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Analysis of Deterministic Cyclic Gene Regulatory Network Models with Delays

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ISSN 2192-6786 ISSN 2192-6794 (electronic)
SpringerBriefs in Electrical and Computer Engineering
ISBN 978-3-319-15605-7 ISBN 978-3-319-15606-4 (eBook)
DOI 10.1007/978-3-319-15606-4

Library of Congress Control Number: 2015931928

Mathematics Subject Classification (2010): 34A34, 34K20, 37C25, 37N25, 92C42, 93C23

Springer Cham Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media
(www.springer.com)

This book is dedicated to
my wife, Emine, M. E. Ahsen
the memory of my father, H. Özbay
my wife, Laura, S.-I. Niculescu

Preface

This brief studies a dynamic model of the vital process of gene regulation. Gene regulation is a way through which cells communicate with their environment. Failures in the gene regulation process may result in serious diseases such as cancer. Moreover, with the newly emerging field of synthetic biology, synthetic circuits are built using gene and gene products. Therefore, accurate modeling and analysis of gene regulatory networks (GRNs) are crucial. In the literature, mathematical models proposed for GRNs include Boolean models, stochastic models, and ordinary differential equation (ODE) based models. In this manuscript, we investigate an ODE-based cyclic GRN model involving static Hill-type nonlinearities and delays. Note that Hill functions are commonly used as nonlinear regulatory functions in the biology literature. An important property of Hill functions is that they have negative Schwarzian derivatives; this fact is exploited in the analysis proposed in the manuscript. The GRN model studied in this volume also contains time delays in the feedback loop, which makes the dynamical system studied an infinite dimensional system. The analysis leads to easily interpretable results for asymptotic stability, oscillations, and bistability.

We investigate the ODE-based GRN model under negative and positive feedback. Negative feedback helps cells respond faster and lower cellular noise. Moreover, it causes oscillations with specific periods. Therefore, negative feedback is a common motif in many biological pathways including those regulating body temperature, blood glucose levels, and circadian cycles. Positive feedback is also common in biological pathways and it is often associated with bistability. Bistability leads to switching behavior, which is important in processes such as cellular differentiation and apoptosis. Given their biological importance, in this manuscript we analyze the GRNs both under negative and positive feedback.

The manuscript is organized as follows. Chapter 1 is devoted to the introduction of various GRN models, starting with Boolean-based models and ending with ODE-based models. Chapter 2 introduces the required mathematical background on systems and control theory as well as the notation that will be used throughout the book. In Chapter 3, a novel analysis of functions with negative Schwarzian derivatives is provided. Readers who are familiar with the control theory and properties of functions with negative Schwarzian derivatives may skip Chapters 2 and 3. Chapter 4 consists of the derivation and basic properties of the model analyzed in the manuscript from the general deterministic ODE-based model of the GRNs introduced in Chapter 1. In Chapter 5, a global stability analysis of the GRN model is performed under negative feedback. A necessary and sufficient condition is derived for the delay-independent global stability of the deterministic ODE-based GRN model. A delay-dependent local stability condition is obtained by extending the so-called secant condition to cyclic systems with delayed feedback. Moreover, lower and upper bounds on the magnitude of the periodic oscillations are given when the system is not stable. Most of the results presented in Chapter 5 are based on our recent work [1]. In Chapter 6, the ODE-based GRN model is studied under positive feedback. It is shown that under positive feedback, generically, the system converges to one of its equilibrium points. Conditions on bistability as well as existence of a unique equilibrium are investigated. Chapter 6 is based on the results of [2] and [3]. Finally, Chapter 7 makes some concluding remarks and points out possible future research directions in this area.

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November 2014	

Acknowledgements

We would like to thank the Managing Editor Dr. Allen Mann, Assistant Editor Chris Tominich, the Series Editors, and anonymous reviewers whose comments helped us improve the manuscript. We also acknowledge discussions with Professor M. Vidyasagar who pointed out important references on this topic. Particular thanks go to some of our collaborators, whose contributions in this subject area helped us directly or indirectly; among them we cite Wim Michiels, B. Misganaw, Constantin-Irinel Morărescu, and Nitin Kumar Singh.

