precise statement is as follows.

Lemma 2.1 *There exists a mapping $\delta:\mathbb{R}^{3n+3}\longrightarrow\mathbb{R}^2$ such that, for each $\kappa,\mathcal{C}$, the map δ restricted to $\mathcal{Z}(\kappa,\mathcal{C})$ is a bijection between the sets $\mathcal{Z}(\kappa,\mathcal{C})$ and $\mathcal{S}(\kappa,\mathcal{C})$.*

Proof. Let us define the mapping $\delta:\mathbb{R}^{3n+3}\longrightarrow\mathbb{R}^2$ as $\delta(x)=(e/f,s_0)$, where

$$x = (s_0,\ldots,s_n,c_0,\ldots,c_{n-1},d_1,\ldots,d_n,e,f).$$

If we can show that δ induces a bijection between $\mathcal{Z}(\kappa,\mathcal{C})$ and $\mathcal{S}(\kappa,\mathcal{C})$, we are done.

First, we claim that $\delta(\mathcal{Z}(\kappa,\mathcal{C}))\subseteq\mathcal{S}(\kappa,\mathcal{C})$. Pick any $x\in\mathcal{Z}(\kappa,\mathcal{C})$, we have that x satisfies (2.3)-(2.5). Moreover, $\Phi_{3n+2}(x,\kappa,\mathcal{C})=0$ yields

$$E_{\rm tot} = e+es_0\varphi_1^\kappa(\frac{e}{f}),$$

and thus

$$e = \frac{E_{\rm tot}}{1+s_0\varphi_1^\kappa(e/f)}. \quad (2.8)$$

Using $\Phi_{3n+1}(x,\kappa,\mathcal{C})=0$ and $\Phi_{3n+2}(x,\kappa,\mathcal{C})=0$, we get

$$\frac{E_{\text{tot}}}{F_{\text{tot}}}=\frac{e(1+s_0\varphi_1^\kappa(e/f))}{f(1+s_0\varphi_2^\kappa(e/f))}, \tag{2.9}$$

which is $G_1^{\kappa,\mathcal{C}}(e/f,s_0)=0$ after multiplying by $1+s_0\varphi_2^\kappa(e/f)$ and rearranging terms.

To check that $G_2^{\kappa,\mathcal{C}}(e/f,s_0)=0$, we start with $\Phi_{3n+3}(x,\kappa,\mathcal{C})=0$, i.e.

$$S_{\text{tot}}=\sum_0^n s_i+\sum_0^{n-1}c_i+\sum_1^n d_i.$$

Using (2.7) and (2.8), this expression becomes

$$\begin{aligned}S_{\text{tot}}&=s_0\varphi_0^\kappa(\frac{e}{f})+\frac{E_{\text{tot}}s_0\varphi_1^\kappa(e/f)}{1+s_0\varphi_1^\kappa(e/f)}+\frac{F_{\text{tot}}s_0\varphi_2^\kappa(e/f)}{1+s_0\varphi_2^\kappa(e/f)}\\&=s_0\varphi_0^\kappa(\frac{e}{f})+\frac{eF_{\text{tot}}s_0\varphi_1^\kappa(e/f)}{f(1+s_0\varphi_2^\kappa(e/f))}+\frac{F_{\text{tot}}s_0\varphi_2^\kappa(e/f)}{1+s_0\varphi_2^\kappa(e/f)},\end{aligned}$$

where the last equality comes from (2.9).

After multiplying by $1+s_0\varphi_2^\kappa(e/f)$, and simplifying, we get

$$\varphi_0^\kappa(\frac{e}{f})\varphi_2^\kappa(\frac{e}{f})s_0^2+\left(\varphi_0^\kappa(\frac{e}{f})-S_{\text{tot}}\varphi_2^\kappa(\frac{e}{f})+\frac{e}{f}F_{\text{tot}}\varphi_1^\kappa(\frac{e}{f})+F_{\text{tot}}\varphi_2^\kappa(u)\right)s_0-S_{\text{tot}}=0,$$

that is, $G_2^{\kappa,\mathcal{C}}(e/f,s_0)=0$. since both $G_1^{\kappa,\mathcal{C}}(e/f,s_0)$ and $G_2^{\kappa,\mathcal{C}}(e/f,s_0)$ are zero, $\delta(x)\in\mathcal{S}(\kappa,\mathcal{C})$.

Next, we will show that $\mathcal{S}(\kappa,\mathcal{C})\subseteq\delta(\mathcal{Z}(\kappa,\mathcal{C}))$. For any $y=(u,v)\in\mathcal{S}(\kappa,\mathcal{C})$, let the coordinates of x be defined as:

$$s_0=v,\quad s_{i+1}=\lambda_i u s_i,\quad e=\frac{E_{\text{tot}}}{1+s_0\varphi_1^\kappa(u)},\quad f=\frac{e}{u},\quad c_i=\frac{es_i}{K_{M_i}},\quad d_{i+1}=\frac{fs_{i+1}}{L_{M_i}},$$

for $i=0,\dots,n-1$. It is easy to see that the vector

$$x=(s_0,\dots,s_n,c_0,\dots,c_{n-1},d_1,\dots,d_n,e,f)$$

satisfies $\Phi_1(x,\kappa,\mathcal{C})=0,\dots,\Phi_{3n+1}(x,\kappa,\mathcal{C})=0$. If $\Phi_{3n+2}(x,\kappa,\mathcal{C})$ and $\Phi_{3n+3}(x,\kappa,\mathcal{C})$ are also zero, then x is an element of $\mathcal{Z}(\kappa,\mathcal{C})$ with $\delta(x)=y$. Given the condition that $G_i^{\kappa,\mathcal{C}}(u,v)=0$ $(i=1,2)$ and $u=e/f, v=s_0$, we have $G_1^{\kappa,\mathcal{C}}(e/f,s_0)=0$, and therefore (2.9) holds. Since

$$e=\frac{E_{\text{tot}}}{1+s_0\varphi_1^\kappa(e/f)}$$

in our construction, we have

$$F_{\text{tot}} = f(1 + s_0\varphi_2^\kappa(e/f)) = f + \sum_1^n d_i.$$

To check $\Phi_{3n+3}(x, \kappa, \mathcal{C}) = 0$, we use

$$\frac{G_2^{\kappa,\mathcal{C}}(e/f, s_0)}{1 + s_0\varphi_2^\kappa(e/f)} = 0,$$

as $G_2^{\kappa,\mathcal{C}}(e/f, s_0) = 0$ and $1 + s_0\varphi_2^\kappa(e/f) > 0$. Applying (2.7)-(2.9), we have

$$\sum_0^n s_i + \sum_0^{n-1} c_i + \sum_1^n d_i = s_0\varphi_0^\kappa(e/f) + \frac{eF_{\text{tot}}s_0\varphi_1^\kappa(e/f)}{f(1 + s_0\varphi_2^\kappa(e/f))} + \frac{F_{\text{tot}}s_0\varphi_2^\kappa(e/f)}{1 + s_0\varphi_2^\kappa(e/f)} = S_{\text{tot}}.$$

It remains for us to show that the map δ is one to one on $\mathcal{Z}(\kappa, \mathcal{C})$. Suppose that $\delta(x^1) = \delta(x^2) = (u, v)$, where

$$x^i = (s_0^i, \ldots, s_n^i, c_0^i, \ldots, c_{n-1}^i, d_1^i, \ldots, d_n^i, e^i, f^i), \quad i = 1, 2.$$

By the definition of δ, we know that $s_0^1 = s_0^2$ and $e^1/f^1 = e^2/f^2$. Therefore, $s_i^1 = s_i^2$ for $i = 0, \ldots, n$. Equation (2.8) gives

$$e^1 = \frac{E_{\text{tot}}}{1 + v\varphi_1^\kappa(u)} = e^2.$$

Thus, $f^1 = f^2$, and $c_i^1 = c_i^2, d_{i+1}^1 = d_{i+1}^2$ for $i = 0, \ldots, n-1$ because of (2.3)-(2.5). Therefore, $x^1 = x^2$, and δ is one to one. ∎

The above lemma ensures that the two sets $\mathcal{Z}(\kappa, \mathcal{C})$ and $\mathcal{S}(\kappa, \mathcal{C})$ have the same number of elements. From now on, we will focus on $\mathcal{S}(\kappa, \mathcal{C})$, the set of positive solutions of equations $G_1^{\kappa,\mathcal{C}}(u, v) = 0, G_2^{\kappa,\mathcal{C}}(u, v) = 0$, i.e.

$$G_1^{\kappa,\mathcal{C}}(u, v) = v\left(u\varphi_1^\kappa(u) - \varphi_2^\kappa(u)E_{\text{tot}}/F_{\text{tot}}\right) - E_{\text{tot}}/F_{\text{tot}} + u = 0, \tag{2.10}$$

$$\begin{aligned} G_2^{\kappa,\mathcal{C}}(u, v) = &\left(\varphi_0^\kappa(u) - S_{\text{tot}}\varphi_2^\kappa(u) + F_{\text{tot}}u\varphi_1^\kappa(u) + F_{\text{tot}}\varphi_2^\kappa(u)\right)v \\ &+ \varphi_0^\kappa(u)\varphi_2^\kappa(u)v^2 - S_{\text{tot}} = 0. \end{aligned} \tag{2.11}$$

2.2.4 Number of positive steady states

Lower bound on the number of positive steady states

One approach to solving (2.10)-(2.11) is to view $G_2^{\kappa,\mathcal{C}}(u, v)$ as a quadratic polynomial in v. Since $G_2^{\kappa,\mathcal{C}}(u, 0) < 0$, equation (2.11) has a unique positive root, namely

$$v = \frac{-H^{\kappa,\mathcal{C}}(u) + \sqrt{H^{\kappa,\mathcal{C}}(u)^2 + 4S_{\text{tot}}\varphi_0^\kappa(u)\varphi_2^\kappa(u)}}{2\varphi_0^\kappa(u)\varphi_2^\kappa(u)}, \tag{2.12}$$

where

$$H^{\kappa,\mathcal{C}}(u) = \varphi_0^\kappa(u) - S_{\rm tot}\varphi_2^\kappa(u) + F_{\rm tot}u\varphi_1^\kappa(u) + F_{\rm tot}\varphi_2^\kappa(u). \tag{2.13}$$

Substituting this expression for v into (2.10), and multiplying by $\varphi_0^\kappa(u)$, we get

$$\begin{aligned} F^{\kappa,\mathcal{C}}(u) := & \frac{-\tilde{H}^{\kappa,\mathcal{C}}(u) + \sqrt{\tilde{H}^{\kappa,\mathcal{C}}(u)^2 + 4S_{\rm tot}\varphi_0^\kappa(u)\varphi_2^\kappa(u)}}{2\varphi_2^\kappa(u)} \left(u\varphi_1^\kappa(u) - \frac{E_{\rm tot}}{F_{\rm tot}}\varphi_2^\kappa(u)\right) \\ & - \frac{E_{\rm tot}}{F_{\rm tot}}\varphi_0^\kappa(u) + u\varphi_0^\kappa(u) = 0. \end{aligned} \tag{2.14}$$

So, any $(u,v) \in \mathcal{S}(\kappa,\mathcal{C})$ should satisfy (2.12) and (2.14). On the other hand, any positive solution u of (2.14) (notice that $\varphi_0^\kappa(u) > 0$) and v given by (2.12) (always positive) provide a positive solution of (2.10)-(2.11), that is, (u,v) is an element in $\mathcal{S}(\kappa,\mathcal{C})$. Therefore, the number of positive solutions of (2.10)-(2.11) is the same as the number of positive solutions of (2.12) and (2.14). But v is uniquely determined by u in (2.12), which further simplifies the problem to one equation (2.14) with one unknown u. Based on this observation, we have the following theorem.

Theorem 2.2 *For each positive numbers S_{tot}, γ, there exist $\varepsilon_0 > 0$ and $\kappa \in \mathbb{R}_+^{6n}$ such that the following property holds. Pick any E_{tot}, F_{tot} such that*

$$F_{tot} = E_{tot}/\gamma < \varepsilon_0 S_{tot}/\gamma, \tag{2.15}$$

then the system $\Sigma(\kappa,\mathcal{C})$ with $\mathcal{C} = (E_{tot}, F_{tot}, S_{tot})$ has at least $n+1$ (n) positive steady states when n is even (odd).

Proof. For each $\kappa, \gamma, S_{\rm tot}$, let us define two functions $\mathbb{R}_+ \times \mathbb{R}_+ \longrightarrow \mathbb{R}$ as follows:

$$\begin{aligned} \tilde{H}^{\kappa,\gamma,S_{\rm tot}}(\varepsilon,u) &= H^{\kappa,(\varepsilon S_{\rm tot}, \varepsilon S_{\rm tot}/\gamma, S_{\rm tot})}(u) \\ &= \varphi_0^\kappa(u) - S_{\rm tot}\varphi_2^\kappa(u) + \varepsilon\frac{S_{\rm tot}}{\gamma}u\varphi_1^\kappa(u) + \varepsilon\frac{S_{\rm tot}}{\gamma}\varphi_2^\kappa(u), \end{aligned} \tag{2.16}$$

and

$$\begin{aligned} \tilde{F}^{\kappa,\gamma,S_{\rm tot}}(\varepsilon,u) &= F^{\kappa,(\varepsilon S_{\rm tot}, \varepsilon S_{\rm tot}/\gamma, S_{\rm tot})}(u) \\ &= \frac{-\tilde{H}^{\kappa,\gamma,S_{\rm tot}}(\varepsilon,u) + \sqrt{\tilde{H}^{\kappa,\gamma,S_{\rm tot}}(\varepsilon,u)^2 + 4S_{\rm tot}\varphi_0^\kappa(u)\varphi_2^\kappa(u)}}{2\varphi_2^\kappa(u)} \\ &\times (u\varphi_1^\kappa(u) - \gamma\varphi_2^\kappa(u)) - \gamma\varphi_0^\kappa(u) + u\varphi_0^\kappa(u). \end{aligned} \tag{2.17}$$

By Lemma 2.1 and the argument before this theorem, it is enough to show that there exist $\varepsilon_0 > 0$ and $\kappa \in \mathbb{R}_+^{6n}$ such that for all $\varepsilon \in (0, \varepsilon_0)$, the equation $\tilde{F}^{\kappa,\gamma,S_{\text{tot}}}(\varepsilon, u) = 0$ has at least $n+1$ (n) positive solutions when n is even (odd). (Then, given S_{tot}, γ, E_{tot}, and F_{tot} satisfying (2.15), we let $\varepsilon = E_{\text{tot}}/S_{\text{tot}} < \varepsilon_0$, and apply the result.)

A straightforward computation shows that when $\varepsilon = 0$,

$$\begin{aligned}
\tilde{F}^{\kappa,\gamma,S_{\text{tot}}}(0,u) &= S_{\text{tot}}\left(u\varphi_1^\kappa(u) - \gamma\varphi_2^\kappa(u)\right) - \gamma\varphi_0^\kappa(u) + u\varphi_0^\kappa(u) \\
&= \lambda_0\cdots\lambda_{n-1}u^{n+1} + \lambda_0\cdots\lambda_{n-2}\left(1 + \frac{S_{\text{tot}}}{K_{M_{n-1}}}(1-\gamma\beta_{n-1}) - \gamma\lambda_{n-1}\right)u^n \\
&+ \cdots + \lambda_0\cdots\lambda_{i-2}\left(1 + \frac{S_{\text{tot}}}{K_{M_{i-1}}}(1-\gamma\beta_{i-1}) - \gamma\lambda_{i-1}\right)u^i + \cdots \qquad (2.18) \\
&+ \left(1 + \frac{S_{\text{tot}}}{K_{M_0}}(1-\gamma\beta_0) - \gamma\lambda_0\right)u - \gamma,
\end{aligned}$$

where the λ_i's and K_{M_i}'s are defined as in (2.6), and $\beta_i = k_{\text{cat}_i}/l_{\text{cat}_i}$. The polynomial $\tilde{F}^{\kappa,\gamma,S_{\text{tot}}}(0,u)$ is of degree $n+1$, so there are at most $n+1$ positive roots. Notice that $u = 0$ is not a root because $\tilde{F}^{\kappa,\gamma,S_{\text{tot}}}(0,u) = -\gamma < 0$, which also implies that when n is odd, there can not be $n+1$ positive roots. Now fix any S_{tot} and γ. We will construct a vector κ such that $\tilde{F}^{\kappa,\gamma,S_{\text{tot}}}(0,u)$ has $n+1$ distinct positive roots when n is even.

Let us pick any $n+1$ positive real numbers $u_1 < \cdots < u_{n+1}$, such that their product is γ, and assume that

$$(u-u_1)\cdots(u-u_{n+1}) = u^{n+1} + a_n u^n + \cdots + a_1 u + a_0, \qquad (2.19)$$

where $a_0 = -\gamma < 0$ keeping in mind that a_i's are given. Our goal is to find a vector $\kappa \in \mathbb{R}_+^{6n}$ such that (2.18) and (2.19) are the same. For each $i = 0, \ldots, n-1$, we pick $\lambda_i = 1$. Comparing the coefficients of u^{i+1} in (2.18) and (2.19), we have

$$\frac{S_{\text{tot}}}{K_{M_i}}(1 + a_0\beta_i) = a_{i+1} - a_0 - 1. \qquad (2.20)$$

Let us pick $K_{M_i} > 0$ such that $\frac{K_{M_i}}{S_{\text{tot}}}(a_{i+1} - a_0 - 1) - 1 < 0$, then take

$$\beta_i = \frac{\frac{K_{M_i}}{S_{\text{tot}}}(a_{i+1} - a_0 - 1) - 1}{a_0} > 0$$

in order to satisfy (2.20). From the given

$$\lambda_0, \ldots, \lambda_{n-1}, K_{M_0}, \ldots, K_{M_{n-1}}, \beta_0, \ldots, \beta_{n-1},$$

we will find a vector

$$\kappa = (k_{\mathrm{on}_0}, \ldots, k_{\mathrm{on}_{n-1}}, k_{\mathrm{off}_0}, \ldots, k_{\mathrm{off}_{n-1}}, k_{\mathrm{cat}_0}, \ldots, k_{\mathrm{cat}_{n-1}},$$
$$l_{\mathrm{on}_0}, \ldots, l_{\mathrm{on}_{n-1}}, l_{\mathrm{off}_0}, \ldots, l_{\mathrm{off}_{n-1}}, l_{\mathrm{cat}_0}, \ldots, l_{\mathrm{cat}_{n-1}}) \in \mathbb{R}_+^{6n}$$

such that $\beta_i = k_{\mathrm{cat}_i}/l_{\mathrm{cat}_i}, i = 0, \ldots, n-1$, and (2.6) holds. This vector κ will guarantee that $\tilde{F}^{\kappa,\gamma,S}\mathrm{tot}\,(0,u)$ has $n+1$ positive distinct roots. When n is odd, a similar construction will give a vector κ such that $\tilde{F}^{\kappa,\gamma,S}\mathrm{tot}\,(0,u)$ has n positive roots and one negative root.

One construction of κ (given $\lambda_i, K_{M_i}, \beta_i, i = 0, \ldots, n-1$) is as follows. For each $i = 0, \ldots, n-1$, we start by defining

$$L_{M_i} = \frac{\lambda_i K_{M_i}}{\beta_i},$$

consistently with the definitions in (2.6). Then, we take

$$k_{\mathrm{on}_i} = 1, \quad l_{\mathrm{on}_i} = 1,$$

and

$$k_{\mathrm{off}_i} = \alpha_i K_{M_i}, \quad k_{\mathrm{cat}_i} = (1-\alpha_i) K_{M_i}, \quad l_{\mathrm{cat}_i} = \frac{1-\alpha_i}{\beta_i} K_{M_i}, \quad l_{\mathrm{off}_i} = L_{M_i} - l_{\mathrm{cat}_i},$$

where $\alpha_i \in (0,1)$ is chosen such that

$$l_{\mathrm{off}_i} = L_{M_i} - \frac{1-\alpha_i}{\beta_i} K_{M_i} > 0.$$

This κ satisfies $\beta_i = k_{\mathrm{cat}_i}/l_{\mathrm{cat}_i}, i = 0, \ldots, n-1$, and (2.6).

In order to apply the Implicit Function Theorem, we now view the functions defined by formulas in (2.16) and (2.17) as defined also for $\varepsilon \leq 0$, i.e. as functions $\mathbb{R} \times \mathbb{R}_+ \longrightarrow \mathbb{R}$. It is easy to see that $\tilde{F}^{\kappa,\gamma,S}\mathrm{tot}\,(\varepsilon,u)$ is C^1 on $\mathbb{R} \times \mathbb{R}_+$ because the polynomial under the square root sign in $\tilde{F}^{\kappa,\gamma,S}\mathrm{tot}\,(\varepsilon,u)$ is never zero. On the other hand, since $\tilde{F}^{\kappa,\gamma,S}\mathrm{tot}\,(0,u)$ is a polynomial in u with distinct roots, $\frac{\partial \tilde{F}^{\kappa,\gamma,S}\mathrm{tot}}{\partial u}(0,u_i) \neq 0$. By the Implicit Function Theorem, for each $i = 1, \ldots, n+1$, there exist open intervals E_i containing 0, open intervals U_i containing u_i, and a differentiable function

$$\alpha_i : E_i \to U_i$$

such that $\alpha_i(0) = u_i$, $\tilde{F}^{\kappa,\gamma,S_{\rm tot}}(\varepsilon, \alpha_i(\varepsilon)) = 0$ for all $\varepsilon \in E_i$, and the images $\alpha_i(E_i)$'s are non-overlapping. If we take

$$(0, \varepsilon_0) := \bigcap_{1}^{n+1} E_i \bigcap (0, +\infty),$$

then for any $\varepsilon \in (0, \varepsilon_0)$, we have $\{\alpha_i(\varepsilon)\}$ as $n+1$ distinct positive roots of $\tilde{F}^{\kappa,\gamma,S_{\rm tot}}(\varepsilon, u)$. The case when n is odd can be proved similarly. ∎

The above theorem shows that when $E_{\rm tot}/S_{\rm tot}$ is sufficiently small, it is always possible for the futile cycle to have $n+1$ (n) steady states when n is even (odd), by choosing appropriate kinetic constants κ. As an example, we consider the following parameter set:

$$n = 2, \quad \lambda_0 = 1, \quad \lambda_1 = 1, \quad \gamma = 6, \quad \beta_0 = \frac{1}{30}, \quad \beta_1 = \frac{1}{4}, \quad K_0 = \frac{1}{20}, \quad K_1 = \frac{1}{2}, \quad S_{\rm tot} = 1.$$

When $\varepsilon = 0$, we have

$$\tilde{F}^{\kappa,\gamma,S_{\rm tot}}(0, u) = u^3 - 6u^2 + 11u - 6 = (u-1)(u-2)(u-3)$$

with three positive roots

$$u_1 = 1, \quad u_2 = 2, \quad u_3 = 3.$$

When $\varepsilon = 0.01$, the equation $\tilde{F}^{\kappa,\gamma,S_{\rm tot}}(\varepsilon, u) = 0$ has three positive roots

$$u_1 = 1.08475, \quad u_2 = 1.77385, \quad u_3 = 3.11988,$$

which are close to the roots of $\tilde{F}^{\kappa,\gamma,S_{\rm tot}}(0, u)$, see Figure 2.3.

We should notice that for arbitrary κ, the derivative of $\tilde{\mathcal{F}}$ at each positive root may become zero, which breaks down the perturbation argument. Here is an example to show that more conditions are needed: with

$$n = 2, \quad \lambda_0 = 1, \quad \lambda_1 = 3, \quad \gamma = 6, \quad \beta_0 = \beta_1 = 1/12, \quad K_0 = 1/8, \quad K_1 = 1/2, \quad S_{\rm tot} = 5,$$

we have that

$$\tilde{F}^{\kappa,\gamma,S_{\rm tot}}(0, u) = 3u^3 - 12u^2 + 15u - 6 = 3(u-1)^2(u-2)$$

has a double root at $u = 1$. In this case, even for $\varepsilon = 0.01$, there is only one positive root of $\tilde{F}^{\kappa,\gamma,S_{\rm tot}}(\varepsilon, u)$, see Figure 2.4.

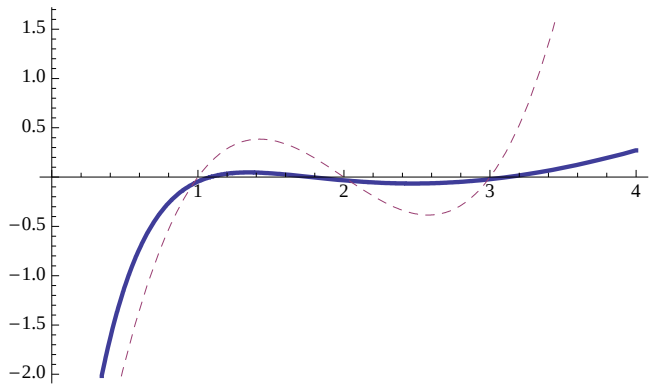

Figure 2.3: The plot of the functions $\tilde{F}^{\kappa,\gamma,S\text{tot}}(0,u)$ and $\tilde{F}^{\kappa,\gamma,S\text{tot}}(0.01,u)$ on $[0,4]$. The dashed line represents the function $\tilde{F}$ when $\varepsilon = 0$. The polynomial $\tilde{F}^{\kappa,\gamma,S\text{tot}}(0,u)$ has three positive roots $u = 1, 2$, and 3. The solid line represents the function $\tilde{F}$ when $\varepsilon = 0.01$. The three positive roots of $\tilde{F}^{\kappa,\gamma,S\text{tot}}(0.01,u)$ are close to the three roots of $\tilde{F}^{\kappa,\gamma,S\text{tot}}(0,u)$ respectively.

However, the following lemma provides a sufficient condition for $\frac{\partial F^{\kappa,\gamma,S\text{tot}}}{\partial u}(0,\bar{u}) \neq 0$, for any positive $\bar{u}$ such that $\tilde{F}^{\kappa,\gamma,S\text{tot}}(0,\bar{u}) = 0$.

Lemma 2.3 *For each positive numbers S_{tot}, γ, and vector $\kappa \in \mathbb{R}_+^{6n}$, if*

$$S_{tot}\left|\frac{1-\gamma\beta_j}{K_{M_j}}\right| \leq \frac{1}{n} \tag{2.21}$$

holds for all $j = 1, \cdots, n-1$, then $\frac{\partial \tilde{F}^{\kappa,\gamma,S}tot}{\partial u}(0,\bar{u}) \neq 0$.

Proof. Recall that (dropping the u's in $\varphi_i^\kappa, i = 0, 1, 2$)

$$\tilde{F}^{\kappa,\gamma,S\text{tot}}(0,u) = u\varphi_0^\kappa + S_{\text{tot}}(u\varphi_1^\kappa - \gamma\varphi_2^\kappa) - \gamma\varphi_0^\kappa.$$

So

$$\frac{\partial \tilde{F}^{\kappa,\gamma,S\text{tot}}}{\partial u}(0,u) = \varphi_0^\kappa + S_{\text{tot}}(u\varphi_1^\kappa - \gamma\varphi_2^\kappa)' - (\gamma - u)(\varphi_0^\kappa)'.$$

Since $\tilde{F}^{\kappa,\gamma,S\text{tot}}(0,\bar{u}) = 0$,

$$S_{\text{tot}}(\bar{u}\varphi_1^\kappa - \gamma\varphi_2^\kappa) = (\gamma - \bar{u})\varphi_0^\kappa,$$

that is,

$$\gamma - \bar{u} = \frac{S_{\text{tot}}(\bar{u}\varphi_1^\kappa - \gamma\varphi_2^\kappa)}{\varphi_0^\kappa}.$$

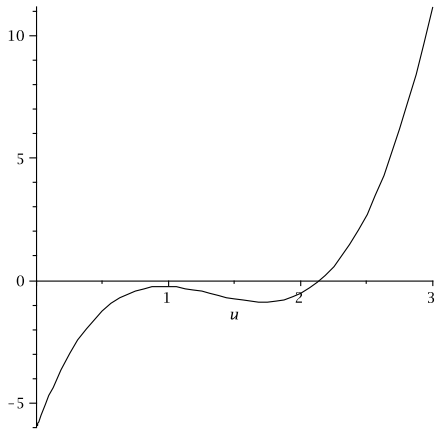

Figure 2.4: The plot of the function $\tilde{F}^{\kappa,\gamma,S_{\text{tot}}}(0.01,u)$ on $[0,3]$. There is a unique positive real solution around $u=2.14$, the double root $u=1$ of $\tilde{F}^{\kappa,\gamma,S_{\text{tot}}}(0,u)$ bifurcates to two complex roots with non-zero imaginary parts.

Therefore, $\frac{\partial \tilde{F}^{\kappa,\gamma,S_{\text{tot}}}}{\partial u}(0,\bar{u})$ equals

$$
\begin{aligned}
&\varphi_0^\kappa + S_{\text{tot}}(u\varphi_1^\kappa - \gamma\varphi_2^\kappa)' - \frac{S_{\text{tot}}(\bar{u}\varphi_1^\kappa - \gamma\varphi_2^\kappa)}{\varphi_0^\kappa}(\varphi_0^\kappa)' \\
&= \varphi_0^\kappa + \frac{S_{\text{tot}}}{\varphi_0^\kappa}\left(\varphi_0^\kappa(u\varphi_1^\kappa - \gamma\varphi_2^\kappa)' - (\bar{u}\varphi_1^\kappa - \gamma\varphi_2^\kappa)(\varphi_0^\kappa)'\right) \\
&= \varphi_0^\kappa + \frac{S_{\text{tot}}}{\varphi_0^\kappa}((1+\lambda_0\bar{u}+\lambda_0\lambda_1\bar{u}^2+\cdots+\lambda_0\cdots\lambda_{n-1}\bar{u}^n)\times \\
&\left(\frac{1}{K_{M_0}}(1-\gamma\beta_0)+2\frac{\lambda_0}{K_{M_1}}(1-\gamma\beta_1)\bar{u}+\cdots+n\frac{\lambda_0\cdots\lambda_{n-2}}{K_{M_{n-1}}}(1-\gamma\beta_{n-1})\bar{u}^{n-1}\right) \\
&-\left(\lambda_0+2\lambda_0\lambda_1\bar{u}+\cdots+n\lambda_0\cdots\lambda_{n-1}\bar{u}^{n-1}\right)\times \\
&\left(\frac{1}{K_{M_0}}(1-\gamma\beta_0)\bar{u}+\frac{\lambda_0}{K_{M_1}}(1-\gamma\beta_1)\bar{u}^2+\cdots+\frac{\lambda_0\cdots\lambda_{n-2}}{K_{M_{n-1}}}(1-\gamma\beta_{n-1})\bar{u}^n\right) \\
&= \varphi_0^\kappa + \frac{S_{\text{tot}}}{\varphi_0^\kappa}\sum_{i=0}^{n}\lambda_0\cdots\lambda_{i-1}\bar{u}^i\left(\sum_{j=0}^{n-1}(j+1-i)\frac{\lambda_0\cdots\lambda_{j-1}}{K_{M_j}}(1-\gamma\beta_j)\bar{u}^j\right) \\
&= \frac{1}{\varphi_0^\kappa}\sum_{i=0}^{n}\lambda_0\cdots\lambda_{i-1}\bar{u}^i\sum_{j=0}^{n}\lambda_0\cdots\lambda_{j-1}\bar{u}^j \\
&+ S_{\text{tot}}\sum_{i=0}^{n}\lambda_0\cdots\lambda_{i-1}\bar{u}^i\left(\sum_{j=0}^{n-1}(j+1-i)\frac{\lambda_0\cdots\lambda_{j-1}}{K_{M_j}}(1-\gamma\beta_j)\bar{u}^j\right) \\
&= \frac{1}{\varphi_0^\kappa}\sum_{i=0}^{n}\lambda_0\cdots\lambda_{i-1}\bar{u}^i \\
&\times\left(\lambda_0\cdots\lambda_{n-1}\bar{u}^n+\sum_{j=0}^{n-1}\lambda_0\cdots\lambda_{j-1}\bar{u}^j\left(1+S_{\text{tot}}(j+1-i)\frac{1-\gamma\beta_j}{K_{M_j}}\right)\right),
\end{aligned}
$$

where the product $\lambda_0 \cdots \lambda_{-1}$ is defined to be 1 for the convenience of notation.

Because of (2.21),

$$S_{\text{tot}} \left| (j+1-i) \frac{1-\gamma\beta_j}{K_{M_j}} \right| \leq 1,$$

we have $\frac{\partial \tilde{F}^{\kappa,\gamma,S}\text{tot}}{\partial u}(0,\bar{u}) > 0$. ∎

Theorem 2.4 *For each positive numbers S_{tot}, γ, and vector $\kappa \in \mathbb{R}_+^{6n}$ satisfying condition* (2.21)*, there exists $\varepsilon_1 > 0$ such that for any F_{tot}, E_{tot} satisfying $F_{tot} = E_{tot}/\gamma < \varepsilon_1 S_{tot}/\gamma$, the number of positive steady states of system $\Sigma(\kappa, \mathcal{C})$ is greater or equal to the number of (positive) roots of $\tilde{F}^{\kappa,\gamma,S}tot(0,u)$.*

Proof. Suppose that $\tilde{F}^{\kappa,\gamma,S}\text{tot}\,(0,u)$ has m roots: $\bar{u}_1, \ldots, \bar{u}_m$. Applying Lemma 2.3, we have

$$\frac{\partial \tilde{F}^{\kappa,\gamma,S}\text{tot}}{\partial u}(0,\bar{u}_k) \neq 0, k = 1, \ldots, m.$$

By the perturbation arguments as in Theorem 2.2, we have that there exists $\varepsilon_1 > 0$ such that $\tilde{F}^{\kappa,\gamma,S}\text{tot}\,(\varepsilon,u)$ has at least m roots for all $0 < \varepsilon < \varepsilon_1$. ∎

The above result depends heavily on a perturbation argument, which only works when $E_{\text{tot}}/S_{\text{tot}}$ is sufficiently small. In the next section, we will give an upper bound of the number of steady states with no restrictions on $E_{\text{tot}}/S_{\text{tot}}$, and independent of κ and $\mathcal{C}$.

Upper bound on the number of positive steady states

Theorem 2.5 *For each $\kappa, \mathcal{C}$, the system $\Sigma(\kappa, \mathcal{C})$ has at most $2n-1$ positive steady states.*

Proof. An alternative approach to solving (2.10)-(2.11) is to first eliminate v from (2.10) instead of from (2.11), i.e.

$$v = \frac{E_{\text{tot}}/F_{\text{tot}} - u}{u\varphi_1^\kappa(u) - (E_{\text{tot}}/F_{\text{tot}})\varphi_2^\kappa(u)} := \frac{A(u)}{B(u)}, \tag{2.22}$$

when $u\varphi_1^\kappa(u) - (E_{\text{tot}}/F_{\text{tot}})\varphi_2^\kappa(u) \neq 0$. Then, we substitute (2.22) into (2.11), and multiply by $(u\varphi_1^\kappa(u) - (E_{\text{tot}}/F_{\text{tot}})\varphi_2^\kappa(u))^2$ to get

$$P^{\kappa,\mathcal{C}}(u) := \varphi_0^\kappa\varphi_2^\kappa\left(\frac{E_{\text{tot}}}{F_{\text{tot}}} - u\right)^2 + (\varphi_0^\kappa - S_{\text{tot}}\varphi_2^\kappa + F_{\text{tot}}u\varphi_1^\kappa + F_{\text{tot}}\varphi_2^\kappa) \tag{2.23}$$
$$\times\left(\frac{E_{\text{tot}}}{F_{\text{tot}}} - u\right)\left(u\varphi_1^\kappa - \frac{E_{\text{tot}}}{F_{\text{tot}}}\varphi_2^\kappa\right) - S_{\text{tot}}\left(u\varphi_1^\kappa - \frac{E_{\text{tot}}}{F_{\text{tot}}}\varphi_2^\kappa\right)^2 = 0.$$

Therefore, if $u\varphi_1^\kappa(u)-(E_{\text{tot}}/F_{\text{tot}})\varphi_2^\kappa(u) \neq 0$, the number of positive solutions of (2.10)-(2.11) is no greater than the number of positive roots of $P^{\kappa,\mathcal{C}}(u)$.

In the special case when $u\varphi_1^\kappa(u) - (E_{\text{tot}}/F_{\text{tot}})\varphi_2^\kappa(u) = 0$, by (2.10), we must have $u = E_{\text{tot}}/F_{\text{tot}}$, and thus $\varphi_1^\kappa(E_{\text{tot}}/F_{\text{tot}}) = \varphi_2^\kappa(E_{\text{tot}}/F_{\text{tot}})$. Substituting into (2.11), we get a unique v defined as in (2.12) with $u = E_{\text{tot}}/F_{\text{tot}}$. Since $u = E_{\text{tot}}/F_{\text{tot}}$ is a root of $P^{\kappa,\mathcal{C}}(u)$, also in this case the number of positive solutions to (2.10)-(2.11) is no greater than the number of positive roots of $P^{\kappa,\mathcal{C}}(u)$.

It is easy to see that $P^{\kappa,\mathcal{C}}(u)$ is divisible by u. Consider the polynomial $Q^{\kappa,\mathcal{C}}(u) := P^{\kappa,\mathcal{C}}(u)/u$ of degree $2n+1$. We will first show that $Q^{\kappa,\mathcal{C}}(u)$ has no more than $2n$ positive roots, then we will prove by contradiction that $2n$ distinct positive roots can not be achieved.

It is easy to see that in the polynomial $Q^{\kappa,\mathcal{C}}(u)$ the coefficient of u^{2n+1} is

$$\frac{(\lambda_0\cdots\lambda_{n-1})^2}{L_{M_{n-1}}} > 0,$$

and the constant term is

$$\frac{E_{\text{tot}}}{F_{\text{tot}}K_{M_0}} > 0.$$

So the polynomial $Q^{\kappa,\mathcal{C}}(u)$ has at least one negative root, and thus has no more than $2n$ positive roots.

Suppose that $\mathcal{S}(\kappa,\mathcal{C})$ has cardinality $2n$, then $Q^{\kappa,\mathcal{C}}(u)$ must have $2n$ distinct positive roots, and each of them has multiplicity one. Let us denote the roots of $Q^{\kappa,\mathcal{C}}(u)$ as $u_1,\ldots,u_{2n}$ in ascending order, and the corresponding v's given by (2.22) as $v_1,\ldots,v_{2n}$. We claim that none of them equals $E_{\text{tot}}/F_{\text{tot}}$. If so, we would have $\varphi_1^\kappa(E_{\text{tot}}/F_{\text{tot}}) = \varphi_2^\kappa(E_{\text{tot}}/F_{\text{tot}})$, and $E_{\text{tot}}/F_{\text{tot}}$ would be a double root of $Q^{\kappa,\mathcal{C}}(u)$, contradiction.

Since $Q^{\kappa,\mathcal{C}}(0) > 0$, $Q^{\kappa,\mathcal{C}}(u)$ is positive on intervals

$$I_0 = (0,u_1), I_1 = (u_2,u_3),\ldots,I_{n-1} = (u_{2n-2},u_{2n-1}), I_n = (u_{2n},\infty),$$

and negative on intervals

$$J_1 = (u_1, u_2), \ldots, J_n = (u_{2n-1}, u_{2n}).$$

As remarked earlier, $\varphi_1^\kappa(E_{\text{tot}}/F_{\text{tot}}) \neq \varphi_2^\kappa(E_{\text{tot}}/F_{\text{tot}})$, the polynomial $Q^{\kappa,\mathcal{C}}(u)$ evaluated at $E_{\text{tot}}/F_{\text{tot}}$ is negative, and therefore, $E_{\text{tot}}/F_{\text{tot}}$ belongs to one of the J intervals, say $J_s = (u_{2s-1}, u_{2s})$, for some $s \in \{1, \ldots, n\}$.

On the other hand, the denominator of v in (2.22), denoted as $B(u)$, is a polynomial of degree n and divisible by u. If $B(u)$ has no positive root, then it does not change sign on the positive axis of u. But v changes sign when u passes $E_{\text{tot}}/F_{\text{tot}}$, thus v_{2s-1} and v_{2s} have opposite signs, and one of (u_{2s-1}, v_{2s-1}) and (u_{2s}, v_{2s}) is not a solution to (2.10)-(2.11), which contradicts the fact that both are in $\mathcal{S}(\kappa, \mathcal{C})$.

Otherwise, there exists a positive root $\bar{u}$ of $B(u)$ such that there is no other positive root of $B(u)$ between $\bar{u}$ and $E_{\text{tot}}/F_{\text{tot}}$. Plugging $\bar{u}$ into $Q^{\kappa,\mathcal{C}}(u)$, we see that $Q^{\kappa,\mathcal{C}}(\bar{u})$ is always positive, therefore, $\bar{u}$ belongs to one of the I intervals, say $I_t = (u_{2t}, u_{2t+1})$ for some $t \in \{0, \ldots, n\}$. There are two cases.

1. $E_{\text{tot}}/F_{\text{tot}} < \bar{u}$. We have

$$u_{2s-1} < E_{\text{tot}}/F_{\text{tot}} < u_{2t} < \bar{u}.$$

   Notice that v changes sign when u passes $E_{\text{tot}}/F_{\text{tot}}$, so the corresponding v_{2s-1} and v_{2t} have different signs, and either $(u_{2s-1}, v_{2s-1}) \notin \mathcal{S}(\kappa, \mathcal{C})$ or $(u_{2t}, v_{2t}) \notin \mathcal{S}(\kappa, \mathcal{C})$, contradiction.