Contents

Acronyms

ARACNE	Algorithm for the Reconstruction of Accurate Cellular Networks
ATP	Adenosine Triphosphate
DM	Delay Margin
DNA	Deoxyribonucleic Acid
EN	Elastic Net
GRN	Gene Regulatory Network
LASSO	Least Absolute Shrinkage and Selection Operator
LTI	Linear Time Invariant
MSE	Mean Square Error
NSD	Negative Schwarzian Derivative
ODE	Ordinary Differential Equations
PM	Phase Margin
RFE	Recursive Feature Selection
RNA	Ribonucleic Acid
mRNA	Messenger RNA
miRNA	Micro RNA
rRNA	Ribosomal RNA
tRNA	Transfer RNA
SISO	Single Input Single Output
SVM	Support Vector Machine
TCGA	The Cancer Genome Atlas

Chapter 1
Introduction

Abstract In this chapter, background material is presented for the gene regulation process and mathematical models of such systems are discussed. In particular, most popular classification and regression methods are briefly mentioned to give an idea on how data collected using microarrays can be used in modeling gene regulatory networks. The chapter ends with the formal definition of the continuous-time ODE-based model with delay to be analyzed in the rest of the book.

Keywords Gene regulatory networks • Modeling • Classification methods Regression methods • ODE-based model • Nonlinear system • Time delay

In this monograph, we will be concerned with one of the most complex processes in nature, namely the gene regulatory mechanism. After decades of research, this subject is not completely well understood. Although we have a system theoretic analysis of gene regulation in this monograph, the authors think that some basic biological knowledge is needed to appreciate the importance of the gene regulation process. The next section gives a brief introduction for this purpose.

1.1 A Brief Glimpse into Biology

Deoxyribonucleic acid (DNA) is the hereditary molecule in almost all living organisms (excluding some viruses). In DNA, genetic information is encoded as a sequence of four nucleotides: adenine (A), guanine (G), cytosine (C), and thymine (T). Most DNA molecules are long polymers which are comprised of double-stranded helical chains coiled round the same axis. These two strands are complementary to each other, i.e. adenine forms hydrogen bonds with thymine and cytosine forms hydrogen bonds with guanine. This double-stranded structure of the DNA was discovered by James Watson and Francis Crick in 1953 [4]. Their seminal paper [4] is only two pages long, but its implications in the study of molecular biology were immense. It is considered one of the greatest scientific achievements in history.

M.E. Ahsen et al., *Analysis of Deterministic Cyclic Gene Regulatory Network Models with Delays*, SpringerBriefs in Electrical and Computer Engineering,
DOI 10.1007/978-3-319-15606-4_1