2. $E_{\text{tot}}/F_{\text{tot}} > \bar{u}$. We have

$$\bar{u} < u_{2t+1} < E_{\text{tot}}/F_{\text{tot}} < u_{2s}.$$

   Since v changes sign when u passes $E_{\text{tot}}/F_{\text{tot}}$, so the corresponding v_{2t+1} and v_{2s} have different signs, and either $(u_{2t+1}, v_{2t+1}) \notin \mathcal{S}(\kappa, \mathcal{C})$ or $(u_{2s}, v_{2s}) \notin \mathcal{S}(\kappa, \mathcal{C})$, contradiction.

Therefore, $\Sigma(\kappa, \mathcal{C})$ has at most $2n - 1$ steady states. ∎

Fine-tuned upper bounds

In the previous section, we have seen that any $(u, v) \in \mathcal{S}(\kappa, \mathcal{C}), u \neq E_{\text{tot}}/F_{\text{tot}}$ must satisfy (2.22)-(2.23), but not all solutions of (2.22)-(2.23) are elements in $\mathcal{S}(\kappa, \mathcal{C})$. Suppose that (u, v) is a solution of (2.22)-(2.23), it is in $\mathcal{S}(\kappa, \mathcal{C})$ if and only if $u, v > 0$. In some special cases, for example, when the enzyme is in excess, or the substrate is in excess, we could count the number of solutions of (2.22)-(2.23) which are not in $\mathcal{S}(\kappa, \mathcal{C})$ to get a better upper bound.

The following is a standard result on continuity of roots; see for instance Lemma A.4.1 in [83].

Lemma 2.6 *Let $g(z) = z^n + a_1 z^{n-1} + \cdots + a_n$ be a polynomial of degree n and complex coefficients having distinct roots*

$$\lambda_1, \ldots, \lambda_q,$$

with multiplicities

$$n_1 + \cdots + n_q = n,$$

respectively. Given any small enough $\delta > 0$ there exists a $\varepsilon > 0$ so that, if

$$h(z) = z^n + b_1 z^{n-1} + \cdots + b_n, \quad |a_i - b_i| < \varepsilon \text{ for } i = 1, \ldots, n,$$

then h has precisely n_i roots in $B_\delta(\lambda_i)$ for each $i = 1, \ldots, q$, where $B_\delta(\lambda_i)$ is the open ball in $\mathbb{C}$ centered at λ_i with radius δ.

Theorem 2.7 *For each $\gamma > 0$ and $\kappa \in \mathbb{R}^{6n}_+$ such that $\varphi^\kappa_1(\gamma) \neq \varphi^\kappa_2(\gamma)$, and each $S_{tot} > 0$, there exists $\varepsilon_2 > 0$ such that for all positive numbers E_{tot}, F_{tot} satisfying $F_{tot} = E_{tot}/\gamma < \varepsilon_2 S_{tot}/\gamma$, the system $\Sigma(\kappa, \mathcal{C})$ has at most $n + 1$ positive steady states.*

Proof. Let us define a function $\mathbb{R}_+ \times \mathbb{C} \longrightarrow \mathbb{C}$ as follows,

$$\tilde{Q}^{\kappa,\gamma,S_{\text{tot}}}(\varepsilon, u) = Q^{\kappa,(\varepsilon S_{\text{tot}}, \varepsilon S_{\text{tot}}/\gamma, S_{\text{tot}})}(u),$$

and a set $\mathcal{B}^{\kappa,\gamma,S_{\text{tot}}}(\varepsilon)$ consisting of the roots of $\tilde{Q}^{\kappa,\gamma,S_{\text{tot}}}(\varepsilon, u)$ which are not positive or the corresponding v's determined by u's as in (2.22) are not positive. Since $\tilde{Q}^{\kappa,\gamma,S_{\text{tot}}}(\varepsilon, u)$ is a polynomial of degree $2n + 1$, if we can show that there exists $\varepsilon_2 > 0$

such that for any $\varepsilon \in (0, \varepsilon_2)$, $\tilde{Q}^{\kappa,\gamma,S}\mathrm{tot}\,(\varepsilon, u)$ has at least n roots counting multiplicities that are in $\mathcal{B}^{\kappa,\gamma,S}\mathrm{tot}\,(\varepsilon)$, then we are done.

In order to apply Lemma 2.6, we regard the function $\tilde{Q}^{\kappa,\gamma,S}\mathrm{tot}$ as defined on $\mathbb{R} \times \mathbb{C}$. At $\varepsilon = 0$:

$$\begin{aligned}
\tilde{Q}^{\kappa,\gamma,S}\mathrm{tot}\,(0, u) &= (\varphi_0^\kappa \varphi_2^\kappa(\gamma - u)^2 + (\varphi_0^\kappa - S_{\mathrm{tot}}\varphi_2^\kappa)(u\varphi_1^\kappa - \gamma\varphi_2^\kappa)(\gamma - u) \\
&\quad - S_{\mathrm{tot}}(u\varphi_1^\kappa - \gamma\varphi_2^\kappa)^2)/u \\
&= (\varphi_0^\kappa(\gamma - u)u(\varphi_1^\kappa - \varphi_2^\kappa) + S_{\mathrm{tot}}u(u\varphi_1^\kappa - \gamma\varphi_2^\kappa)(\varphi_2^\kappa - \varphi_1^\kappa))/u \\
&= (\varphi_2^\kappa - \varphi_1^\kappa)(u\varphi_0^\kappa + S_{\mathrm{tot}}(u\varphi_1^\kappa - \gamma\varphi_2^\kappa) - \gamma\varphi_0^\kappa) \\
&= (\varphi_2^\kappa - \varphi_1^\kappa)\tilde{F}^{\kappa,\gamma,S}\mathrm{tot}\,(0, u)
\end{aligned}$$

Let us denote the distinct roots of $\tilde{Q}^{\kappa,\gamma,S}\mathrm{tot}\,(0, u)$ as

$$u_1, \ldots, u_q,$$

with multiplicities

$$n_1 + \cdots + n_q = 2n + 1,$$

and the roots of $\varphi_1^\kappa - \varphi_2^\kappa$ as

$$u_1, \ldots, u_p, \quad p \leq q,$$

with multiplicities

$$m_1 + \cdots + m_p = n, \quad n_i \geq m_i, \text{ for } i = 1, \ldots, p.$$

For each $i = 1, \ldots, p$, if u_i is real and positive, then there are two cases ($u_i \neq \gamma$ as $\varphi_1^\kappa(\gamma) \neq \varphi_2^\kappa(\gamma)$).

1. $u_i > \gamma$. We have

$$u_i\varphi_1^\kappa(u_i) - \gamma\varphi_2^\kappa(u_i) > \gamma(\varphi_1^\kappa(u_i) - \varphi_2^\kappa(u_i)) = 0.$$

2. $u_i < \gamma$. We have

$$u_i\varphi_1^\kappa(u_i) - \gamma\varphi_2^\kappa(u_i) < \gamma(\varphi_1^\kappa(u_i) - \varphi_2^\kappa(u_i)) = 0.$$

In both cases, $u_i\varphi_1^\kappa(u_i) - \gamma\varphi_2^\kappa(u_i)$ and $\gamma - u_i$ have opposite signs, i.e.

$$(u_i\varphi_1^\kappa(u_i) - \gamma\varphi_2^\kappa(u_i))(\gamma - u_i) < 0.$$

Let us pick $\delta > 0$ small enough such that the following conditions hold.

1. For all $i = 1, \ldots, p$, if u_i is not real, then $B_\delta(u_i)$ has no intersection with the real axis.

2. For all $i = 1, \ldots, p$, if u_i is real and positive, the following inequality holds for any real $u \in B_\delta(u_i)$,
$$(u\varphi_1^\kappa(u) - \gamma\varphi_2^\kappa(u))(\gamma - u) < 0. \tag{2.24}$$

3. For all $i = 1, \ldots, p$, if u_i is real and negative, then $B_\delta(u_i)$ has no intersection with the imaginary axis.

4. $B_\delta(u_j) \bigcap B_\delta(u_k) = \emptyset$ for all $j \neq k = 1, \ldots, q$.

By Lemma 2.6, there exists $\varepsilon_2 > 0$ such that for all $\varepsilon \in (0, \varepsilon_2)$, polynomial $\tilde{Q}^{\kappa,\gamma,S}\mathrm{tot}\,(\varepsilon, u)$ has exactly n_j roots in each $B_\delta(u_j), j = 1, \ldots, q$, denoted by $u_j^k(\varepsilon), k = 1, \ldots, n_j$.

We pick one such ε, and we claim that none of the roots in $B_\delta(u_i), i = 1, \ldots, p$ with the v defined as in (2.22) will be an element in $\mathcal{S}$. If so, we are done, since there are $\sum_1^p n_i \geq \sum_1^p m_i = n$ such roots of $\tilde{Q}^{\kappa,\gamma,S}\mathrm{tot}\,(\varepsilon, u)$ which are in $\mathcal{B}^{\kappa,\gamma,S}\mathrm{tot}\,(\varepsilon)$.

For each $i = 1, \ldots, p$, there are two cases.

1. u_i is not real. Then condition 1 guarantees that $u_i^k(\varepsilon)$ is not real for each $k = 1, \ldots, n_i$, and thus is in $\mathcal{B}^{\kappa,\gamma,S}\mathrm{tot}\,(\varepsilon)$.

2. u_i is real and positive. Pick any root $u_i^k(\varepsilon) \in B_\delta(u_i), k = 1, \ldots, n_i$, the corresponding $v_i^k(\varepsilon)$ equals
$$\frac{\gamma - u_i^k(\varepsilon)}{u_i^k(\varepsilon)\varphi_1^\kappa(u_i^k(\varepsilon)) - \gamma\varphi_2^\kappa(u_i^k(\varepsilon))} < 0$$
followed from (2.24). So $(u_i^k(\varepsilon), v_i^k(\varepsilon)) \notin \mathcal{S}(\kappa, \mathcal{C})$, and $u_i^k(\varepsilon) \in \mathcal{B}^{\kappa,\gamma,S}\mathrm{tot}\,(\varepsilon)$.

3. u_i is real and negative. By condition 3, $u_i^k(\varepsilon)$ is not positive for all $k = 1, \ldots, n_i$.

∎

The next theorem considers the case when enzyme is in excess.

Theorem 2.8 *For each $\gamma > 0, \kappa \in \mathbb{R}^{6n}_+$ such that $\varphi^\kappa_1(\gamma) \neq \varphi^\kappa_2(\gamma)$, and each $E_{tot} > 0$, there exists $\varepsilon_3 > 0$ such that for all positive numbers F_{tot}, S_{tot} satisfying $F_{tot} = E_{tot}/\gamma > S_{tot}/(\varepsilon_3\gamma)$, the system $\Sigma(\kappa, \mathcal{C})$ has at most one positive steady state.*

Proof. For each $\gamma > 0, \kappa \in \mathbb{R}^{6n}_+$ such that $\varphi^\kappa_1(\gamma) \neq \varphi^\kappa_2(\gamma)$, and each $E_{\text{tot}} > 0$, we define a function $\mathbb{R}_+ \times \mathbb{C} \longrightarrow \mathbb{C}$ as follows:

$$\bar{Q}^{\kappa,\gamma,E}\text{tot}\,(\varepsilon, u) = Q^{\kappa,(E\text{tot},E\text{tot}/\gamma,\varepsilon E\text{tot})}(u).$$

Let us define the set $\mathcal{C}^{\kappa,\gamma,E}\text{tot}\,(\varepsilon)$ as the set of roots of $\bar{Q}^{\kappa,\gamma,E}\text{tot}\,(\varepsilon, u)$ which are not positive or the corresponding v's determined by u's as in (2.22) are not positive. If we can show that there exists $\varepsilon_3 > 0$ such that for any $\varepsilon \in (0, \varepsilon_3)$ there is at most one positive root of $\bar{Q}^{\kappa,\gamma,E}\text{tot}\,(\varepsilon, u)$ that is not in $\mathcal{C}^{\kappa,\gamma,E}\text{tot}\,(\varepsilon)$, we are done.

In order to apply Lemma 2.6, we now view the function $\bar{Q}^{\kappa,\gamma,E}\text{tot}$ as defined on $\mathbb{R} \times \mathbb{C}$. At $\varepsilon = 0$, $\bar{Q}^{\kappa,\gamma,E}\text{tot}\,(0, u)$ equals

$$\begin{aligned}&(\gamma - u)\left((\gamma - u)\,\varphi^\kappa_0\varphi^\kappa_2 + \left(\varphi^\kappa_0 + \frac{E_{\text{tot}}}{\gamma}u\varphi^\kappa_1 + \frac{E_{\text{tot}}}{\gamma}\varphi^\kappa_2\right)(u\varphi^\kappa_1 - \gamma\varphi^\kappa_2)\right)/u\\ &:= (\gamma - u)\,R^{\kappa,\gamma,E}\text{tot}\,(u).\end{aligned}$$

Let us denote the distinct roots of $\bar{Q}^{\kappa,\gamma,E}\text{tot}\,(0, u)$ as

$$u_1(=\gamma), u_2, \ldots, u_q,$$

with multiplicities

$$n_1 + \cdots + n_q = 2n + 1,$$

and $u_2, \ldots, u_q$ are the roots of $R^{\kappa,\gamma,E}\text{tot}\,(u)$ other than γ.

Since $\varphi^\kappa_1(\gamma) \neq \varphi^\kappa_2(\gamma)$, $R^{\kappa,\gamma,E}\text{tot}\,(u)$ is not divisible by $u - \gamma$, and thus $n_1 = 1$.

For each $i = 2, \ldots, q$, we have $(\gamma - u_i)\,\varphi^\kappa_0(u_i)\varphi^\kappa_2(u_i)$ equals

$$-\left(\varphi^\kappa_0(u_i) + \frac{E_{\text{tot}}}{\gamma}u_i\varphi^\kappa_1(u_i) + \frac{E_{\text{tot}}}{\gamma}\varphi^\kappa_2(u_i)\right)(u_i\varphi^\kappa_1(u_i) - \gamma\varphi^\kappa_2(u_i))\,.$$

If $u_i > 0$, then $\varphi_0^\kappa(u_i)\varphi_2^\kappa(u_i)$ and $\varphi_0^\kappa(u_i) + \frac{E_{\text{tot}}}{\gamma} u_i\varphi_1^\kappa(u_i) + \frac{E_{\text{tot}}}{\gamma}\varphi_2^\kappa(u_i)$ are both positive. Since $u_i\varphi_1^\kappa(u_i) - \gamma\varphi_2^\kappa(u_i)$ and $\gamma - u_i$ are non zero, $u_i\varphi_1^\kappa(u_i) - \gamma\varphi_2^\kappa(u_i)$ and $\gamma - u_i$ must have opposite signs, that is

$$(u_i\varphi_1^\kappa(u_i) - \gamma\varphi_2^\kappa(u_i))(\gamma - u_i) < 0.$$

Let us pick $\delta > 0$ small enough such that the following conditions hold for all $i = 2, \ldots, q$,

1. If u_i is not real, then $B_\delta(u_i)$ has no intersection with the real axis.

2. If u_i is real and positive, then for any real $u \in B_\delta(u_i)$, the following inequality holds,
$$(u\varphi_1^\kappa(u) - \gamma\varphi_2^\kappa(u))(\gamma - u) < 0. \tag{2.25}$$

3. If u_i is real and negative, then $B_\delta(u_i)$ has no intersection with the imaginary axis.

4. $B_\delta(u_j) \bigcap B_\delta(u_k) = \emptyset$ for all $i \neq k = 2, \ldots, q$.

By Lemma 2.6, there exists $\varepsilon_3 > 0$ such that for all $\varepsilon \in (0, \varepsilon_3)$, the polynomial

$$\bar{Q}^{\kappa,\gamma,E_{\text{tot}}}(\varepsilon, u)$$

has exactly n_j roots in each $B_\delta(u_j), j = 1, \ldots, q$, denoted by $u_j^k(\varepsilon), k = 1, \ldots, n_j$.

We pick one such ε, and if we can show that all of the roots in $B_\delta(u_i), i = 2, \ldots, q$ are in $\mathcal{C}^{\kappa,\gamma,E_{\text{tot}}}(\varepsilon)$, then we are done, since the only roots that may not be in $\mathcal{C}^{\kappa,\gamma,E_{\text{tot}}}(\varepsilon)$ are the roots in $B_\delta(u_1)$, and there is one root in $B_\delta(u_1)$.

For each $i = 2, \ldots, p$, there are three cases.

1. u_i is not real. Then condition 1 guarantees that $u_i^k(\varepsilon)$ is not real for all $k = 1, \ldots, n_i$.

2. u_i is real and positive. Pick any root $u_i^k(\varepsilon), k = 1, \ldots, n_i$, the corresponding $v_i^k(\varepsilon)$ equals
$$\frac{\gamma - u_i^k(\varepsilon)}{u_i^k(\varepsilon)\varphi_1^\kappa(u_i^k(\varepsilon)) - \gamma\varphi_2^\kappa(u_i^k(\varepsilon))} < 0.$$
So, $u_i^k(\varepsilon)$ is in $\mathcal{C}^{\kappa,\gamma,E_{\text{tot}}}(\varepsilon)$.

3. u_i is real and negative. By conditions 3, $u_i^k(\varepsilon)$ is not positive for all $k = 1, \ldots, n_i$.

∎

2.2.5 Conclusions

To summarize, we have set up a mathematical model for multisite phosphorylation-dephosphorylation cycles of size n, and studied the number of positive steady states based on this model. We reformulated the question of number of positive steady states to question of the number of positive roots of certain polynomials, through which we also applied perturbation techniques. Our theoretical results depend on the assumption of mass action kinetics and distributive sequential mechanism, which are customary in the study of multisite phosphorylation and dephosphorylation.

An upper bound of $2n-1$ positive steady states is obtained for arbitrary parameter combinations. Biologically, when the substrate concentration greatly exceeds that of the enzyme, there are at most $n+1$ (n) positive steady states if n is even (odd). And this upper bound can be achieved under proper kinetic conditions, see Theorem 2.2 for the construction. On the other extreme, when the enzyme is in excess, there is a unique positive steady state.

As a special case of $n = 2$, which can be applied to a single level of MAPK cascades, see Figure 2.2. Our results guarantee that there are no more than three positive steady states, consistent with numerical simulations in [58].

2.3 Another Motif in Biological Networks

2.3.1 Introduction

In this section, we study multistationarity of another futile cycle of size two, see Figure 2.5. This module is different from the one in Figure 2.2 of Section 2.2. In Figure 2.2, the same enzyme E converted S_0 to S_1 and S_1 to S_2, whereas in Figure 2.5, the step from S_0 to S_1 is facilitated by the enzyme E_1, and the transformation from S_1 to S_2 is completed by the enzyme E_2. Similarly, the reversed reactions are also different.

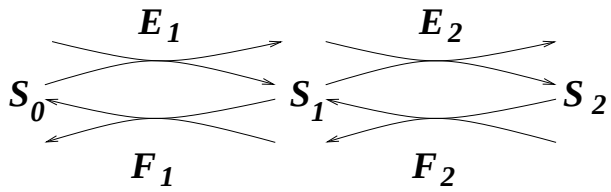

Figure 2.5: Another "futile cycle" motif. The protein S_0 is transformed by the enzyme E_1 to S_1, which is in turn brought to S_2 by another enzyme E_2. Conversely, the protein S_2 is changed to S_1 with the help of the enzyme F_2, and S_1 is converted back to S_0 through a different enzyme F_1.

The difference between these two modules affects their asymptotic behaviors. We will show in this section that the module, with different enzymes in the S_0 to S_2 direction and different enzymes in the transformation from S_2 back to S_0, admits a unique positive steady state for any values of kinetic parameters involved in the system. This suggests that the competition between S_0 and S_1 for the enzyme E is crucial for multistationarity in the module shown in Figure 2.2. Regarding the dynamical behavior of the motif in Figure 2.5, we show in Section 3.2 that the unique positive steady state is globally asymptotically stable in $\mathbb{R}^7_{\geq 0}$.

2.3.2 Mathematical formalism

Let us first write down all of the chemical reactions involved in Figure 2.5:

$$S_0 + E_1 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C_1 \overset{k_2}{\to} S_1 + E_1$$

$$S_1 + E_2 \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} C_2 \overset{k_4}{\to} S_2 + E_2$$

$$S_2 + F_2 \underset{k_{-5}}{\overset{k_5}{\rightleftarrows}} C_3 \overset{k_6}{\to} S_1 + F_2$$

$$S_1 + F_1 \underset{k_{-7}}{\overset{k_7}{\rightleftarrows}} C_4 \overset{k_8}{\to} S_0 + F_1.$$

Here, k_1, etc., are kinetic parameters for binding and unbinding; C_1 denotes the complex consisting of the enzyme E_1 and substrate S_0; C_2 denotes the complex formed by the enzyme E_2 and substrate S_1; the complex consisting of the enzyme F_2 and substrate

S_2 is denoted by C_3, and the complex formed by the enzyme F_1 and substrate S_1 is denoted by C_4.

These reactions can be modeled by differential equations according to mass action kinetics:

$$\begin{aligned}
\frac{ds_0}{dt} &= -k_1 s_0 e_1 + k_{-1} c_1 + k_8 c_4 \\
\frac{ds_1}{dt} &= -k_3 s_1 e_2 + k_{-3} c_2 - k_7 s_1 f_1 + k_{-7} c_4 + k_6 c_3 + k_2 c_1 \\
\frac{ds_2}{dt} &= -k_5 s_2 f_2 + k_{-5} c_3 + k_4 c_2 \\
\frac{dc_1}{dt} &= k_1 s_0 e_1 - (k_{-1} + k_2) c_1 \\
\frac{dc_2}{dt} &= k_3 s_1 e_2 - (k_{-3} + k_4) c_2 \\
\frac{dc_3}{dt} &= k_5 s_2 f_2 - (k_{-5} + k_6) c_3 \\
\frac{dc_4}{dt} &= k_7 s_1 f_1 - (k_{-7} + k_8) c_4,
\end{aligned} \tag{2.26}$$

together with the algebraic "conservation equations":

$$\begin{aligned}
E_{1,\text{tot}} &= e_1 + c_1 \\
E_{2,\text{tot}} &= e_2 + c_2 \\
F_{1,\text{tot}} &= f_1 + c_4 \\
F_{2,\text{tot}} &= f_2 + c_3 \\
S_{\text{tot}} &= \sum_{i=0}^{2} s_i + \sum_{i=1}^{4} c_i.
\end{aligned} \tag{2.27}$$

The variables $s_0, s_1, s_2, c_1, \ldots, c_4, e_1, e_2, f_1, f_2$ stand for the concentrations of

$$S_0, S_1, S_n, C_1, \ldots, C_4, E_1, E_2, F_1, F_2,$$

respectively. The total amount of the chemicals are denoted by

$$E_{1,\text{tot}}, E_{2,\text{tot}}, F_{1,\text{tot}}, F_{2,\text{tot}}, \text{ and } S_{\text{tot}},$$

which are positive constants.

2.3.3 Positive steady states

At the steady state of system (2.26), we have the right hand side of (2.26) equal zero:

$$0 = -k_1 s_0 e_1 + k_{-1} c_1 + k_8 c_4 \tag{2.28}$$

$$0 = -k_3 s_1 e_2 + k_{-3} c_2 - k_7 s_1 f_1 + k_{-7} c_4 + k_6 c_3 + k_2 c_1 \tag{2.29}$$

$$0 = -k_5 s_2 f_2 + k_{-5} c_3 + k_4 c_2 \tag{2.30}$$

$$0 = k_1 s_0 e_1 - (k_{-1} + k_2) c_1 \tag{2.31}$$

$$0 = k_3 s_1 e_2 - (k_{-3} + k_4) c_2 \tag{2.32}$$

$$0 = k_5 s_2 f_2 - (k_{-5} + k_6) c_3 \tag{2.33}$$

$$0 = k_7 s_1 f_1 - (k_{-7} + k_8) c_4. \tag{2.34}$$

In this section, the analysis is restricted to the steady states, that is solutions of (2.28)-(2.34) together with (2.27). We will prove that the positive solutions of (2.28)-(2.34) and (2.27) are unique. The strategy is to first express variables

$$s_0, s_2, c_1, \ldots, c_4, e_1, e_2, f_1, f_2$$

in terms of s_1, then to show that there is a unique positive s_1 satisfying (2.28)-(2.34) and (2.27). It thus follows that the steady state value of other variables are also unique.

We start by solving equations (2.31) to (2.34), from which we obtain $c_1, \ldots, c_4$ as functions of s_0, s_1, and s_2:

$$\begin{aligned} c_1 &= \frac{s_0 E_{1,\text{tot}}}{K_{m1} + s_0} \\ c_2 &= \frac{s_1 E_{2,\text{tot}}}{K_{m2} + s_1} \\ c_3 &= \frac{s_2 F_{2,\text{tot}}}{K_{m3} + s_2} \\ c_4 &= \frac{s_1 F_{1,\text{tot}}}{K_{m4} + s_1}. \end{aligned} \tag{2.35}$$

Here, $K_{m1}, K_{m2}, K_{m3}, K_{m4}$ are the Michaelis-Menten constants defined as

$$K_{m1} = \frac{k_{-1} + k_2}{k_1}, \quad K_{m2} = \frac{k_{-3} + k_4}{k_3},$$

$$K_{m3} = \frac{k_{-5} + k_6}{k_5}, \quad K_{m4} = \frac{k_{-7} + k_7}{k_8}.$$

If we could find relations to link s_0 to s_1 and to link s_2 to s_1, then $c_1, \ldots, c_4$ can be written as functions of s_1 solely. We achieve this by first adding up equation (2.28) and equation (2.31), which gives

$$k_2 c_1 = k_8 c_4. \tag{2.36}$$

Invoking (2.35), we have

$$k_2 \frac{s_0 E_{1,\text{tot}}}{K_{m1} + s_0} = k_8 \frac{s_1 F_{1,\text{tot}}}{K_{m4} + s_1}, \tag{2.37}$$

from where we can solve s_0 as a function of s_1. Let us denote by $s_0 = \gamma_1(s_1)$ the solution of equation (2.37). Notice that γ_1 is an strictly increasing function of s_1 with $\gamma_1(0) = 0$.

Similarly, by adding up equation (2.30) and equation (2.33), we have

$$k_4 c_2 = k_6 c_3,$$

which leads to $s_2 = \gamma_2(s_1)$, where γ_2 is an strictly increasing function of s_1 with $\gamma_2(0) = 0$.

Now, we can rewrite $c_1, \ldots, c_4$ as functions of s_1:

$$\begin{aligned}
c_1 &= \frac{\gamma_1(s_1) E_{1,\text{tot}}}{K_{m1} + \gamma_1(s_1)} := \varphi_1(s_1) \\
c_2 &= \frac{s_1 E_{2,\text{tot}}}{K_{m2} + s_1} := \varphi_2(s_1) \\
c_3 &= \frac{\gamma_2(s_1) F_{2,\text{tot}}}{K_{m3} + \gamma_2(s_1)} := \varphi_3(s_1) \\
c_4 &= \frac{s_1 F_{1,\text{tot}}}{K_{m4} + s_1} := \varphi_4(s_1).
\end{aligned} \tag{2.38}$$

It is easy to see that $\varphi_1, \ldots, \varphi_4$ are all strictly increasing functions of s_1 with $\varphi_i(0) = 0, i = 1, \ldots, 4$.

On the hand, by the conservation relations in (2.27), we have

$$S_{\text{tot}} = \gamma_1(s_1) + s_1 + \gamma_2(s_1) + \sum_{i=1}^{4} \varphi_i(s_1) := \alpha(s_1). \tag{2.39}$$

The function $\alpha(s_1)$ is thus strictly increasing in s_1 and satisfies $\alpha(0) = 0$. As a result, for any given $S_{\text{tot}} > 0$, equation (2.39) has a unique positive solution of s_1.

Once we have the steady state value of s_1, by (2.38), the steady state values of $c_1, \ldots, c_4$ are uniquely determined. The steady state value of s_0 and s_2 are given by

$\gamma_1(s_1)$ and $\gamma_2(s_1)$ respectively. Moreover, the conservation relations in (2.27) yield the steady state values of e_1, e_2, f_1, and f_2.

To summarize, for any given positive numbers $S_{\text{tot}}, E_{1,\text{tot}}, E_{2,\text{tot}}, F_{1,\text{tot}}$, and $F_{2,\text{tot}}$, system (2.26)-(2.27) admits a unique positive steady state.

2.4 Arkin's Example

In [74], Samoilov, Plyasunov, and Arkin provided an example of a set of chemical reactions whose full stochastic (Master Equation) model exhibits bistable behavior, but the deterministic (mean field) version yields a unique positive steady state.

The reactions that they introduced consist of a futile cycle of size one driven by a second reaction which induces "deterministic noise" on the concentration of the forward enzyme. The model is as follows:

$$
\begin{aligned}
N + N &\underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} N + E \\
N &\underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} E \\
S + E &\underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} C_1 \xrightarrow{k_4} P + E \\
P + F &\underset{k_{-5}}{\overset{k_5}{\rightleftarrows}} C_2 \xrightarrow{k_6} S + F\,.
\end{aligned}
$$

In fact [74] does not prove mathematically that this reaction's deterministic model has a single-steady state property, but shows numerically that, for a particular value of the kinetic constants k_i, a unique steady state (subject to stoichiometric constraints) exists. In this section, we provide a proof of uniqueness valid for all possible parameter values.

We use lower case letters n, e, s, c_1, p, c_2, f to denote the concentrations of the corresponding chemicals, as functions of t. The reactions can be modeled by the following

differential equations:

$$\begin{aligned}
\frac{dn}{dt} &= -k_1 n^2 + k_{-1} ne - k_2 n + k_{-2} e \\
\frac{de}{dt} &= -k_3 se + k_{-3} c_1 + k_4 c_1 + k_1 n^2 - k_{-1} ne + k_2 n - k_{-2} e \\
\frac{ds}{dt} &= -k_3 se + k_{-3} c_1 + k_6 c_2 \\
\frac{dc_1}{dt} &= k_3 se - k_{-3} c_1 - k_4 c_1 \\
\frac{dp}{dt} &= k_4 c_1 - k_5 pf + k_{-5} c_2 \\
\frac{dc_2}{dt} &= k_5 pf - k_{-5} c_2 - k_6 c_2 \\
\frac{df}{dt} &= -k_5 pf + k_{-5} c_2 + k_6 c_2.
\end{aligned} \tag{2.40}$$

Observe the following conservation relations hold:

$$\begin{aligned}
E_{\text{tot}} &= e + n + c_1 \\
F_{\text{tot}} &= f + c_2 \\
S_{\text{tot}} &= s + c_1 + c_2 + p.
\end{aligned}$$

Theorem 2.9 *For any given positive numbers $E_{tot}, F_{tot}, S_{tot}$, k_1 and so on, system* (2.40) *has a unique positive steady state.*

Proof. Existence follows from the Brower fixed point theorem, since the reduced system evolves on a compact convex set (intersection of the positive orthant and the affine subspace given by the stoichiometry class).

We now fix one stoichiometry class and a set of kinetic parameters to prove uniqueness. At the steady states, we have the right hand side of (2.40) equal zero. The idea is to express the steady state values of variables e, s, c_1, c_2, and p in terms of n, and then show that there is a unique steady state value of n.

From $dn/dt = 0$, we obtain that:

$$e = \frac{k_1 n^2 + k_2 n}{k_{-1} n + k_{-2}} := \alpha(n).$$

From $dc_1/dt = 0$, we have:

$$s = \frac{(k_{-3} + k_4) c_1}{k_3 e} = \frac{(k_{-3} + k_4) c_1}{k_3 \alpha(n)}\,. \tag{2.41}$$

Solving $dc_2/dt = 0$ for p and then substituting $f = F_{\text{tot}} - c_2$ gives:

$$p = \frac{(k_{-5} + k_6)c_2}{k_5(F_{\text{tot}} - c_2)}\,. \tag{2.42}$$

Finally, from $d(p - f)/dt = 0$, we obtain:

$$c_2 = \frac{k_4}{k_6}c_1\,. \tag{2.43}$$

The derivative of $\alpha(n)$ is

$$\alpha'(n) = \frac{k_1k_{-1}n^2 + 2k_1k_{-2}n + k_2k_{-2}}{(k_{-2} + k_{-1}n)^2} > 0 \text{ for all } n \geq 0,$$

and thus $\alpha(n)$ is a strictly increasing function on $[0, +\infty)$.

Notice that

$$c_1 = E_{\text{tot}} - (e + n) = E_{\text{tot}} - (\alpha(n) + n).$$

As a result the steady state value of c_1 as a function of n is strictly decreasing on $[0, +\infty)$. Following from (2.41)-(2.43), the steady state values of c_2, s, and p are also strictly decreasing in n for $n \geq 0$.

Recall that

$$S_{\text{tot}} = s + c_1 + c_2 + p.$$

The right hand side of the above equation is strictly decreasing in n. Therefore, for any given $S_{\text{tot}} > 0$, there is a unique positive steady state value of n. The steady state values of other variables are now functions of n, and it follows that the steady state is unique and positive. ∎

Chapter 3

Singularly Perturbed Monotone Systems

3.1 Introduction

Monotone dynamical systems constitute a rich class of models, for which global and almost-global convergence properties can be established. They are particularly useful in biochemical applications and also appear in areas like coordination ([60]) and other problems in control ([16]). One of the fundamental results in monotone systems theory is Hirsch's Generic Convergence Theorem ([36, 37, 38, 39, 82]). Informally stated, Hirsch's result says that almost every bounded solution of a strongly monotone system converges to the set of equilibria. There is a rich literature regarding the application of this powerful theorem, as well as of other results dealing with everywhere convergence when equilibria are unique ([19, 44, 82]), to models of biochemical systems.

Unfortunately, many models in biology are not monotone, at least with respect to any standard orthant order. This is because in monotone systems (with respect to orthant orders) every net feedback loop should be positive, but, on the other hand, in many systems negative feedback loops often appear as well, as they are required for adaptation and precision. In order to address this drawback, as well as to study properties of large systems which are monotone but which are hard to analyze in their entirety, a recent line of work introduced an input/output approach that is based on the analysis of interconnections of monotone systems. For example, the approach allows one to view a *non*-monotone system as a negative feedback loop around a monotone open-loop system, thus leading to results on global stability provided that the loop gain is small enough (small-gain theorem for monotone systems) and to the emergence of oscillations under transmission delays, and to the construction of relaxation oscillators by slow adaptation rules on feedback gains. See [84, 85] for expositions and many

references.

The method presented here is in the same character. Intuitively, negative loops that act at a comparatively fast time scale should not affect the main characteristics of monotone behavior. The main purpose of this chapter is to show that this is indeed the case, in the sense that singularly perturbed strongly monotone systems inherit generic convergence properties. A system that is not monotone may become monotone once that fast variables are replaced by their steady-state values.

A trivial linear example that illustrates this point is

$$\dot{x} = -x - y,$$

$$\varepsilon\dot{y} = -y + x,$$

with $\varepsilon > 0$. This system is not monotone with respect to any orthant cone. On the other hand, for $\varepsilon \ll 1$, the fast variable y tracks x, so the slow dynamics is well-approximated by $\dot{x} = -2x$, which is strongly monotone, because every scalar system is; hence, for $\varepsilon \ll 1$ one may expect that convergence still holds. (In this example, there is global convergence to zero for all ε.) In order to prove a precise time-separation result, we employ tools from geometric singular perturbation theory.

This point of view is of special interest in the context of biochemical systems; for example, Michaelis Menten kinetics are mathematically justified as singularly perturbed versions of mass action kinetics ([21, 65]). One particular example of great interest in view of current systems biology research is that of dual "futile cycle" motifs, as illustrated in Figure 2.2. This is a special case of Figure 1.1 when $n = 2$, see Section 2.2.1 for discussions of its biological applications.

Numerical simulations suggested that the above system could be either monostable or bistable, see [58]. The latter will give rise to switch-like behavior, which is ubiquitous in cellular pathways ([28, 70, 78, 79]). In either case, the system under meaningful biological parameters shows convergence, not other dynamical properties such as periodic behavior or even chaotic behavior. Analytical studies done for the quasi-steady-state version of the model (slow dynamics), which is a monotone system, indicate that the reduced system is indeed monostable or bistable, see [67]. Thus, it is of great interest

to show that, at least in certain parameter ranges (as required by singular perturbation theory), the full system inherits convergence properties from the reduced system, and this is what we do as an application of our results. We remark that the simplified system consisting of a unary conversion cycle (no S_2) is known to admit a unique equilibrium (subject to mass conservation constraints) which is a global attractor, see [7].

A feature of our approach is the use of geometric invariant manifold theory [24, 45, 66]. There is a manifold M_ε, invariant for the full dynamics of a singularly perturbed system, which attracts all near-enough solutions. However, we need to exploit the full power of the theory, and especially the fibration structure and an asymptotic phase property. The system restricted to the invariant manifold M_ε is a regular perturbation of the slow (ε=0) system. As remarked in Theorem 1.2 in Hirsch's early paper [37], a C^1 regular perturbation of a flow with eventually positive derivatives also has generic convergence properties. So, solutions in the manifold will generally be well-behaved, and asymptotic phase implies that solutions near M_ε track solutions in M_ε, and hence also converge to equilibria if solutions on M_ε do. A key technical detail is to establish that the tracking solutions also start from the "good" set of initial conditions, for generic solutions of the large system.

A preliminary version of these results by Wang and Sontag in [97] deals with the special case of singularly perturbed systems of the form:

$$\begin{aligned} \dot{x} &= f(x,y) \\ \varepsilon\dot{y} &= Ay + h(x) \end{aligned} \tag{3.1}$$

on a product domain, where A is a constant Hurwitz matrix and the reduced system $\dot{x} = f(x, -A^{-1}h(x))$ is strongly monotone. However, for the application to the above futile cycle, there are two major problems with that formulation: first, the dynamics of the fast system have to be allowed to be nonlinear in y, and second, it is crucial to allow for an ε-dependence on the right-hand side as well as to allow the domain to be a convex polytope depending on ε. We provide a much more general formulation here.

We note that no assumptions are imposed regarding global convergence of the reduced system, which is essential because of the intended application to multi-stable

systems. This seems to rule out the applicability of Lyapunov-theoretic and input-to-state stability tools ([17, 89]).

3.2 Monotone Systems for Ordinary Differential Equations

In this section, we review several useful definitions and theorems regarding monotone systems. As we wish to provide results valid for arbitrary orders, not merely orthants, and some of these results, though well-known, are not readily available in a form needed for reference, we provide some technical proofs.

Definition 3.1 *A nonempty, closed set $C \subset \mathbb{R}^N$ is a cone if*

1. $C + C \subset C$,
2. $\mathbb{R}_+ C \subset C$,
3. $C \bigcap (-C) = \{0\}$.

We always assume $C \neq \{0\}$. Associated to a cone C is a partial order on $\mathbb{R}^N$. For any $x, y \in \mathbb{R}^N$, we define

$$x \geq y \Leftrightarrow x - y \in C$$
$$x > y \Leftrightarrow x - y \in C, x \neq y.$$

When Int$C$ is not empty, we can define

$$x \gg y \Leftrightarrow x - y \in \text{Int}C.$$

The most typical example would be $C = \mathbb{R}^N_{\geq 0}$, in which case $x \geq y$ means that each coordinate of x is bigger or equal than the corresponding coordinate of y. This order gives rise to the class of "cooperative systems" (to be defined later) which is a special class of the systems discussed below. Other orthant orders in $\mathbb{R}^N$ also arise naturally in biological systems, as we will see in our applications.

Definition 3.2 *The dual cone of C is defined as*

$$C^* = \{\lambda \in (\mathbb{R}^N)^* \mid \lambda(C) \geq 0\}.$$

An immediate consequence is

$$x \in C \Leftrightarrow \lambda(x) \geq 0, \forall \lambda \in C^*$$

$$x \in \mathrm{Int}C \Leftrightarrow \lambda(x) > 0, \forall \lambda \in C^* \setminus \{0\}.$$

With this partial order on $\mathbb{R}^N$, we analyze certain features of the dynamics of an ordinary differential equation

$$\frac{dz}{dt} = F(z), \tag{3.2}$$

where $F : \mathbb{R}^N \to \mathbb{R}^N$ is a C^1 vector field. For any $z \in \mathbb{R}^N$, we denote the maximally defined solution of (3.2) with initial condition z by $t \to \phi_t(z), t \in I(z)$, where $I(z)$ is an open interval in $\mathbb{R}$ that contains zero. For each $t \in \mathbb{R}$, the set of $z \in \mathbb{R}^N$ for which $\phi_t(z)$ is defined is an open set $W(t) \subseteq \mathbb{R}^N$, and $\phi_t : W(t) \to W(-t)$ is a C^1 diffeomorphism. The collection of maps $\phi_t, t \in \mathbb{R}$ is called the *flow* of (3.2). We also write just $z(t)$ for the solution of (3.2), if the initial condition $z(0)$ is clear from the context. The forward trajectory of $z \in \mathbb{R}^N$ is a parametrized curve $t \to \phi_t(z)$ $(t \geq 0, t \in I(x))$. Its image is the forward orbit of z, denoted as $O_+(z)$. The backward trajectory and the backward orbit $O_-(z)$ are defined analogously.

Definition 3.3 *A set $U \subseteq \mathbb{R}^N$ is called positively (respectively, negatively) invariant if $O_+(U) \subseteq U$ (respectively, $O_-(U) \subseteq U$). It is called invariant if it is both positively and negatively invariant.*

We are interested in a special class of ordinary differential equations that preserve the order along the trajectories. For simplicity, the solutions of (3.2) are assumed to exist for all $t \geq 0$ in the sets considered in the following.

Definition 3.4 *The flow ϕ_t of (3.2) is said to have (eventually) positive derivatives on a set $V \subseteq \mathbb{R}^N$ with respect to a cone C, if $[D_z\phi_t(z)]x \in \mathrm{Int}C$ for all $x \in C \setminus \{0\}, z \in V$, and $t \geq 0$ ($t \geq t_0$ for some $t_0 > 0$ independent of z).*

Next, we give a special class of systems with the property of positive derivatives.

Definition 3.5 *A square $N \times N$ matrix $A = (a_{ij})$ is said to be reducible if the indices $1, 2, \ldots, N$ can be divided into two disjoint nonempty sets $i_1, i_2, \ldots, i_u$ and $j_1, j_2, \ldots, j_v$ (with $u + v = N$) such that $a_{i_\alpha j_\beta} = 0$ for all $\alpha = 1, 2, \ldots, u$ and $\beta = 1, 2, \ldots, v$. A square matrix that is not reducible is said to be irreducible.*

Proposition 3.6 *A matrix is reducible if and only if it can be placed into block upper-triangular form by simultaneous row and column permutations. In addition, a matrix is reducible if and only if its associated digraph is not strongly connected.*

Definition 3.7 *System* (3.2) *is called irreducible on a set $V \subseteq \mathbb{R}^N$ if the Jacobian matrix $DF(z)$ is irreducible for all $z \in V$.*

Definition 3.8 *System* (3.2) *is cooperative (respectively, competitive) with respect to the nonnegative orthant $\mathbb{R}^N_{\geq 0}$ on a set $V \subseteq \mathbb{R}^N$ if*

$$\frac{\partial F_i}{\partial z_j}(z) \geq 0 \, (respectively, \leq 0)$$

for all $i \neq j$ and $z \in V$. Moreover, system (3.2) *is called a strictly cooperative (resp. competitive) system with respect to the nonnegative orthant on V, if* (3.2) *is cooperative and the partial derivatives are never zero.*

Hereafter in this thesis, without special mentioning of the cone, "cooperative" means cooperative with respect to the nonnegative orthant.

Systems that are irreducible and cooperative have nice properties. For example:

Lemma 3.9 *(Theorem 1.1 in [36]). If* (3.2) *is irreducible and cooperative on a convex open set $W \subseteq \mathbb{R}^N$, then the flow of* (3.2) *has positive derivatives.*

A careful reading of the proof in [36] of the above result leads to the following lemma:

Lemma 3.10 *If* (3.2) *is strictly cooperative on a convex open set $W \subseteq \mathbb{R}^N$, then the flow of* (3.2) *has positive derivatives.*

It is worth noticing that $[D_z\phi_t(z)]x \in \mathrm{Int}C$ is equivalent to $\lambda([D_z\phi_t(z)]x) > 0$ for all $\lambda \in C^*$ with $|\lambda| = 1$, where $|\cdot|$ denotes the Euclidean norm. We will use this fact in

the proofs of the following lemmas. Lemma 3.11 deals with "regular" perturbations in the dynamics. It generalizes Theorem 1.2 of [36] from the nonnegative orthant $\mathbb{R}^N_{\geq}$ to an arbitrary cone C.

Lemma 3.11 *Assume $V \subset \mathbb{R}^N$ is a compact set in which the flow ϕ_t of (3.2) has eventually positive derivatives. Then there exists $\delta > 0$ with the following property. Let ψ_t denote the flow of a C^1 vector field G such that the C^1 norm of $F(z) - G(z)$ is less than δ for all z in V. Then there exists $t_* > 0$ such that if $\psi_s(z) \in V$ for all $s \in [0, t]$ where $t \geq t_*$, then $[D_z\psi_t(z)]x \in Int\, C$ for all $z \in V$ and $x \in C \setminus \{0\}$. If, in addition, V is positively invariant under the flow ψ_t, then ψ_t has eventually positive derivatives in V.*

Proof. Pick $t_* = t_0 > 0$, so that $\lambda([D_z\phi_t(z)]x) > 0$ for all $t \geq t_0, z \in V, \lambda \in C^*, x \in C$ with $|\lambda| = 1, |x| = 1$. Then there exists $\delta > 0$ with the property that when the C^1 norm of $F(z) - G(z)$ is less than δ, we have $\lambda([D_z\psi_t(z)]x) > 0$ for $t_0 \leq t \leq 2t_0$.

For $t > 2t_0$, we write $t = r + kt_0$, where $t_0 \leq r < 2t_0$ and $k \in \mathbb{N}$. If $\psi_s(z) \in V$ for all $s \in [0, t]$, we can define $z_j := \psi_{jt_0}(z)$ for $j = 0, \ldots, k$. For any $x \in C \setminus \{0\}$, using the chain rule, we have

$$[D_z\psi_t(z)]x = [D_z\psi_r(z_k)][D_z\psi_{t_0}(z_{k-1})] \cdots [D_z\psi_{t_0}(z_0)]x,$$

and thus $[D_z\psi_t(z)]x \in \mathrm{Int}C$.

Furthermore, if V is positively invariant under the flow ψ_t, then for any $z \in V$ the condition $\psi_s(z) \in V$ for $s \in [0, t]$ is satisfied for all $t \geq 0$. As a result, the flow ψ_t has eventually positive derivatives in V. ∎

Definition 3.12 *The system (3.2) or the flow ϕ_t of (3.2) is called monotone (resp. strongly monotone) in a set $W \subseteq \mathbb{R}^N$, if for all $t > 0$ and $z_1, z_2 \in W$,*

$$z_1 \geq z_2 \Rightarrow \phi_t(z_1) \geq \phi_t(z_2)$$
$$(\textit{resp. } \phi_t(z_1) \gg \phi_t(z_2) \textit{ when } z_1 \neq z_2).$$

It is eventually (strongly) monotone if there exists $t_0 > 0$ such that ϕ_t is (strongly) monotone for all $t \geq t_0$.

If the partial derivatives $\partial F_i/\partial z_j$ have constant sign for all $i \neq j$, system (3.2) is said to have "definite feedback relations". For such systems, we can define their "signed influence digraphs" as follows. Vertexes of the signed influence digraph associated with system (3.2) consist of the dependent variables $z_i, i = 1, \ldots, N$. There is no directed edge from z_j to z_i if $\partial F_i/\partial z_j \equiv 0$. The edge from z_j to z_i is positive if $\partial F_i/\partial z_j \geq 0$, and the edge from z_j to z_i is negative if $\partial F_i/\partial z_j \leq 0$.

System (3.2) is monotone with respect to some orthant order if there is no negative (non-oriented) loops in the signed influence digraph, see [49, 82, 92]. If further the graph is strongly connected, meaning that there is a path from z_i to z_j for each $i \neq j$, then system (3.2) is strongly monotone with respect to some orthant order.

As an example, let us recall the motif introduced in Section 2.3. The signed influence digraph of its ordinary differential equation model (2.26) is depicted in Figure 3.1. It is clear from Figure 3.1 that the signed influence digraph contains no negative edges, as a result system (2.26) is monotone. Moreover, the digraph is strongly connected, so system (2.26) is strongly monotone with respect to the nonnegative orthant $\mathbb{R}^7_{\geq 0}$. On the other hand, system (2.26) has a unique positive equilibrium, see Section 2.3. By a result in [19], we know that every bounded solution in $\mathbb{R}^7_{\geq 0}$ of system (2.26) converges to that unique equilibrium.

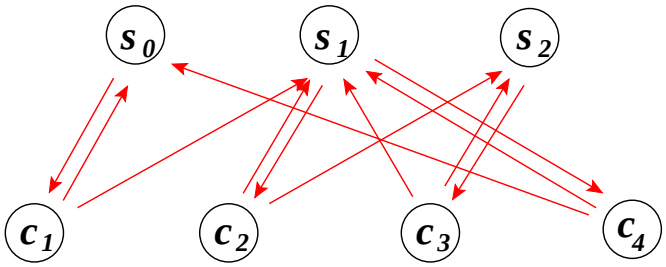

Figure 3.1: The signed influence digraph of system (2.26). In a signed influence digraph, we use solid lines to represent edges with positive signs, and dashed lines to denote edges with negative signs. In this digraph, there are no edges with negative signs.

In Section 3.5.2, we show that the double phosphorylation dephosphorylation motif admits a signed influence digraph that contains negative loops, and therefore is not monotone.

Definition 3.13 *An set $W \subseteq \mathbb{R}^N$ is called p-convex, if W contains the entire line*

segment joining x and y whenever $x \leq y$, $x, y \in W$.

Proposition 3.14 *Let $W \subseteq \mathbb{R}^N$ be p-convex. If the flow ϕ_t has (eventually) positive derivatives in W, then it is (eventually) strongly monotone in W.*

Proof. For any $z_1 > z_2 \in W, \lambda \in C^* \setminus \{0\}$ and $t > 0$ ($t \geq t_0$ for some $t_0 > 0$), we have

$$\lambda(\phi_t(z_1) - \phi_t(z_2)) = \int_0^1 \lambda\big([D_z\phi_t(sz_1 + (1-s)z_2)](z_1 - z_2)\big)ds > 0.$$

Therefore, ϕ_t is (eventually) strongly monotone in W. ∎

The following two lemmas are variations of Hirsch's Generic Convergence Theorem.

Lemma 3.15 *Suppose that the flow ϕ_t of (3.2) has eventually positive derivatives in a p-convex open set $W \subseteq \mathbb{R}^N$. Let $W^c \subseteq W$ be the set of points whose forward orbit has compact closure in W. If the set of equilibria is totally disconnected (e.g., countable), then the forward trajectory starting from almost every point in W^c converges to an equilibrium.*

Lemma 3.15 is a generalization of Theorem 4.1 in [37] to arbitrary cones, done in the same manner as in Lemma 3.11, so we omit the proof here. In our futile cycle example, as well as in most biochemical systems after reduction by elimination of stoichiometric constraints, the set of equilibria is discrete, and thus Lemma 3.15 will apply. However, the more general result in Lemma 3.17 is also true, and applies even when the set of equilibria is not discrete. It follows as a direct application of Theorem 2.26 in [39].

Definition 3.16 *A point x in a set $W \subseteq \mathbb{R}^N$ is called strongly accessible from below (respectively, above) if there exists a sequence $\{y_n\}$ in W converging to x such that $y_n < y_{n+1} < x$ (respectively, $y_n > y_{n+1} > x$).*

Lemma 3.17 *Suppose that the flow ϕ_t of (3.2) has compact closure and eventually positive derivatives in a p-convex open set $W \subseteq \mathbb{R}^N$. If any point in W can be strongly accessible either from above or from below in W, then the forward trajectory from every point, except for initial conditions in a nowhere dense set, converges to an equilibrium.*

3.3 Geometric Singular Perturbation Theory

The theory of geometric singular perturbation can be traced back to the works of Fenichel [24] , Mitropolsky and Lykova [59] in 1970's. Later on, the works by Henry [35], Knobloch and Aulbach [48], Nipp [66], and Sakamoto [73] also presented results similar to [24]. By now, the theory is fairly standard, and there have been enormous applications to traveling waves of partial differential equations, see [45] and the references there. For control theoretical applications, see [1, 43].

In this section, we revisit Fenichel's theorems on geometric singular perturbation, in the form stated in [73] by Sakamoto.

Consider a general singular perturbation system,

$$\begin{aligned} \frac{dx}{dt} &= f(x, y, \varepsilon) \\ \varepsilon \frac{dy}{dt} &= g(x, y, \varepsilon), \end{aligned} \tag{3.3}$$

where $x \in \mathbb{R}^n, y \in \mathbb{R}^m$, and $\varepsilon \in [0, \varepsilon_0]$ for some $\varepsilon_0 > 0$. We shall denote various hypotheses about system (3.3) with the letter S.

S1 The functions f and g, defined on $(x, y, \varepsilon) \in \mathbb{R}^n \times \mathbb{R}^m \times I$, are of class C_b^r for some positive integer r. (A function h is of class C_b^r if it is in C^r, and its derivatives up to order r as well as f itself are bounded.)

S2 There is a function m_0 whose derivatives up through order r are bounded (except for the function itself), such that $g(x, m_0(x), 0) = 0$.

The manifold, defined as

$$M_0 := \{(x, y) \mid y = m_0(x), x \in \mathbb{R}^n\}$$

is often called the limiting slow manifold.

When $\varepsilon = 0$, system (3.3) degenerates to

$$\begin{aligned} \frac{dx}{dt} &= f(x, m_0(x), 0) \\ y &= m_0(x), \end{aligned} \tag{3.4}$$

where trajectories are restricted to the limiting slow manifold M_0.

When $\varepsilon \neq 0$, we may change the time scale to $\tau = t/\varepsilon$, and study the following equivalent system of (3.3):

$$\begin{aligned} \frac{dx}{d\tau} &= \varepsilon f(x, y, \varepsilon) \\ \frac{dy}{d\tau} &= g(x, y, \varepsilon). \end{aligned} \tag{3.5}$$

System (3.5) is often referred to as the fast time scale representation in contrast to the slow time scale representation (3.3).

In the limit of $\varepsilon = 0$, system (3.5) becomes:

$$\begin{aligned} \frac{dx}{d\tau} &= 0 \\ \frac{dy}{d\tau} &= g(x, y, 0). \end{aligned} \tag{3.6}$$

We remark that system (3.6) focuses on the beginning part of a trajectory, while system (3.4) gives the asymptotic behavior of a trajectory. One of the main goals of Geometric Singular Perturbation theory is to connect these two limiting systems using ideas from invariant manifold to gain deeper insights of the full system (3.3), see Figure 3.2.

Definition 3.18 *We say that M_0 is normally hyperbolic relative to (3.5) if all eigenvalues of the matrix $D_y g(x, m_0(x), 0)$ have nonzero real part for every $x \in \mathbb{R}^n$.*

The notation $D_y g(x, m_0(x), 0)$ means the partial derivatives of $g(x, y, \varepsilon)$ with respect to the y coordinate evaluated at the point $(x, m_0(x), 0)$. Fenichel studied manifolds that are normally hyperbolic. For our purpose of application, we are solely interested in manifolds with the following property.

S3 All eigenvalues of the matrix $D_y g(x, m_0(x), 0), x \in \mathbb{R}^n$ have negative real parts smaller than $-\mu$, where μ is a positive constant.

Theorem 3.19 *Under assumptions* **S1** *to* **S3**, *there exists a positive number $\varepsilon_1 < \varepsilon_0$ such that for every $\varepsilon \in (0, \varepsilon_1]$ the following properties hold.*

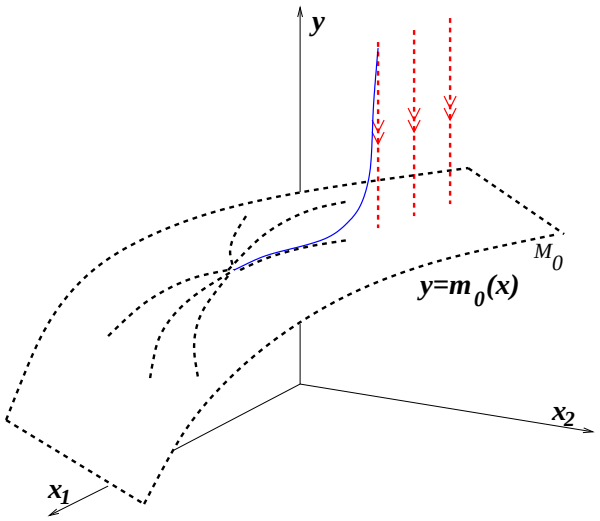

Figure 3.2: An illustration of the two limiting systems (3.6) and (3.4). The surface bounded by dashed curves is the limiting slow manifold M_0. The dashed trajectories on M_0 are solutions to system (3.4). The dashed straight lines perpendicular to M_0 are the solutions to system (3.6). The solid curve is the true trajectory of system (3.3) when $\varepsilon \neq 0$.

1. *There is a* C_b^{r-1} *function*

$$m : \mathbb{R}^n \times [0, \varepsilon_1] \to \mathbb{R}^m$$

such that the set M_ε *defined by*

$$M_\varepsilon := \{\big(x, m(x, \varepsilon)\big) \,|\, x \in \mathbb{R}^n\}$$

is invariant under the flow generated by (3.17). Moreover,

$$\sup_{x \in \mathbb{R}^n} |m(x, \varepsilon) - m_0(x)| = O(\varepsilon), \ \text{as } \varepsilon \to 0.$$

In particular, we have $m(x, 0) = m_0(x)$ *for all* $x \in \mathbb{R}^n$.

2. *The stable manifold of* M_ε*, consisting of all points* (x_0, y_0) *such that*

$$\sup_{\tau \geq 0} |y(\tau; x_0, y_0) - m\big(x(\tau; x_0, y_0), \varepsilon\big)| e^{\frac{\mu\tau}{4}} < \infty,$$

where $\big(x(\tau; x_0, y_0), y(\tau; x_0, y_0)\big)$ *is the solution of* (3.3) *passing through* (x_0, y_0) *at* $\tau = 0$*, is a* C^{r-1}*-immersed submanifold in* $\mathbb{R}^n \times \mathbb{R}^m$ *of dimension* $n + m$*, denoted by* $W^s(M_\varepsilon)$.

3. *There is a positive constant δ_0 such that if*

$$\sup_{\tau\geq 0}|y(\tau;x_0,y_0)-m\big(x(\tau;x_0,y_0),\varepsilon\big)|<\delta_0,$$

then $(x_0,y_0)\in W^s(M_\varepsilon)$.

4. *The manifold $W^s(M_\varepsilon)$ is a disjoint union of C^{r-1}-immersed manifolds $W^s_\varepsilon(\xi)$ of dimension m:*

$$W^s(M_\varepsilon)=\bigcup_{\xi\in\mathbb{R}^n}W^s_\varepsilon(\xi).$$

For each $\xi\in\mathbb{R}^n$, let $H_\varepsilon(\xi)(\tau)$ be the solution for $\tau\geq 0$ of

$$\frac{dx}{d\tau}=\varepsilon f(x,m(x,\varepsilon),\varepsilon),\quad x(0)=\xi\in\mathbb{R}^n.$$

Then, the manifold $W^s_\varepsilon(\xi)$ is the set

$$\{(x_0,y_0)\,|\,\sup_{\tau\geq 0}|\tilde{x}(\tau)|e^{\frac{\mu\tau}{4}}<\infty,\sup_{\tau\geq 0}|\tilde{y}(\tau)|e^{\frac{\mu\tau}{4}}<\infty\},$$

where

$$\tilde{x}(\tau)=x(\tau;x_0,y_0)-H_\varepsilon(\xi)(\tau),$$
$$\tilde{y}(\tau)=y(\tau;x_0,y_0)-m\big(H_\varepsilon(\xi)(\tau),\varepsilon\big).$$

5. *The fibers are "positively invariant" in the sense that $W^s_\varepsilon(H_\varepsilon(\xi)(\tau))$ is the set*

$$\{\big(x(\tau;x_0,y_0),y(\tau;x_0,y_0)\big)\,|\,(x_0,y_0)\in W^s_\varepsilon(\xi)\}$$

for each $\tau\geq 0$, see Figure 3.3.

6. *The fibers restricted to the δ_0 neighborhood of M_ε, denoted by $W^s_{\varepsilon,\delta_0}$, can be parametrized as follows. Let $[-\delta_0,\delta_0]$ denote the cube $\{(\eta_1,\ldots,\eta_n)\mid|\eta_i|\leq\delta_0\}$. There are two C^{r-1}_b functions*

$$P_{\varepsilon,\delta_0}:\mathbb{R}^n\times[-\delta_0,\delta_0]\to\mathbb{R}^n$$

$$Q_{\varepsilon,\delta_0}:\mathbb{R}^m\times[-\delta_0,\delta_0]\to\mathbb{R}^m,$$

and a map

$$T_{\varepsilon,\delta_0}:\mathbb{R}^n\times[-\delta_0,\delta_0]\to\mathbb{R}^n\times\mathbb{R}^m$$

mapping (ξ, η) *to* (x, y)*, where*

$$x = \xi + P_{\varepsilon,\delta_0}(\xi, \eta), \quad y = m(x, \varepsilon) + Q_{\varepsilon,\delta_0}(\xi, \eta)$$

such that

$$W^s_{\varepsilon,\delta_0}(\xi) = T_{\varepsilon,\delta_0}(\xi, [-\delta_0, \delta_0]).$$

Remark 3.20 *The* δ_0 *in property 3 can be chosen uniformly for* $\varepsilon \in (0, \varepsilon_0]$*.*

Notice that property 4 ensures that for each $(x_0, y_0) \in W^s(M_\varepsilon)$*, there exists a* ξ *such that*

$$|x(\tau; x_0, y_0) - H_\varepsilon(\xi)(\tau)| \to 0,$$

$$|y(\tau; x_0, y_0) - m\big(H_\varepsilon(\xi)(\tau), \varepsilon\big)| \to 0.$$

as $\tau \to \infty$*. This is often referred to as the "asymptotic phase" property, see Figure 3.3.*

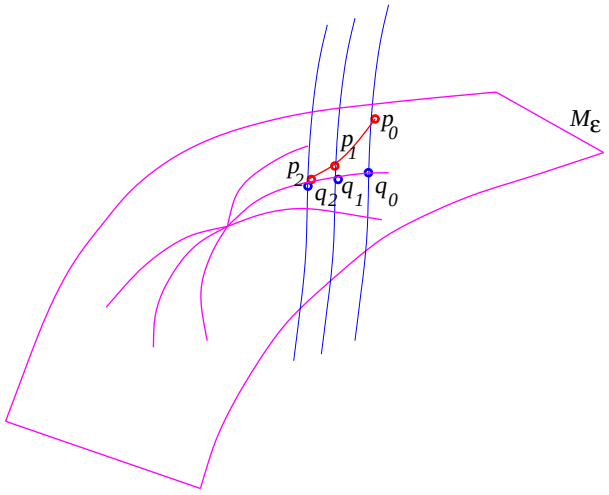

Figure 3.3: An illustration of the "positive invariant" and "asymptotic phase" properties. Let p_0 be a point on the fiber $W^s_\varepsilon(q_0)$ (vertical curve). Suppose the solution of (3.17) starting from $q_0 \in M_\varepsilon$ evolves to $q_1 \in M_\varepsilon$ after time τ_1, then the solution of (3.17) starting from p_0 will evolve to $p_1 \in W^s_\varepsilon(q_1)$ after time τ_1. At time τ_2, q_1 and p_1 evolve to q_2, p_2 respectively. These two solutions at any given time are always on the same fiber. If the solution starting from q_0 converges to an equilibrium, then the solution starting from p_0 also converges to an equilibrium.

3.4 Main Results on Singularly Perturbed Monotone Systems

3.4.1 Statement of the main theorem

Consider the following system:

$$\begin{aligned} \frac{dx}{dt} &= f_0(x, y, \varepsilon) \\ \varepsilon \frac{dy}{dt} &= g_0(x, y, \varepsilon), \end{aligned} \tag{3.7}$$

where $x \in \mathbb{R}^n, y \in \mathbb{R}^m$, and $\varepsilon \in [0, \varepsilon_0]$ for some $\varepsilon_0 > 0$. We will be interested in the dynamics of this system on an ε-dependent domain D_ε. The corresponding fast system is:

$$\begin{aligned} \frac{dx}{d\tau} &= \varepsilon f_0(x, y, \varepsilon) \\ \frac{dy}{d\tau} &= g_0(x, y, \varepsilon). \end{aligned} \tag{3.8}$$

We make the following assumptions, where the integer $r > 1$ is fixed from now on:

A1 Let $U \subset \mathbb{R}^n$ and $V \subset \mathbb{R}^m$ be open and bounded. The functions

$$f_0 : U \times V \times [0, \varepsilon_0] \to \mathbb{R}^n$$

$$g_0 : U \times V \times [0, \varepsilon_0] \to \mathbb{R}^m$$

are both of class C^r.

A2 There is a function

$$m_0 : U \to V$$

of class C^r, such that $g_0(x, m_0(x), 0) = 0$ for all x in U.

It is often helpful to consider $z = y - m_0(x)$, and the fast system (3.8) in the new coordinates becomes:

$$\begin{aligned} \frac{dx}{d\tau} &= \varepsilon f_1(x, z, \varepsilon) \\ \frac{dz}{d\tau} &= g_1(x, z, \varepsilon), \end{aligned} \tag{3.9}$$

where

$$f_1(x,z,\varepsilon) = f_0(x, z + m_0(x), \varepsilon),$$
$$g_1(x,z,\varepsilon) = g_0(x, z + m_0(x), \varepsilon) - \varepsilon[D_x m_0(x)] f_1(x,z,\varepsilon).$$

When $\varepsilon = 0$, the system (3.9) degenerates to

$$\frac{dz}{d\tau} = g_1(x,z,0), \quad x(\tau) \equiv x_0 \in U, \tag{3.10}$$

seen as equations on $\{z \,|\, z + m_0(x_0) \in V\}$.

A3 The steady state $z = 0$ of (3.10) is globally asymptotically stable on $\{z \,|\, z + m_0(x_0) \in V\}$ for all $x_0 \in U$.

A4 All eigenvalues of the matrix $D_y g_0(x, m_0(x), 0)$ have negative real parts for every $x \in U$, i.e. the matrix $D_y g_0(x, m_0(x), 0)$ is Hurwitz on U.

A5 There exists a family of convex compact sets $D_\varepsilon \subset U \times V$, which depend continuously on $\varepsilon \in [0, \varepsilon_0]$, such that (3.7) is positively invariant on D_ε for $\varepsilon \in (0, \varepsilon_0]$.

A6 The flow ψ_t^0 of the limiting system (set $\varepsilon = 0$ in (3.7)):

$$\frac{dx}{dt} = f_0(x, m_0(x), 0) \tag{3.11}$$

has eventually positive derivatives on K_0 with respect to some cone C. Here K_0 is the projection of

$$D_0 \bigcap \{(x,y) \,|\, y = m_0(x), x \in U\}$$

onto the x-axis.

A7 The set of equilibria of (3.7) on D_ε is totally disconnected.

Remark 3.21 *In mass-action chemical kinetics, the vector fields are polynomials, so* **A1** *follows naturally. Assumption* **A3** *implies that $y = m_0(x)$ is a unique solution of $g_0(x,y,0) = 0$ on U. Continuity in* **A5** *is understood with respect to the Hausdorff metric.*

Our main theorem is:

Theorem 3.22 *Under assumptions* **A1** *to* **A7**, *there exists a positive constant* $\varepsilon^* < \varepsilon_0$ *such that for each* $\varepsilon \in (0, \varepsilon^*)$, *the forward trajectory of* (3.7) *starting from almost every point in* D_ε *converges to some equilibrium.*

Remark 3.23 *A variant of this result is to assume that the reduced system (3.11) has a unique equilibrium. In this case, one may improve the conclusions of the theorem to global (not just generic) convergence, by appealing to results of Hirsch and others that apply when equilibria are unique. The proof is simpler in that case, since the foliation structure given by Fenichel's theory is not required.*

3.4.2 Extensions of the vector fields

Our approach to solve the varying domain problem is motivated by Nipp [66]. The idea is to extend the vector fields from $U \times V \times [0, \varepsilon]$ to $\mathbb{R}^n \times \mathbb{R}^m \times [0, \varepsilon_0]$, then apply Theorem 3.19 on $\mathbb{R}^n \times \mathbb{R}^m \times [0, \varepsilon_0]$, and finally restrict the flows to D_ε for the generic convergence result.

Under assumption **A5** (continuity of D_ε in ε), we can pick a compact set $K \subset U$ such that $K_\varepsilon \subseteq K$ for ε sufficiently small. Without loss of generality, we assume $K_\varepsilon \subseteq K$ for all $\varepsilon \in [0, \varepsilon_0]$. The following is a routine "smooth patching" result.

Lemma 3.24 *For any* $q \in C^r(U)$, *there exists a compact-supported function* $\bar{q}(x) \in C^r(\mathbb{R}^n)$ *such that* $\bar{q}(x) \equiv q(x)$ *on* K. *In particular, this implies that* $\bar{q}(x) \in C^r_b(\mathbb{R}^n)$.

Proof. It is a standard procedure to use convolution of the characteristic function and mollifiers to obtain a C^∞ cutoff function φ such that $\varphi = 1$ on K and $\varphi^{(k)} = 0$ outside of U for all $k = 0, 1, \ldots$.

We define

$$\bar{q}(x) = \begin{cases} q(x)\varphi(x) & x \in U; \\ 0 & x \notin U. \end{cases}$$

By the definition above, $\bar{q}(x) \equiv q(x)$ on K. Moreover, it is easy to see that $\bar{q}(x)$ has compact support and $\bar{q}(x) \in C^r(\mathbb{R}^n)$. ∎

We will be focusing on extending the vector field of system (3.9) in the (x, z) coordinates, then transform it back to the (x, y) coordinates. First, we look at the x

direction, and restrict z in the following set

$$L_{d_0} := \{z \in \mathbb{R}^m \,|\, |z| \le d_0\} \subset \bigcap_{x \in K} \{z \,|\, z + m_0(x) \in V\}.$$

for some fixed d_0.

Lemma 3.25 *Under assumptions* **A1** *and* **A4**, *there exist functions* $\bar{f}_1, \bar{g}_1$, *and* $\bar{m}_0$ *with the following properties:*

$$\bar{f}_1 \in C_b^r(\mathbb{R}^n \times L_{d_0} \times [0, \varepsilon_0]), \quad \bar{f}_1 \equiv f_1 \text{ on } K \times L_{d_0} \times [0, \varepsilon_0], \tag{3.12}$$

$$\bar{g}_1 \in C_b^{r-1}(\mathbb{R}^n \times L_{d_0} \times [0, \varepsilon_0]), \quad \bar{g}_1 \equiv g_1 \text{ on } K \times L_{d_0} \times [0, \varepsilon_0], \tag{3.13}$$

$$\bar{m}_0 \in C_b^r(\mathbb{R}^n), \quad \bar{m}_0 \equiv m_0 \text{ on } K. \tag{3.14}$$

Moreover, all eigenvalues of the matrix $D_z \bar{g}_1(x, 0, 0)$ *have negative real parts less than* $-\mu$ *for every* $x \in \mathbb{R}^n$.

Proof. Applying Lemma 3.24, we obtain functions $\bar{f}_1$ and $\bar{m}_0$ satisfying properties in (3.12) and (3.14), respectively. The extension of g_1 requires more work since we need to make sure that the Jacobian of the extended function is Hurwitz on all of $\mathbb{R}^n$. Let us rewrite the differential equation for z in system (3.9) as:

$$\frac{dz}{d\tau} = \big(B(x) + C(x, z)\big)z + \varepsilon H(x, z, \varepsilon) - \varepsilon [D_x m_0(x)] f_1(x, z, \varepsilon),$$

where

$$B(x) = D_y g_0(x, m_0(x), 0) \text{ and } C(x, 0) = 0.$$

Applying Lemma 3.24, we can extend the functions C and H to $\bar{C}$ and $\bar{H}$, respectively. Let μ be a positive constant such that the real parts of all eigenvalues of $B(x)$ is less than $-\mu$ for every $x \in K$. The extension of B is defined as:

$$\bar{B}(x) = \begin{cases} B(x)\varphi(x) - \mu(1 - \varphi(x))I_n & x \in U; \\ -\mu(1 - \varphi(x))I_n & x \notin U, \end{cases}$$

where the function φ is defined as in the proof of Lemma 3.24. It is easy to see that $\bar{B}(x) \in C_b^r(\mathbb{R}^n)$, and that all eigenvalues of $\bar{B}(x)$ will have negative real parts less than $-\mu$ for every $x \in \mathbb{R}^n$.

Finally, the extension $\bar{g}_1$ is defined as:

$$\bar{g}_1(x,z,\varepsilon) = \big(\bar{B}(x) + \bar{C}(x,z)\big)z + \varepsilon\bar{H}(x,z,\varepsilon) - \varepsilon[D_x\bar{m}_0(x)]\bar{f}_1(x,z,\varepsilon).$$

The function $\bar{g}_1$ satisfies property (3.13) and all eigenvalues of the matrix $D_z\bar{g}_1(x,0,0) = \bar{B}(x)$ have negative real parts less than $-\mu$ for every $x \in \mathbb{R}^n$. ∎

Lemma 3.26 *For any $0 < d_1 < d_0$, under assumptions* **A1** *and* **A4**, *there exist functions $\tilde{f}_1$ and $\tilde{g}_1$, with the following properties:*

$$\tilde{f}_1 \in C_b^r(\mathbb{R}^n \times \mathbb{R}^m \times [0,\varepsilon_0]), \quad \tilde{f}_1 \equiv \bar{f}_1 \text{ on } \mathbb{R}^n \times L_{d_1} \times [0,\varepsilon_0], \tag{3.15}$$

$$\tilde{g}_1 \in C_b^{r-1}(\mathbb{R}^n \times \mathbb{R}^m \times [0,\varepsilon_0]), \quad \tilde{g}_1 \equiv \bar{g}_1 \text{ on } \mathbb{R}^n \times L_{d_1} \times [0,\varepsilon_0]. \tag{3.16}$$

Proof. To extend functions $\bar{f}_1$ and $\bar{g}_1$ in the z direction from L_{d_0} to $\mathbb{R}^m$, we consider the restriction of those functions to the interior of L_{d_0}, then follow the same procedure as in Lemma 3.24 to extend them in the z direction and to keep the extended functions in agreement with the original ones on L_{d_1}. Let us denote the extensions of $\bar{f}_1, \bar{C}$, and $\bar{H}$ in the z direction by $\tilde{f}_1, \tilde{C}$, and $\tilde{H}$, respectively. Then property (3.15) is satisfied trivially. In the product $\big(\bar{B}(x)+\bar{C}(x,z)\big)z$, we view the factor z as an identity function of z, and denote by $\tilde{z}$ the extension of this identity function to $\mathbb{R}^m$ done in the same manner as in Lemma 3.24. The function $\tilde{g}_1$ defined as

$$\tilde{g}_1 = \big(\bar{B}(x) + \tilde{C}(x,z)\big)\tilde{z}(z) + \varepsilon\tilde{H}(x,z,\varepsilon) - \varepsilon[D_x\bar{m}_0(x)]\tilde{f}_1(x,z,\varepsilon)$$

satisfies property (3.16). ∎

Now we transform back to the (x,y) coordinate, and define

$$f(x,y,\epsilon) = \tilde{f}_1(x, y - \bar{m}_0(x), \varepsilon),$$

$$g(x,y,\epsilon) = \tilde{g}_1(x, y - \bar{m}_0(x), \varepsilon).$$

Property (3.16) implies $\tilde{g}_1(x,0,0) = 0$, and thus $g(x,\bar{m}_0(x),0) = 0$. To summarize, the system

$$\begin{aligned} \frac{dx}{d\tau} &= \varepsilon f(x,y,\varepsilon) \\ \frac{dy}{d\tau} &= g(x,y,\varepsilon), \end{aligned} \tag{3.17}$$

satisfies the following properties:

E1 The functions

$$f \in C_b^r(\mathbb{R}^n \times \mathbb{R}^m \times [0, \varepsilon_0]),$$

$$g \in C_b^{r-1}(\mathbb{R}^n \times \mathbb{R}^m \times [0, \varepsilon_0]).$$

E2 The function $\bar{m}_0 \in C_b^r(\mathbb{R}^n)$ satisfies

$$g(x, \bar{m}_0(x), 0) = 0, \ \forall x \in \mathbb{R}^n.$$

E3 All eigenvalues of the matrix $D_y g(x, \bar{m}_0(x), 0)$ have negative real parts less than $-\mu$ for every $x \in \mathbb{R}^n$.

E4 The function $\bar{m}_0$ coincides with m_0 on K, and the functions f and g coincide with f_0 and g_0 respectively on

$$\Omega_{d_1} := \{(x, y) \,|\, x \in K, \, |y - m_0(x)| \leq d_1\}.$$

Properties **E1** to **E3** are the assumptions for geometric singular perturbation theorems, and property **E4** ensures that on Ω_{d_1} the flow of (3.8) coincides with the flow of (3.17). If we apply geometric singular perturbation theorems to (3.17) on $\mathbb{R}^n \times \mathbb{R}^m \times [0, \varepsilon_0]$, the exact same results are true for (3.8) on Ω_{d_1}. For the rest of the paper, we identify the flow of (3.17) and the flow of (3.8) on Ω_{d_1} without further mentioning this fact.

3.4.3 Further analysis of the dynamics and the proof of Theorem 3.22

Properties **E1** to **E3** correspond to assumptions **S1** to **S3** in Lemma 3.19. As a result, there exists a positive number $\varepsilon_1 < \varepsilon_0$ such that conclusions in Lemma 3.19 hold.

The first property of Lemma 3.19 concludes the existence of an invariant manifold M_ε. There are two reasons to introduce M_ε. First, on M_ε the x-equation is decoupled from the y-equation:

$$\begin{aligned} \frac{dx}{dt} &= f(x, m(x, \varepsilon), \varepsilon) \\ y(t) &= m(x(t), \varepsilon). \end{aligned} \tag{3.18}$$

This reduction allows us to analyze a lower dimensional system, whose dynamics may have been well studied. Second, when ε approaches zero, the limit of (3.18) is (3.11). If

(3.11) has some desirable property, it is natural to expect that this property is inherited by (3.18). An example of this principle is provided by the following Lemma:

Lemma 3.27 *There exists a positive constant $\varepsilon_2 < \varepsilon_1$, such that for each $\varepsilon \in (0, \varepsilon_2)$, the flow ψ_t^ε of* (3.18) *has eventually positive derivatives on K_ε, which is the projection of $M_\varepsilon \bigcap D_\varepsilon$ to the x-axis.*

Proof. Assumption **A6** states that the flow ψ_t^0 of the limiting system (3.11) has eventually positive derivatives on K_0. By the continuity of $m(x, \varepsilon)$ and D_ε at ε=0, we can pick ε_2 small enough such that the flow ψ_t^0 has eventually positive derivatives on K_ε for all $\varepsilon \in (0, \varepsilon_2)$. Applying Lemma 3.11, we conclude that the flow ψ_t^ε of (3.18) has eventually positive derivatives on K_ε provided K_ε is positively invariant under (3.18), which follows easily from the fact that (3.17) is positively invariant on D_ε and M_ε is an invariant manifold. ∎

The next lemma asserts that the generic convergence property is preserved for (3.18).

Lemma 3.28 *For each $\varepsilon \in (0, \varepsilon_2)$, there exists a set $C_\varepsilon \subseteq K_\varepsilon$ such that the forward trajectory of* (3.18) *starting from any point of C_ε converges to some equilibrium, and the Lebesgue measure of $K_\varepsilon \setminus C_\varepsilon$ is zero.*

Proof. There exists a convex open set W_ε containing K_ε such that flow ψ_t^ε of (3.18) has eventually positive derivatives on W_ε. Assumption **A5** assures that $K_\varepsilon \subseteq W_\varepsilon^c$. The proof is completed by applying Lemma 3.15 under the assumption **A7**. ∎

By now, we have discussed flows restricted to the invariant manifold M_ε, see Figure 3.4. Next, we will explore conditions for a point to be on $W^s(M_\varepsilon)$, the stable manifold of M_ε. Property 3 of Lemma 3.19 provides a sufficient condition, namely, any point (x_0, y_0) such that

$$\sup_{\tau \geq 0} |y(\tau; x_0, y_0) - m\big(x(\tau; x_0, y_0), \varepsilon\big)| < \delta_0 \tag{3.19}$$

is on $W^s(M_\varepsilon)$. In fact, if we know that the difference between y_0 and $m(x_0, \varepsilon)$ is sufficiently small, then the above condition is always satisfied. This follows from the proof of Claim 1 in [66].

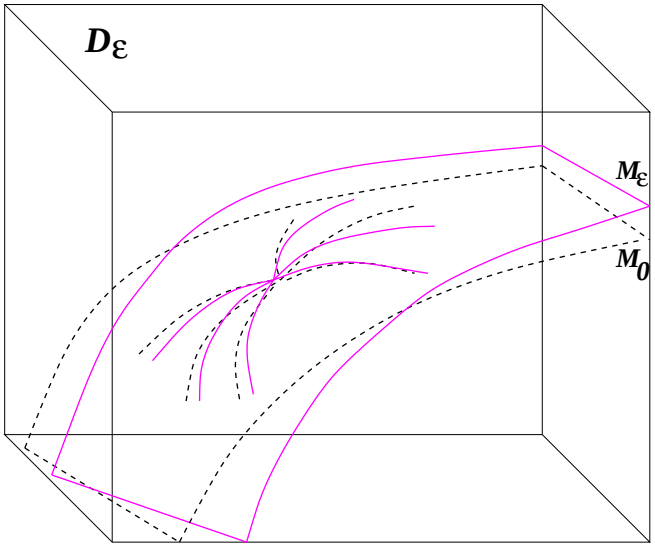

Figure 3.4: This is a sketch of the manifolds M_0 (surface bounded by dashed curves), M_ε (surface bounded by solid curves), and D_ε (the cube). It highlights two major characters of M_ε. First, M_ε is close to M_0. Second, the trajectories on M_ε converge to equilibria if those on M_0 do.

Lemma 3.29 *There exists $\varepsilon_3 > 0, \delta_0 > d > 0$, such that for each $\varepsilon \in (0, \varepsilon_3)$, if the initial condition satisfies $|y_0 - m(x_0, \varepsilon)| < d$, then (3.19) holds, i.e. $(x_0, y_0) \in W^s(M_\varepsilon)$.*

See Figure 3.5 for a graphical interpretation of Lemma 3.29.