Within cells, DNA is packaged into compartments called chromosomes. For example, humans have 23 pairs of chromosomes, out of which one pair contains the sex chromosomes responsible for the determination of the sex. The whole set of chromosomes in a cell is called the genome. It is estimated that the human genome consists of 3 billion base pairs of DNA. Ribonucleic acid (RNA) is a biological molecule which has an important role in protein synthesis. Unlike DNA, most RNA is single stranded and consists of the nucleic acids: adenine (A), guanine (G), cytosine (C), and uracil (U). Moreover, RNA contains the sugar ribose, whereas DNA contains the sugar deoxyribose. The three major types of RNA that are of interest to us are: 1) Messenger RNA (mRNA), 2) Transfer RNA (tRNA), and 3) Ribosomal RNA (rRNA), each of which has a role in protein synthesis. Genes are the parts of the DNA that code for proteins and RNA. Proteins are biological molecules consisting of chains of amino acids that are bound together by peptide bonds. The functions of proteins include (i) catalyzers of metabolic reactions, (ii) receptors to environmental stimuli, (iii) building blocks of organelles, and (iv) transportation of molecules. As an example of their catabolic function, we may consider energy production. Cells need energy to perform metabolic processes. The mechanism to produce energy in cells is *ATP synthesis*. In cells, ATP is synthesized in the presence of an enzyme called ATP synthase. In humans, around 1000 ATP molecules are produced per second. Without the presence of the enzyme ATP synthase, it would take days to produce a single molecule of ATP. Therefore, without enzymes the cell would not be able to sustain life. Protein synthesis starts with a process called *transcription*, during which the protein-coding gene is copied into pre-mRNA with the help of an enzyme called RNA polymerase. Then, the coding parts of the gene are separated from the non-coding parts by a process called *RNA splicing*. After the splicing process, the mature mRNA leaves the nucleus. In the mRNA sequence, three nucleotides correspond to a codon. Each codon sequence specifies a unique amino acid, but more than one codon sequence may correspond to the same amino acid. This can also be inferred from the fact that there are only 20 different amino acids but 64 different codon sequences. The amino acids are linked to each other by peptide bonds by ribosomes with the help of tRNA.

According to [5], the human genome has approximately 20 000 protein-coding genes. The human genome project was the first attempt to determine the complete human genome. The project started in 1989, and the first complete genome was announced in 2003. The project cost around 3 billion dollars and it took nearly 14 years to complete. Today, the cost of sequencing has dropped to several thousand dollars, thanks to the advances in the sequencing technology. This decrease in the cost of sequencing resembles Moore’s law for transistors. Owing to this decrease, vast amounts of data have been produced by several researchers across the world. Most of those data is available in public databases such as TCGA (The Cancer Genome Atlas) [6].

The fact that only a portion of the genome codes for protein (it is estimated that less than 2% of the genome codes for protein) poses a challenge in gene regulation: identifying parts of the gene that code for protein is a challenging task, and there is a huge literature devoted to this problem. Mutations in the DNA provide an additional challenge. It is thought that mutations in the protein-coding genes may lead to genetic diseases such as cancer; hence, the determination of the gene sequence is important for the cure of some diseases. Today, researchers are comparing gene sequences of healthy and tumorous tissues to determine which mutations may lead to cancer. The ultimate aim of these studies is to provide personalized medicine, as reasons leading to a disease are generally different between patients. Therefore, understanding gene regulation and the effects of specific genetic mutations on gene regulation are important factors in disease development and cure.

1.2 Gene Regulatory Networks

A Gene Regulatory Network (GRN) can be defined as the interaction of DNA segments (genes) with themselves and with regulatory proteins in the cell. Cells use this regulatory mechanism to communicate with their environment. They respond to environmental stimuli by expressing certain genes. As an example, when bacteria cells are grown in a glucose-deficient but lactose-rich environment, the genes that are responsible for the digestion of lactose are expressed [7]. This way, lactose is digested into glucose; hence, cells are able to produce the energy required for the metabolism from glucose. Another example is the p53 gene, which plays an important role in various vital processes including the initiation of *apoptosis* (the process of programmed cell death). If there exists irreparable damage in the DNA, p53 initiates apoptosis which leads to the death of the infected cell [8]. Therefore, p53 is generally known as tumor suppressor. Thus, in order to cure diseases, gene regulation has to be understood correctly, and reliable mathematical models facilitate this endeavor.