Before we get further into the technical details, let us give an outline of the proof of the main theorem. The proof can be decomposed into three steps. First, we show that almost every trajectory on $D_\varepsilon \bigcap M_\varepsilon$ converges to some equilibrium. This is precisely Lemma 3.28. Second, we show that almost every trajectory starting from $W^s(M_\varepsilon)$ converges to some equilibrium. This follows from Lemma 3.28 and the "asymptotic phase" property in Lemma 3.19, but we still need to show that the set of non-convergent initial conditions is of measure zero. The last step is to show that all trajectories in D_ε will eventually stay in $W^s(M_\varepsilon)$, which is our next lemma:

Lemma 3.30 *There exist two positive numbers τ_0 and $\varepsilon_4 < \varepsilon_3$, such that*

$$(x(\tau_0), y(\tau_0)) \in W^s(M_\varepsilon)$$

for all $\varepsilon \in (0, \varepsilon_4)$, where $(x(\tau), y(\tau))$ is the solution to (3.8) with the initial condition $(x_0, y_0) \in D_\varepsilon$.

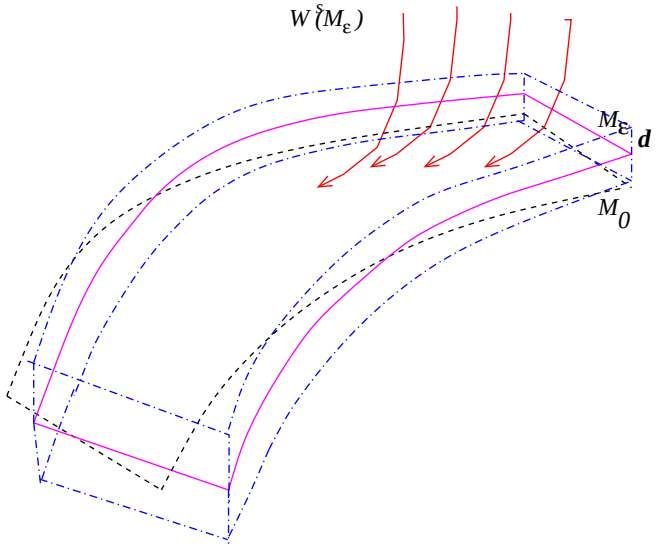

Figure 3.5: In this picture, the box bounded by the dashed-dot curves consists of all points (x_0, y_0) with the property $|y_0 - m(x_0, \varepsilon)| < d$. The surface bounded by dashed curves is the limiting slow manifold M_0. The surface bounded by the solid curve is the invariant manifold M_ε. Trajectories in the stable manifold $W^s(M_\varepsilon)$ are attracted to M_ε. Lemma 3.29 states that all the points inside the dashed-dot box belong to in the stable manifold $W^s(M_\varepsilon)$.

Proof. It is convenient to consider the problem in the (x, z) coordinates. Let $(x(\tau), z(\tau))$ be the solution to (3.9) with initial condition (x_0, z_0), where $z_0 = y_0 - m(x_0, 0)$. We first show that there exists a τ_0 such that $|z(\tau_0)| \le d/2$.

Expanding $g_1(x, z, \varepsilon)$ at the point $(x_0, z, 0)$, the equation of z becomes

$$\frac{dz}{d\tau} = g_1(x_0, z, 0) + \frac{\partial g_1}{\partial x}(\xi, z, 0)(x - x_0) + \varepsilon R(x, z, \varepsilon)$$

for some $\xi(\tau)$ between x_0 and $x(\tau)$ (where $\xi(\tau)$ can be picked continuously in τ). Let us write

$$z(\tau) = z^0(\tau) + w(\tau),$$

where $z^0(\tau)$ is the solution to (3.10) with the initial condition $z^0(0) = z_0$, and $w(\tau)$ satisfies

$$\begin{aligned} \frac{dw}{d\tau} &= g_1(x_0, z, 0) - g_1(x_0, z^0, 0) + \frac{\partial g_1}{\partial x}(\xi, z, 0)(x - x_0) + \varepsilon R(x, z, \varepsilon) \qquad (3.20) \\ &= \frac{\partial g_1}{\partial z}(x_0, \zeta, 0)w + \varepsilon \frac{\partial g_1}{\partial x}(\xi, z, 0) \int_0^\tau f_1(x(s), z(s), \varepsilon)\, ds + \varepsilon R(x, z, \varepsilon), \end{aligned}$$

with the initial condition $w(0) = 0$ and some $\zeta(\tau)$ between $z^0(\tau)$ and $z(\tau)$ (where $\zeta(\tau)$ can be picked continuously in τ).

By assumption **A3**, there exists a positive τ_0 such that $|z^0(\tau)| \leq d/4$ for all $\tau \geq \tau_0$. Notice that we are working on the compact set D_ε, so τ_0 can be chosen uniformly for all initial conditions in D_ε.

We write the solution of (3.20) as:

$$w(\tau) = \int_0^\tau \frac{\partial g_1}{\partial z}(x_0, \zeta, 0) w \, ds + \varepsilon \int_0^\tau \Big(\frac{\partial g_1}{\partial x}(\xi, z, 0) \int_0^{s'} f_1(x, z, \varepsilon) \, ds' + R(x, z, \varepsilon) \Big) \, ds.$$

Since the functions f_1, R and the derivatives of g_1 are bounded on D_ε, we have:

$$|w(\tau)| \leq \int_0^\tau L|w| \, ds + \varepsilon \int_0^\tau \left(M_1 \int_0^{s'} M_2 \, ds' + M_3 \right) ds,$$

for some positive constants L, M_i, $i = 1, 2, 3$. The notation $|w|$ means the Euclidean norm of $w \in \mathbb{R}^m$. Moreover, if we define

$$\alpha(\tau) = \int_0^\tau \left(M_1 \int_0^{s'} M_2 \, ds' + M_3 \right) ds,$$

then

$$|w(\tau)| \leq \int_0^\tau L|w| \, ds + \varepsilon \alpha(\tau_0),$$

for all $\tau \in [0, \tau_0]$ as α is increasing in τ. Applying Gronwall's inequality ([83]), we have:

$$|w(\tau)| \leq \varepsilon \alpha(\tau_0) e^{L\tau},$$

which holds in particular at $\tau = \tau_0$. Finally, we choose ε_4 small enough such that $\varepsilon \alpha(\tau_0) e^{L\tau_0} < d/4$ and $|m(x, \varepsilon) - m(x, 0)| < d/2$ for all $\varepsilon \in (0, \varepsilon_4)$. Then we have:

$$\begin{aligned} |y(\tau_0) - m(x(\tau_0), \varepsilon)| &\leq |y(\tau_0) - m(x(\tau_0), 0)| + |m(x(\tau_0), \varepsilon) - m(x(\tau_0), 0)| \\ &< |z(\tau_0)| + d/2 \\ &\leq |z^0(\tau_0)| + |w(\tau_0)| + d/2 \\ &< d/4 + d/4 + d/2 = d. \end{aligned}$$

That is, $(x(\tau_0), y(\tau_0)) \in W^s(M_\varepsilon)$ by Lemma 3.29. ∎

By now, we have completed all three steps, and are ready to prove Theorem 3.22, see Figure 3.6 for a graphical illustration of the proof.

Proof. (Theorem 3.22). Let $\varepsilon^* = \min\{\varepsilon_2, \varepsilon_4\}$. For $\varepsilon \in (0, \varepsilon^*)$, it is equivalent to prove the result for the fast system (3.8). Pick an arbitrary point (x_0, y_0) in D_ε, and there are three cases:

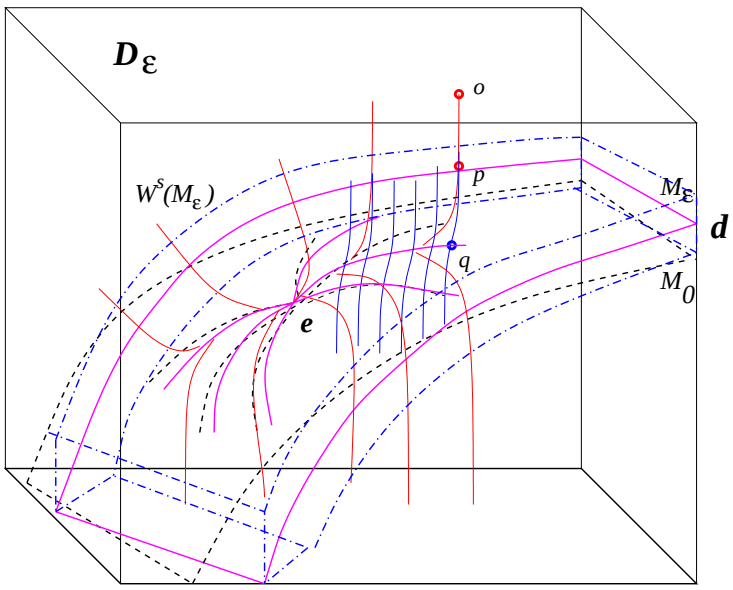

Figure 3.6: This picture illustrates the key points in the proof of Theorem 3.22. Notations follow from Figure 3.4 and Figure 3.5. The point q is on the invariant manifold M_ε, and it converges to the equilibrium e. The point p belongs to the stable manifold $W^s(M_\varepsilon)$, and p is on the same fiber (vertical curve) with q. As a result, p also converges to the equilibrium e. The point o in this picture represents an arbitrary point in D_ε. After time τ_0, the trajectory starting from o evolves to the point q, and eventually converges to the equilibrium e.

1. $y_0 = m(x_0, \varepsilon)$, that is, $(x_0, y_0) \in M_\varepsilon \bigcap D_\varepsilon$. By Lemma 3.28, the forward trajectory converges to an equilibrium except for a set of measure zero.

2. $0 < |y_0 - m(x_0, \varepsilon)| < d$. By Lemma 3.29, we know that (x_0, y_0) is in $W^s(M_\varepsilon)$. Then, property 4) of Lemma 3.19 guarantees that the point (x_0, y_0) is on some fiber $W^s_{\varepsilon,d}(\xi)$, where $\xi \in K_\varepsilon$. If $\xi \in C_\varepsilon$, that is, the forward trajectory of ξ converges to some equilibrium, then by the "asymptotic phase" property of Lemma 3.19, the forward trajectory of (x_0, y_0) also converges to an equilibrium. To deal with the case when ξ is not in C_ε, it is enough to show that the set

$$B_{\varepsilon,d} = \bigcup_{\xi \in K_\varepsilon \setminus C_\varepsilon} W^s_{\varepsilon,d}(\xi)$$

has measure zero in $\mathbb{R}^{m+n}$. Define

$$S_{\varepsilon,d} = (K_\varepsilon \setminus C_\varepsilon) \times L_d.$$

By Lemma 3.28, $K_\varepsilon \setminus C_\varepsilon$ has measure zero in $\mathbb{R}^n$, thus $S_{\varepsilon,d}$ has measure zero in $\mathbb{R}^n \times \mathbb{R}^m$. On the other hand, Property 6) in Lemma 3.19 implies $B_{\varepsilon,d} = T_{\varepsilon,d}(S_{\varepsilon,d})$.

Since Lipschitz maps send measure zero sets to measure zero sets, $B_{\varepsilon,d}$ is of measure zero.

3. $|y_0 - m(x_0, \varepsilon)| \geq d$. By Lemma 3.30, the point $\big(x(\tau_0), y(\tau_0)\big)$ is in $W^s(M_\varepsilon)$ and we are back to case 2. The proof is completed if the set $\phi^{\varepsilon}_{-\tau_0}(B_{\varepsilon,d})$ has measure zero, where $\phi^{\varepsilon}_{\tau}$ is the flow of (3.8). This is true because $\phi^{\varepsilon}_{\tau}$ is a diffeomorphism for any finite τ.

∎

3.5 Applications

Several applications using Theorem 3.22 are worked out in detail. These applications cover common biological examples ranging from molecular level enzymatic reactions to macro level networks.

3.5.1 Enzyme competitive inhibition

Enzymes are proteins that act as catalysts. They help convert other molecules called substrates into products, but they themselves are not changed by the reaction. The size of an enzyme is usually large compared to the substrate molecules whose reaction it catalyzes. Embedded on an enzyme are active sites, where the substrate can bind to form a complex. Active sites are usually highly specific, and they bind only substrates with similar structures.

Enzyme inhibitors are those molecules that can bind to the active sites of an enzyme and decrease the enzyme's activity. Here we focus on one class of enzyme inhibitors called competitive inhibitors. Competitive inhibitors closely resemble the chemical structure and molecular geometry of the substrate. The inhibitors compete for the same active site with the substrate molecules. When a inhibitor occupies the active site of the enzyme, it prevents any substrate molecules from reacting with the enzyme, see Figure 3.7. Many drugs are designed using competitive inhibitors, for example, Allegra®.

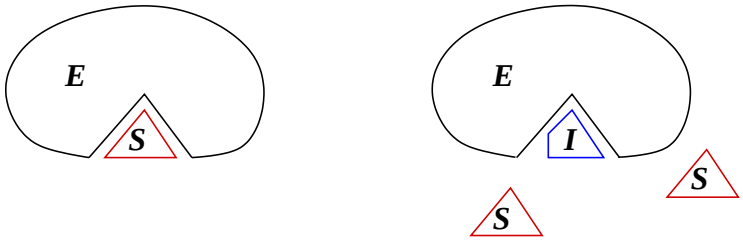

Figure 3.7: An illustration for competitive inhibition. The substrate, inhibitor, and enzyme are denoted by S, I, and E, respectively. To the left, the enzyme E is bound with the substrate S so as to form a complex. To the right, the inhibitor I occupies the active site on the enzyme E and prevents the substrate S from binding to E.

The simplest model of enzyme competitive inhibition can be described by the following reactions:

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C_1 \overset{k_2}{\to} P + E$$

$$I + E \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} C_2$$

Here, C_1 is the complex formed by the substrate S and the enzyme E; C_2 is the complex consisting of the inhibitor I and the enzyme E.

According to the law of mass action, we can model the above reactions using the following set ordinary differential equations:

$$\begin{aligned} \frac{ds}{d\tau} &= -k_1 se + k_{-1} c_1 \\ \frac{dc_1}{d\tau} &= k_1 se - (k_{-1} + k_2) c_1 \\ \frac{dc_2}{d\tau} &= k_3 ie - k_{-3} c_2, \end{aligned} \tag{3.21}$$

together with three conservation relations:

$$\begin{aligned} S_{\text{tot}} &= s + c_1 + p, \\ E_{\text{tot}} &= e + c_1 + c_2, \\ I_{\text{tot}} &= i + c_2. \end{aligned}$$

Here, small letters denote the concentrations of their corresponding molecules; the total amount of the substrate, the enzyme, and the inhibitor are denoted by $S_{\text{tot}}, E_{\text{tot}}$, and I_{tot}, respectively. The constants $S_{\text{tot}}, E_{\text{tot}}$, and I_{tot} are assumed to have positive

values. System (3.21) consists of differential equations of three variables s, c_1, and c_2. The rest of the variables e, p, and i can be solved from the conservation relations once s, c_1, and c_2 are determined through system (3.21).

Let us first solve for the steady states of (3.21). At the steady states, we have the right hand side of system (3.21) equal zero, that is,

$$0 = -k_1 se + k_{-1} c_1 \tag{3.22}$$

$$0 = k_1 se - (k_{-1} + k_2) c_1 \tag{3.23}$$

$$0 = k_3 ie - k_{-3} c_2. \tag{3.24}$$

Adding up equation (3.22) and equation (3.23), we have $c_1 = 0$. Plugging $c_1 = 0$ back into equation (3.22), we have either $s = 0$ or $e = 0$. If $e = 0$, then by equation (3.24), $c_2 = 0$ too. As a result, $E_{\text{tot}} = e + c_1 + c_2 = 0$ also, which contradicts $E_{\text{tot}} > 0$. So $s = 0$.

On the other hand, because $c_1 = 0$, we have

$$e = E_{\text{tot}} - c_1 - c_2 = E_{\text{tot}} - c_2. \tag{3.25}$$

Plugging $i = I_{\text{tot}} - c_2$ and (3.25) into equation (3.24), we obtain a quadratic equation for c_2:

$$c_2^2 - (I_{\text{tot}} + E_{\text{tot}} + K_{\text{eq}}) c_2 + I_{\text{tot}} E_{\text{tot}} = 0. \tag{3.26}$$

Here,

$$K_{\text{eq}} = \frac{k_{-3}}{k_3}$$

is called the equilibrium constant, which relates to the relative preference for the chemicals to be in the combined state C_2 compared to the dissociated state I and E.

Let us rewrite equation (3.26) as

$$c_2^2 - A c_2 + B = 0 \tag{3.27}$$

where

$$A = I_{\text{tot}} + E_{\text{tot}} + K_{\text{eq}} > 0, \quad B = I_{\text{tot}} E_{\text{tot}} > 0.$$

It is easy to see that both roots of equation (3.26) are positive. However, the larger root is greater than I_{tot} because the right hand side of equation (3.26) evaluated at $c_2 = I_{\text{tot}}$ is

$$-E_{\text{tot}}K_{\text{eq}} < 0.$$

If c_2 takes a value greater than I_{tot}, then $i = I_{\text{tot}} - c_2$ is negative, which has no biological meaning. Therefore, system (3.21) has a unique nonnegative steady state

$$(s^*, c_1^*, c_2^*) = (0, 0, \frac{A - \sqrt{A^2 - 4B}}{2}).$$

The signed influence digraph of system (3.21) is shown in Figure 3.8.

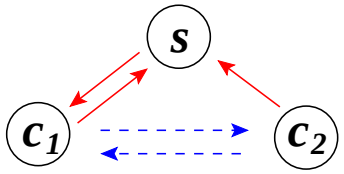

Figure 3.8: The signed influence digraph of system (3.21). We use solid lines to represent edges with positive signs, and dashed line to denote edges with negative signs. The loops formed by s, c_1, and c_2 are negative.

It is clear from Figure 3.8 that system (3.21) is not monotone with respect to any orthant order, and thus the flows of (3.21) do not have positive derivatives by Lemma 3.14. However, in enzymatic reactions, it is often true that the amount of enzymes is much less than the amount of substrates. Thus, it is reasonable to rescale the system by defining

$$x = \frac{s}{S_{\text{tot}}}, \quad y_1 = \frac{c_1}{E_{\text{tot}}}, \quad y_2 = \frac{c_2}{E_{\text{tot}}},$$
$$\varepsilon = \frac{E_{\text{tot}}}{S_{\text{tot}}}, \quad c = \frac{I_{\text{tot}}}{S_{\text{tot}}}, \quad t = \tau\varepsilon,$$

and to assume that $\varepsilon \ll 1$ and c is a positive constant of order one.

System (3.21) in the new coordinates becomes

$$\begin{aligned} \frac{dx}{dt} &= -k_1 x S_{\text{tot}}(1 - y_1 - y_2) + k_{-1}y_1 \\ \varepsilon\frac{dy_1}{dt} &= k_1 x S_{\text{tot}}(1 - y_1 - y_2) - (k_{-1} + k_2)y_1 \\ \varepsilon\frac{dy_2}{dt} &= k_3 S_{\text{tot}}(c - \varepsilon y_2)(1 - y_1 - y_2) - k_{-3}y_2. \end{aligned} \tag{3.28}$$

These equations are in the form of (3.7). The conservation relations suggest taking $\varepsilon_0 = c$ and

$$D = D_\varepsilon := \{(x, y_1, y_2) \,|\, 0 \le x \le 1, 0 \le y_1 + y_2 \le 1, y_1, y_2 \ge 0\}.$$

The set D is positively invariant for all $\varepsilon \in (0, \varepsilon_0]$ with respect to the flow of (3.28). This is because the inner product of the outward-pointing normal vector at every point of the boundary ∂D and the vector fields of (3.7) at that point is always nonnegative.

Corresponding to the steady state (s^*, c_1^*, c_2^*) of system (3.21), we have the steady state

$$(x^*, y_1^*, y_2^*) = (0, 0, \frac{2cS_{\text{tot}}}{\alpha + \sqrt{\alpha^2 - 4\beta}})$$

of system (3.28), where

$$\alpha = cS_{\text{tot}} + \varepsilon S_{\text{tot}} + K_{\text{eq}}, \quad \beta = \varepsilon c S_{\text{tot}}^2.$$

Assumption **A7** thus holds.

The projection of D to the x coordinate is defined as the set

$$K = K_\varepsilon := \{x \,|\, 0 \le x \le 1\},$$

for all $\varepsilon \in [0, \varepsilon_0]$.

Setting $\varepsilon = 0$ in (3.28), the y equations degenerate to algebraic equations:

$$0 = k_1 x S_{\text{tot}}(1 - y_1 - y_2) - (k_{-1} + k_2) y_1$$
$$0 = k_3 S_{\text{tot}} c(1 - y_1 - y_2) - k_{-3} y_2.$$

Solving for y_1 and y_2, we obtain

$$y_1 = \frac{\frac{K_{\text{eq}}}{K_m} x}{\frac{K_{\text{eq}}}{S_{\text{tot}}} + c + \frac{K_{\text{eq}}}{K_m} x} := m_0^1(x)$$
$$y_2 = \frac{c}{\frac{K_{\text{eq}}}{S_{\text{tot}}} + c + \frac{K_{\text{eq}}}{K_m} x} := m_0^2(x).$$

Here,

$$K_m = \frac{k_{-1} + k_2}{k_1}$$

is the Michaelis-Menten constant, which relates to the relative preference for the chemicals to be in the combined state C_1 compared to the two dissociated states.

The reduced system ($\varepsilon = 0$ in (3.28))

$$\frac{dx}{dt} = -k_1 x S_{\text{tot}}(1 - m_0^1(x) - m_0^2(x)) + k_{-1} m_0^1(x)$$

is of dimension one, and thus flows of the reduced system have positive derivatives on K_0 automatically. Therefore, assumption **A6** also holds.

Consider the matrix

$$B(x) := D_y g_0(x, m_0(x), 0) = \begin{pmatrix} -k_1 \left(x S_{\text{tot}} + K_m\right) & -k_1 x S_{\text{tot}} \\ -k_3 S_{\text{tot}} c & -k_3 \left(S_{\text{tot}} c + K_{\text{eq}}\right) \end{pmatrix}$$

and a set U in the following form:

$$U = \{x \mid -\sigma < x < 1 + \sigma\}.$$

The determinant of $B(x)$ is

$$\det(B(x)) = k_1 k_3 (K_m S_{\text{tot}} c + K_{\text{eq}} x S_{\text{tot}} + K_m K_{\text{eq}}),$$

and the trace of $B(x)$ is

$$-k_1 \left(x S_{\text{tot}} + K_m\right) - k_3 \left(S_{\text{tot}} c + K_{\text{eq}}\right).$$

As a result, for

$$\sigma < \min \left\{ \frac{K_m S_{\text{tot}} c + K_m K_{\text{eq}}}{K_{\text{eq}} S_{\text{tot}}}, \frac{k_1 K_m + k_3 \left(S_{\text{tot}} c + K_{\text{eq}}\right)}{k_1 S_{\text{tot}}} \right\},$$

the determinant of $B(x)$ is positive, and trace of $B(x)$ is negative for any $x \in (-\sigma, 1 + \sigma)$. Therefore, the matrix $B(x)$ is Hurwitz on U, which guarantees assumption **A4**. Assumptions **A1** and **A2** follows naturally for sufficiently small σ.

It remains to check assumption **A3**. Notice that the fast limiting system corresponding to (3.9) is:

$$\frac{dz}{d\tau} = B(x_0) z, \quad x_0 \in U.$$

This system is linear in z with a Hurwitz matrix $B(x_0)$ for every $x_0 \in U$. Therefore, assumption **A3** also holds.

Now we have checked assumptions **A1** to **A7**, applying Theorem 3.22, we have:

Theorem 3.31 *There exists a positive $\varepsilon^* < \varepsilon_0$ such that for each $\varepsilon \in (0, \varepsilon^*)$, the forward trajectory of* (3.28) *starting from almost every point in D converges to some equilibrium.*

In fact, for this example every trajectory not merely *almost* every trajectory converges to a equilibrium, see Remark 3.23.

3.5.2 Double phosphorylation dephosphorylation futile cycle

In section 2.2, we introduced an important motif called the futile cycle. Typically, the enzymatic activation and de-activation in a futile cycle are given by phosphorylation and dephosphorylation, and the cycle is thus called phosphorylation and dephosphorylation futile cycle. In this section, we study the dynamical property of a phosphorylation dephosphorylation futile cycle of size two, see Figure 2.2.

Let us first write down the chemical reactions involved:

$$S_0 + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C_1 \overset{k_2}{\to} S_1 + E \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} C_2 \overset{k_4}{\to} S_2 + E$$

$$S_2 + F \underset{h_{-1}}{\overset{h_1}{\rightleftarrows}} C_3 \overset{h_2}{\to} S_1 + F \underset{h_{-3}}{\overset{h_3}{\rightleftarrows}} C_4 \overset{h_4}{\to} S_0 + F.$$

Based on mass action kinetics, we have the following set of ordinary differential equations:

$$\begin{aligned}
\frac{ds_0}{d\tau} &= h_4 c_4 - k_1 s_0 e + k_{-1} c_1 \\
\frac{ds_2}{d\tau} &= k_4 c_2 - h_1 s_2 f + h_{-1} c_3 \\
\frac{dc_1}{d\tau} &= k_1 s_0 e - (k_{-1} + k_2) c_1 \\
\frac{dc_2}{d\tau} &= k_3 s_1 e - (k_{-3} + k_4) c_2 \\
\frac{dc_4}{d\tau} &= h_3 s_1 f - (h_{-3} + h_4) c_4 \\
\frac{dc_3}{d\tau} &= h_1 s_2 f - (h_{-1} + h_2) c_3,
\end{aligned} \tag{3.29}$$

together with three conservation relations:

$$S_{\text{tot}} = s_0 + s_1 + s_2 + c_1 + c_2 + c_4 + c_3,$$
$$E_{\text{tot}} = e + c_1 + c_2,$$
$$F_{\text{tot}} = f + c_4 + c_3,$$

where small letters denote the concentrations of their corresponding molecules. We can draw the signed influence digraph of system (3.29), see Figure 3.9.

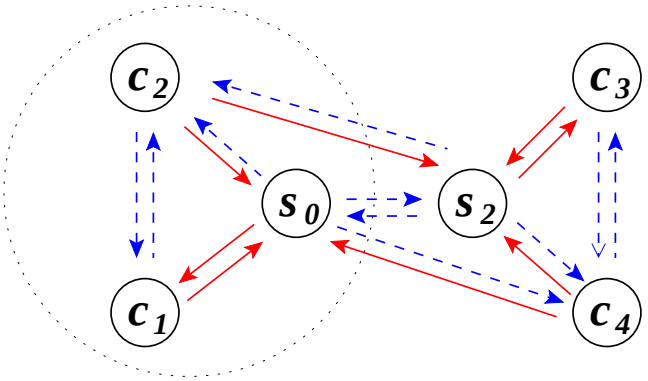

Figure 3.9: The signed influence digraph of the full system (3.29). We use solid lines to represent edges with positive signs, and dashed line to denote edges with negative signs. The subgraph inside the dotted circle is not sign consistent.

Inside the dotted circle of Figure 3.9, the loops formed by nodes c_1, c_2, and s_0 are sign inconsistent. This is because on one hand, we have c_2 directly inhibits c_1; on the other hand, c_2 activates c_1 through s_0. As a result, the full system (3.29) is not monotone.

However, as in Section 3.5.1, we can assume that the amount of enzyme is much less than the amount of substrate, which in our case corresponds to $E_{\text{tot}} \ll S_{\text{tot}}$ and $F_{\text{tot}} \ll S_{\text{tot}}$. Time scale separation arises under this assumption. We introduce new variables:

$$x_1 = \frac{s_0}{S_{\text{tot}}}, \quad x_2 = \frac{s_2}{S_{\text{tot}}}, \quad y_1 = \frac{c_1}{E_{\text{tot}}}, \quad y_2 = \frac{c_2}{E_{\text{tot}}},$$
$$y_3 = \frac{c_4}{F_{\text{tot}}}, \quad y_4 = \frac{c_3}{F_{\text{tot}}}, \quad \varepsilon = \frac{E_{\text{tot}}}{S_{\text{tot}}}, \quad c = \frac{F_{\text{tot}}}{E_{\text{tot}}}, \quad t = \tau\varepsilon.$$

In terms of the new variables (rescaling the concentrations and time), system (3.29)

becomes:

$$\begin{aligned}
\frac{dx_1}{dt} =& - k_1 S_{\text{tot}} x_1(1 - y_1 - y_2) + k_{-1} y_1 + h_4 c y_3 \\
\frac{dx_2}{dt} =& - h_1 S_{\text{tot}} c x_2(1 - y_3 - y_4) + h_{-1} c y_4 + k_4 y_2 \\
\varepsilon \frac{dy_1}{dt} =& k_1 S_{\text{tot}} x_1(1 - y_1 - y_2) - (k_{-1} + k_2) y_1 \\
\varepsilon \frac{dy_2}{dt} =& k_3 S_{\text{tot}}(1 - x_1 - x_2 - \varepsilon y_1 - \varepsilon y_2 - \varepsilon c y_3 - \varepsilon c y_4)(1 - y_1 - y_2) - (k_{-3} + k_4) y_2 \\
\varepsilon \frac{dy_3}{dt} =& h_3 S_{\text{tot}}(1 - x_1 - x_2 - \varepsilon y_1 - \varepsilon y_2 - \varepsilon c y_3 - \varepsilon c y_4)(1 - y_3 - y_4) - (h_{-3} + h_4) y_3 \\
\varepsilon \frac{dy_4}{dt} =& h_1 S_{\text{tot}} x_2(1 - y_3 - y_4) - (h_{-1} + h_2) y_4.
\end{aligned} \tag{3.30}$$

These equations are in the form of (3.7). The conservation laws suggest taking $\varepsilon_0 = 1/(1 + c)$ and

$$\begin{aligned}
D_\varepsilon =& \{(x_1, x_2, y_1, y_2, y_3, y_4) \,|\, 0 \le y_1 + y_2 \le 1, \\
& 0 \le y_3 + y_4 \le 1, x_1, x_2, y_1, y_2, y_3, y_4 \ge 0, \\
& 0 \le x_1 + x_2 + \varepsilon(y_1 + y_2 + c y_3 + c y_4) \le 1\}.
\end{aligned}$$

For $\varepsilon \in (0, \varepsilon_0]$, taking the inner product of the normal of ∂D_ε and the vector fields, it is easy to check that (3.30) is positively invariant on D_ε, so **A5** holds. We want to emphasize that in this example the domain D_ε is a convex polytope varying with ε.

In Section 2.2, we study the number of positive steady states of a general phosphorylation dephosphorylation futile cycle of size n, and provide explicit lower and upper bounds of the number of positive steady states. In any case, system (3.30) has at most a finite number of positive steady states, and thus **A7** holds.

The projection of D_ε to the x coordinate is defined as

$$K = K_\varepsilon = \{(x_1, x_2) \,|\, 0 \le x_1 + x_2 \le 1\}$$

for all $\varepsilon \in [0, \varepsilon_0]$.

At $\varepsilon = 0$, solving $g_0(x, y, 0) = 0$, we get

$$\begin{aligned}
y_1 &= \frac{x_1}{\frac{K_{m1}}{S_{\text{tot}}} + \frac{K_{m1}(1-x_1-x_2)}{K_{m2}} + x_1}, \\
y_2 &= \frac{\frac{K_{m1}(1-x_1-x_2)}{K_{m2}}}{\frac{K_{m1}}{S_{\text{tot}}} + \frac{K_{m1}(1-x_1-x_2)}{K_{m2}} + x_1}, \\
y_3 &= \frac{\frac{K_{m3}(1-x_1-x_2)}{K_{m4}}}{\frac{K_{m3}}{S_{\text{tot}}} + \frac{K_{m3}(1-x_1-x_2)}{K_{m4}} + x_2}, \\
y_4 &= \frac{x_2}{\frac{K_{m3}}{S_{\text{tot}}} + \frac{K_{m3}(1-x_1-x_2)}{K_{m4}} + x_2},
\end{aligned}$$

where K_{m1}, K_{m2}, K_{m3} and K_{m4} are the Michaelis-Menten constants defined as

$$K_{m1} = \frac{k_{-1} + k_2}{k_1}, \quad K_{m2} = \frac{k_{-3} + k_4}{k_3}, \quad K_{m3} = \frac{h_{-1} + h_2}{h_1}, \quad K_{m4} = \frac{h_{-3} + h_4}{h_3}.$$

Now, we need to find a proper set $U \subset \mathbb{R}^2$ satisfying assumptions **A1**-**A4**. Suppose that U has the form

$$U = \{(x_1, x_2) \,|\, x_1 > -\sigma,\, x_2 > -\sigma,\, x_1 + x_2 < 1 + \sigma\},$$

for some positive σ, and V is any bounded open set such that D_ε is contained in $U \times V$, then **A1** follows naturally. Moreover, if

$$\sigma \leq \sigma_0 := \min\left\{ \frac{K_{m1}K_{m2}}{S_{\text{tot}}(K_{m1} + K_{m2})},\, \frac{K_{m3}K_{m4}}{S_{\text{tot}}(K_{m3} + K_{m4})} \right\},$$

A2 also holds. To check **A4**, let us look at the matrix:

$$B(x) := D_y g_0(x, m_0(x), 0) = \begin{pmatrix} B_1(x) & 0 \\ 0 & B_2(x) \end{pmatrix},$$

where the column vectors of $B_1(x)$ are

$$B_1^1(x) = \begin{pmatrix} -k_1 S_{\text{tot}} x_1 - (k_{-1} + k_2) \\ -k_3 S_{\text{tot}}(1 - x_1 - x_2) \end{pmatrix},$$

$$B_1^2(x) = \begin{pmatrix} -k_1 S_{\text{tot}} x_1 \\ -k_3 S_{\text{tot}}(1 - x_1 - x_2) - (k_{-3} + k_4) \end{pmatrix},$$

and the column vectors of $B_2(x)$ are

$$B_2^1(x) = \begin{pmatrix} -h_3 S_{\text{tot}}(1 - x_1 - x_2) - (h_{-3} + h_4) \\ -h_1 S_{\text{tot}} x_2 \end{pmatrix},$$

$$B_2^2(x) = \begin{pmatrix} -h_3 S_{\text{tot}}(1 - x_1 - x_2) \\ -h_1 S_{\text{tot}} x_2 - (h_{-1} + h_2) \end{pmatrix}.$$

If both matrices B_1 and B_2 have negative traces and positive determinants, then **A4** holds. The trace of B_1 is

$$-k_1 S_{\text{tot}} x_1 - (k_{-1} + k_2) - k_3 S_{\text{tot}}(1 - x_1 - x_2) - (k_{-3} + k_4).$$

It is negative provided that

$$\sigma \leq \frac{k_{-1} + k_2 + k_{-3} + k_4}{S_{\text{tot}}(k_1 + k_3)}.$$

The determinant of B_1 is

$$k_1(k_{-3} + k_4) S_{\text{tot}} x_1 + k_3(k_{-1} + k_2) S_{\text{tot}}(1 - x_1 - x_2) + (k_{-1} + k_2)(k_{-3} + k_4).$$

It is positive if

$$\sigma \leq \frac{(k_{-1} + k_2)(k_{-3} + k_4)}{S_{\text{tot}}\big(k_1(k_{-3} + k_4) + k_3(k_{-1} + k_2)\big)}.$$

The condition for B_2 can be derived similarly. To summarize, if we take

$$\sigma = \min\left\{\sigma_0,\ \frac{k_{-1} + k_2 + k_{-3} + k_4}{S_{\text{tot}}(k_1 + k_3)},\ \frac{(k_{-1} + k_2)(k_{-3} + k_4)}{S_{\text{tot}}\big(k_1(k_{-3} + k_4) + k_3(k_{-1} + k_2)\big)},\right.$$
$$\left.\frac{h_{-1} + h_2 + h_{-3} + h_4}{S_{\text{tot}}(h_1 + h_3)},\ \frac{(h_{-1} + h_2)(h_{-3} + h_4)}{S_{\text{tot}}\big(h_1(h_{-3} + h_4) + h_3(h_{-1} + h_2)\big)}\right\},$$

then the assumptions **A1**, **A2** and **A4** will hold.

Notice that dy/dt in (3.30) is linear in y when $\varepsilon = 0$, so g_1 (defined as in (3.9)) is linear in z, and hence the equation for z can be written as:

$$\frac{dz}{d\tau} = B(x_0)z, \quad x_0 \in U,$$

where the matrix $B(x_0)$ is Hurwitz for every $x_0 \in U$. Therefore, **A3** also holds.

It remains to show that assumption **A6** is satisfied. Let us look at the reduced system ($\varepsilon = 0$ in (3.30)):

$$\frac{dx_1}{dt} = -\frac{k_2 x_1}{\frac{K_{m1}}{S_{\text{tot}}} + \frac{K_{m1}(1-x_1-x_2)}{K_{m2}} + x_1} + \frac{h_4 c \frac{K_{m3}(1-x_1-x_2)}{K_{m4}}}{\frac{K_{m3}}{S_{\text{tot}}} + \frac{K_{m3}(1-x_1-x_2)}{K_{m4}} + x_2} := F_1(x_1, x_2) \quad (3.31)$$

$$\frac{dx_2}{dt} = -\frac{h_2 c x_2}{\frac{K_{m3}}{S_{\text{tot}}} + \frac{K_{m3}(1-x_1-x_2)}{K_{m4}} + x_2} + \frac{k_4 \frac{K_{m1}(1-x_1-x_2)}{K_{m2}}}{\frac{K_{m1}}{S_{\text{tot}}} + \frac{K_{m1}(1-x_1-x_2)}{K_{m2}} + x_1} := F_2(x_1, x_2).$$

It is easy to see that F_1 is strictly decreasing in x_2, and F_2 is strictly decreasing in x_1 on

$$K_0 = \{(x_1, x_2) \,|\, x_1 \geq 0, x_2 \geq 0, x_1 + x_2 \leq 1\}.$$

The reduced system (3.31) is a strictly competitive system, see Figure 3.10.

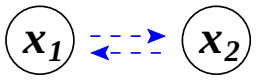

Figure 3.10: The "signed influence digraph" of the reduced system (3.31). The nodes x_1 and x_2 inhibit each other, and the overall effect is positive.

Under the change of variable $x_1^* = x_1, x_2^* = -x_2$, system (3.31) becomes irreducible and cooperative on the set K_0. Moreover, because the partial derivatives are strictly decreasing for (3.31), system (3.31) in the new coordinates (x_1^*, x_2^*) is irreducible and cooperative on the set U (defined before) for sufficiently small σ. It then follows from Lemma 3.9 that flow of (3.31) in the new coordinates has positive derivatives with respect to the nonnegative orthant, and flow in the original coordinates has positive derivatives with respect to the orthant

$$\{(x_1, x_2) \,|\, x_1 \leq 0, x_2 \geq 0\}.$$

As a result assumption **A6** is satisfied for U with sufficiently small $\sigma > 0$ independent of ε. Applying Theorem 3.22, we have:

Theorem 3.32 *There exists a positive $\varepsilon^* < \varepsilon_0$ such that for each $\varepsilon \in (0, \varepsilon^*)$, the forward trajectory of* (3.30) *starting from almost every point in D_ε converges to some equilibrium.*

Parameter	Value
k_1	0.02
k_{-1}	1
k_2	0.01
k_3	0.032
k_{-3}	1
k_4	15
h_1	0.045
h_{-1}	1
h_2	0.092
h_3	0.01
h_{-3}	1
h_4	0.5
S_{tot}	500
E_{tot}	50
F_{tot}	100

Table 3.1: Parameters used in the simulation of system (3.29).

We simulate system (3.29) with parameters given in Table 3.1. The simulation result confirms our conclusion in Theorem 3.32 and shows the key feature of singular perturbation systems, that is, trajectories quickly converge to the invariant manifold, then track trajectories on the invariant manifold, see Figure 3.11.

It is worth pointing out that the conclusion we obtained from the above theorem is only valid for small enough ε; that is, the concentration of the enzyme should be much smaller than the concentration of the substrate. Unfortunately, this is not always true in biological systems, especially when feedbacks are present. However, if the sum of the Michaelis-Menten constants and the total concentration of the substrate are much larger than the concentration of enzyme, a different scaling:

$$x_1 = \frac{s_0}{A}, \quad x_2 = \frac{s_2}{A}, \quad \varepsilon' = \frac{E_{\text{tot}}}{A}, \quad t = \tau\varepsilon',$$

where $A = S_{\text{tot}} + K_{m1} + K_{m2} + K_{m3} + K_{m4}$ will allow us to obtain the same convergence result.