With the help of recent advances in the microarray technology, we can obtain accurate measurements of gene expression levels at a reasonable price. Without going into details, we can simply say that microarrays allow simultaneous measurements of the gene expression levels, under certain experimental conditions. By using the gene expression levels and using statistical tools, researchers try to find biomarkers which may be associated with a specific disease. The most important problem with this approach is the lack of samples (patients). Microarray data consists of expression levels of approximately 20000 genes, whereas the number of samples available is generally several hundreds. This situation is generally referred to as $p \ll n$ case. Depending on the nature of the biological problem, available data can be analyzed using various machine learning methods such as regression, classification, or clustering. In this chapter, we want to give a brief introduction to *classification* and *regression methods* due to their wide range of applications in biology and engineering, as well as other fields such as finance and business management. If the outcome of the events associated with our problem is real valued, we can use available regression methods. Two biological applications of interests are the problems of predicting the time to tumor recurrence from gene expression data, and predicting the response of a patient to the drug. These type of applications have very important benefits to the community such as predicting which treatment will be the best for the patient, especially for a cancer patient, for whom the timely treatment is crucial. One of the most popular regression algorithms is Lasso (Least Absolute Shrinkage and Selection Operator) [9]. It is a linear regression algorithm which minimizes the sum of squares subject to the sum of the absolute value of the coefficients being less than a constant value. Sum of the absolute value of the coefficients is usually known as the ℓ_1-norm. Hence, Lasso formulation is as follows: given A, y, c, find

$$\hat{x}_{Lasso} = \arg\min_x \|y - Ax\|_2 \quad \text{s.t.} \quad \|x\|_1 \leq c. \tag{1.1}$$

The ℓ_1-norm constraint in the Lasso formulation gives it the ability to automatically shrink some coefficients to zero; thus, providing feature selection. The constant c in (1.1) is used as a mean to balance the MSE (Mean Square Error) and the sparsity of the vector x. In the above formulation, the matrix $A \in \mathbb{R}^{m \times n}$ is the measurement matrix, whereas $y \in \mathbb{R}^m$ is the observation vector. In biology, depending on the application, the matrix A can denote continuous variables such as gene expression levels, miRNA (micro RNA), protein expression levels as well as binary variables such as the mutation status of genes. Similarly, the vector y denotes continuous variables such as the time for a tumor to recur after being treated, or binary values such as the stage of a cancer. There are several limitations of Lasso including the number of genes selected is generally less than the number of training points, and the instability of the final feature set when the variables are highly correlated. These deficiencies have been discussed in [10], and to overcome the shortcomings of Lasso the authors introduced EN (Elastic Net) algorithm. The EN formulation is given as follows:

$$\hat{x}_{EN} = \arg\min_x \|y - Ax\|_2 \quad \text{s.t.} \quad \mu\|x\|_1 + (1-\mu)\|x\|_2^2 \leq c. \tag{1.2}$$

In general, the EN algorithm chooses more features than the number of training points. More importantly, among a given set of high correlated variables, EN tends to assign similar weights, whereas Lasso chooses one at random. This makes Lasso very sensitive to measurement noise. In other words, if the data is perturbed slightly, the final feature set selected by Lasso may change dramatically, whereas the final feature set selected by EN remains pretty much the same. This is a clear advantage of EN over Lasso. Moreover, in most practical applications, EN seems to provide more accurate results than Lasso. The Lasso and EN are mostly used in regression problems, where the vector y takes continuous values.

In classification problems, the vector y takes finitely many discrete values (usually binary). To clarify the basic idea in classification we will assume that y is binary valued. A typical formulation of a classification problem is as follows: Suppose we are given a measurement matrix $A \in \mathbb{R}^{m \times n}$, where each row a^i of A consists of measurements of features corresponding to sample i. Moreover, suppose that the m samples are grouped into two sets, which we denote as $\mathcal{M}_1$ and $\mathcal{M}_2$. Without loss of generality, we can assume that the first set $\mathcal{M}_1$ consists of the vectors $a^1, \cdots, a^{m_1}$, and the second set $\mathcal{M}_2$ consists of the vectors $a^{m_1+1}, \cdots, a^{m_1+m_2}$. Here we implicitly assume that $m_1+m_2 = m$. We then construct the vector y by assigning the label $y_i = +1$ to the vectors in the set $\mathcal{M}_1$ and $y_i = -1$ to the vectors in the set $\mathcal{M}_2$. The purpose of a classification problem is to find a *discrimination function* $f : \mathbb{R}^n \to \mathbb{R}$ such that $f(x^i)$ has the same sign as y_i for all i. Moreover, in a biological problem, where one wants to find which features are mostly related to the observed output, one desires to find an appropriate mapping $f(x)$ that uses relatively few features while still maintaining the discriminative ability between the groups $\mathcal{M}_1$ and $\mathcal{M}_2$. The most popular classification algorithm is the well-known Support Vector Machine (SVM), which is introduced in [11]. The basic idea behind SVM can be described as follows. Suppose we are given a set of labeled vectors $\{(a^i, y_i), a^i \in \mathbb{R}^n, y_i \in \{-1, 1\}\}$, we want to find a support vector $w \in \mathbb{R}^n$ and a threshold $\theta \in \mathbb{R}$ such that the discriminant function $f(x) = xw - \theta$ linearly separates the data. In other words, we have