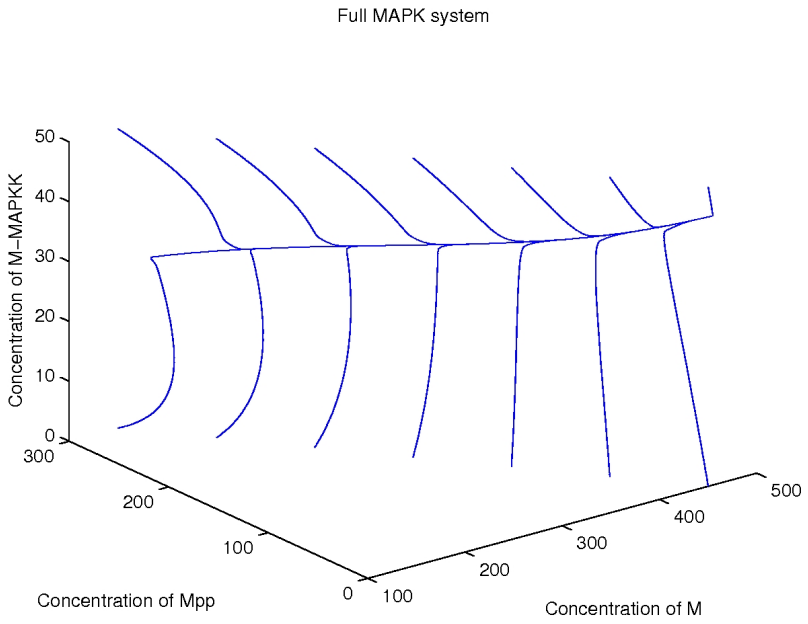

Figure 3.11: This picture shows the projection of trajectories to the $s_0 s_2 c_1$-plane. The vertical axis is the concentration of the complex ("M-MAPKK" in the picture) formed by the protein MAPK and the enzyme MAPKK, denoted by c_1 in the equations. The horizontal axises consist of concentrations of the protein MAPK ("M" in the picture) and the doubly phosphorylated form MAPK ("Mpp" in the picture), which correspond to s_0 and s_2 respectively in system (3.29). The vertical curves are solutions to system (3.29) with different initial conditions. The central horizontal curve is the projection of the invariant manifold M_ε to the $s_0 s_2 c_1$-plane. We can see from this picture that solutions converge quickly to the invariant manifold, then follow trajectories on the invariant manifold.

3.5.3 A genetic circuit example

Separation of time scales also arises in gene regulatory networks. Processes like binding and unbinding of transcription factors often occur at a much faster time scale (usually seconds) than other processes such as transcription and translation (usually several minutes), see also Alon's book [3] and references there. Protein-protein interaction often depends on the size of the proteins and number of intimidate steps, and it could be slow in some cases.

Consider a genetic circuit consisting of one gene a, which transcribes protein A. The protein A can then form dimers, called B, and bind to the upstream regulatory sites controlling the transcription of gene a, see Figure 3.12.

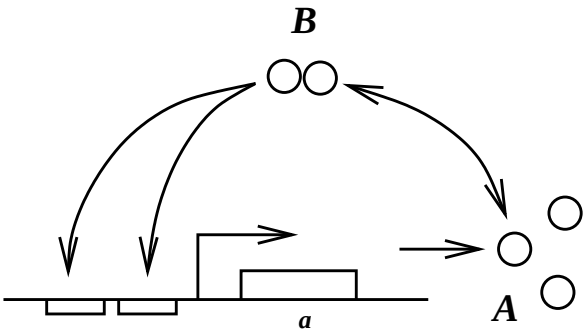

Figure 3.12: A genetic circuit. The transcription of gene a is regulated by two promoter binding sites. The product of gene a, protein A, can form dimer B, which in turn binds to the promoter site to activate the transcription of gene a.

We make the following assumptions about the system.

1. Binding and unbinding of the transcription factors occur at a much faster time scale than other processes such as transcription, translation, degradation, and protein-protein interaction.

2. The transcription rate of gene a is dramatically increased when the dimer B binds to one of the regulatory sites.

3. The two regulatory sites are symmetric in terms of binding and unbinding of B as well as the transcription rate.

4. The transcription rate is greater when both sites are bound with B than only one site is occupied.

Reactions in the system are listed as follows:

$$M \xrightarrow{\sigma} M + A, \quad A + A \underset{k_-}{\overset{k_+}{\rightleftarrows}} B,$$

$$B + D_0 \underset{\beta_-^1}{\overset{\beta_+^1}{\rightleftarrows}} D_B \xrightarrow{\alpha_1} D_B + M,$$

$$B + D_B \underset{\beta_-^2}{\overset{\beta_+^2}{\rightleftarrows}} D_{BB} \xrightarrow{\alpha_2} D_{BB} + M,$$

$$D_0 \xrightarrow{\alpha_0} M, \quad A \xrightarrow{\gamma_a} \emptyset, \quad M \xrightarrow{\gamma_m} \emptyset,$$

Symbol	Biological Meaning
D_0	DNA without binding of B
D_B	DNA bound with one B
D'_{BB}	DNA bound with two Bs
A	Protein A
B	Dimmer formed by two As
M	mRNA of gene a
σ	Translation rate for protein A
k_+	Dimerization rate of two As
k_-	Disassociation rate of B
β_+^1	Binding rate of B to D_0
β_-^1	Unbinding rate of B from D_B
β_+^2	Binding rate of B to D_B
β_-^2	Unbinding rate of B from D_{BB}
α_0	Basal transcription rate of gene a
α_1	Transcription rate of gene a when DNA is bound with one B
α_2	Transcription rate of gene a when DNA is bound with two Bs

Table 3.2: Explanation of notations used in the genetic circuit.

The notations are given in Table 3.2.

According to our assumptions 2 to 4, we have

$$\alpha_0 \ll \alpha_1 < \alpha_2. \tag{3.32}$$

Based on mass action kinetics, the dynamics of this system can be modeled by the following ordinary differential equations:

$$\begin{aligned}
a' &= 2k_-b - 2k_+a^2 + \sigma m - \gamma_a a,\\
b' &= k_+a^2 - k_-b - \beta_+^1 d_0 b + \beta_-^1 d_b - \beta_+^2 d_b b + \beta_-^2 d_{bb},\\
m' &= \alpha_0 d_0 + \alpha_1 d_b + \alpha_2 d_{bb} - \gamma_m m,\\
d_b' &= \beta_+^1 d_0 b - \beta_-^1 d_b,\\
d_{bb}' &= \beta_+^2 d_b b - \beta_-^2 d_{bb},
\end{aligned} \tag{3.33}$$

with the conservation relation

$$D_{\text{tot}} = d_0 + d_b + d_{bb}.$$

Here prime stands for d/dt, and the small letter represents the concentration of the chemical in the corresponding capital letter.

The signed influence digraph of system (3.33) is given in Figure 3.13. It is clear

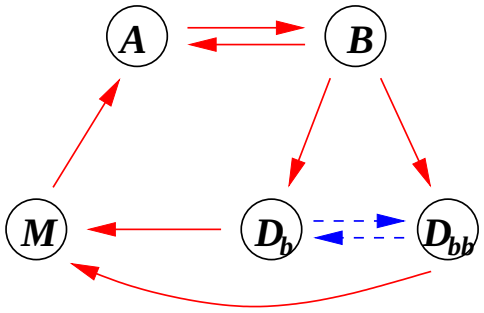

Figure 3.13: The signed influence digraph of system (3.33). We use solid lines to denote edges with positive signs, and dashed lines to represent edges with negative signs. The loop formed by B, D_b, and D_{bb} is not sign consistent.

from Figure 3.13 that system (3.33) is not monotone, and thus flows of system (3.33) do not have positive derivatives.

However, under assumption 1, we can regard $1/\beta_+^1$ as a small quantity, and define

$$\varepsilon = \frac{1}{\beta_+^1}, \quad \delta_1 = \frac{\beta_-^1}{\beta_+^1}, \quad c = \frac{\beta_+^2}{\beta_+^1}, \quad \delta_2 = \frac{\beta_-^2}{\beta_+^2}.$$

We thus assume that $\varepsilon \ll 1$ and δ_1, δ_2 and c are of order one. It is clear that d_b and d_{bb} are fast variables, whereas a and m are slow variables. Variable b is involved in both fast and slow reactions, but the total amount of dimmer B, defined as

$$\bar{b} := b + d_b + d_{bb}$$

is a slow variable.

In the new coordinates $(a, \bar{b}, m, d_b, d_{bb})$, system (3.33) becomes

$$\begin{aligned}
a' &= 2k_-(\bar{b} - d_b - d_{bb}) - 2k_+a^2 + \sigma m - \gamma_a a, \\
\bar{b}' &= k_+a^2 - k_-(\bar{b} - d_b - d_{bb}), \\
m' &= \alpha_0(D_{\text{tot}} - d_b - d_{bb}) + \alpha_1 d_b + \alpha_2 d_{bb} - \gamma_m m, \\
\varepsilon d_b' &= (D_{\text{tot}} - d_b - d_{bb})(\bar{b} - d_b - d_{bb}) - \delta_1 d_b, \\
\varepsilon d_{bb}' &= c d_b(\bar{b} - d_b - d_{bb}) - c\delta_2 d_{bb}.
\end{aligned} \tag{3.34}$$

System (3.34) is now in the standard singular perturbation form, and we can apply Theorem 3.22 provided assumptions **A1** to **A7** in Section 3.4 hold.

It is straightforward to check that the set D, defined as

$$D = D_\varepsilon := \{(a, \bar{b}, m, d_b, d_{bb}) \mid 0 \le a + \bar{b} \le \frac{\sigma M_0}{\gamma_a}, 0 \le m \le M_0,$$
$$a, \bar{b}, d_b, d_{bb} \ge 0, 0 \le d_b + d_{bb} \le \min\{\bar{b}, D_{\text{tot}}\}\},$$

is positive invariant, and thus **A5** holds. Here M_0 is any number greater than

$$\frac{\alpha_2 D_{\text{tot}}}{\gamma_m}.$$

The projection of D to the $(a, \bar{b}, m)$-plane is

$$K = K_\varepsilon = \{(a, \bar{b}, m) \mid 0 \le a + \bar{b} \le \frac{\sigma M_0}{\gamma_a}, 0 \le m \le M_0,\ a, \bar{b} \ge 0\}.$$

Setting $\varepsilon = 0$ in (3.34), the equations for d_b and d_{bb} degenerate to:

$$\begin{aligned} 0 &= (D_{\text{tot}} - d_b - d_{bb})(\bar{b} - d_b - d_{bb}) - \delta_1 d_b, \\ 0 &= d_b(\bar{b} - d_b - d_{bb}) - \delta_2 d_{bb}. \end{aligned} \tag{3.35}$$

It is not straightforward to solve for d_b, d_{bb} as functions of $\bar{b}$ from equations in (3.35). We use the following approach to achieve this. Recall that

$$b = \bar{b} - d_b - d_{bb}. \tag{3.36}$$

Substituting (3.36) into (3.35), we have

$$\begin{aligned} 0 &= (D_{\text{tot}} - d_b - d_{bb})b - \delta_1 d_b, \\ 0 &= d_b b - \delta_2 d_{bb}. \end{aligned} \tag{3.37}$$

Solving equations in (3.37), we get

$$\begin{aligned} d_b &= \frac{D_{\text{tot}} b}{\delta_1 + b + b^2/\delta_2} := \varphi_1(b), \\ d_{bb} &= \frac{D_{\text{tot}} b^2/\delta_2}{\delta_1 + b + b^2/\delta_2} := \varphi_2(b). \end{aligned}$$

For each $i = 1, 2$, φ_i is a strictly increasing and C^∞ function of b on $[0, +\infty)$. As a result,

$$\bar{b} = b + \varphi_1(b) + \varphi_2(b) \tag{3.38}$$

is also a strictly increasing and C^∞ function of b on $[0,+\infty)$. By the Implicit Function Theorem, there exists a function $b = f(\bar{b})$ such that (3.38) holds and $f' > 0$ on $[0,+\infty)$.

Now, we can write the solution of (3.35) as

$$d_b = \varphi_1(f(\bar{b})) := h_1(\bar{b})$$
$$d_{bb} = \varphi_2(f(\bar{b})) := h_2(\bar{b}).$$

The reduced system ($\varepsilon = 0$ in (3.34)) is

$$\begin{aligned} a' &= 2k_- f(\bar{b}) - 2k_+ a^2 + \sigma m - \gamma_p a := F_1(a,\bar{b},m), \\ \bar{b}' &= k_+ a^2 - k_- f(\bar{b}) := F_2(a,\bar{b},m), \\ m' &= \frac{D_{\text{tot}}(\alpha_0\delta_1 + \alpha_1 f(\bar{b}) + \alpha_2 f(\bar{b})^2/\delta_2)}{\delta_1 + f(\bar{b}) + f(\bar{b})^2/\delta_2} - \gamma_m m := F_3(a,\bar{b},m). \end{aligned} \tag{3.39}$$

The partial derivatives are

$$\frac{\partial F_1}{\partial \bar{b}} > 0, \quad \frac{\partial F_1}{\partial m} > 0, \quad \frac{\partial F_2}{\partial a} \geq 0,$$

on K with $\partial F_2/\partial a = 0$ only when $a = 0$;

$$\frac{\partial F_3}{\partial \bar{b}} = D_{\text{tot}}\delta_2 \frac{(\alpha_2-\alpha_1)f(\bar{b})^2 + 2(\alpha_2-\alpha_0)\delta_1 f(\bar{b}) + (\alpha_1-\alpha_0)\delta_1\delta_2}{(\delta_1\delta_2 + \delta_2 f(\bar{b}) + f(\bar{b})^2)^2} > 0$$

under the condition (3.32). As a result, we can find an open set U of the following form

$$U := \{(a,\bar{b},m) \mid -\sigma < a + \bar{b} < \frac{\sigma M_0}{\gamma_a} + \sigma, -\sigma < m < M_0 + \sigma\}$$

and all of the partial derivatives, except $\partial F_2/\partial a$, are positive on U for small enough σ. We next extend the function $F_2(a,\bar{b})$ to zero on the set $U \setminus K$. It is easy to see that F_2 is C^1 with nonnegative partial derivatives. The signed influence digraph of system (3.39) clearly shows that (3.39) is irreducible and cooperative, see Figure 3.14. By Lemma 3.9, flow of (3.39) has positive derivatives with respect to the nonnegative orthant on U for sufficiently small $\sigma > 0$.

To check **A3**, we fix the slow variables $a, \bar{b}, m$, and show that the equilibrium $d_b = h_1(\bar{b}), d_{bb} = h_2(\bar{b})$ of fast limiting system

$$\begin{aligned} \frac{d_b}{d\tau} &= (D_{\text{tot}} - d_b - d_{bb})(\bar{b} - d_b - d_{bb}) - \delta_1 d_b, \\ \frac{d_{bb}}{d\tau} &= c d_b(\bar{b} - d_b - d_{bb}) - c\delta_2 d_{bb} \end{aligned} \tag{3.40}$$

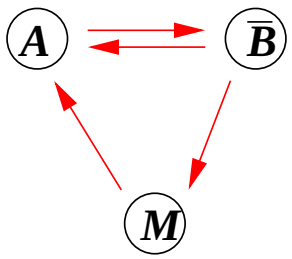

Figure 3.14: The signed influence digraph of system (3.33). We use solid lines to denote edges with positive signs, and dashed lines to represent edges with negative signs. There are no edges with negative signs. The digraph is strongly connected.

is globally asymptotically stable. Notice that the set

$$O := \{(d_b, d_{bb}) \,|\, d_b \geq 0, d_{bb} \geq 0, 0 \leq d_b + d_{bb} \leq \min\{D_{\text{tot}}, \bar{b}\}\}$$

is positively invariant, and the Jacobian matrix J of the system (3.40) is

$$\begin{pmatrix} -(\bar{b} - d_b - d_{bb}) - (D_{\text{tot}} - d_b - d_{bb}) - \delta_1 & -(\bar{b} - d_b - d_{bb}) - (D_{\text{tot}} - d_b - d_{bb}) \\ c(\bar{b} - d_b - d_{bb}) - cd_b & -cd_b - c\delta_2 \end{pmatrix}.$$

It is clear that the divergence of does not change sign on the the simply connected domain O. By the Bendixson's criterion, there are no periodic orbits inside O.

The determinant of J,

$$\det(J) = c((A + B + \delta_1)\delta_2 + \delta_1 d_b + (A + B)A),$$

is positive, where

$$A := \bar{b} - d_b - d_{bb}, \quad B := D_{\text{tot}} - d_b - d_{bb}.$$

The trace of J,

$$\text{trace}(J) = -(A + B + \delta_1 + cd_b + c\delta_2)$$

is negative. Therefore, the matrix J is Hurwitz, and the unique steady state $(d_b, d_{bb}) = (h_1(\bar{b}), h_2(\bar{b}))$ is asymptotically stable. Since we have ruled out the existence of periodic orbits, by the Poincare-Bendixson Theorem, this unique steady state is also globally asymptotically stable.

On the other hand, because the matrix J is Hurwitz, assumption **A4** is also satisfied. It remains to check **A7**. As in the futile cycle example in Section 2.2, the number of

positive steady states is bounded by the number of roots of certain polynomials. So assumption **A7** holds.

Applying Theorem 3.22, we have:

Theorem 3.33 *There exists a positive $\varepsilon^* < \varepsilon_0$ such that for each $\varepsilon \in (0, \varepsilon^*)$, the forward trajectory of* (3.34) *starting from almost every point in D converges to some equilibrium.*

3.5.4 A network example

Consider the following system:

$$\begin{aligned} \frac{dx_i}{dt} &= \gamma_i(y_1, \ldots, y_m) - \beta_i(x_1, \ldots, x_n), \quad i = 1, \ldots, n, \\ \varepsilon \frac{dy_j}{dt} &= -d_j y_j - \alpha_j(x_1, \ldots, x_n), \quad d_j > 0, \quad j = 1, \ldots, m, \end{aligned} \tag{3.41}$$

where α_j, β_i and γ_i are smooth functions. We assume that

1. The reduced system

   $$\frac{dx_i}{dt} = \gamma_i(-\frac{\alpha_1}{d_1}, \ldots, -\frac{\alpha_m}{d_m}) - \beta_i(x_1, \ldots, x_n) := F_i(x_1, \ldots, x_n), \quad i = 1, \ldots, n$$

   has partial derivatives that satisfy:

   $$\frac{\partial F_i}{\partial x_k} = \sum_{l=1}^{m} -\frac{1}{d_i} \frac{\partial \gamma_i}{\partial y_l} \frac{\partial \alpha_l}{\partial x_k} - \frac{\partial \beta_i}{\partial x_k} > 0 \text{ for } i \neq k. \tag{3.42}$$

2. For each i,

   $$\lim_{u \to +\infty} \min_{x \in S_i(u)} \beta_i(x) = +\infty \tag{3.43}$$

   and

   $$\lim_{u \to -\infty} \max_{x \in S_i(u)} \beta_i(x) = -\infty \tag{3.44}$$

   where $S_i(u)$ is the set of vectors in $\mathbb{R}^n$ whose ith coordinate is u. (For $n = 1$, this means simply that $\lim_{x \to \pm\infty} \beta_i(x) = \pm\infty$.)

3. There exists a positive constant M_j such that $|\alpha_j(x)| \leq M_j$ for all $x \in \mathbb{R}^n$.

4. The number of roots of the system of equations

$$\gamma_i(\alpha_1(x), \ldots, \alpha_m(x)) = \beta_i(x), \quad i = 1, \ldots, m$$

is countable.

We are going to show that on any large enough region, and provided that ε is sufficiently small, almost every trajectory converges to some equilibrium. To emphasize the need for small ε, we also show that when $\varepsilon > 1$, a limit cycle could appear.

Assumption 4 implies **A7**, and because of the form of (3.41), **A3** and **A4** follow naturally. Under condition 1, **A6** holds by applying Lemma 3.10.

We define

$$V = \{\, y \in \mathbb{R}^m \mid |y_j| < b_j,\ j = 1, \ldots, m \,\},$$

where b_j is an arbitrary positive number greater than M_j/d_j. Picking such b_j assures $y_j dy_j/dt < 0$ for all $x \in \mathbb{R}$ and $|y_j| = b_j$, i.e. the vector field points transversely inside on the boundary of V. Let

$$U = \{\, x \in \mathbb{R}^n \mid -a_{i,2} < x_i < a_{i,1},\ i = 1, \ldots, n \,\}$$

where $a_{i,1}$ can be any positive number such that

$$\beta_i(x) > N_i := \max_{|y_j| \le b_j} |\gamma_i(y_1, \ldots, y_m)|$$

for all $x \in \mathbb{R}^n$ whose ith coordinate satisfies $x_i \ge a_{i,1}$; the number $a_{i,2}$ can be chosen as any positive number such that

$$\beta_i(x) < -N_i$$

for all $x \in \mathbb{R}^n$ whose ith coordinate satisfies $x_i \le -a_{i,2}$. All large enough $a_{i,j}$'s satisfy this condition, because of the unboundedness assumption on β.

By the definition of U, it is easy to see that $x_i dx_i/dt < 0$ on the boundary of U for all $y \in V$, and thus the vector field will point to the interior of $U \times V$. Let $D = D_\varepsilon$ be any convex compact set in $U \times V$. It follows that D satisfies assumption **A5**. Assumptions **A1** and **A2** follow naturally by the definition of U and V. Since all assumptions **A1**-**A7**

are satisfied, by By our main theorem, for sufficiently small ε, the forward trajectory of (3.41) starting from almost every point in D converges to some equilibrium.

On the other hand, convergence does not hold for large ε. Let

$$n = 1, \beta(x) = \frac{x^3}{3} - x,\ \alpha_1(x) = 2\tanh x,$$

$$m = 1,\ \gamma(y) = y,\ d = 1.$$

System (3.41) becomes

$$\begin{aligned} \frac{dx}{dt} &= y - \frac{x^3}{3} + x \\ \varepsilon\frac{dy}{dt} &= -y - 2\tanh x. \end{aligned} \tag{3.45}$$

It is easy to verify that $(0,0)$ is the only equilibrium, and the Jacobian matrix at $(0,0)$ is

$$\begin{pmatrix} 1 & 1 \\ -2/\varepsilon & -1/\varepsilon \end{pmatrix}.$$

When $\varepsilon > 1$, the trace of the above matrix is $1 - 1/\varepsilon > 0$, its determinant is $1/\varepsilon > 0$, so the (only) equilibrium in D is repelling.

On the other hand, the set D is chosen such that the vector fields point transversely inside on the boundary of D. By the Poincaré-Bendixson Theorem, there exists a limit cycle in D, see Figure 3.15.

For the same system (3.45), when $\varepsilon > 0$ is small, forward trajectory starting from almost every point in D converges to the origin, see Figure 3.16.

Remark 3.34 *The conditions (3.42), (3.43), and (3.44) are satisfied, in particular, if one assumes the following easier to check conditions on the functions β_i's, α_j's, and γ_i's. The functions β_i are required to satisfy:*

$$\frac{\partial \beta_i}{\partial x_k}(x) < 0$$

for all $i \neq k = 1, \ldots, n$ (strict cooperativity condition among x_i variables), and also so that:

$$\lim_{x_1 \to +\infty, \ldots, x_n \to +\infty} \beta_i(x_1, \ldots, x_n) = +\infty \tag{3.46}$$

$$\lim_{x_1 \to -\infty, \ldots, x_n \to -\infty} \beta_i(x_1, \ldots, x_n) = -\infty. \tag{3.47}$$

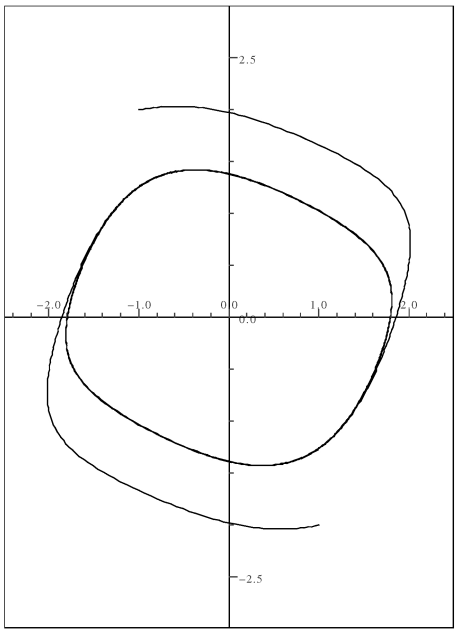

Figure 3.15: A simulation of system (3.45) with $\varepsilon = 5$. The horizontal axis is x, and the vertical axis is y. Limit cycle appears.

These last conditions are very natural. They are satisfied, for example, if there is a linear decay term $-\lambda_i x_i$ in the differential equation for each x_i, and all other variables appear saturated in this rate. Since $\frac{\partial \beta_i}{\partial x_k}(x) < 0$ for all $i \neq k$, (3.46)-(3.47) imply that conditions (3.43) and (3.44) both hold.

Regarding the remaining functions, we ask:

$$\sum_{l=1}^{m} \frac{\partial \gamma_i}{\partial y_l} \frac{\partial \alpha_l}{\partial x_k} \leq 0 \tag{3.48}$$

for all $i \neq k = 1, \ldots, n$. This condition can be guaranteed to hold based only upon the signs of the partial derivatives: it holds true if there is no indirect negative effect (through the variables y_l) of any variable x_k on any other variable x_i. The diagram shown in Figure 3.17 illustrates one such influence graph (signs indicate signs of partial derivatives), for $n = m = 2$. Observe that this example cannot describe a monotone system (with respect to any orthant cone, i.e., it is not cooperative under any possible change of coordinates of the type $x_i \to -x_i$ or $y_l \to -y_l$). An entirely analogous example

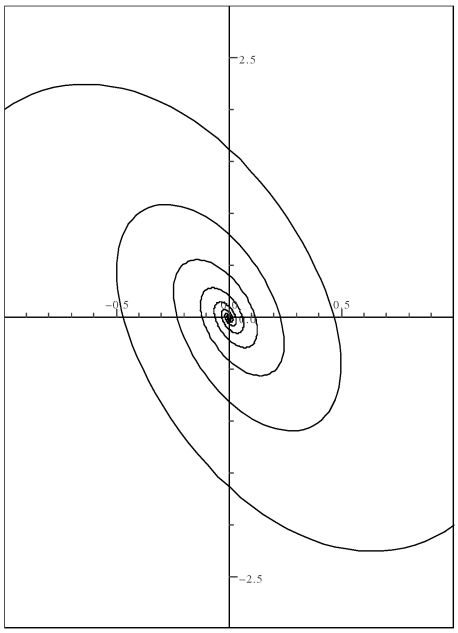

Figure 3.16: A simulation of system (3.45) with $\varepsilon = 0.5$. The horizontal axis is x, and the vertical axis is y. Solutions converge to the equilibrium at the origin.

can be done for any $n = m$, the key property being that each variable x_i "represses" its associated variables y_i and the y_l's "enhance" some or all other variables.

3.6 Conclusions

Singular perturbation techniques are routinely used in the analysis of biological systems. The *geometric* approach is a powerful tool for global analysis, since it permits one to study the behavior for finite ε on a manifold in which the dynamics is "close" to the slow dynamics. Moreover, and most relevant to us, a suitable fibration structure allows the "tracking" of trajectories and hence the lifting to the full system of the exceptional set of non-convergent trajectories, if the slow system satisfies the conditions of Hirsch's Theorem. Using the geometric approach, we were able to provide a global convergence theorem for singularly perturbed strongly monotone systems, in a form that makes it applicable to the study of double futile cycles and other biochemical processes.

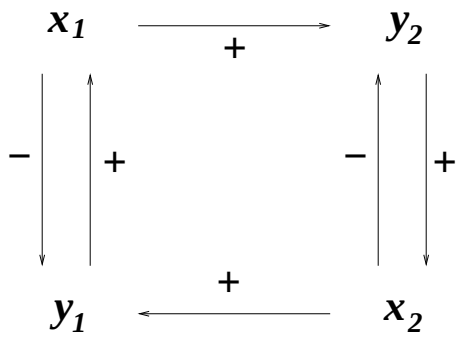

Figure 3.17: The sign diagram of an example satisfying condition (3.48)*. The negative sign means one inhibits the other, and the positive sign means one enhances the other.*

Chapter 4

Monotone Tridiagonal Systems with Negative Feedback

4.1 Introduction

Tridiagonal systems are those in which each of the state variables $x_1, \ldots, x_n$ is only allowed to interact with its "neighbors", see Figure 4.1. The dynamical behaviors of

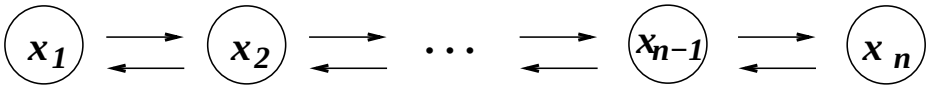

Figure 4.1: An example of tridiagonal system of size n.

tridiagonal systems have been well studied; see, for instance, Smillie [80] and Smith [81].

Such systems arise in many models in biology. In biochemical networks, examples include those in which a set of genes g_i control the production of proteins P_i, each of which acts as a transcription factor for the next gene g_{i+1} (binding and unbinding to the promoter region of g_{i+1} affects the concentration of free protein P_i as well as the transcription rate of g_{i+1}). Somewhat different, though mathematically similar, biological examples arise from sequences of protein post-translational modifications such as phosphorylations and (providing the backward interaction) dephosphorylations.

Especially in biology, it is usual to find situations involving feedback from the last to the first component. A very common situation involves negative (repressive) feedback, which allows set-point regulation of protein levels, or which enables the generation of oscillations. A specific and classical instance of this is the Goldbeter model for circadian clocks in the *Drosophila* PER ("period") protein ([30]). In all such examples, it is of interest to find conditions that characterize oscillatory versus non-oscillatory regimes.

In this chapter, we provide sufficient conditions for global asymptotic stability of monotone tridiagonal systems with negative feedback. Of course, when negated, we also

have then necessary conditions on parameters that must hold in order for oscillations to exist.

4.2 Preliminaries

In this section, we introduce some definitions and notions that are used throughout this chapter. A key result about systems with Poincaré-Bendixson property is also presented.

By default, all matrices and vectors considered in this chapter have real values.

Definition 4.1 *A square matrix A is called quasi-monotone if it has nonnegative off-diagonal entries.*

Definition 4.2 *A real vector is called nonnegative (positive) if all its components are nonnegative (positive).*

Definition 4.3 *A square matrix A is called Hurwitz if all eigenvalues of the matrix A have negative real parts.*

If A and B are two $n \times n$ matrices such that $A_{ij} \leq B_{ij}$ for all $i, j = 1, \ldots, n$, then we denote this by $A \leq B$.

For an arbitrary $n \times n$ matrix A, we let $|A|$ be the $n \times n$ matrix defined as

$$|A_{ij}| = \begin{cases} A_{ij}, \text{ if } i = j \\ |A_{ij}|, \text{ if } i \neq j. \end{cases}$$

Consider a general ordinary differential equation

$$\dot{y} = G(y), \quad y \in U, \tag{4.1}$$

where U is an open set in $\mathbb{R}^n$, and the vector field G is of class C^1. Suppose that system (4.1) has a periodic solution $p(t)$.

Definition 4.4 *The periodic solution $p(t)$ is said to be orbitally (Lyapunov) stable if for an arbitrarily small neighborhood W of $p(t)$, all forward trajectories which start in a sufficiently small neighborhood of $p(t)$ do not emerge from W.*

Definition 4.5 *The periodic solution $p(t)$ is said to be orbitally asymptotically stable if it is orbitally Lyapunov stable and if all the phase curves with initial condition sufficiently close to $p(t)$ approach $p(t)$ asymptotically as $t \to +\infty$.*

We remark that a non-constant periodic solution can not be asymptotically stable because solutions with initial conditions at different points of the cycle do not approach one another as $t \to +\infty$.

Definition 4.6 *A set K is called absorbing in U for (4.1) if the solution $y(t)$ with initial condition in K_1 stays in K for each compact set $K_1 \subset U$ and t sufficiently large.*

A tridiagonal system with feedback has the form:

$$\begin{aligned} \dot{x}_i &= f_i(x_{i-1}, x_i, x_{i+1}), \quad i = 1, \ldots, n-1 \\ \dot{x}_n &= f_n(x_{n-1}, x_n), \end{aligned} \tag{4.2}$$

where x_0 is identified with x_n, and the vector field $F = (f_1, \ldots, f_n)$ is defined on an open set U.

In typical applications, the variables x_i represent nonnegative physical quantities, such as concentrations of chemical species. In such cases, the equations describing the system are initially only specified for vectors x belonging to the nonnegative orthant $\mathbb{R}^n_{\geq 0}$. However, in most cases, one may restrict the system to the interior of $\mathbb{R}^n_{\geq 0}$, or one may also view any such system as a system defined on a slightly larger open set U. This is done by appropriately extending the functions f_i to a neighborhood of the orthant.

Definition 4.7 *System (4.2) is called a monotone tridiagonal feedback system if there exist scalars $\delta_i \in \{+1, -1\}$, $i = 1, \ldots, n$, such that for all $1 \leq i \leq n-1$,*

$$\delta_i \frac{\partial f_i(x_{i-1}, x_i, x_{i+1})}{\partial x_{i-1}} > 0, \ \textit{and} \ \frac{\partial f_i(x_{i-1}, x_i, x_{i+1})}{\partial x_{i+1}} \geq 0, \tag{4.3}$$

for all $x \in U$, and

$$\delta_n \frac{\partial f_n(x_{n-1}, x_n)}{\partial x_{n-1}} > 0 \tag{4.4}$$

for all $x \in U$.

Monotone tridiagonal feedback systems are known to have the Poincaré-Bendixson property ([56]), that is, any nonempty compact omega limit set that contains no equilibria is a closed orbit. There are two types of monotone tridiagonal feedback systems depending on the sign of the product $\delta_1 \cdots \delta_n$. If the sign is positive (negative), then system (4.2) is called a monotone tridiagonal system with positive (negative) feedback. In this paper, we focus on the negative feedback case, and from now on we assume without loss of generality (after suitable rescaling of the state components with scaling factor $+1$ or -1) that system (4.2) satisfies conditions (4.3) and (4.4) with

$$\delta_1 = -1 \text{ and } \delta_i = +1, \text{ for } i = 2, \ldots, n. \tag{4.5}$$

For a system with the Poincaré-Bendixson property, if it has an absorbing set K and a unique equilibrium x^*, we can obtain global stability of x^* by ruling out the existence of periodic orbits. To achieve this, the following argument is used, see also [55].

We assume that every periodic orbit is orbitally asymptotically stable. Then the boundary of the region of attraction of x^* must contain a periodic orbit since it is invariant. As a result, there exist points in the region of attraction of x^* whose orbit converges to the periodic orbit, which is impossible. More precisely,

Theorem 4.8 *(Theorem 2.2 in [55]) For a general ordinary differential equation system* (4.1) *with Poincaré-Bendixson property, if the following assumptions hold:*

1. *There exists a compact absorbing set $K \subset U$.*
2. *There is a unique equilibrium point x^*, and it is locally asymptotically stable.*
3. *Each periodic orbit is orbitally asymptotically stable.*

Then x^* is globally asymptotically stable in U.

4.3 Ruling out periodic orbits

In this section, we use the theory of second compound matrices to show that all periodic orbits are orbitally asymptotically stable. This is inspired by the work of Sanchez in [75],

which studied the globally asymptotic stability of cyclic systems with negative feedback. Cyclic systems are those for which

$$\frac{\partial f_i(x_{i-1}, x_i, x_{i+1})}{\partial x_{i+1}} \equiv 0 \quad \text{for all } x \in U,\ i = 1, \ldots, n$$

in (4.2). Cyclic systems are known to have Poincaré-Bendixson property ([57]) and other nice properties ([29]).

Recall the definition of the second additive compound matrix:

Definition 4.9 *Let A be a matrix of order n. The second compound matrix $A^{[2]}$ is a matrix of order $\binom{n}{2}$ which is defined as follows:*

$$A^{[2]}_{ij} = \begin{cases} A_{i_1 i_1} + A_{i_2 i_2}, & \text{if } (i) = (j), \\ (-1)^{r+s} A_{i_r j_s} & \text{if exactly one entry } i_r \text{ of } (i) \text{ does} \\ & \text{not occur in } (j) \text{ and } j_s \text{ does not} \\ & \text{occur in } (i), \text{ for some } r, s \in \{1, 2\}, \\ 0 & \text{if } (i) \text{ differs from } (j) \text{ in both entries.} \end{cases}$$

Here, $(i) = (i_1, i_2)$ is the ith member of the lexicographic order of integer pairs for which $1 \le i_1 < i_2 \le n$.

For future reference we state the following well-known facts from the theory of second compound matrices, see [12, 54].

Lemma 4.10 *Let the eigenvalues of a real $n \times n$ matrix A be denoted by λ_i, $i = 1, \ldots, n$. Then the eigenvalues of $A^{[2]}$ are given by $\lambda_i + \lambda_j$ for $i < j$ with $i = 1, \ldots, n$ and $j = 2, \ldots, n$.*

Lemma 4.11 *A matrix A of order n is Hurwitz if and only if $A^{[2]}$ is Hurwitz and the sign of $\det(A)$ is $(-1)^n$.*

Let us denote by $DF(x)$ the Jacobian of system (4.2). The following observation is crucial to our proof.

Lemma 4.12 *The second additive compound matrix $DF^{[2]}(x)$ is quasi-monotone for any $x \in U$.*

Proof. Recall that the only non-zero off-diagonal entries of $DF(x)$ are

$$DF(x)_{ii-1} > 0, \quad DF(x)_{ii+1} \geq 0$$

for $i = 2, \ldots, n-1$,

$$DF(x)_{12} \geq 0, \quad DF(x)_{nn-1} > 0, \quad DF(x)_{1n} < 0.$$

Thus the off-diagonal entries of $DF^{[2]}(x)$ are non-zero only when one of the following five cases happens:

1. The pairs $i = (i_1, i_2), j = (i_1, i_2 - 1)$ for some $i_2 > i_1 + 1$. In this case

$$DF^{[2]}_{ij}(x) = (-1)^{2+2} DF(x)_{i_2 i_2 - 1} > 0.$$

2. The pairs $i = (i_1, i_2), j = (i_1, i_2 + 1)$ for some $i_2 > i_1$. In this case

$$DF^{[2]}_{ij}(x) = (-1)^{2+2} DF(x)_{i_2 i_2 + 1} \geq 0.$$

3. The pairs $i = (i_1, i_2), j = (i_1 - 1, i_2)$ for some $i_2 > i_1$. In this case

$$DF^{[2]}_{ij}(x) = (-1)^{1+1} DF(x)_{i_1 i_1 - 1} > 0.$$

4. The pairs $i = (i_1, i_2), j = (i_1 + 1, i_2)$ for some $i_2 > i_1 + 1$. In this case

$$DF^{[2]}_{ij}(x) = (-1)^{1+1} DF(x)_{i_1 i_1 + 1} \geq 0.$$

5. The pairs $i = (1, i_2), j = (i_2, n)$ for some $1 < i_2 < n$. In this case

$$DF^{[2]}_{ij}(x) = (-1)^{1+2} DF(x)_{1n} > 0.$$

Therefore, the second additive compound matrix $DF^{[2]}(x)$ has only nonnegative off-diagonal entries. ∎

Second additive compound matrices can be used to study the stability of periodic orbits. The following lemma states a result by Muldowney ([53, 63]), also used in [55, 75, 76].

Lemma 4.13 *A given periodic solution $p(t)$ of (4.1) is orbitally asymptotically stable provided the linear system*

$$\dot{z} = DG^{[2]}(p(t))z$$

is asymptotically stable.

By Lemma 4.12 we know that for system the matrix of $DF^{[2]}(p(t))$ is quasi-monotone for all times. In this case, it turns out that to establish asymptotic stability for

$$\dot{z} = DF^{[2]}(p(t))z, \tag{4.6}$$

it is enough to check that for all t, the matrix $DF^{[2]}(p(t))$ is bounded above (componentwise) by a quasi-monotone and Hurwitz matrix B. This follows for instance from Proposition 3 in [76]. Here we use a lemma by De Leenheer in [96].

Lemma 4.14 *(De Leenheer). Let $\dot{x} = A(t)x$ be a linear time-varying system where $A(t)$ is a continuous function. If there are positive vectors c, d such that $|A(t)|d \leq -c$ (componentwise) for all t, then $x = 0$ is asymptotically stable.*

Now, we are ready to state and proof our main theorem.

Theorem 4.15 *Assume that the first two conditions in Theorem 4.8 hold, and that there exists a quasi-monotone Hurwitz matrix B such that $B \geq DF^{[2]}(x)$ for all $x \in K$. Then x^* is globally asymptotically stable for system (4.2).*

Proof. Let us assume that $p(t)$ is a nontrivial periodic solution and show that it must be orbitally asymptotically stable. Since B is quasi-monotone and Hurwitz, it follows that there exist positive vectors c and d such that $Bd \leq -c$ (componentwise) by Theorem 15.1.1 in [40]. On the other hand, we have $B - DF^{[2]}(p(t)) \geq 0$, and thus $\left(B - DF^{[2]}(p(t))\right) d \geq 0$.

Notice that $|DF^{[2]}(p(t))| = DF^{[2]}(p(t))$ for all $t > 0$ since $DF^{[2]}(p(t))$ is quasi-monotone. We thus have

$$|DF^{[2]}(p(t))|d \leq Bd \leq -c,$$

which by Lemma 4.14 yields that (4.6) is asymptotically stable. Applying Lemma 4.13, we know that $p(t)$ is orbitally asymptotically stable for system (4.2). The conclusion now follows from an application of Theorem 4.8. ∎

4.4 Applications

4.4.1 Linear Monotone Tridiagonal Systems with Nonlinear Negative Feedback

We restrict our attention to systems of the form:

$$\begin{aligned} \dot{x}_1 &= -d_1 x_1 + \beta_1 x_2 + g(x_n) \\ \dot{x}_i &= \alpha_i x_{i-1} - d_i x_i + \beta_i x_{i+1}, \quad i = 2, \ldots, n-1 \\ \dot{x}_n &= \alpha_n x_{n-1} - d_n x_n. \end{aligned} \tag{4.7}$$

We denote by $F = (f_1, \ldots, f_n)$ the vector field of system (4.7). The following assumptions are made about system (4.7).

A1 For each $i = 1, \ldots, n, j = 2, \ldots, n$, and $k = 1, \ldots, n-1$, d_i, α_j, and β_k are positive numbers.

A2 The function $g : \mathbb{R}_{\geq 0} \to \mathbb{R}_{\geq 0}$ is smooth and strictly decreasing with $g(0) > 0$.

A3 The matrix T, defined as

$$T = \begin{pmatrix} -d_1 & \beta_1 & 0 & \cdots & 0 \\ \alpha_2 & -d_2 & \beta_2 & \cdots & 0 \\ \cdots & \cdots & \cdots & \cdots & \cdots \\ 0 & \cdots & 0 & \alpha_n & -d_n \end{pmatrix},$$

is Hurwitz.

It is clear from assumptions **A1** and **A2** that system (4.7) is a monotone tridiagonal system with negative feedback on the interior of $\mathbb{R}^n_{\geq 0}$. Moreover, the nonnegative orthant is forward invariant for system (4.7).

Lemma 4.16 *Under assumptions* **A1** *to* **A3**, *system* (4.7) *has a unique steady state* $x^* \in \mathbb{R}^n_{>0}$.

Proof. The steady state x^* satisfies

$$Tx^* + G(x_n^*) = 0.$$

Let us start from solving the nth equation of $T\bar{x}^* + G(x_n^*) = 0$, which gives

$$\alpha_n \bar{x}_{n-1}^* = d_n \bar{x}_n^*, \text{ that is, } \bar{x}_{n-1}^* = \frac{d_n}{\alpha_n}\bar{x}_n^*.$$

Substituting $\bar{x}_{n-1}^* = d_n \bar{x}_n^*/\alpha_n$ in the $(n-1)$th equation

$$\alpha_{n-1}\bar{x}_{n-2}^* + \beta_{n-1}x_n^* = d_{n-1}x_{n-1}^*,$$

we obtain

$$\bar{x}_{n-2}^* = \frac{1}{\alpha_{n-1}\alpha_n}\det(T_{n-1\,n,n-1\,n})\bar{x}_n^*.$$

Here $T_{i_1,\ldots,i_k,i_1,\ldots,i_k}$ denote the $k \times k$ submatrix of T consisting of rows and columns from i_1 to i_k. Repeating this procedure for other equations of $T\bar{x}^* + G(x_n^*) = 0$ in backward order, we have

$$x_j^* = \frac{1}{\prod_{i=j+1}^{n}\alpha_i}(-1)^{n-j}\det(T_{j+1,\ldots,n,j+1,\ldots,n})x_n^*, \quad j = 1,\ldots,n-1. \tag{4.8}$$

The coefficient in front of $\bar{x}_n^*$ in equation (4.8) is positive for each j since the matrix T is Hurwitz (assumption **A3**). By substituting (4.8) to the equation

$$d_1 x_1^* - \beta_1 x_2^* = g(x_n^*),$$

we obtain

$$\frac{1}{\prod_{i=2}^{n}\alpha_i}(-1)^n \det(T)x_n^* = g(x_n^*).$$

Under assumption **A3**, the left-hand side is a linear increasing function in x_n^*. The right hand side is a decreasing function with $g(0) > 0$. So there is a unique root x_n^* in $(0,\infty)$. The other components are also positive and unique because of (4.8). ∎

Define a vector function

$$G(x_n) = (g(x_n)\ 0\ \ldots\ 0)^T.$$

System (4.7) can be rewritten as

$$\dot{x} = Tx + G(x_n).$$

Lemma 4.17 *Under assumptions* **A1** *to* **A3**, *system* (4.7) *has a compact absorbing set* $K \subset \mathbb{R}^n_{>0}$, *defined as*

$$K = \{x \,|\, \underline{x} - \delta \leq x \leq \bar{x} + \delta\},$$

where $\bar{x} = (\bar{x}_1, \ldots, \bar{x}_n) = -T^{-1}G(0)$, $\underline{x} = (\underline{x}_1, \ldots, \underline{x}_n) = -T^{-1}G(\bar{x}_n)$, *and* δ *is a positive vector such that* $\underline{x} - \delta > 0$.

Proof. By a similar argument as in the proof of Lemma 4.16, it is easy to see that $\bar{x}$ and $\underline{x}$ are both in the interior of $\mathbb{R}^n_{\geq 0}$. As a result, there exists a positive vector δ such that $\underline{x} - \delta > 0$. We pick such a δ from now on.

Let K_1 be any compact subset of $\mathbb{R}^n_{>0}$ and $x(t)$ be the solution to system (4.7) with an arbitrary initial condition $x_0 \in K_1$. We first show that $x(t)$ is bounded from above by the constant $\bar{x}$ for large enough t.

Consider the following system:

$$\dot{u} = Tu + G(0). \tag{4.9}$$

Let $u(t)$ be the solution of (4.9) with the initial condition $u(0) = x_0$. The point $\bar{x}$ is the steady state of the linear system (4.9), and it is globally asymptotically stable.

On the other hand, since $g(x_n)$ is strictly decreasing in x_n on $[0, +\infty)$, we have $\dot{x} \leq Tx + G(0)$. By the comparison principle for monotone systems ([82]), it follows that the solution $x(t)$ of (4.7) is bounded from above by $u(t)$ for all $t \geq 0$, that is, $x(t) \leq u(t)$ for all $t \geq 0$. As a result,

$$\limsup_{t \geq 0} x(t) \leq \lim_{t \to 0} u(t) = \bar{x},$$

which implies that there exists a positive constant t_0 such that $x(t) \leq \bar{x} + \delta$ for all $t > t_0$. This t_0 can be chosen uniformly for all $x_0 \in K_1$.

Similarly, we can consider the system

$$\dot{v} = Tv + G(\bar{x}_n), \tag{4.10}$$

and let $v(t)$ be the solution of (4.10) with an arbitrary initial condition $x_0 \in K_1$. Since $g(x_n)$ is strictly decreasing in x_n, and $x_n(t)$ is bounded from above by $\bar{x}_n$ for all $t > t_0$,

as a result we have $\dot{x} \geq Tx + G(\bar{x}_n)$ for all $t > t_0$. Applying again the comparison principle for monotone systems, we get $x(t) \geq v(t)$ for all $t > t_0$. It thus follows that

$$\liminf_{t>t_0} x(t) \geq \lim_{t\to 0} y(t) = \underline{x}.$$

That is, there exists a positive constant $t_1 > t_0$ such that $x(t) \geq \underline{x} - \delta$ for all $t > t_1$.

To summarize, we have established that for any initial condition $x_0 \in K_1$, the following inequality:

$$\underline{x} - \delta \leq x(t) \leq \bar{x} + \delta$$

holds for all $t > t_1$. Therefore K is an absorbing set in $\mathbb{R}^n_{>0}$. ∎

Remark 4.18 *Using this result, the existence of the steady states of system* (4.7) *can be derived directly from the fact that K is homeomorphic to a ball. However, the algebraic approach given in the proof of Lemma 4.16 guarantees both existence and uniqueness.*

The Jacobian matrix of system (4.7) is

$$DF(x) = \begin{pmatrix} -d_1 & \beta_1 & 0 & \cdots & g'(x_n) \\ \alpha_2 & -d_2 & \beta_2 & \cdots & 0 \\ \cdots & \cdots & \cdots & \cdots & \cdots \\ 0 & \cdots & 0 & \alpha_n & -d_n \end{pmatrix}.$$

We define another matrix

$$D = \begin{pmatrix} -d_1 & \beta_1 & 0 & \cdots & g_0 \\ \alpha_2 & -d_2 & \beta_2 & \cdots & 0 \\ \cdots & \cdots & \cdots & \cdots & \cdots \\ 0 & \cdots & 0 & \alpha_n & -d_n \end{pmatrix},$$

where

$$g_0 = \min\{\, g'(x_n) \mid x_n \in \text{ projection of } K \text{ to its } n\text{th coordinate}\}$$

is a negative number. Based on the proof of Lemma 4.12, it is easy to see that for all $x \in K$,

$$DF^{[2]}(x) \leq D^{[2]},$$

and $DF^{[2]}(x)$ and $D^{[2]}$ are quasi-monotone.

We make the following assumption about the matrix D.

A4 The matrix D is Hurwitz.

Lemma 4.19 *Under assumptions* **A1** *to* **A4**, *the steady state x^* obtained in Lemma 4.16 is asymptotically stable.*

Proof. By Lemma 4.10, if D is Hurwitz, then so is $D^{[2]}$. Moreover, $DF^{[2]}(x^*)$ is also Hurwitz because $\lambda_{PF}(DF^{[2]}(x^*)) \leq \lambda_{PF}(D^{[2]})$ ([10]).

The steady state x^* is asymptotically stable if $DF(x^*)$ is Hurwitz. It follows from Lemma 4.11 that this will be the case if $DF^{[2]}(x^*)$ is Hurwitz and the determinant of $DF(x^*)$ has the sign of $(-1)^n$. Indeed, this is true under the condition that the matrix T is Hurwitz. To see this, we compute $\det(DF(x^*))$, and it equals

$$(-1)^n(-g'(x_n^*))\alpha_2\alpha_3\cdots\alpha_n + \det(T).$$

It is clear that $\det(DF(x^*))$ has the sign of $(-1)^n$, which completes the proof. ∎

Now we have checked all conditions in Theorem 4.15. Applying Theorem 4.15 to system (4.7), we have the following result.

Theorem 4.20 *Under assumptions* **A1** *to* **A4**, *the steady state x^* is globally asymptotically stable for system (4.7) on $\mathbb{R}^n_{>0}$.*

4.4.2 Goldbeter Model

Circadian rhythms are pervasive features found in diverse organisms ranging from bacteria to humans. The rhythm is robust over a range of temperatures, and persists in constant conditions (for example constant darkness) with a period of about 24 hours. Disturbance in the human circadian rhythm could lead to many health problems such as delayed sleep phase syndrome and various sleep disorders.

In this section, we consider one of the simplest and classical models of circadian rhythms by Goldbeter ([30, 31]), and present conditions under which the rhythm is disrupted, more precisely, there is a globally asymptotically stable steady state. The

model is given as follows:

$$\begin{aligned}
\dot{M} &= \frac{v_s K_I^n}{K_I^n + P_N^n} - \frac{v_m M}{k_m + M} \\
\dot{P}_0 &= k_s M - \frac{V_1 P_0}{K_1 + P_0} + \frac{V_2 P_1}{K_2 + P_1} \\
\dot{P}_1 &= \frac{V_1 P_0}{K_1 + P_0} - \frac{V_2 P_1}{K_2 + P_1} - \frac{V_3 P_1}{K_3 + P_1} + \frac{V_4 P_2}{K_4 + P_2} \\
\dot{P}_2 &= \frac{V_3 P_1}{K_3 + P_1} - \frac{V_4 P_2}{K_4 + P_2} - k_1 P_2 + k_2 P_N - \frac{v_d P_2}{k_d + P_2} \\
\dot{P}_N &= k_1 P_2 - k_2 P_N.
\end{aligned} \tag{4.11}$$

Here, all the parameters are positive, and all variables are nonnegative. The variable M represents the mRNA concentration of PER; variables P_0, P_1, and P_2 represent the concentrations of PER in the cytoplasm with no phosphate groups, one phosphate group, and two phosphate groups, respectively; the variable P_N denotes the concentration of PER in the nucleus.

System (4.11) considered on a slightly larger open set U containing $\mathbb{R}^n_{\geq 0}$ is a tridiagonal system with a negative feedback from P_N to M. It clearly satisfies conditions (4.3) and (4.4) with values of the δ_i as in (4.5). We next state a result by Angeli and Sontag in [6] for this system:

Lemma 4.21 *Assume the following conditions hold:*

- $0 < \frac{v_s k_m}{v_m - v_s} < \frac{v_d}{k_s}$;
- $v_d + V_2 < V_1$;
- $V_1 + V_4 < V_2 + V_3$;
- $V_4 + v_d < V_3$.

Then there exist positive numbers $\bar{M}, \bar{P}_0, \bar{P}_1, \bar{P}_2, \bar{P}_N$ such that system (4.11) has a compact absorbing set

$$\begin{aligned}
B = \{x \,|\, 0 \leq M \leq \bar{M}, 0 \leq P_0 \leq \bar{P}_0, 0 \leq P_1 \leq \bar{P}_1, \\
0 \leq P_2 \leq \bar{P}_2, 0 \leq P_N \leq \bar{P}_N\}.
\end{aligned}$$

Moreover, there is a unique steady state x^* inside B.

Observe that the vector field of (4.11) contains functions of Michaelis-Menten form, that is,

$$h(y) = \frac{vy}{K+y}, \quad y \in [0, \bar{y}].$$

Taking the derivative of h, we have

$$h'(y) = \frac{vK}{(K+y)^2} > 0.$$

As a result, the maximum and minimum of $h'(x)$ on $[0, \bar{y}]$ are $h'(0)$ and $h'(\bar{y})$, respectively. Based on this observation, it is easy to see that the second additive compound matrix $DF^{[2]}(x)$ is bounded by the matrix $D^{[2]}$, where the matrix D is defined as the sum of the diagonal matrix

$$\begin{aligned}\mathrm{diag}\{&-\frac{v_m k_m}{(k_m+\bar{M})^2}, -\frac{V_1 K_1}{(K_1+\bar{P}_0)^2}, -\frac{V_2 K_2}{(K_2+\bar{P}_1)^2} - \frac{V_3 K_3}{(K_3+\bar{P}_1)^2},\\ &-\frac{V_4 K_4}{(K_4+\bar{P}_2)^2} - \frac{v_d k_d}{(k_d+\bar{P}_2)^2} - k_1, -k_2\}\end{aligned}$$

and the matrix

$$\begin{pmatrix} 0 & 0 & 0 & 0 & g_0 \\ k_s & 0 & \frac{V_2}{K_2} & 0 & 0 \\ 0 & \frac{V_1}{K_1} & 0 & \frac{V_4}{K_4} & 0 \\ 0 & 0 & \frac{V_3}{K_3} & 0 & k_2 \\ 0 & 0 & 0 & k_1 & 0 \end{pmatrix}.$$

Here

$$g_0 = -\frac{v_s (n-1)^{\frac{n-1}{n}} (n+1)^{\frac{n+1}{n}}}{4nK_I}$$

is the minimum of $v_s K_I^n/(K_I^n + P_N^n)$ on $[0, \infty)$. Following a similar argument as in the proof of Lemma 4.19, we have:

Lemma 4.22 *Suppose that the assumptions in Lemma 4.21 hold and that the matrix D is Hurwitz. If the sign of $det(DF(x^*))$ is -1, then x^* is asymptotically stable.*

Applying Theorem 4.15, we obtain the following result for system (4.11).

Theorem 4.23 *Suppose that the assumptions in Lemma 4.21 hold and that the matrix D is Hurwitz. If the sign of $det(DF(x^*))$ is -1, then x^* is globally asymptotically stable.*

This result provides conditions under which oscillations will be blocked. On the other hand, when there is a oscillation, conditions in Theorem 4.23 fail to hold for that set of kinetic parameters.

Chapter 5

A Class of Reaction Diffusion Systems with Interconnected Structure

5.1 Introduction

Spatial information is crucial in many physiological processes such as cell division, motility, and migration. During evolution, cells developed mechanism to detect spatial localization of signaling proteins. A classical example is the process of morphogenesis. In a human embryo, the change from a cluster of nearly identical cells to different tissues and organs is controlled by chemicals that can be modified by environmental factors. In MAPK cascades introduced in Chapter 2 and Chapter 3, the spatial gradient of phosphorylated mitogen-actived protein kinase kinase (MEK) also plays important roles.

Chemicals in an organism usually move around in a random way. This random motion can be regarded as a diffusion process. On the other hand, chemicals may react with each other, and thus form a reaction diffusion system. We shall point out that the addition of diffusion could destabilize constant steady states. The phenomenon of Turing's pattern formation is a well known example of diffusion driven instability, see [21, 64, 68, 93] for more on this subject. In this Chapter, in contrast to the loss of stability, we show that if a passivity-based stability condition (will be defined precisely later) holds for the ordinary differential equation system, then the global stability of the same constant steady state is preserved when diffusion is added.

In their novel work [8] , Arcak and Sontag developed a passivity-based stability condition for general interconnected systems without incorporating spatial factors (diffusion). The structure of a general interconnected system can be described by a directed graph without self-loops. We use node $i \in \{1, \ldots, N\}$ to represent

the ith subsystem. If there is a directed link l from node i to node j, we write $i = s(l), j = t(l), l \in \mathcal{L}_i^- \subseteq \{1, \ldots, M\}$, and $l \in \mathcal{L}_j^+$. See Figure 5.1 for an example of such graphs. Let x_i represent the state of the ith subsystem. Different subsystems

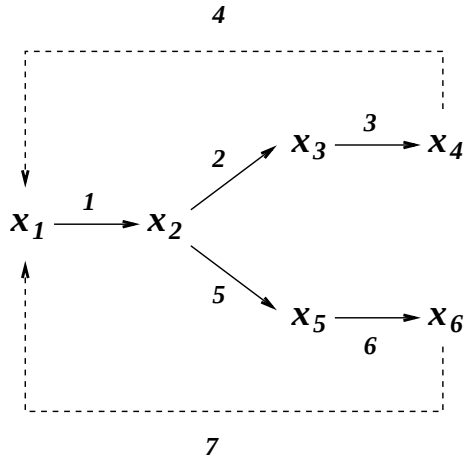

Figure 5.1: An example of branched network in [8]. The dashed lines 4 and 7 indicate negative feedback signals, and the solid lines indicate the positive influence.

are connected according to the structure of the graph. The set of ordinary differential equations describing the dynamics is given by:

$$\dot{x}_i = -f_i(x_i) + g_i(x_i) \sum_{l \in \mathcal{L}_i^+} h_l(x_{s(l)}), \quad i = 1, \ldots, N. \tag{5.1}$$

A key notion used in [8] is *passivity*, which evolved from the idea of energy conservation and dissipation in electrical and mechanical systems ([101]). To determine the stability of the whole system, Arcak and Sontag constructed a *dissipativity matrix* (denoted by E below). The matrix E carries information about the structure of the network, the signs of the interconnected terms, and the passivity properties of the subsystems.

Under the assumption that system (5.1) has a unique steady state and that the matrix E is diagonally stable, that is, there exists a diagonal matrix

$$Q = \text{diag}\{q_1, \ldots, q_M\} > 0$$

such that

$$E^T Q + QE < 0,$$

Arcak and Sontag proved globally stability of the steady state by constructing a Lyapunov function V. Similar stability tests based on dissipativity were developed in the earlier work of Vidyasagar [95], Moylan and Hill *et al* [62], and Sundareshan and Vidyasagar [88].

The work by Jovanović, Arcak, and Sontag in [46] studied the effect of diffusion to a special class of systems whose interconnected graphs are cyclic, see Figure 5.2. The

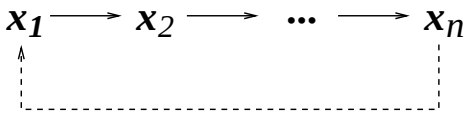

Figure 5.2: A cyclic system of size n. The dashed line represents the inhibition from x_n to x_1.

present work extends the results in [46] to encompass the broader class of interconnected systems represented by graphs, see Figure 5.1.

The effect of diffusion to the persistence of stability is one of the important questions to the theory of reaction diffusion equations. Let us consider a general ordinary differential equation system:

$$\frac{dz_i}{dt} = F_i(z) \tag{5.2}$$

$$z_i(0) = z_i^0, \tag{5.3}$$

and its corresponding partial differential equation system:

$$\frac{\partial u_i}{\partial t} = A_i u_i + F_i(u) \qquad \text{on } \Omega \times (0, +\infty), \tag{5.4}$$

$$\frac{\partial u_i}{\partial \nu} = 0 \qquad \text{on } \partial\Omega \times (0, +\infty), \tag{5.5}$$

$$u_i(\xi, 0) = u_i^0(\xi) \qquad \text{on } \Omega, \tag{5.6}$$

for each $i = 1, \dots N$. Here A_i is a second order differential operator defined as

$$A_i = \sum_{j,k=1}^{N} a_{jk}^i(\xi) \frac{\partial}{\partial \xi_j} \frac{\partial}{\partial \xi_k} + \sum_{k=1}^{N} a_k^i(\xi) \frac{\partial}{\partial \xi_k}.$$

In the work [71] by Redheffer, Redlinger, and Walter, the authors used the convexity of a Lyapunov function associated with (5.2) to prove the global existence of classical solutions to (5.4)-(5.5) under the assumption that the elliptic operator A_i's are the same

for all $i = 1, \ldots, N$. Moreover, they obtained La Salle type of result for systems with strictly convex Lyapunov functions. Morgan in [61] studied the case when the elliptic operator

$$A_i \equiv a_i \frac{\partial}{\partial \xi_i} \frac{\partial}{\partial \xi_i},$$

where a_i is a positive constant. He made the assumption that there exists a Lyapunov structure function $H \in C^2(M, \mathbb{R}_{\geq 0})$, where M is a unbounded region of $\mathbb{R}^N$ for which (5.4) is invariant. Under various assumptions on the function H, Morgan in [61] obtained boundedness and stability results for (5.4). Along these lines is the paper [26] by Fitzgibbon, Hollis, and Morgan with an emphasis on stability in L^∞.

In the present work, instead of assuming conditions on the Lyapunov function, we provide a specific form of the Lyapunov function, and present a self-contained proof for the global stability of the constant equilibrium without the restriction of constant diffusion coefficients.

5.2 Review of the Ordinary Differential Equation Case

In [8], the authors made the following assumptions about system (5.1).

A1 For each $i = 1, \ldots, N$ and $l = 1, \ldots, M$, the functions f_i, g_i, and h_l are locally Lipschitz with $f_i(0) = 0$, and $g_i(\theta) > 0, h_l(\theta) \geq 0$ for all $\theta \geq 0$.

A2 System (5.1) admits an equilibrium $x^* \in \mathbb{R}^N_{\geq 0}$.

A3 For each node i, the function f_i/g_i satisfies the sector property

$$(\theta - x_i^*) \left(\frac{f_i(\theta)}{g_i(\theta)} - \frac{f_i(x_i^*)}{g_i(x_i^*)} \right) > 0, \quad \forall \theta \in \mathbb{R}_{\geq 0} \setminus \{x_i^*\}. \tag{5.7}$$

A4 For each node i, and for each link $l \in \mathcal{L}_i^-$, the function h_l satisfies one of the following sector properties for all $\theta \in \mathbb{R}_{\geq 0} \setminus \{x_i^*\}$

$$(\theta - x_i^*)(h_l(\theta) - h_l(x_i^*)) > 0, \tag{5.8}$$

$$(\theta - x_i^*)(h_l(\theta) - h_l(x_i^*)) < 0. \tag{5.9}$$

We define κ_l to be $+1$ when (5.8) holds, and -1 when (5.9) holds. The above conditions (5.8) and (5.9) can be unified as

$$\kappa_l(\theta - x_i^*)(h_l(\theta) - h_l(x_i^*)) > 0. \tag{5.10}$$

A5 For each node i, and for each link $l \in \mathcal{L}_i^-$, there exists a constant $\gamma_l > 0$ such that

$$\kappa_l \frac{h_l(\theta) - h_l(x_i^*)}{\frac{f_i(\theta)}{g_i(\theta)} - \frac{f_i(x_i^*)}{g_i(x_i^*)}} \leq \gamma_l, \quad \forall \theta \in \mathbb{R}_{\geq 0} \setminus \{x_i^*\}. \tag{5.11}$$

The following is a restatement of the main result in [8].

Theorem 5.1 *Suppose that the system* (5.1) *satisfies assumptions* **A1** *to* **A5**, *and the* $M \times M$ *dissipativity matrix*

$$E_{lk} = \begin{cases} -1/\gamma_l & \text{if } k = l \\ \kappa_k & \text{if } s(l) = t(k) \\ 0 & \text{otherwise.} \end{cases}$$

is diagonally stable with the diagonal matrix $Q = diag\{q_1, \ldots, q_M\} > 0$ *such that*

$$E^T Q + QE < 0.$$

Then the equilibrium x^* *is asymptotically stable. If further, for each node* i, *one of the following two conditions holds, then* x^* *is globally asymptotically stable in* $\mathbb{R}_{\geq 0}^N$*:*

1. $\mathcal{L}_i^-$ *is nonempty and there exists at least one link* $l \in \mathcal{L}_i^-$ *such that*

$$\lim_{\theta \to +\infty} \int_{x_i^*}^{\theta} \frac{h_l(\sigma) - h_l(x_i^*)}{g_i(\sigma)} \, d\sigma = \infty. \tag{5.12}$$

2. $\mathcal{L}_i^-$ *is empty and*

$$\lim_{\theta \to +\infty} \int_{x_i^*}^{\theta} \frac{\sigma - x_i^*}{g_i(\sigma)} \, d\sigma = \infty, \tag{5.13}$$

and there exists a class-$\mathcal{K}_\infty$ *function* $\omega(\cdot)$ *such that*

$$(\theta_i - x_i^*) \left(\frac{f_i(\theta)}{g_i(\theta)} - \frac{f_i(x_i^*)}{g_i(x_i^*)} \right) \geq |\theta - x_i^*| \, \omega(|\theta - x_i^*|), \quad \forall \theta \in \mathbb{R}_{\geq 0}. \tag{5.14}$$

Recall that $\omega : \mathbb{R}_{\geq 0} \to \mathbb{R}_{\geq 0}$ is a class-$\mathcal{K}_\infty$ function if ω is continuous, strictly increasing, unbounded, and satisfies $\omega(0) = 0$.

The above theorem is proved by constructing a composite Lyapunov function $V : \mathbb{R}^N_{\geq 0} - x^* \to [0, \infty)$ defined as:

$$V(x - x^*) = \sum_{l=1}^{M} q_l V_l(x_{s(l)} - x^*_{s(l)}), \tag{5.15}$$

where

$$V_l(x_{s(l)} - x^*_{s(l)}) = \kappa_l \int_{x^*_{s(l)}}^{x_{s(l)}} \frac{h_l(\sigma) - h_l(x^*_{s(l)})}{g_i(\sigma)}\, d\sigma. \tag{5.16}$$

Here, the set $\mathbb{R}^N_{\geq 0} - x^*$ consists of all vectors of the form $c - x^*, c \in \mathbb{R}^N_{\geq 0}$. Under assumptions **A1** to **A5**, the following properties of V are shown in [8]:

1. V is continuously differentiable.

2. V is positive definite, meaning that $V(x-x^*) \geq 0$ for all $x \in \mathbb{R}^N_{\geq 0}$, and $V(x-x^*) = 0$ if and only if $x = x^*$.

3. Let $x(t) = (x_1(t), \ldots, x_N(t))$ be a solution to system (5.1). For each $l = 1, \ldots, M$, the derivative of V_l along $x_{s(l)}(t) - x^*_{s(l)}$ with respect to t is

$$\frac{dV_l(x_{s(l)}(t) - x^*_{s(l)})}{dt} \leq y_l(t) \sum_{k=1}^{M} E_{lk} y_k(t),$$

where

$$y_l(t) := \kappa_l\big(h_l(x(t)) - h_l(x^*_{s(l)})\big).$$

Stability of x^* can be derived from the above properties plus other conditions, see [8] as well as below (for the more general PDE case) for details. The work here concerns the persistence of the stability of x^* when diffusion is added to the system. To simplify our discussion, we hereafter assume that the graph, describing interconnections among different subsystems, has no leaves, that is, the set $\mathcal{L}_i^-$ is nonempty for all $i = 1, \ldots, N$.

5.3 Extension to Partial Differential Equation Systems

5.3.1 Notations and assumptions

Throughout this paper, we shall denote by Ω a bounded, open, and connected set in $\mathbb{R}^r$ with smooth boundary, $\partial\Omega$. By smooth, we mean that the boundary $\partial\Omega$ can be locally described as the level set of a function of class C^4 with nonvanishing gradient. We denote by $\bar{\Omega}$ the union of Ω and $\partial\Omega$. Let $t \in [0, +\infty)$ represent time and $\xi = (\xi_1, \ldots, \xi_r) \in \mathbb{R}^r$ represent the spatial coordinate. For each $i = 1, \ldots, N$, the function $\psi_i : \Omega \times [0, T_{\max}) \to \mathbb{R}_{\geq 0}$ describes the state of the ith subsystem at spatial coordinate ξ and time t. Here, $[0, T_{\max})$ is the maximal interval of existence of the solution to the following initial boundary value problem:

$$\frac{\partial \psi_i}{\partial t} = \nabla \cdot (d_i(\xi)\nabla\psi_i) - f_i(\psi_i) + g_i(\partial\psi_i) \sum_{l \in \mathcal{L}_i^+} h_l(\psi_{\text{source}(l)}) \qquad \text{on } \Omega \times (0, +\infty), \tag{5.17}$$

$$\frac{\partial \psi_i}{\partial \nu} = 0 \qquad \text{on } \partial\Omega \times (0, +\infty), \tag{5.18}$$

$$\psi_i(\xi, 0) = \psi_i^0(\xi) \qquad \text{on } \Omega, \tag{5.19}$$

for each $i = 1, \ldots, N$. (More details on existence and uniqueness are given below.) The notation $\frac{\partial}{\partial \nu}$ above represents the derivative with respect to the outward normal on $\partial\Omega$. Let ∇ be the gradient operator, and by convention,

$$\nabla \cdot u = \frac{\partial u}{\partial \xi_1} + \cdots + \frac{\partial u}{\partial \xi_r},$$
$$\nabla \cdot \nabla u = \Delta u = \frac{\partial^2 u}{\partial \xi_1^2} + \cdots + \frac{\partial^2 u}{\partial \xi_r^2}.$$

In what follows, the range of the function $\psi(\xi, t)$ is in $\mathbb{R}^N_{\geq 0}$. We denote by $L^{1,N}(\Omega)$ the set

$$\{(\phi_1, \ldots, \phi_N) \mid \phi_i \in L^1(\Omega), i = 1, \ldots, N\},$$

and denote by $L^{1,N}_{\geq 0}(\Omega)$ the set

$$\{\phi \in L^{1,N}(\Omega) \mid \phi_i(\xi) \geq 0 \text{ for all } \xi \in \Omega, i = 1, \ldots, N\}.$$

The norm $\|\phi\|_{1,\Omega}$ for any $\phi \in L^{1,N}(\Omega)$ is defined as

$$\|\phi\|_{1,\Omega} = \sum_{i=1}^{N} \int_{\Omega} |\phi_i(\xi)|\, d\xi.$$

By abuse of notation, we use $\|\phi_i\|_{1,\Omega}$ for $\int_{\Omega} |\phi_i(\xi)|\, d\xi$.

For the PDE system (5.17)-(5.19), we make the following assumptions.

A6 For each $i = 1, \dots N$, the function d_i is of class $C^3(\bar{\Omega})$, and there exists a positive constant d_0 such that $d_i(\xi) \geq d_0$ for all $\xi \in \bar{\Omega}$.

I1 For each $i = 1, \dots N$, the function ψ_i^0 is of class $C(\bar{\Omega})$, and $\psi_i^0(\xi) \geq 0$ for all $\xi \in \bar{\Omega}$.

We also assume stronger regularities of the reaction part, and hereafter assumption **A1** will be replaced by

A1′ For each $i = 1, \dots, N$ and $l = 1, \dots, M$, the functions f_i, g_i, and h_l are of class C^2 with $f_i(0) = 0$ and $g_i(\theta) > 0, h_l(\theta) \geq 0$ for all $\theta \geq 0$. The functions f_i, g_i, and h_l can be extended as C^2 functions to an open subset of $\mathbb{R}$ which contains $\mathbb{R}_{\geq 0}$.

Remark 5.2 *If we know that the functions f_i, g_i, and h_l can be extended as C^2 functions to an open set containing $\mathbb{R}_{\geq 0}$, then the functions f_i, g_i, and h_l can be extended as C^2 functions on all of $\mathbb{R}$. Let $p : U \to \mathbb{R}$ be a C^2 function, where U is an open interval containing $\mathbb{R}_{\geq 0}$. Let φ be a C^∞ function such that $\varphi = 1$ on $\mathbb{R}_{\geq 0}$, and $\varphi = 0$ outside of U. The function P, defined as*

$$P(\theta) = \begin{cases} \varphi(\theta)p(\theta) & \theta \in U \\ 0 & \theta \notin U, \end{cases}$$

is a C^2 function on all of $\mathbb{R}$, and it satisfies $P = p$ on $\mathbb{R}_{\geq 0}$.

This extension from $\mathbb{R}_{\geq 0}$ to $\mathbb{R}$ is used when we apply Lemma 5.3, see assumption **R3** *introduced later.*

In order to lift the Lyapunov function from the ODE system to the PDE system, we need an additional assumption which insures convexity of the Lyapunov function V for the ODE system (5.1).

A7 For each note i, and for each link $l \in \mathcal{L}_i^-$,

$$\kappa_l \left(\frac{h_l(\theta) - h_l(x_i^*)}{g_i(\theta)} \right)' \geq 0, \quad \forall \theta \in \mathbb{R}_{\geq 0} \setminus \{x_i^*\}. \tag{5.20}$$

Convex Lyapunov functions have been used in studies of boundedness, global existence, and stability of reaction diffusion systems, see, for instance, [61] and [71].

5.3.2 Existence and uniqueness

Next, we use results from Chapter 7 of [82] to derive the existence and uniqueness of classical solutions of system (5.17)-(5.19), as well as the positive invariance property of $\mathbb{R}^N_{\geq 0}$ in the state space.

Consider the general reaction diffusion system (5.4)-(5.6) with the following assumptions.

R1 For each $i, j, k = 1, \ldots, N$, $a^i_{jk} = a^i_{kj}$; a^i_{jk} and a^i_k is of class $C^2(\bar{\Omega})$.

R2 The differential operator A_i is *uniformly elliptic* in Ω in the sense that there exists a constant $\alpha > 0$ such that for all $v \in \mathbb{R}^N$,

$$\sum_{j,k=1}^{N} a^i_{jk}(\xi) v_j v_k \geq \alpha \|v\|^2, \quad \xi \in \Omega.$$

R3 For each $i = 1, \ldots, N$, the function $F_i : \mathbb{R} \to \mathbb{R}$ is of class C^2, and satisfies

$$F_i(u) \geq 0 \text{ whenever } u \in \mathbb{R}^N_{\geq 0} \text{ and } u_i = 0.$$

R4 For each $i = 1, \ldots N$, the function u_i^0 is of class $C(\bar{\Omega})$, and $u_i^0(\xi) \geq 0$ for all $\xi \in \bar{\Omega}$.

Theorem 3.1 and Corollary 3.2 in Chapter 7 of [82] are developed by expressing system (5.4)-(5.5) as an abstract ordinary differential equation in an appropriate Banach space. We next quote the existence and uniqueness results.

Recall that a solution $u(x,t)$, where $(x,t) \in \Omega \times [0, T_{\max})$, is a called a *classical solution* of (5.4)-(5.5), if $u(x,t)$ satisfies (5.4)-(5.5), and

$$u(x,t) \in C^2(\Omega \times [0, T_{\max})) \bigcap C^1(\bar{\Omega} \times [0, T_{\max})).$$

Lemma 5.3 *(Theorem 3.1 and Corollary 3.2 in Chapter 7 of [82]). Let the assumptions* **R1** *to* **R3** *hold for the system* (5.4)-(5.5). *Then, for each initial condition* u^0 *satisfying* **R4**, *the system* (5.4)-(5.6) *has a unique noncontinuable classical solution* $u(\xi,t) \in \mathbb{R}^N_{\geq 0}$, *defined on* $[0, T_{max})$ *where* $T_{max} = T_{max}(u^0) \leq +\infty$. *If* $T_{max} < +\infty$, *then* $u(\xi,t)$ *is unbounded as* $t \to T_{max}$.

We emphasize that Lemma 5.3 implies that $\mathbb{R}^N_{\geq 0}$ is positively invariant for system (5.4)-(5.5). Hence after, we will focus on solutions in $\mathbb{R}^N_{\geq 0}$.

For our system (5.17)-(5.18), it is easy to see that assumption **A6** implies **R1** and **R2**; assumptions **A1′** and **I1** imply **R3** and **R4**, respectively. Applying Lemma 5.3, we have the following theorem.

Theorem 5.4 *Under assumptions* **A1′** *to* **A7**, *for every initial condition* ψ^0 *satisfying assumption* **I1**, *system* (5.17)-(5.19) *has a unique noncontinuable classical solution* $\psi \in \mathbb{R}^N_{\geq 0}$, *defined on* $[0, T_{max})$. *If* $T_{max} < +\infty$, *then* ψ *is unbounded as* $t \to T_{max}$.

To simplify our discussion, we hereafter assume that solutions obtained in Theorem 5.4 are all bounded, and thus exist for all $t \in [0, +\infty)$. Separate analysis is usually required to prove the boundedness of solutions using properties of the Lyapunov function and a priori estimates. In the special case when all the elliptic operators are the same, one may invoke the result in [71] to conclude boundedness. In this Chapter, we assume boundedness of solutions and focus on stability analysis.

5.3.3 Stability of the homogeneous equilibrium

The equilibrium x^* of the ODE system (5.1) corresponds to the homogeneous equilibrium

$$\psi^*(\xi) \equiv \ x^*, \forall \xi \in \Omega$$

of the PDE system (5.17)-(5.18). Before we state our main result, we shall clarify some terms that will be used later.

Definition 5.5 *The equilibrium* ψ^* *of system* (5.17)-(5.18) *is said to be*

- *stable in the L^1 sense if for any $\varepsilon > 0$, there exists $\delta(\varepsilon) > 0$, such that for any initial condition ψ^0 satisfying* **I1** *and $\|\psi^0 - \psi^*\|_{1,\Omega} < \delta(\varepsilon)$, the solution $\psi(x,t)$ of* (5.17)-(5.18) *stays within ε distance from ψ^*, that is, $\|\psi(\cdot,t) - \psi^*\|_{1,\Omega} < \varepsilon$ for all $t \geq 0$;*

- *asymptotically stable in the L^1 sense if it is stable and there exists some $\delta_0 > 0$ such that for any initial condition ψ^0 satisfying* **I1** *and $\|\psi^0 - \psi^*\|_{1,\Omega} < \delta_0$, we have $\lim_{t\to+\infty} \|\psi(\cdot,t) - \psi^*\|_{1,\Omega} = 0$;*

- *globally asymptotically stable in the L^1 sense if it is stable and for any initial condition ψ^0 satisfying* **I1**, *we have*

$$\lim_{t\to+\infty} \|\psi(\cdot,t) - \psi^*\|_{1,\Omega} = 0.$$

Here, we choose to work in L^1 because it is convenient to apply Jensen's inequality, see the proof in Lemma 5.10. Notice that once we have stability in L^1, we can obtain stability in L^p for all $p \in (1, +\infty)$ using Hölder's inequality and the assumption that all solutions are bounded.

Theorem 5.6 *Suppose that system* (5.17)-(5.19) *satisfies assumptions* **A1′** *through* **A7**, *and the dissipativity matrix E is diagonally stable with the diagonal matrix $Q = diag\{q_1, \ldots, q_M\} > 0$ such that*

$$E^T Q + QE < 0.$$

Then ψ^* is globally asymptotically stable in the $L^1(\Omega)$ sense.

We denoted by $L^{1,N}_{\geq 0}(\Omega) - \psi^*$ the set of all functions of the form $\phi - \psi^*, \phi \in L^{1,N}_{\geq 0}$. Define a Lyapunov function candidate $W : L^{1,N}_{\geq 0}(\Omega) - \psi^* \to \mathbb{R}_{\geq 0}$:

$$W(\phi - \psi^*) = \sum_{l=1}^{M} q_l W_l(\phi_{s(l)} - \psi^*_{s(l)}), \tag{5.21}$$

where $W_l : L^1_{\geq 0}(\Omega) - \psi^*_{s(l)} \to \mathbb{R}_{\geq 0}$ is given by:

$$W_l(\phi_{s(l)} - \psi^*_{s(l)}) = \kappa_l \int_\Omega \int_{\psi^*_{s(l)}(\xi)}^{\phi_{s(l)}(\xi)} \frac{h_l(\sigma) - h_l(x^*_{s(l)})}{g_{s(l)}(\sigma)}\, d\sigma d\xi = \int_\Omega V\big(\phi_{s(l)}(\xi) - \psi^*_{s(l)}(\xi)\big)\, d\xi. \tag{5.22}$$

We next explore some properties of W in the following lemmas.

Lemma 5.7 *Under assumption* **A4**, *the function* W *is positive definite, that is, for any* $\phi \in L^{1,N}_{\geq 0}$,

$$W(\phi - \psi^*) \geq 0,$$

and

$$W(\phi - \psi^*) = 0 \text{ if and only if } \|\phi - \psi^*\|_{1,\Omega} = 0.$$

Proof. Pick any $\phi \in L^{1,N}_{\geq 0}(\Omega)$. The inequality (5.10) in assumption **A4** implies that $W_l(\phi_{s(l)} - \psi^*_{s(l)}) \geq 0$ for all $l = 1, \ldots, M$. As a result $W(\phi - \psi^*) \geq 0$. Furthermore, for each l, $W_l(\phi_{s(l)} - \psi^*_{s(l)}) = 0$ only when $\phi_{s(l)}$ differs from $\psi^*_{s(l)}$ on a measure zero subset of Ω, that is, $\|\phi_{s(l)} - \psi^*_{s(l)}\|_{1,\Omega} = 0$. Since $\mathcal{L}_i^-$ is not empty for each node i, it follows that $W(\phi - \psi^*) = 0$ if and only if $\|\phi - \psi^*\|_{1,\Omega} = 0$. ∎

Lemma 5.8 *Under the assumptions in Theorem 5.6,* W *is continuous at* ψ^* *in the following sense. Let* $\{\phi^n\}, n = 1, 2, \ldots,$ *be a sequence of* $L^{1,N}_{\geq 0}(\Omega)$ *functions. If*

$$\lim_{n \to +\infty} \|\phi^n - \psi^*\|_{1,\Omega} = 0,$$

then

$$\lim_{n \to +\infty} W(\phi^n - \psi^*) = 0.$$

In order to prove Lemma 5.8, we use the following result.