$$a^i w > \theta, \forall i \in \mathcal{M}_1, \quad a^i w < \theta, \forall i \in \mathcal{M}_2.$$

If the data is linearly separable [12], then there exist infinitely many support vectors that separate the data. The SVM chooses the support vector w that minimizes $\|w\|$ for a given norm $\|\cdot\|$ of w. In the original formulation of SVM [11], this norm is chosen as the ℓ_2-norm. Hence, mathematically we can formulate the SVM as follows:

$$\min_w \|w\| \;\text{ s.t. }\; a^i w \geq 1,\ \forall i \in \mathcal{M}_1, \quad a^i w \leq -1,\ \ \forall i \in \mathcal{M}_2. \tag{1.3}$$

The SVM formulation in (1.3) is a convex optimization problem. In particular, if the norm in (1.3) is the ℓ_2-norm, then the problem becomes a quadratic programming problem which can be solved efficiently. Due to the structure of the ℓ_2-norm, the ℓ_2-norm SVM produces a support vector w such that every entry of the vector w is nonzero [12]. Hence, automatic feature selection is not possible in the original SVM formulation. The paper [13] suggests RFE (Recursive Feature Selection) for feature selection in SVM with ℓ_2 in the regularizer, where the authors propose to order the features using their corresponding coordinates in the weight vector w and then remove the one with the least weight. Similar to Lasso, which uses ℓ_1-norm in its regularizer, the ℓ_1-norm SVM, introduced in [14], produces a sparse classifier that linearly separates data. One recent classification algorithm, which takes advantage of the ℓ_1-norm SVM, is called ℓ_1-StaR (Lone Star) [15]. The Lone Star is a two-class classification algorithm which selects statistically significant genes, the number of such genes is generally far less than the number of samples. Similar to ℓ_2-norm SVM RFE algorithm in [13], Lone Star uses RFE with ℓ_1-norm to provide feature selection. The automatic feature selection ability of the ℓ_1-norm

SVM provides reduction of several features in one iteration of Lone Star. Hence, Lone Star converges much faster than its ℓ_2-norm counterpart given in [13].

In both regression and classification problems, a known problem is *overfitting*. Overfitting generally arises when the number of features n is larger than the number of samples m, which is the case for almost all the available biological data sets. When the number of features is larger the number of samples, we can easily find a discriminant function that performs well on the training data but very poorly on test data. In fact, one reason for feature selection is to reduce the risk of overfitting by explaining the data in as few dimensions as possible. Feature selection algorithms that use regularization, including SVM, Lasso, and EN, avoid overfitting to some extent even without feature reduction [13]. Two biological reasons for feature selection are as follows: (i) it is believed that a biological process is affected by a small number of molecules; (ii) we need to generate hypothesis that can be practically validated experimentally.