Lemma 5.9 *Let* $\{q_n(\cdot)\}, n = 1, 2, \ldots,$ *be a family of* $L^1_{\geq 0}(\Omega)$ *functions. Let* $G(\cdot)$ *be a continuous function from* $\mathbb{R}_{\geq 0}$ *to* $\mathbb{R}_{\geq 0}$ *with* $G(s) = 0$ *if and only if* $s = s_0$. *Moreover, we assume that* $G(s) \to +\infty$ *as* $s \to +\infty$. *Then*

$$\lim_{n \to +\infty} \int_\Omega |q_n(\xi) - s_0| \, d\xi = 0$$

implies

$$\lim_{n \to +\infty} \int_\Omega G(q_n(\xi)) \, d\xi = 0.$$

Proof. Let $\mu(\cdot)$ denote the Lebesgue measure. Since $\mu(\Omega)$ is finite, Lebesgue's dominated convergence theorem also holds if almost everywhere convergence is replaced by convergence in measure. As a result, it is enough to show that for any $c > 0$,

$$\lim_{n \to \infty} \mu\big(\{\xi \in \Omega \mid G(q_n(\xi)) > c\}\big) = 0.$$

Define

$$\alpha_c := \inf\{|s - s_0| \mid G(s) \geq c\}.$$

Since $G(s) \to +\infty$ as $s \to +\infty$, we know that α_c is well-defined for each $c > 0$. Moreover, because G is continuous and $G(s) = 0$ only when $s = s_0$, we have $\alpha_c > 0$. The set

$$\{\xi \in \Omega \mid G(q_n(\xi)) > c\} \subset \left\{\xi \in \Omega \mid |q_n(\xi) - s_0| \geq \frac{\alpha_c}{2}\right\},$$

and thus,

$$\mu\big(\{\xi \in \Omega \mid G(q_n(\xi)) > c\}\big) \leq \mu\left(\left\{\xi \in \Omega \mid |q_n(\xi) - s_0| \geq \frac{\alpha_c}{2}\right\}\right). \tag{5.23}$$

On the other hand,

$$\int_\Omega |q_n(\xi) - s_0|\, d\xi \;\geq\; \mu\left(\left\{\xi \in \Omega \mid |q_n(\xi) - s_0| \geq \frac{\alpha_c}{2}\right\}\right)\frac{\alpha_c}{2}.$$

If $\lim_{n\to+\infty} \int_\Omega |q_n(\xi) - s_0|\, d\xi = 0$, then $\lim_{n\to+\infty} \mu\big(\{\xi \in \Omega \mid |q_n(\xi) - s_0| \geq \alpha_c/2\}\big) = 0$. Combining with (5.23), we have

$$\lim_{n\to+\infty} \mu\big(\{\xi \in \Omega \mid G(q_n(\xi)) > c\}\big) = 0,$$

which completes the proof. ∎

Proof. (Lemma 5.8). It is enough to show that for each l,

$$\lim_{n\to+\infty} \|\phi^n_{s(l)} - \psi^*_{s(l)})\|_{1,\Omega} = 0 \tag{5.24}$$

implies

$$\lim_{n\to+\infty} W_l(\phi^n_{s(l)} - \psi^*_{s(l)}) = 0.$$

For each $l = 1, \ldots, M$, let

$$q_n = \phi^n_{s(l)}, \quad s_0 = x^*_{s(l)}, \quad G(s) = \kappa_l \int_{s_0}^{s} \frac{h_l(\sigma) - h_l(x^*_{s(l)})}{g_{s(l)}(\sigma)}\, d\sigma.$$

By the definition of G, we have $G(s) = 0$ only when $s = s_0$. Using the inequality (5.10) in assumption **A4**, we know that $G(s) \geq 0$ on $\mathbb{R}_{\geq 0}$ and $G(s)$ is a strictly increasing function for $s \geq s_0$. Moreover, G is a convex function for $s \geq s_0$ because of assumption **A7**. Therefore $G(s) \to +\infty$ as $s \to +\infty$. Equation (5.24) says that

$$\lim_{n\to+\infty} \int_\Omega |q_n(\xi) - s_0|\, d\xi = 0.$$

Applying Lemma 5.9, we have

$$\lim_{n\to+\infty} W_l(\phi^n_{s(l)} - \psi^*_{s(l)}) = \lim_{n\to+\infty} \int_\Omega G(q_n(\xi))\, d\xi = 0.$$

∎

The next lemma is a generalization of Appendix A in [46] to the general graph case.

Lemma 5.10 *Under the assumptions in Theorem 5.6, there exists a class $\mathcal{K}_\infty$ function $\alpha(\cdot)$ such that*

$$W(\phi - \psi^*) \geq \alpha(\|\phi - \psi^*\|_{1,\Omega}) \text{ for any } \phi \in L^{1,N}_{\geq 0}(\Omega). \tag{5.25}$$

Proof. For each $l = 1, \ldots, N$, we define two functions $p^+_{s(l)} : \mathbb{R}_{\geq 0} \to \mathbb{R}_{\geq 0}$ and $p^-_{s(l)} : [0, x^*_{s(l)}] \to \mathbb{R}_{\geq 0}$:

$$p^+_{s(l)}(s) = \kappa_l \int_0^s \frac{h_l(\sigma' + x^*_{s(l)}) - h_l(x^*_{s(l)})}{g_{s(l)}(\sigma' + x^*_{s(l)})}\, d\sigma',$$
$$p^-_{s(l)}(s) = \kappa_l \int_0^{-s} \frac{h_l(\sigma' + x^*_{s(l)}) - h_l(x^*_{s(l)})}{g_{s(l)}(\sigma' + x^*_{s(l)})}\, d\sigma'.$$

Because of inequality (5.10) in assumption **A4**, the first derivatives of $p^\pm_{s(l)}$ are positive,

$$(p^+_{s(l)})'(s) = \kappa_l \frac{h_l(s + x^*_{s(l)}) - h_l(x^*_{s(l)})}{g_{s(l)}(s + x^*_{s(l)})} > 0,$$
$$(p^-_{s(l)})'(s) = -\kappa_l \frac{h_l(-s + x^*_{s(l)}) - h_l(x^*_{s(l)})}{g_{s(l)}(-s + x^*_{s(l)})} > 0.$$

The second derivatives are nonnegative under assumption **A7**,

$$(p^+_{s(l)})''(s) = \kappa_l \left(\frac{h_l(s + x^*_{s(l)}) - h_l(x^*_{s(l)})}{g_{s(l)}(s + x^*_{s(l)})} \right)' \geq 0,$$
$$(p^-_{s(l)})''(s) = \kappa_l \left(\frac{h_l(-s + x^*_{s(l)}) - h_l(x^*_{s(l)})}{g_{s(l)}(-s + x^*_{s(l)})} \right)' \geq 0.$$

Therefore, the function $p^+_{s(l)}$ is of class $\mathcal{K}_\infty$ and convex; the function $p^-_{s(l)}$ is convex, continuous, strictly increasing, and $p^-_{s(l)}(0) = 0$. For any $\phi \in L^{1,N}_{\geq 0}(\Omega)$, using Jensen's

inequality, we have

$$W_l(\phi_{s(l)} - \psi^*_{s(l)}) = \int_{\Omega^+_{s(l)}} p^+_{s(l)}(|\phi_{s(l)}(\xi) - \psi^*_{s(l)}(\xi)|)\, d\xi \tag{5.26}$$

$$+ \int_{\Omega^-_{s(l)}} p^-_{s(l)}(|\phi_{s(l)}(\xi) - \psi^*_{s(l)}(\xi)|)\, d\xi,$$

$$\geq \mu(\Omega^+_{s(l)})\; p^+_{s(l)} \left(\frac{\|\phi_{s(l)} - \psi^*_{s(l)}\|_{1,\Omega^+_{s(l)}}}{\mu(\Omega^+_{s(l)})} \right) \tag{5.27}$$

$$+ \mu(\Omega^-_{s(l)})\; p^-_{s(l)} \left(\frac{\|\phi_{s(l)} - \psi^*_{s(l)}\|_{1,\Omega^-_{s(l)}}}{\mu(\Omega^-_{s(l)})} \right), \tag{5.28}$$

and

$$\Omega^+_{s(l)} := \{\xi \in \Omega \mid \phi_{s(l)}(\xi) - \psi^*_{s(l)}(\xi) \geq 0\}$$

$$\Omega^-_{s(l)} := \{\xi \in \Omega \mid \phi_{s(l)}(\xi) - \psi^*_{s(l)}(\xi) < 0\}.$$

We claim that, for each i, there exists a convex $\mathcal{K}_\infty$ function $p_{s(l)} : \mathbb{R}_{\geq 0} \to \mathbb{R}_{\geq 0}$ satisfying

$$p_{s(l)}(s) \leq \begin{cases} \min\{p^+_{s(l)}(s), p^-_{s(l)}(s)\} & \text{if } 0 \leq s \leq x^*_{s(l)}; \\ p^+_{s(l)}(s) & \text{if } s > x^*_{s(l)}. \end{cases} \tag{5.29}$$

The existence of the function $p_{s(l)}$ will be deferred to the end of the proof. Applying Jensen's inequality to (5.26) again, we have

$$\begin{aligned} W_l(\phi_{s(l)} - \psi^*_{s(l)}) &\geq \mu(\Omega^+_{s(l)})\; p_{s(l)} \left(\frac{\|\phi_{s(l)} - \psi^*_{s(l)}\|_{1,\Omega^+_{s(l)}}}{\mu(\Omega^+_{s(l)})} \right) \\ &+ \mu(\Omega^-_{s(l)})\; p_{s(l)} \left(\frac{\|\phi_{s(l)} - \psi^*_{s(l)}\|_{1,\Omega^-_{s(l)}}}{\mu(\Omega^-_{s(l)})} \right) \\ &\geq \mu(\Omega)\; p_{s(l)} \left(\frac{\|\phi_{s(l)} - \psi^*_{s(l)}\|_{1,\Omega^+_{s(l)}} + \|\phi_{s(l)} - \psi^*_{s(l)}\|_{1,\Omega^-_{s(l)}}}{\mu(\Omega)} \right) \\ &= \mu(\Omega)\; p_{s(l)} \left(\frac{\|\phi_{s(l)} - \psi^*_{s(l)}\|_{1,\Omega}}{\mu(\Omega)} \right). \end{aligned}$$

We define two functions $\beta_0 : \mathbb{R}^N_{\geq 0} \to \mathbb{R}_{\geq 0}$ and $\alpha_0 : \mathbb{R}_{\geq 0} \to \mathbb{R}_{\geq 0}$:

$$\beta_0(v) = \mu(\Omega) \sum_{l=1}^{M} q_l\, p_{s(l)} \left(\frac{v_{s(l)}}{\mu(\Omega)} \right),$$

$$\alpha_0(r) = \min_{\sum_1^N v_i = r} \beta_0(v).$$

The function α_0 is well-defined because the set $\{v \in \mathbb{R}^N_{\geq 0} \mid \sum_1^N v_i = r\}$ is compact for each $r \geq 0$. For any $\phi \in L^{1,N}_{\geq 0}(\Omega)$, let $v_i = \|\phi_i - \psi_i^*\|_{1,\Omega}, i = 1, \ldots, N$, then

$$W(\phi - \psi^*) \geq \beta_0(v) \geq \alpha_0(\sum_1^N v_i) = \alpha_0(\|\phi - \psi^*\|_{1,\Omega}). \tag{5.30}$$

Next, we will show the existence of a $\mathcal{K}_\infty$ function $\alpha(\cdot)$ such that (5.25) holds. First, it is easy to see that $\alpha_0(0) = 0$ since $p_i(0) = 0$ for each $i = 1, \ldots, N$. Moreover, the function α_0 is radially unbounded because the functions p_i's are. Now we have a radially unbounded function α_0 with $\alpha_0(0) = 0$. It is a standard exercise to show that there exists a $\mathcal{K}_\infty$ function α such that $\alpha_0(r) \geq \alpha(r)$ for all $r \geq 0$. Combining with (5.30), our conclusion follows.

Next, we show the existence of the function p_i for each $i = 1, \ldots, N$. First, we denote by P_i^- the convex and $\mathcal{K}_\infty$ function satisfying $p_i^- = P_i^-$ on $[0, x_i^*]$. Then, we define

$$p_i(s) := \int_0^s \int_0^{r_1} \min\{(p_i^+(s))'', (P_i^-(s))''\}\, dr_2\, dr_1.$$

It is straightforward to check that p_i is a convex and $\mathcal{K}_\infty$ function. Finally, p_i satisfies (5.29) because

$$p_i^+(s) = (p_i^+)'(0) + \int_0^s \int_0^{r_1} (p_i^+(s))''\, dr_2\, dr_1 \geq p_i(s),$$

$$P_i^-(s) = (P_i^-)'(0) + \int_0^s \int_0^{r_1} (P_i^-(s))''\, dr_2\, dr_1 \geq p_i(s).$$

∎

Lemma 5.11 *Under the assumptions in Theorem 5.6, for any solution $\psi(\xi, t)$ of system* (5.17)-(5.18)*, the derivative of W along $\psi(\cdot, t) - \psi^*$ with respect to t is nonnegative, and the derivative equals zero only for those t's such that $\|\psi(\cdot, t) - \psi^*\|_{1,\Omega} = 0$.*

Proof. In this proof, for the convenience of notations, we may drop the dependence on ξ and t when there is no confusion. For each $l = 1, \ldots, M$, by the definition of W_l, we have

$$\frac{dW_l\big(\psi_{s(l)}(\cdot, t) - \psi^*_{s(l)}\big)}{dt} = \frac{d}{dt} \int_\Omega V\big(\psi_{s(l)}(\xi, t) - \psi^*_{s(l)}(\xi)\big)\, d\xi.$$

Since ψ is a classical solution, V is C^2, and Ω is compact, using a similar argument as in Leibniz Integral Rule, we can exchange the order differentiation and integration to get

$$\begin{aligned}\frac{dW_l\big(\psi_{s(l)}(\cdot,t)-\psi^*_{s(l)}\big)}{dt} &= \int_\Omega \left.\frac{dV_l(x_{s(l)}-x^*_{s(l)})}{dx_{s(l)}}\right|_{x_{s(l)}=\psi_{s(l)}(\xi,t)} \frac{\partial\psi_{s(l)}(\xi,t)}{\partial t}\,d\xi \qquad (5.31)\\ &= \kappa_l\int_\Omega\left(\frac{h_l(\psi_{s(l)}(\xi,t))-h_l(\psi^*_{s(l)}(\xi))}{g_{s(l)}(\psi_{s(l)}(\xi,t))}\right)\frac{\partial\psi_{s(l)}}{\partial t}(\xi,t)\,d\xi\\ &= \kappa_l\int_\Omega\left(\frac{h_l(\psi_{s(l)})-h_l(\psi^*_{s(l)})}{g_{s(l)}(\psi_{s(l)})}\right)\nabla\cdot(d_{s(l)}\nabla\psi_{s(l)})\,d\xi\\ &+\kappa_l\int_\Omega\Big(h_l(\psi_{s(l)})-h_l(\psi^*_{s(l)})\Big)\left(-\frac{f_{s(l)}(\psi_{s(l)})}{g_{s(l)}(\psi_{s(l)})}+u_{s(l)}\right)d\xi,\end{aligned}$$

where

$$u_{s(l)}(\xi,t) := \sum_{k\in\mathcal{L}^+_{s(l)}} h_k(\psi_{s(k)}(\xi,t)). \qquad (5.32)$$

Using integration by parts and the Neumann boundary condition on $\psi_{s(l)}$, we have

$$\begin{aligned}\frac{dW_l(\psi_{s(l)}(\cdot,t)-\psi^*_{s(l)})}{dt} &= -\kappa_l\int_\Omega\left(\frac{h_l(\psi_{s(l)})-h_l(\psi^*_{s(l)})}{g_{s(l)}(\psi_{s(l)})}\right)'\nabla\psi^T_{s(l)}\,d_{s(l)}\nabla\psi_{s(l)}\,d\xi\\ &+\kappa_l\int_\Omega\Big(h_l(\psi_{s(l)})-h_l(\psi^*_{s(l)})\Big)\left(-\frac{f_{s(l)}(\psi_{s(l)})}{g_{s(l)}(\psi_{s(l)})}+u_{s(l)}\right)d\xi\\ &\le \kappa_l\int_\Omega\Big(h_l(\psi_{s(l)})-h_l(\psi^*_{s(l)})\Big)\left(-\frac{f_{s(l)}(\psi_{s(l)})}{g_{s(l)}(\psi_{s(l)})}+u_{s(l)}\right)d\xi.\end{aligned}$$

The last inequality is obtained using (5.20). By (5.32),

$$u^*_{s(l)} = \sum_{k\in\mathcal{L}^+_{s(l)}} h_k(\psi^*_{s(k)}) = \frac{f_{s(l)}(\psi^*_{s(l)})}{g_{s(l)}(\psi^*_{s(l)})},$$

and thus,

$$\begin{aligned}\frac{dW_l(\psi_{s(l)}(\cdot,t)-\psi^*_{s(l)})}{dt} \le{}&\kappa_l\int_\Omega\Big(h_l(\psi_{s(l)})-h_l(\psi^*_{s(l)})\Big)\times\\ &\left(-\frac{f_{s(l)}(\psi_{s(l)})}{g_{s(l)}(\psi_{s(l)})}+\frac{f_{s(l)}(\psi^*_{s(l)})}{g_{s(l)}(\psi^*_{s(l)})}+u_{s(l)}-u^*_{s(l)}\right)d\xi.\end{aligned}$$

Next, using (5.11) and the fact that $\kappa_l(h_l(\psi_{s(l)})-h_l(\psi^*_{s(l)}))$ and $-\frac{f_{s(l)}(\psi_{s(l)})}{g_{s(l)}(\psi_{s(l)})}+\frac{f_{s(l)}(\psi^*_{s(l)})}{g_{s(l)}(\psi^*_{s(l)})}$ have opposite signs due to (5.7) and (5.10), we obtain

$$\kappa_l\Big(h_l(\psi_{s(l)})-h_l(\psi^*_{s(l)})\Big)\left(-\frac{f_{s(l)}(\psi_{s(l)})}{g_{s(l)}(\psi_{s(l)})}+\frac{f_{s(l)}(\psi^*_{s(l)})}{g_{s(l)}(\psi^*_{s(l)})}\right)\le-\frac{1}{\gamma_l}\Big(h_l(\psi_{s(l)})-h_l(\psi^*_{s(l)})\Big)^2.$$

Defining

$$y_l(\xi,t) := \kappa_l\big(h_l(\psi_{s(l)}(\xi,t)) - h_l(\psi^*_{s(l)}(\xi,t))\big), \tag{5.33}$$

we have

$$u_{s(l)} - u^*_{s(l)} = \sum_{k\in\mathcal{L}^+_{s(l)}} \big(h_k(\psi_{s(k)}) - h_k(\psi^*_{s(k)})\big) = \sum_{k\in\mathcal{L}^+_{s(l)}} \kappa_k y_k.$$

It follows that,

$$\begin{aligned}
\frac{dW_l(\psi_{s(l)}(\cdot,t) - \psi^*_{s(l)})}{dt} &\le -\frac{1}{\gamma_l}\int_\Omega y_l^2(\xi,t)\,d\xi + \int_\Omega y_l(\xi,t)\left(u_{s(l)}(\xi,t) - u^*_{s(l)}\right)\,d\xi \\
&= -\frac{1}{\gamma_l}\int_\Omega y_l^2(\xi,t)\,d\xi + \int_\Omega y_l(\xi,t)\sum_{k\in\mathcal{L}^+_{s(l)}} \kappa_k y_k(\xi,t)\,d\xi \\
&= \int_\Omega y_l(\xi,t)\sum_{k=1}^{M} E_{lk} y_k(\xi,t)\,d\xi.
\end{aligned}$$

The last equality holds because of the definition of the dissipativity matrix E.

For the derivative of the composite Lyapunov function, we have

$$\begin{aligned}
\frac{dW(\psi(\cdot,t) - \psi^*)}{dt} &\le \sum_{l=1}^{M} q_l \int_\Omega y_l(\xi,t)\sum_{k=1}^{M} E_{lk} y_k(\xi,t) \\
&= \frac{1}{2}\int_\Omega y^T(\xi,t)(E^TQ + QE)y(\xi,t)\;d\xi \\
&\le -\frac{\lambda}{2}\int_\Omega y^T(\xi,t)y(\xi,t)\;d\xi \le 0,
\end{aligned} \tag{5.34}$$

where $-\lambda < 0$ is the largest eigenvalue of the matrix $E^TQ + QE$. The equality in (5.34) holds for those t's such that $\int_\Omega y_l^2(\xi,t)\,d\xi = 0$ for all $l = 1,\ldots,M$. By the definition of y_l (equation (5.33)) and the inequality (5.10) in assumption A4, $\int_\Omega y_l^2(\xi,t)\,d\xi = 0$ if and only if $\|\psi_{s(l)}(\cdot,t) - \psi^*_{s(l)}\|_{1,\Omega} = 0$. As a result, the equality holds in (5.34) for those t's such that $\|\psi(\cdot,t) - \psi^*\|_{1,\Omega} = 0$. ∎

Lemma 5.12 *Let $\{p_n(\cdot)\}, n = 1,2,\ldots,$ be a family of L^1 functions from Ω to $\mathbb{R}_{\ge 0}$. Assume that for every $n = 1,2,\ldots,$ the function $p_n(\cdot)$ satisfies $p_n(\xi) \le K$ for all $\xi \in \Omega$, where K is a positive constant. Let $H(\cdot)$ be a continuous function from $\mathbb{R}_{\ge 0}$ to $\mathbb{R}_{\ge 0}$ with $H(r) = 0$ if and only if $r = r_0$. Then*

$$\lim_{n\to+\infty}\int_\Omega H(p_n(\xi))\,d\xi = 0$$

implies

$$\lim_{n\to+\infty}\int_\Omega |p_n(\xi)-r_0|\,d\xi = 0.$$

Proof. Since $\mu(\Omega)$ is finite, Lebesgue's dominated convergence theorem also holds if almost everywhere convergence is replaced by convergence in measure. As a result, it is enough to show that p_n converges to r_0 in measure, that is, for any $c>0$, $\lim_{n\to\infty}\mu\big(\{\xi\in\Omega \mid |p_n(\xi)-r_0|>c\}\big)=0$. We assume without loss of generality that $K>2r_0$. Pick any $c>0$. If $c>K-r_0$, then $\mu\big(\{\xi\in\Omega \mid |p_n(\xi)-r_0|>c\}\big)=0$, and we are done. For $c\le K-r_0$, we define

$$\gamma_c := \min\{H(r) \mid |r-r_0|\in[c,K-r_0]\}.$$

Since $H(r)=0$ only when $r=r_0$, γ_c has to be positive. Moreover,

$$\{\xi\in\Omega \mid |p_n(\xi)-r_0|>c\}\subset\left\{\xi\in\Omega \mid H(p_n(\xi))\ge\frac{\gamma_c}{2}\right\},$$

and thus,

$$\mu\big(\{\xi\in\Omega \mid |p_n(\xi)-r_0|>c\}\big)\le\mu\left(\left\{\xi\in\Omega \mid H(p_n(\xi))\ge\frac{\gamma_c}{2}\right\}\right). \tag{5.35}$$

On the other hand,

$$\int_\Omega H(p_n(\xi))\,d\xi \;\ge\; \mu\left(\left\{\xi\in\Omega \mid H(p_n(\xi))\ge\frac{\gamma_c}{2}\right\}\right)\frac{\gamma_c}{2}.$$

If $\lim_{n\to+\infty}\int_\Omega H(p_n(\xi))\,d\xi=0$, then $\lim_{n\to+\infty}\mu\big(\{\xi\in\Omega \mid H(p_n(\xi))\ge\gamma_c/2\}\big)=0$. Combining with (5.35), we have

$$\lim_{n\to+\infty}\mu\big(\{\xi\in\Omega \mid |p_n(\xi)-r_0|>c\}\big)=0.$$

∎

Now we are ready to prove our main result, Theorem 5.6.

Proof. [Theorem 5.6] We first show that ψ^* is stable. By Lemma 5.8, for any given $\varepsilon>0$, there exists $\delta>0$ such that for any ψ^0 satisfying **I1**, $\|\psi^0-\psi^*\|_{1,\Omega}<\delta$ implies $W(\psi^0-\psi^*)<\varepsilon$. Let $\psi(\xi,t)$ be the solution of (5.17)-(5.19) with initial condition ψ^0. By Lemma 5.11, we have

$$W(\psi(\cdot,t)-\psi^*)\le W(\psi(\cdot,0)-\psi^*)\le W(\psi^0-\psi^*)<\varepsilon \text{ for all } t\in(0,\infty).$$

On the other hand, Lemma 5.10 says

$$W(\psi(\cdot,t)-\psi^*) \geq \alpha(\|\psi(\cdot,t)-\psi^*\|_{1,\Omega}).$$

As a result, $\|\psi(\cdot,t)-\psi^*\|_{1,\Omega} < \alpha^{-1}(\varepsilon)$ for all $t \in (0,\infty)$, which gives the stability of ψ^*.

Next, we show that for any ψ^0 satisfying **I1**, the solution $\psi(\xi,t)$ of (5.17)-(5.19) satisfies $\lim_{t\to+\infty}\|\psi(\cdot,t)-\psi^*\|_{1,\Omega}=0$. By Lemma 5.11, $W(\psi(\cdot,t)-\psi^*)$ is nonincreasing in time, and it is bounded from below by zero, thus it has a limit $c \geq 0$ as $t \to +\infty$. If $c=0$, using Lemma 5.10 we obtain the asymptotic stability of ψ^*. If $c>0$, that is, $\lim_{t\to+\infty} W(\psi(\cdot,t)-\psi^*) = c > 0$, then by the continuity and positive definiteness property of W, there exists a $d>0$ such that $\|\psi(\cdot,t)-\psi^*)\|_{1,\Omega} > d$ for all large t.

Next, we claim that this implies that there exists a constant $m>0$ such that

$$\sup_{t>0}\int_\Omega y^T(\xi,t)y(\xi,t)\,d\xi > m.$$

We will prove this claim by contradiction. Suppose that there is a sequence $\{t_n\}, n = 1,2,\ldots,$ such that

$$\lim_{n\to+\infty}\int_\Omega y_l^2(\xi,t_n)\,d\xi = 0$$

for all $l=1,\ldots,M$. By the definition of y_l in (5.33),

$$\lim_{n\to+\infty}\int_\Omega \big(h_l(\psi_{s(l)}(\xi,t_n)) - h_l(\psi^*_{s(l)}(\xi))\big)^2\,d\xi = 0. \tag{5.36}$$

Letting $p_n(\xi)=\psi_{s(l)}(\xi,t_n), H(r)=\big(h_l(r)-h_l(x^*_{s(l)})\big)^2$, and $r_0 = x^*_{s(l)}$, equation (5.36) says that

$$\lim_{n\to+\infty}\int_\Omega H\big(p_n(\xi)\big)\,d\xi = 0.$$

Therefore, applying Lemma 5.12, we have that

$$\lim_{n\to+\infty}\int_\Omega |\psi_{s(l)}(\xi,t_n)-\psi^*_{s(l)}(\xi)|\,d\xi = 0 \quad \text{for all } l=1,\ldots,M.$$

Notice that in the above argument we used the assumption that $\psi(\xi,t)$ is bounded. Since $\mathcal{L}_i^-$ is not empty for each node i, we thus have $\lim_{n\to+\infty}\|\psi(\cdot,t_n)-\psi^*\|_{1,\Omega}=0$, which contradicts $\|\psi(\cdot,t)-\psi^*)\|_{1,\Omega} > d > 0$ for all large t. This proves the claim.

Pick an $m>0$ so that $\sup_{t>0}\int_\Omega y^T(\xi,t)y(\xi,t)\,d\xi > m > 0$. Inequality (5.34) implies

$$\frac{dW(\psi(\xi,t)-\psi^*)}{dt} \leq -\frac{\lambda m}{2}.$$

It then follows from

$$W(\psi(\cdot,t)-\psi^*) \leq W(\psi(\cdot,0)-\psi^*) - \frac{\lambda m}{2}t$$

that $W(\psi(\cdot,t)-\psi^*)$ will eventually become negative. This contradicts the nonnegativity of W. ∎

Chapter 6

Retroactivity in Biological Networks

6.1 Introduction

The concept of retroactivity comes from engineering studies of electrical systems. In electrical engineering, complex electronic devices are built by assembling independent functional units called modules. Fundamental challenges include to understand how a signal affects the dynamics of the module from which the signal is sent and to find ways to reduce this effect. Engineers solve this problem usually by changing the input and output strengths. For example, in electronic amplifiers, retroactivity is reduced by a large amplification gain of the operational amplifier and a large negative feedback that regulates the output voltage.

In their novel work [94], Del Vecchio, Ninfa, and Sontag developed a theoretical framework to formulate the notion of retroactivity and to quantify the effect of retroactivity in biological settings. The effect of retroactivity arises widely in biological networks. Consider a cell containing a network that can produce certain protein Z. This protein Z in turn activates another downstream system. The terms "upstream" and "downstream" refer to the direction in which we think the signal is traveling. Let us consider one possible way that Z could activate its downstream system. The protein Z can bind to some promoter site in the downstream system to produce a new protein and thus start a chain of reactions, see Figure 6.1. However, upon binding to the promoter region, the amount of the "free" protein Z is changed, and the dynamic of the upstream system might be affected. We call this phenomena of a upstream system affected by the connection to a downstream system as retroactivity. In general, when two modules are connected, such a phenomena is expected.

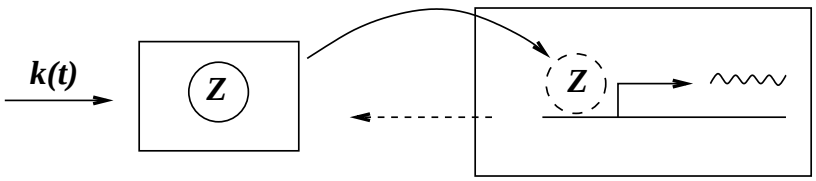

Figure 6.1: The box to the left is the upstream system. We assume that it is driven by some external signal $k(t)$. The product Z goes to the down stream system (right box), and binds to the promoter region of some promoter to produce new proteins. The retroactivity from the downstream system to upstream system is represented by the dashed line.

In this chapter, a more complicated case of three connected components are considered. We study the retroactivity effect on the overall dynamics, and propose designs and conditions under which the overall retroactivity can be attenuated. Let us denote by U the upper system and by u its internal state. The function $k(t)$ represents the outside signal driving the system U. The downstream system of U is denoted by X with internal state x. Let i be the input from U to X and r be the retroactivity from X to U. The downstream system of X is denoted by Y with internal state y. The symbol o stands for the output from X to Y, and the symbol s stands for the retroactivity from Y to X. See Figure 6.2 for a schematic diagram of such systems. Throughout this chapter, we shall refer to u as the input variable, x as the state variable, and y as the output variable.

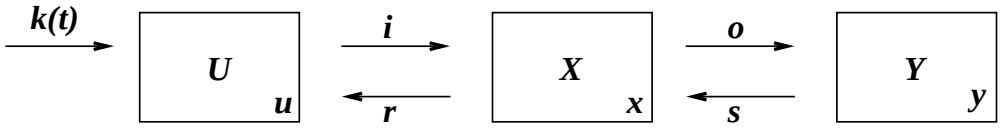

Figure 6.2: A diagram of three interconnected systems with input $k(t)$. The signal travels from U to X and then to Y.

To analyze the retroactivity from Y to X, we disconnect Y from the system, see Figure 6.3. We focus on the change of the dynamics in module X when module Y is removed.

Finally, we remove both module X and module Y (Figure 6.4), and study the dynamics of module U in isolation. We compare the dynamics of U in Figure 6.4 with the dynamics of U in Figure 6.2, and provide conditions under which the dynamics in U is not affected when modules X and Y are connected to the system.

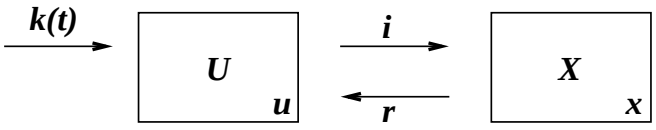

Figure 6.3: A diagram of the remaining system when we disconnect module Y from the system shown in Figure 6.2.

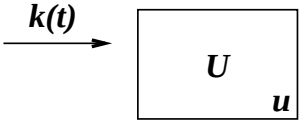

Figure 6.4: A diagram of the remaining system when we disconnect module X from the system shown in Figure 6.3.

The dynamics of the whole system in Figure 6.2 can be modeled by a set of ordinary differential equations:

$$\begin{aligned} \frac{du}{dt} &= f(t,u,x) \\ \frac{dx}{dt} &= g(u,x,y) \\ \frac{dy}{dt} &= h(x,y). \end{aligned} \tag{6.1}$$

We remark that the stability analysis for monotone tridiagonal systems with negative feedback, as done in Chapter 4, does not apply here since the right hand side of (6.1) depends on t.

In [94], Vecchio, Ninfa, and Sontag proposed two designs to attenuate retroactivity. One is transcriptional activation, and the other is phosphorylation. The phosphorylation design uses the assumption that reactions in Y are on the same time scale as those in X, and are much faster than reactions in U. In this chapter, we show that even when the reactions in Y are on a slower time scale, we would still be able to design a mechanism to attenuate the retroactivity.

For this reason, a key to our design is to have reactions in X act on a much faster time scale than the reactions in U and Y. We emphasize that under this assumption it is still possible that u and y are fast variables, since they may be affected by reactions in the X component through retroactivity r and input o, respectively. Following this

design, system (6.1) takes a special form:

$$\begin{aligned} \frac{du}{dt} &= F(t,u,x) + \frac{1}{\varepsilon}K_\alpha(u,x,y,\varepsilon), & u(0) &= u_0 \\ \varepsilon\frac{dx}{dt} &= G_\alpha(u,x) + \varepsilon L(x,y), & x(0) &= x_0 \\ \frac{dy}{dt} &= H(x,y), & y(0) &= y_0, \end{aligned} \tag{6.2}$$

where $0 \leq \varepsilon \leq \varepsilon_0, 0 < \alpha < \alpha_0$ for some positive numbers ε_0 and α_0. The point (u,x,y) belongs to a compact set $U \times X \times Y \subset \mathbb{R}^l \times \mathbb{R}^n \times \mathbb{R}^m$, and $K_\alpha(u,x,y,0)$ is not identically zero on $U \times X \times Y$.

The functions

$$\begin{aligned} F &: (0,\infty) \times U \times X \to \mathbb{R}^l \\ K_\alpha &: U \times X \times Y \times [0,\varepsilon_0] \to \mathbb{R}^l \text{ for each } \alpha \\ G_\alpha &: U \times X \to \mathbb{R}^n \text{ for each } \alpha \\ L &: X \times Y \to \mathbb{R}^n \\ H &: X \times Y \to \mathbb{R}^m. \end{aligned}$$

Retroactivity effects r and s are encoded in functions K_α/ε and εL, respectively. Our goal is to provide conditions under which retroactivity r and s can be neglected. This chapter is organized as follows. In Section 6.2, we study the retroactivity s to the state variable x. Section 6.3 focuses on retroactivity r to the input variable u. In Section 6.4, we workout a common biological example in detail to illustrate how retroactivity can be attenuated using our theoretical results.

6.2 Retroactivity from the output to the state variables

Let us fix α from now on, and vary it in the next section when we consider the retroactivity from the state variable x to the input variable u.

Notice that the equation of u in (6.2) contains a fast-varying term $K_\alpha(u,x,y,\varepsilon)/\varepsilon$. In order to eliminate it, we perform a change of variables. Consider a diffeomorphism

$$V : U \times X \times Y \to O \times Y,$$

where O is a compact subset of $\mathbb{R}^l \times \mathbb{R}^n$. The map V has a special form

$$V(u,x,y) = (V_1(u,x),x,y)$$

keeping the x and y coordinates unchanged, and

$$V_1 : U \times X \to P_u O,$$

where $P_u O$ is the projection of O to its u-coordinate. We denote by W the inverse of V and write $\tilde{u} = V_1(u,x)$, so that $u = W_1(\tilde{u},x)$ (the first coordinate of W). Suppose that:

$$\frac{\partial V_1}{\partial(u,x)}(u,x) \begin{pmatrix} K_\alpha(u,x,y,\varepsilon) \\ G_\alpha(u,x) + \varepsilon L(x,y) \end{pmatrix} = 0_{l,1}, \tag{6.3}$$

where $0_{l,1}$ is a zero matrix of size l times 1. Then, in the coordinates $(\tilde{u},x,y) = V(u,x,y)$ obtained under the change of variables V, the equations for system (6.2) become:

$$\begin{aligned} \frac{d\tilde{u}}{dt} &= \frac{\partial V_1}{\partial u}(W_1(\tilde{u},x),x)F(t,W_1(\tilde{u},x),x) := f(t,\tilde{u},x), && \tilde{u}(0) = \tilde{u}_0 \\ \varepsilon\frac{dx}{dt} &= G_\alpha(W_1(\tilde{u},x),x) + \varepsilon L(x,y), && x(0) = x_0 \\ \frac{dy}{dt} &= H(x,y), && y(0) = y_0, \end{aligned} \tag{6.4}$$

where $\tilde{u}_0 = V_1(u_0,x_0)$. From now on, we assume that $G_\alpha(W_1(\tilde{u},x),x) = 0$ has a unique solution $x = \gamma_\alpha(\tilde{u})$ in the set O.

Next, we consider the system when module Y is removed, see Figure 6.3.

The set of ordinary differential equations describes the dynamics of the remaining system is

$$\begin{aligned} \frac{d\tilde{u}^*}{dt} &= f(t,\tilde{u}^*,x^*), && \tilde{u}^*(0) = \tilde{u}_0 \\ \varepsilon\frac{dx^*}{dt} &= G_\alpha(W_1(\tilde{u}^*,x^*),x^*), && x^*(0) = x_0. \end{aligned} \tag{6.5}$$

Our purpose in this section is to compare the x-coordinate of the solutions of (6.4) and (6.5). We will achieve this in two steps, by first comparing (6.4) to its reduced system when $\varepsilon = 0$ and then comparing (6.5) with its reduced system.

We remark that the result in this section can be obtained by applying a *regular* perturbation argument to system (6.4) directly. We take a detour here since we need the

results, for example Theorem 6.1, in the next section when considering the retroactivity from the state variable x to the input variable u.

The reduced algebraic-differential system of (6.4) ($\varepsilon = 0$ in (6.4) with the initial condition of x ignored) is:

$$\begin{aligned} \frac{d\bar{\tilde{u}}}{dt} &= f(t, \bar{\tilde{u}}, \gamma_\alpha(\bar{\tilde{u}})), & \bar{\tilde{u}}(0) &= \tilde{u}_0 \\ \bar{x} &= \gamma_\alpha(\bar{\tilde{u}}), & & \\ \frac{d\bar{y}}{dt} &= H(\gamma_\alpha(\bar{\tilde{u}}), \bar{y}), & \bar{y}(0) &= y_0. \end{aligned} \tag{6.6}$$

We assume that

I The reduced system (6.6) has a unique continuous solution $(\bar{\tilde{u}}(t), \bar{x}(t), \bar{y}(t))$ on some interval $0 \le t \le T_\alpha$.

II The function f has continuous derivatives up to order two with respect to its arguments $(t, \tilde{u}, x)$ in some neighborhood of the points $(t, \bar{\tilde{u}}(t), \bar{x}(t)), 0 \le t \le T_\alpha$. Similar conditions hold for functions G_α, L and H.

III The eigenvalues of the matrix $\frac{\partial G_\alpha}{\partial x}(W_1(\bar{\tilde{u}}(t), \bar{x}(t)), \bar{x}(t))$ have real parts smaller than a fixed negative number $-\eta$ on the interval $[0, T_\alpha]$.

The standard result in singular perturbation theory on finite intervals ([41, 51, 91]) for systems of the form

$$\begin{aligned} \frac{dx}{dt} &= f(t, x, y, \varepsilon) \\ \varepsilon\frac{dy}{dt} &= g(t, x, y, \varepsilon) \end{aligned}$$

leads to the following theorem.

Theorem 6.1 *Given functions f, G_α, L, H as in* (6.4) *and initial conditions $\tilde{u}_0, y_0$ as in* (6.6)*, let the assumptions I through III hold. For any $t_1 \in (0, T_\alpha)$, there exist three positive numbers $r_\alpha, \varepsilon_\alpha$, and K_α with the following property. If $|x_0 - \gamma_\alpha(\tilde{u}_0)| < r_\alpha$ and $0 < \varepsilon < \varepsilon_\alpha$, then the initial value problem* (6.4) *has a unique solution $(\tilde{u}(t), x(t), y(t))$ such that*

$$|\tilde{u}(t) - \bar{\tilde{u}}(t)| \le K_\alpha\varepsilon, \quad |x(t) - \gamma_\alpha(\bar{\tilde{u}}(t))| \le K_\alpha\varepsilon, \quad |y(t) - \bar{y}(t))| \le K_\alpha\varepsilon \tag{6.7}$$

for any $t \in [t_1, T_\alpha]$*, where* $|\cdot|$ *denotes the Euclidean norm.*

For the isolated U, X-system (6.5), notice that it is already in the standard singular perturbation form. The corresponding reduced algebraic-differential system is:

$$\begin{aligned} \frac{d\bar{\tilde{u}}^*}{dt} &= f(t, \bar{\tilde{u}}^*, \gamma_\alpha(\bar{\tilde{u}}^*)), \qquad & \bar{\tilde{u}}^*(0) &= \tilde{u}_0 \\ \bar{x}^* &= \gamma_\alpha(\bar{\tilde{u}}^*). \end{aligned} \tag{6.8}$$

We assume that

I* The reduced system (6.8) has a unique continuous solution $(\bar{\tilde{u}}^*(t), \bar{x}^*(t))$ on some interval $0 \le t \le T_\alpha^*$.

II* The function f has continuous derivatives up to order two with respect to its arguments $(t, \tilde{u}^*, x^*)$ in some neighborhood of the points $(t, \bar{\tilde{u}}^*(t), \bar{x}^*(t)), 0 \le t \le T_\alpha^*$. Similar conditions hold for the function G_α.

III* The eigenvalues of the matrix $\frac{\partial G_\alpha}{\partial x}(W_1(\bar{\tilde{u}}^*(t), \bar{x}^*(t)), \bar{x}^*(t))$ have real parts smaller than a fixed negative number $-\eta^*$ on the interval $[0, T_\alpha^*]$.

Again, applying singular perturbation theorems on finite time interval, we have the following theorem.

Theorem 6.2 *Given functions* f *and* G_α *as in* (6.5) *and initial conditions* $\tilde{u}_0$ *as in* (6.8)*, let the assumptions I** *through III** *hold. For any* $t_1^* \in (0, T_\alpha^*)$*, there exist three positive numbers* $r_\alpha^*, \varepsilon_\alpha^*$*, and* K_α^* *with the following property. If* $|x_0 - \gamma_\alpha(\tilde{u}_0)| < r_\alpha^*$ *and* $0 < \varepsilon < \varepsilon_\alpha^*$*, then the initial value problem* (6.5) *has a unique solution* $(\tilde{u}^*(t), x^*(t))$ *such that*

$$|u^*(t) - \bar{\tilde{u}}^*(t))| \le K_\alpha^* \varepsilon, \quad |x^*(t) - \gamma_\alpha(\bar{\tilde{u}}^*(t))| \le K_\alpha^* \varepsilon \tag{6.9}$$

for any $t \in [t_1^*, T_\alpha^*]$.

Remark 6.3 *Notice that system* (6.8) *is a subsystem of* (6.6)*,* $\bar{\tilde{u}}^*$ *and* $\bar{\tilde{u}}$ *share the same initial condition, and the output* $\bar{y}$ *of the* $\bar{\tilde{u}}, \bar{x}$*-system in* (6.6) *does not affect the dynamics of* $\bar{\tilde{u}}$ *and* $\bar{x}$*. If assumption I holds for system* (6.6)*, then* $(\bar{\tilde{u}}^*(t), \bar{x}^*(t)) = (\bar{\tilde{u}}(t), \bar{x}(t))$ *on* $[0, T_\alpha]$ *is the unique solution to* (6.8)*. For the same reason, assumptions II and III imply assumptions II** *and III** *with* $T_\alpha^* = T_\alpha$.

Based on the above observation, we can combine Theorem 6.1 and Theorem 6.2 to compare the x-coordinate of the solutions of (6.4) and (6.5).

Theorem 6.4 *Given functions f, G_α, L, H as in (6.4), $\tilde{u}_0$ as in (6.6). Let the assumptions I through III hold. For any $t_1 \in (0, T_\alpha)$, there exist three positive numbers $r'_\alpha, \varepsilon'_\alpha$, and K'_α with the following property. If $|x_0 - \gamma_\alpha(\tilde{u}_0)| < r'_\alpha$ and $0 < \varepsilon < \varepsilon'_\alpha$, then the initial value problems (6.4) and (6.5) have unique solutions $(\tilde{u}(t), x(t), y(t))$ and $(\tilde{u}^*(t), x^*(t))$, respectively, such that*

$$|x(t) - x^*(t)| \leq K'_\alpha \varepsilon$$

for any $t \in [t_1, T_\alpha]$.

Proof. Let $r_\alpha, \varepsilon_\alpha$ and K_α be as in Theorem 6.1 and $r^*_\alpha, \varepsilon^*_\alpha$ and K^*_α be as in Theorem 6.2. Define

$$r'_\alpha = \min\{r_\alpha, r^*_\alpha\}, \quad \varepsilon'_\alpha = \min\{\varepsilon_\alpha, \varepsilon^*_\alpha\}, \quad K'_\alpha = K_\alpha + K^*_\alpha.$$

By Theorem 6.1 and Theorem 6.2, if $|x_0 - \gamma_\alpha(\tilde{u}_0)| < r'_\alpha$ and $0 < \varepsilon < \varepsilon'_\alpha$, then the initial value problems (6.4) and (6.5) have unique solutions $(\tilde{u}(t), x(t), y(t))$ and $(\tilde{u}^*(t), x^*(t))$, respectively. Moreover, they satisfy

$$\begin{aligned} |x(t) - x^*(t)| &\leq |x(t) - \gamma_\alpha(\bar{\tilde{u}}(t))| + |\gamma_\alpha(\bar{\tilde{u}}^*(t)) - x^*(t)| \\ &\leq K_\alpha \varepsilon + K^*_\alpha \varepsilon = K'_\alpha \varepsilon \end{aligned}$$

for any $t \in [t_1, T_\alpha]$. ∎

The above theorem shows that when ε is sufficiently small, the retroactivity from y to x can be ignored.

6.3 Retroactivity from the state to the input variables

In this section, we assume for simplicity that the function F in (6.2) does not depend on x, that is,

$$\begin{aligned} \frac{du}{dt} &= F(t,u) + \frac{1}{\varepsilon}K_\alpha(u,x,y,\varepsilon), & u(0) &= u_0 \\ \varepsilon\frac{dx}{dt} &= G_\alpha(u,x) + \varepsilon L(x,y), & x(0) &= x_0 \\ \frac{dy}{dt} &= H(x,y), & y(0) &= y_0. \end{aligned} \tag{6.10}$$

In order to study the retroactivity from x to u,we disconnect the system X from U, see Figure 6.4.

The corresponding ordinary differential equation describing the dynamics in Figure 6.4 is

$$\frac{d\hat{u}}{dt} = F(t,\hat{u}), \quad \hat{u}(0) = u_0. \tag{6.11}$$

The goal in this section is to find conditions under which the u-coordinate of the solution $(u(t),x(t),y(t))$ of (6.10) can be approximated on some time interval $[0,\hat{T})$ (to be defined later) by the solution $\hat{u}(t)$ of (6.11).

Let $(\bar{\tilde{u}}(t),\bar{x}(t),\bar{y}(t))$ be the solution of (6.6) on $[0,T_\alpha]$ (as in assumption I). We introduce a new function $\bar{u}(t)$, defined as $W_1(\bar{\tilde{u}}(t),\bar{x}(t))$. We next compare $\bar{u}(t)$ to $\hat{u}(t)$. Differentiating $\bar{u}(t)$ with respect to t, we have (to simplify notations, we drop the t in functions such as $\bar{\tilde{u}}(t)$):

$$\begin{aligned} \frac{d\bar{u}}{dt} &= \frac{\partial W_1}{\partial\tilde{u}}(\bar{\tilde{u}},\bar{x})\frac{d\bar{\tilde{u}}}{dt} + \frac{\partial W_1}{\partial x}(\bar{\tilde{u}},\bar{x})\frac{d\bar{x}}{dt} \\ &= \frac{\partial W_1}{\partial\tilde{u}}(\bar{\tilde{u}},\bar{x})\frac{\partial V_1}{\partial u}(\bar{u},\bar{x})F(t,\bar{u}) + \frac{\partial W_1}{\partial x}(\bar{\tilde{u}},\bar{x})\frac{\partial\gamma_\alpha}{\partial\tilde{u}}(\bar{\tilde{u}})\frac{\partial V_1}{\partial u}(\bar{u},\bar{x})F(t,\bar{u}) \\ &= F(t,\bar{u}) + \frac{\partial W_1}{\partial x}(\bar{\tilde{u}},\bar{x})\frac{\partial\gamma_\alpha}{\partial\tilde{u}}(\bar{\tilde{u}})\frac{\partial V_1}{\partial u}(\bar{u},\bar{x})F(t,\bar{u}) \\ &= \left(I + \frac{\partial W_1}{\partial x}(\bar{\tilde{u}},\bar{x})\frac{\partial\gamma_\alpha}{\partial\tilde{u}}(\bar{\tilde{u}})\frac{\partial V_1}{\partial u}(\bar{u},\bar{x})\right)F(t,\bar{u}), \end{aligned}$$

which we rewrite as

$$\frac{d\bar{u}}{dt} = \beta_\alpha(t)F(t,\bar{u}), \tag{6.12}$$

where

$$\beta_\alpha(t) = I + \frac{\partial W_1}{\partial x}(\bar{\tilde{u}},\bar{x})\frac{\partial\gamma_\alpha}{\partial\tilde{u}}(\bar{\tilde{u}})\frac{\partial V_1}{du}(\bar{u},\bar{x}). \tag{6.13}$$

The function $\bar{u}(t)$ can be viewed as the solution of (6.12) with the initial condition $W_1(\tilde{u}_0, \gamma_\alpha(\tilde{u}_0))$. We shall keep in mind that $\bar{u}(t)$ and equation (6.12) do not involve ε, since $\bar{u}(t)$ is defined through $\bar{\tilde{u}}(t)$ and $\bar{x}(t)$, which are solutions of (6.6).

We make the following assumption.

IV There exists a positive constant K_0 such that for all $\alpha \in [0, \alpha_0]$,

$$\sup_{t\in[0,T_\alpha]} ||\beta_\alpha(t) - I|| \leq K_0\rho(\alpha),$$

where $||\cdot||$ is the induced L^2 matrix norm and ρ is an increasing function in α with $\rho(0) = 0$.

By continuity of solutions as a function of initial conditions and inputs (see for instance, Theorem 6.1 in [83]), for any $\delta > 0$, there exist two positive numbers α_δ and r_0 such that for that fixed α_δ, if the initial conditions satisfy

$$|u_0 - W_1(\tilde{u}_0, \gamma_{\alpha_\delta}(\tilde{u}_0))| < r_0,$$

then

$$|\hat{u}(t) - \bar{u}(t)| \leq \delta/2 \text{ on } [0, T_0],$$

where $T_0 = \min\{T_{\alpha_\delta}, \hat{T}\}$. Let us fix this α_δ from now on, and let $(u(t), x(t), y(t))$ be the solution of (6.10) on $[0, T_0]$. We next study the difference between $\bar{u}(t)$ and $u(t)$. When considering $u(t)$, ε is not zero, and we expect to specify a range of ε such that $u(t)$ is close to $\bar{u}(t)$ on some time interval.

Pick any $t_1 \in (0, T_0]$, and fix it. Let us denote

$$\tilde{u}(t) = \bar{\tilde{u}}(t) + g^1_{\alpha_\delta}(t), \quad x(t) = \bar{x}(t) + g^2_{\alpha_\delta}(t),$$

where $\bar{x}(t) = \gamma(\bar{\tilde{u}}(t))$. By Theorem 6.1, the function $g^i_{\alpha_\delta}(t), i = 1, 2$ satisfies

$$|g^i_{\alpha_\delta}(t)| \leq K_{\alpha_\delta}\varepsilon \text{ on } [t_1, T_0].$$

Therefore, using the Mean Value Theorem, we have

$$\begin{aligned} u(t) &= W_1(\tilde{u}(t), x(t)) \\ &= W_1(\bar{\tilde{u}}(t) + g^1_{\alpha_\delta}(t), \bar{x}(t) + g^2_{\alpha_\delta}(t)) \\ &= W_1(\bar{\tilde{u}}, \bar{x}(t)) + \frac{\partial W_1}{\partial \tilde{u}}(\xi^u(t), \xi^x(t)) g^1_{\alpha_\delta}(t) + \frac{\partial W_1}{\partial x}(\xi^u(t), \xi^x(t)) g^2_{\alpha_\delta}(t) \\ &= \bar{u}(t) + \frac{\partial W_1}{\partial \tilde{u}}(\xi^u(t), \xi^x(t)) g^1_{\alpha_\delta}(t) + \frac{\partial W_1}{\partial x}(\xi^u(t), \xi^x(t)) g^2_{\alpha_\delta}(t) \end{aligned}$$

for some $\xi^u(t) \in \tilde{U}$ and $\xi^x(t) \in X$. Thus,

$$|u(t) - \bar{u}(t)| \leq 2MK_{\alpha_\delta}\varepsilon,$$

where M is the upper bound of the partial derivatives of W_1 on $\tilde{U} \times X$. If we choose

$$0 < \varepsilon < \frac{\delta}{4MK_{\alpha_\delta}},$$

then

$$|u(t) - \bar{u}(t)| < \delta/2 \text{ on } [t_1, T_0].$$

To summarize, we have established the following result.

Theorem 6.5 *Let assumption IV hold for all $\alpha \in [0, \alpha_0]$. For any $\delta > 0$, there exist two positive numbers α_δ and r_0 with the following property. Let assumptions I to III hold for $\alpha = \alpha_\delta$. For any $t_1 \in (0, T_0]$ where $T_0 = \min\{T_{\alpha_\delta}, \hat{T}\}$, there exists $\varepsilon_1 > 0$ such that for any $0 < \varepsilon < \varepsilon_1$ and initial conditions with $|u_0 - W_1(\tilde{u}_0, \gamma_{\alpha_\delta}(\tilde{u}_0))| < r_0$, the u-coordinate of the solution $(u(t), x(t), y(t))$ of (6.10) and the solution $\hat{u}(t)$ of (6.11) satisfy*

$$|u(t) - \hat{u}(t)| \leq \delta \text{ on } [t_1, T_0].$$

Theorem 6.5 says that we could make the solution of (6.11) as close as we want to the u solution of (6.10) by choosing small enough ε. In another word, when subsequent modules X and Y are connected to the system, the retroactivity r can be controlled at a low level by speeding up the dynamics in X. In the next section, we illustrate this idea using a common biological example.

6.4 Applications

Consider the "futile cycle" motif introduced in Chapter 2. Here for simplicity, we use the "futile cycle" of size one and connect to it with input and output systems. More precisely, we have a kinase Z, which can phosphorylate the substrate X to X_p. The phosphatase Y then dephosphorylates X_p and brings it back to X. We assume that the total amount of the phosphatase Y is conserved. The protein X_p can bind to a promoter denoted by P in the downstream system. Upon binding, transcription initiates. This is a typical signaling mechanism proposed in [94].

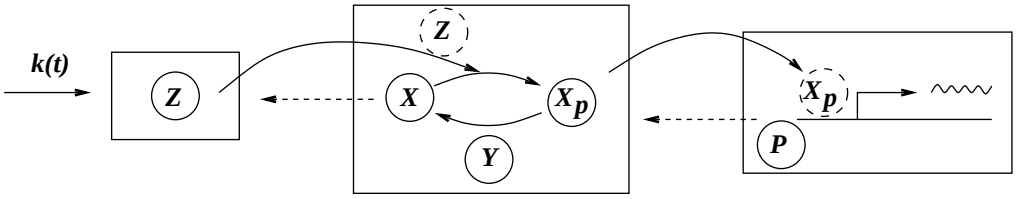

Figure 6.5: We use circles to represent proteins and promoters to distinguish from systems and state variables in other figures. To the left is the module used to produce the protein Z. In the center, we have the "futile cycle" of size one. The substrate X is phosphorylated by the kinase Z. Their product X_p goes into the downstream system and binds to the promoter region of promoter P. Retroactivity is represented by dashed lines.

The corresponding reactions are given as follows.

$$\xrightarrow{k(t)} Z \xrightarrow{\kappa} \emptyset$$

$$X + Z \underset{\beta_2}{\overset{\beta_1}{\rightleftarrows}} C_1 \xrightarrow{k_1} X_p + Z$$

$$X_p + Y \underset{\alpha_2}{\overset{\alpha_1}{\rightleftarrows}} C_2 \xrightarrow{k_2} X + Y$$

$$X_p + P \underset{k_{\text{off}}}{\overset{k_{\text{on}}}{\rightleftarrows}} C,$$

where $k(t)$ is a smooth function from $[0, \infty)$ to $[0, M]$ for some positive constant M.

The differential equations modeling the dynamics of these reactions are:

$$\begin{aligned}
\frac{dZ}{dt} &= k(t) - \kappa Z - \beta_1 Z X_{\text{tot}} \left(1 - \frac{X_p}{X_{\text{tot}}} - \frac{C_1}{X_{\text{tot}}} - \frac{C_2}{X_{\text{tot}}} - \frac{C}{X_{\text{tot}}}\right) + (\beta_2 + k_1) C_1 \\
\frac{dC_1}{dt} &= -(\beta_2 + k_1) C_1 + \beta_1 Z X_{\text{tot}} \left(1 - \frac{X_p}{X_{\text{tot}}} - \frac{C_1}{X_{\text{tot}}} - \frac{C_2}{X_{\text{tot}}} - \frac{C}{X_{\text{tot}}}\right) \\
\frac{dC_2}{dt} &= -(\alpha_2 + k_2) C_2 + \alpha_1 X_p Y_{\text{tot}} \left(1 - \frac{C_2}{Y_{\text{tot}}}\right) \\
\frac{dX_p}{dt} &= k_1 C_1 + \alpha_2 C_2 - \alpha_1 X_p Y_{\text{tot}} \left(1 - \frac{C_2}{Y_{\text{tot}}}\right) + k_{\text{off}} C - k_{\text{on}} X_p (P_{\text{tot}} - C) \\
\frac{dC}{dt} &= -k_{\text{off}} C + k_{\text{on}} X_p (P_{\text{tot}} - C).
\end{aligned} \tag{6.14}$$

Concentrations of X, Y and P are determined through the conservation relations

$$\begin{aligned}
X_{\text{tot}} &= X + X_p + C_1 + C_2 + C \\
Y_{\text{tot}} &= Y + C_2 \\
P_{\text{tot}} &= P + C.
\end{aligned} \tag{6.15}$$

With the assumption $P_{\text{tot}} \ll X_{\text{tot}}$, system (6.14) can be approximated by a system obtained by removing the term C_2/X_{tot} in Z and C_1 equations of (6.14):

$$\begin{aligned}
\frac{dZ}{dt} &= k(t) - \kappa Z - \beta_1 Z X_{\text{tot}} \left(1 - \frac{X_p}{X_{\text{tot}}} - \frac{C_1}{X_{\text{tot}}} - \frac{C_2}{X_{\text{tot}}}\right) + (\beta_2 + k_1) C_1 \\
\frac{dC_1}{dt} &= -(\beta_2 + k_1) C_1 + \beta_1 Z X_{\text{tot}} \left(1 - \frac{X_p}{X_{\text{tot}}} - \frac{C_1}{X_{\text{tot}}} - \frac{C_2}{X_{\text{tot}}}\right) \\
\frac{dC_2}{dt} &= -(\alpha_2 + k_2) C_2 + \alpha_1 X_p Y_{\text{tot}} \left(1 - \frac{C_2}{Y_{\text{tot}}}\right) \\
\frac{dX_p}{dt} &= k_1 C_1 + \alpha_2 C_2 - \alpha_1 X_p Y_{\text{tot}} \left(1 - \frac{C_2}{Y_{\text{tot}}}\right) + k_{\text{off}} C - k_{\text{on}} X_p (P_{\text{tot}} - C) \\
\frac{dC}{dt} &= -k_{\text{off}} C + k_{\text{on}} X_p (P_{\text{tot}} - C).
\end{aligned} \tag{6.16}$$

Before proceeding, let us first rescale the system. We define

$$z = \frac{Z}{Z_0}, \quad Z_0 = \frac{M}{\delta}, \quad c_1 = \frac{C_1}{Z_0}, \quad c_2 = \frac{C_2}{Y_{\text{tot}}}, \quad x_p = \frac{X_p}{X_{\text{tot}}}, \quad c = \frac{C}{P_{\text{tot}}},$$

$$\varepsilon = \frac{1}{\alpha_2 + k_2}, \quad \sigma = \frac{\beta_2 + k_1}{\alpha_2 + k_2}, \quad \alpha = \frac{\beta_1 X_{\text{tot}}}{\beta_2 + k_1}, \quad \theta = \frac{\alpha_1 X_{\text{tot}}}{\alpha_2 + k_2}, \quad k_1 = \mu_1 \beta_2, \quad k_2 = \mu_2 \alpha_2.$$

System (6.16) in terms of the new variables and parameters becomes

$$\begin{aligned}
\frac{dz}{dt} &= \frac{k(t)}{Z_0} - \kappa z + \frac{1}{\varepsilon}\left[-\sigma\alpha z\left(1 - x_p - \frac{Z_0}{X_{\text{tot}}}c_1 - \frac{Y_{\text{tot}}}{X_{\text{tot}}}c_2\right) + \sigma c_1\right] \\
\varepsilon\frac{dc_1}{dt} &= -\sigma c_1 + \sigma\alpha z\left(1 - x_p - \frac{Z_0}{X_{\text{tot}}}c_1 - \frac{Y_{\text{tot}}}{X_{\text{tot}}}c_2\right) \\
\varepsilon\frac{dc_2}{dt} &= -c_2 + \theta x_p(1 - c_2) \\
\varepsilon\frac{dx_p}{dt} &= \frac{\sigma\mu_1 Z_0}{(1+\mu_1)X_{\text{tot}}}c_1 + \frac{Y_{\text{tot}}}{(1+\mu_2)X_{\text{tot}}}c_2 - \frac{Y_{\text{tot}}\theta}{X_{\text{tot}}}x_p(1 - c_2) \\
&\quad + \varepsilon\left(\frac{k_{\text{off}}P_{\text{tot}}}{X_{\text{tot}}}c - k_{\text{on}}x_p P_{\text{tot}}(1 - c)\right) \\
\frac{dc}{dt} &= -k_{\text{off}}c + k_{\text{on}}x_p X_{\text{tot}}(1 - c).
\end{aligned} \tag{6.17}$$

System (6.17) is defined on $U \times X \times Y$, where $U = [0,1], X = [0,1]\times[0,1]\times[0,1], Y = [0,1]$. We consider the solution to (6.17) with the initial condition

$$(u(0), c_1(0), c_2(0), x_p(0), c(0)) = (u^0, c_1^0, c_2^0, x_p^0, c^0) \in U \times X \times Y. \tag{6.18}$$

System (6.17) with initial condition (6.18) is now in the form of (6.2). Here, z is the input, c_1, c_2, and x_p are the state variables, and c is the output. We define V as

$$V(z, c_1, c_2, x_p, c) = (z + c_1, c_1, c_2, x_p, c),$$

and thus,

$$\tilde{z} = V_1(z, c_1, c_2, x_p) = z + c_1, \quad z = W_1(\tilde{z}, c_1, c_2, x_p) = \tilde{z} - c_1.$$

The transformation V satisfies condition (6.3), and the new system under the change of variables is

$$\begin{aligned}
\frac{d\tilde{z}}{dt} &= \frac{k(t)}{Z_0} - \kappa\tilde{z}, & \tilde{z}(0) &= \tilde{z}^0 := z^0 + c_1^0, \\
\varepsilon\frac{dc_1}{dt} &= -\sigma c_1 + \sigma\alpha(\tilde{z} - c_1)\left(1 - x_p - \frac{Z_0}{X_{\text{tot}}}c_1 - \frac{Y_{\text{tot}}}{X_{\text{tot}}}c_2\right), & c_1(0) &= c_1^0, \\
\varepsilon\frac{dc_2}{dt} &= -c_2 + \theta x_p(1 - c_2), & c_2(0) &= c_2^0, \\
\varepsilon\frac{dx_p}{dt} &= \frac{\sigma\mu_1 Z_0}{(1+\mu_1)X_{\text{tot}}}c_1 + \frac{Y_{\text{tot}}}{(1+\mu_2)X_{\text{tot}}}c_2 - \frac{Y_{\text{tot}}\theta}{X_{\text{tot}}}x_p(1 - c_2) \\
&\quad + \varepsilon\left(\frac{k_{\text{off}}P_{\text{tot}}}{X_{\text{tot}}}c - k_{\text{on}}x_p P_{\text{tot}}(1 - c)\right), & x_p(0) &= x_p^0, \\
\frac{dc}{dt} &= -k_{\text{off}}c + k_{\text{on}}x_p X_{\text{tot}}(1 - c), & c(0) &= c^0.
\end{aligned} \tag{6.19}$$

It is defined on $O \times Y$, where

$$O = \{(\tilde{z}, c_1, c_2, x_p) \,|\, 0 \le \tilde{z}, c_2, x_p \le 1, 0 \le c_1 \le \tilde{z}\}.$$

From the conservation relations in (6.15), it is easy to see that system (6.19) is forward invariant in $O \times Y$, that is, solutions starting inside $O \times Y$ never leave $O \times Y$ in forward times. An immediate consequence is that the solution $(\tilde{z}(t), c_1(t), c_2(t), x_p(t), c(t))$ to the initial value problem (6.19) exists for all $t \in [0, \infty)$.

Setting $\varepsilon = 0$, the differential equations of c_1, c_2, and x_p degenerate to algebraic equations:

$$\begin{aligned} 0 &= -\sigma c_1 + \sigma\alpha(\tilde{z} - c_1)\left(1 - x_p - \frac{Z_0}{X_{\text{tot}}}c_1 - \frac{Y_{\text{tot}}}{X_{\text{tot}}}c_2\right) \\ 0 &= -c_2 + \theta x_p(1 - c_2) \\ 0 &= \frac{\sigma\mu_1 Z_0}{(1+\mu_1)X_{\text{tot}}}c_1 + \frac{Y_{\text{tot}}}{(1+\mu_2)X_{\text{tot}}}c_2 - \frac{Y_{\text{tot}}\theta}{X_{\text{tot}}}x_p(1 - c_2). \end{aligned} \tag{6.20}$$

Lemma 6.6 *For any $\theta \in (0, 1], \alpha \in (0, \infty)$, the algebraic equations* (6.20) *on the set $O \times Y$ admit a unique solution $(c_1, c_2, x_p) = (\gamma_1(\tilde{z}), \gamma_2(\tilde{z}), \gamma_3(\tilde{z})) := \gamma(\tilde{z})$, where the vector function γ depends on θ, α, and other parameters in system* (6.19)*. (We do not write these parameters explicitly in γ to simplify the notations.)*

Proof. From the last two equations of (6.20), we can solve c_1 and c_2 in terms of x_p, and get

$$c_1 = \chi\frac{\theta x_p}{1 + \theta x_p}, \quad c_2 = \frac{\theta x_p}{1 + \theta x_p}, \tag{6.21}$$

where the constant

$$\chi = \frac{(1+\mu_1)\mu_2 X_{\text{tot}}}{\mu_1(1+\mu_2)\sigma Z_0}.$$

Plugging (6.21) into the first equation of (6.20) with $z = \tilde{z} - c_1$, we obtain a quadratic equation in x_p (viewing z as a constant):

$$\alpha\theta z x_p^2 + B(z)x_p - \alpha z = 0, \tag{6.22}$$

where

$$B(z) = \chi\theta + \alpha z - \alpha\theta z + \frac{Y_{\text{tot}}}{X_{\text{tot}}}\alpha\theta z + \frac{Z_0}{X_{\text{tot}}}\chi\alpha\theta z.$$

When $z = 0$, equation (6.22) becomes a linear equation, and the solution is $x_p = 0$, which in turn gives $c_1 = c_2 = \tilde{z} = 0$. In the case of $z \neq 0$, notice that when $\theta \in (0, 1]$, $B(z)$ is an increasing function in z, and thus $B(z) > 0$ for $z > 0$. Moreover, the y-intercept of the quadratic (left hand side of (6.22)) is $-\alpha z < 0$. Therefore, (6.22) has a unique positive root

$$x_p(z) = \frac{-B(z) + \Delta^{1/2}(z)}{2\alpha\theta z} := \zeta_3(z), \tag{6.23}$$

where

$$\Delta(z) = B^2(z) + 4\alpha^2\theta z^2.$$

By L'Hospital's Rule,

$$\lim_{z\to 0} x_p(z) = \frac{-B'(0) + \Delta^{-1/2}(0)B(0)B'(0)}{2\alpha\theta} = 0,$$

so the function $\zeta_3(z)$ defined in (6.23) is continuous at $z = 0$. Plugging (6.23) into (6.21), we obtain c_1 and c_2 as functions of z, denoted as $c_1 = \zeta_1(z), c_2 = \zeta_2(z)$. Next, we show that the function $\zeta_3(z)$ has continuous derivative with $\zeta_3'(z) > 0$ for all z on $[0, \infty)$. To see this, we differentiate $\zeta_3(z)$ with respect to z and obtain

$$\zeta_3'(z) = \frac{-zB'(z) + z\Delta^{-1/2}(z)(B(z)B'(z) + 4\alpha^2\theta z) + B(z) - \Delta^{1/2}(z)}{2\alpha\theta z^2}. \tag{6.24}$$

Using the equality

$$B(z) = B'(z)z + \chi\theta,$$

the numerator of (6.24) becomes

$$\begin{aligned}
&\Delta^{-1/2}(z)(\chi\theta\Delta^{1/2}(z) + z(B(z)B'(z) + 4\alpha^2\theta z) - \Delta(z)) \\
&= \Delta^{-1/2}(z)(\chi\theta\Delta^{1/2}(z) + B(z)(zB'(z) - B(z))) \\
&= \Delta^{-1/2}(z)(\chi\theta\Delta^{1/2}(z) + \chi\theta B(z)) \\
&= \chi\theta(1 + B(z)\Delta^{-1/2}(z)).
\end{aligned}$$

So,

$$\zeta_3'(z) = \frac{\chi(1 - B(z)\Delta^{-1/2}(z))}{2\alpha z^2} > 0 \text{ for all } z > 0. \tag{6.25}$$

Using the Binomial Formula, we have

$$\lim_{z\to 0^+} \zeta_3'(z) = \frac{\chi\left(1 - \left(1 - \frac{2\alpha^2\theta z^2}{B^2(0)}\right)\right)}{2\alpha z^2} = \frac{\alpha}{\chi\theta},$$

and

$$\lim_{z\to 0^+}\frac{\zeta_3(z)}{z}=\frac{B(0)\left(-1+1+\frac{2\alpha^2\theta z^2}{B(0)^2}\right)}{2\alpha\theta z^2}=\frac{\alpha}{\chi\theta}.$$

Therefore, $\zeta_3'(z)$ is continuous at $z=0$. To summarize, we have proved that the function $\zeta_3(z)$ has continuous derivative with $\zeta_3'(z)>0$ for all z on $[0,\infty)$.

Because of (6.21), the function $\zeta_1(z)$ also has continuous derivative with $\zeta_1'(z)>0$ for all z on $[0,\infty)$. Recall that $\tilde{z}=z+c_1=z+\zeta_1(z)$. By the Inverse Function Theorem, we can solve z as a function of $\tilde{z}$, denoted as $z=\omega(\tilde{z})$. Therefore,

$$\gamma(\tilde{z})=(\zeta_1\circ\omega(\tilde{z}),\zeta_2\circ\omega(\tilde{z}),\zeta_3\circ\omega(\tilde{z}))$$

is a solution of (6.20) on $O\times Y$, and the uniqueness of ω and ζ's implies the uniqueness of γ. ∎

Now the reduced algebraic-differential equations of (6.19) can be written as

$$\begin{aligned}
&\frac{d\bar{\tilde{z}}}{dt}=\frac{k(t)}{Z_0}-\kappa\bar{\tilde{z}}, && \bar{\tilde{z}}(0)=\tilde{z}^0,\\
&\bar{c}_1=\gamma_1(\bar{\tilde{z}}),\\
&\bar{c}_2=\gamma_2(\bar{\tilde{z}}), && (6.26)\\
&\bar{x}_p=\gamma_3(\bar{\tilde{z}}),\\
&\frac{d\bar{c}}{dt}=-k_{\text{off}}\bar{c}+k_{\text{on}}\gamma_3(\bar{\tilde{z}})X_{\text{tot}}(1-\bar{c}), && \bar{c}(0)=c^0.
\end{aligned}$$

Since (6.26) is forward invariant on $[0,1]\times Y$, the solution $(\bar{\tilde{z}}(t),\bar{c}_1,\bar{c}_2,\bar{x}_p,\bar{c})$ to (6.26) is defined for all $t>0$. As a result, assumption I holds for any positive real number T independent of α. It is easy to see that assumption II holds trivially.

Lemma 6.7 *Assumption III holds for all $\alpha\in(0,\infty)$.*

Proof. Let us write the Jacobian matrix $\frac{\partial G_\alpha}{\partial x}$ evaluated along

$$(\bar{\tilde{z}}(t),\bar{c}_1(t),\bar{c}_2(t),\bar{x}_p(t),\bar{c}(t))$$

as

$$J(t)=\begin{pmatrix}
-A(t) & -\frac{Y_{\text{tot}}}{X_{\text{tot}}}\sigma\alpha(\tilde{z}(t)-c_1(t)) & -\sigma\alpha(\tilde{z}(t)-c_1(t))\\
0 & -1-\theta x_p(t) & \theta(1-c_2(t))\\
\frac{\sigma\mu_1 Z_0}{(1+\mu_1)X_{\text{tot}}} & \frac{Y_{\text{tot}}}{(1+\mu_2)X_{\text{tot}}}+\frac{Y_{\text{tot}}}{X_{\text{tot}}}\theta x_p(t) & -\frac{Y_{\text{tot}}}{X_{\text{tot}}}\theta(1-c_2(t))
\end{pmatrix},$$

where

$$A(t) = \sigma + \frac{Z_0}{X_{\text{tot}}}\sigma\alpha(\tilde{z}(t) - c_1(t)) + \sigma\alpha(1 - x_p(t) - \frac{Z_0}{X_{\text{tot}}}c_1(t) - \frac{Y_{\text{tot}}}{X_{\text{tot}}}c_2(t)) > 0.$$

We first show that all the eigenvalues of $J(t)$ have negative real parts. It is enough to show that all the eigenvalues of $J^*(t)$ have negative real parts, where $J^*(t)$ is defined as

$$\begin{aligned} J^*(t) &= \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & \frac{X_{\text{tot}}}{Y_{\text{tot}}} \end{pmatrix} J(t) \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & \frac{Y_{\text{tot}}}{X_{\text{tot}}} \end{pmatrix} \\ &= \begin{pmatrix} -A(t) & -\frac{Y_{\text{tot}}}{X_{\text{tot}}}\sigma\alpha(\tilde{z}(t) - c_1(t)) & -\frac{Y_{\text{tot}}}{X_{\text{tot}}}\sigma\alpha(\tilde{z}(t) - c_1(t)) \\ 0 & -1 - \theta x_p(t) & \frac{Y_{\text{tot}}}{X_{\text{tot}}}\theta(1 - c_2(t)) \\ \frac{\sigma\mu_1 Z_0}{(1+\mu_1)Y_{\text{tot}}} & \frac{1}{1+\mu_2} + \theta x_p(t) & -\frac{Y_{\text{tot}}}{X_{\text{tot}}}\theta(1 - c_2(t)) \end{pmatrix}. \end{aligned}$$

Let us rewrite the matrix $J^*(t)$ as

$$J^*(t) = \begin{pmatrix} -A(t) & -I(t) & -I(t) \\ 0 & -R(t) & D(t) \\ E & F(t) & -D(t) \end{pmatrix},$$

where E is a positive constant; A, R, D, F are positive functions of t; I, D are nonnegative functions of t defined in the obvious way. Hereafter, we drop the t to simplify the notations. By Routh-Hurwitz criterion. All the eigenvalues of $J^*(t)$ have negative real part if the first column of the Routh-Hurwitz table does not change signs. The elements in the first column of the Routh-Hurwitz table are

$$\begin{aligned} a_1 &= 1, \\ a_2 &= D + R + A, \\ b_1 &= \frac{1}{a_2}\left(\frac{\mu_2}{1+\mu_2}(D^2 + RD) + D^2A + 2DRA + R^2A + AEI + DA^2 + RA^2\right), \\ c_1 &= EID + EIR + AD\frac{\mu_2}{1+\mu_2}. \end{aligned}$$

It is straightforward to see that a_1, a_2, b_1 are positive, and $c_1 \geq 0$. The last element c_1 is zero only when both I and D are zero, that is, $c_2 = 1, \tilde{z} = c_1$. However, this

would not happen, since $\tilde{z} = c_1$ gives $z = 0$, and thus, $x_p = 0$. By (6.21), both c_1 and c_2 are zero, which contradicts $c_2 = 1$. So, the last element is always positive too. By Routh-Hurwitz criterion, all the eigenvalues of $J^*(t)$ have negative real part, and so does $J(t)$. It follows that for any $T > 0$, we can find a positive number η such that all the eigenvalues of $J(t)$ have negative real part smaller than $-\eta$ on $[0, T]$.

∎

Lemma 6.8 *Assumption IV holds for all $\alpha \in (0, \infty)$ and $\theta \in (0, 1]$.*

Proof. The function $\beta_\alpha(t)$ defined as in (6.13) equals $1 - \gamma_1'(\tilde{z}(t))$. Thus, $|\beta_\alpha(t) - 1| = |\gamma_1'(\tilde{z}(t))| \leq |\zeta_1'(z)\omega'(\tilde{z})|$. Notice that,

$$0 < \omega'(\tilde{z}) = \frac{1}{1 + \zeta_1'(z)} < 1 \text{ and } \zeta_1'(z) > 0,$$

so,

$$|\beta_\alpha(t) - 1| < \zeta_1'(z) = \frac{\chi\theta\zeta_3'(z)}{(1 + \theta\zeta_3)^2} \leq \chi\theta\zeta_3'(z).$$

Applying the Binomial Formula to (6.25), we have

$$\zeta_3'(z) \leq \frac{\chi\left(1 - \left(1 - \frac{2\alpha^2\theta z^2}{B^2(z)}\right)\right)}{2\alpha z^2} = \frac{\chi\alpha\theta}{B^2(z)} \leq \frac{\alpha}{\chi\theta}.$$

Therefore,

$$|\beta_\alpha(t) - 1| \leq \alpha,$$

which satisfies assumption IV. ∎

Now we have checked assumptions I to IV on system (6.17). Applying Theorem 6.5, we have

Theorem 6.9 *Let $\theta \in (0, 1]$. For any $\delta > 0$, there exists two positive numbers α_δ and r_0 with the following property. For any $T \in (0, \infty)$, and any $t_1 \in [0, T]$, there exists $\varepsilon_1 > 0$ such that for any $0 < \varepsilon < \varepsilon_1$ and initial conditions with $|z^0 - (\tilde{z}^0 - \gamma_1(\tilde{z}^0))| < r_0$, that is, $|\gamma_1(\tilde{z}^0) - c_1^0| < r_0$, the z-coordinate of the solution $(z(t), c_1(t), c_2(t), x_p(t), c(t))$ of (6.17) and the solution $\hat{z}(t)$ of*

$$\frac{d\hat{z}}{dt} = \frac{k(t)}{Z_0} - \kappa\hat{z}, \quad \hat{z}(0) = z^0$$

satisfy

$$|z(t) - \hat{z}(t)| \leq \delta, \ \ on \ [t_1, T].$$

Chapter 7

Future Work

In Section 2.2, we have provided upper and lower bounds on the number of positive steady states of a "futile cycle" of size n. However, there are still many questions remained to answer. For instance, is the upper bound $2n-1$ always achievable? How many positive steady states are there if the activation and deactivation follow mechanisms other than distributive and sequential?

Notice also that there is an apparent gap between the upper bound $2n-1$ for any parameters and the upper bound of $n+1$ (n) if n is even (odd) when the substrate is in excess. If we think the ratio $E_{\text{tot}}/F_{\text{tot}}$ as a parameter ε, then when $\varepsilon \ll 1$, there are at most $n+1$ (n) steady states when n is even (odd), which coincides with the largest possible lower bound. When $\varepsilon \gg 1$, there is a unique steady state. If the number of steady states changes "continuously" with respect to ε, then we do not expect the number of steady states to exceed $n+1$ (n) if n is even (odd). A natural conjecture would be that the number of steady states never exceeds $n+1$ under any condition.

We also plan to develop an abstract approach generalizing the results for "futile cycles", so that we may also handle multistationarity of other ubiquitous motifs arising in biochemistry networks.

In Chapter 3, we showed that multi-timescale non-monotone systems can become monotone once the fast variables are replaced by their steady state values. There are many directions to explore.

The first is to fill in the details when the reduced system has a unique steady state. As mentioned in Remark 3.23, one may obtain convergence, not merely *generic* convergence to the steady state. The proof is simpler in that case since the foliation structure is not needed.

In the other direction, we could drop the assumption of countability and instead provide theorems on generic convergence to the set of equilibria, or even to equilibria if hyperbolicity conditions are satisfied, in the spirit of what is done in the theory of strongly monotone systems.

It will be also interesting to incorporate spatial factors in singularly perturbed monotone systems and to study the associated reaction diffusion systems. On the other hand, numerical approximation would also be useful in applications.

Another exciting direction is to study the system where the fast dynamics is monotone, but not the slow system. In that case, one has almost every or even every trajectory converges to the limiting slow manifold. This is a very appealing property compared to the usual local stability property, since the latter only yields perturbation result for initial conditions close to the limiting slow manifold.

It is also important to see how the results in Chapter 3 can be extended to systems with oscillations.

In Chapter 5, for simplicity we assume boundedness of solutions. However, by the virtue of Lyapunov function, a bootstrap argument could be applied to obtain boundedness of the solutions.

The other direction is to extend the global stability of the constant equilibrium to C^2 norm. In Chapter 5, the stability is in terms of L^1, using a priori estimates for parabolic equations, it is possible to obtain global stability in C^2.

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