Another way of using microarray technology is getting time series data for genes of interest and trying to fit a continuous-time model accounting for the dynamical behavior of the regulatory mechanism between the selected genes. Such a specific need is in the design of synthetic networks. In [16], a synthetic oscillatory network has been produced by using gene products, which could be considered as one of the early achievements of the newly emerging field *synthetic biology*. By using tools from synthetic biology, it is envisioned that one will be able to use plants as sensor chemicals in order to produce clean and renewable fuels, or even to recognize cancer cells and destroy them [17]. Therefore, accurate modeling and analysis of gene regulatory networks (GRNs) are important for building synthetic networks. Synthetic networks are designed to serve a specific purpose; for example, they may be designed to function similar to components in the electrical circuits (such as inverters and gates) or they may have oscillations with predefined periods. For all such purposes, techniques from feedback control and system theory can be applied to gene regulatory network models. The study of gene regulation can be divided into two parts. The first part deals with finding accurate models compatible with the biological evidence. This includes estimating the parameters of a dynamical network model from experimental data. The second part deals with the analysis of these models, which includes the robustness analysis of a network with respect to perturbations in the system parameters. This book is about the second part, for a deterministic continuous-time dynamical model. In order to give a bigger picture, in the next section we discuss various GRN models.

1.3 Models for Gene Regulatory Networks

The decrease in the cost of obtaining gene expression data, and the improvement in measurement techniques resulted in vast amounts of data produced by various researchers around the globe. Proper analysis of gene expression data may give us useful insights about the changes in the regulatory networks that may lead to diseases. For example, parts of the regulatory network between a normal person and a cancer patient might be quite different. A differential analysis of the GRN between a healthy person and a patient might give us clues about the treatment of that particular disease. In this section, we will briefly summarize three methods on the analysis of gene regulatory networks: (1) Boolean Networks (2) Reverse Engineering Methods (3) Continuous-Time models. Out of these three methods, Reverse Engineering Methods are used to identify which genes are interacting with each other by using the available steady state gene expression data. In this way, it serves as a guide for building dynamical models from time series data using the other two methods mentioned. The focus of the current monograph will be on the continuous-time models, i.e. ODE models representing GRNs. Let us now briefly describe these three methods.

1.3.1 Boolean Networks

Boolean networks consist of Boolean variables which take only discrete values. Usually, when the biological entity is active it takes the value "1" and when inactive it takes the value "0." The input to each Boolean variable is a Boolean function of a subset of the variables. The output of this Boolean function determines the output (state) of the corresponding Boolean variable. In Boolean networks, the time is discretized as well; hence, the states of each variable is updated at each step according to its Boolean function. In the Boolean network representation of GRNs, each node corresponds to a gene or gene product. Therefore, the number of nodes (N) in the network is equal to the number of genes or gene products under consideration. At any time, the state of a node is "1" if the corresponding gene is expressed and it is "0" otherwise. Therefore, at any given time instant the network is at one of its 2^N possible states. Due to its discrete nature, Boolean networks do not capture the dynamic nature of the underlying GRN. So, when the GRN under consideration is small and only qualitative information is available, Boolean networks can provide researchers useful knowledge about the existence and nature of steady states. Therefore, if one needs to analyze dynamical features of the GRN such as the period of oscillations or effect of delays on the network, it will be better to use continuous-time models. Usually, Boolean networks are used when the regulatory network is not known or partially known. Boolean networks are easier to analyze as they have a predetermined number of states. The regulatory relationships between the genes can be found using the experimental data. Also, note that we may choose to analyze the global network or we may focus on a local part of the network. For an in-depth analysis of Boolean gene regulatory network models, see, for instance, [18].

1.3.2 Reverse Engineering Gene Regulatory Networks

The primary aim of reverse engineering GRN modeling is to understand how gene and gene products within a cell interact with each other. In this method, gene and gene products are considered as random variables. The network is then constructed by using the dependence structure between these random variables (genes and gene products). The joint probability distribution functions between genes are estimated using the experimental steady state data. Depending on the algorithm used, we may end up with a directed or an indirected network. Reverse engineering allows deducing dependence structure between genes and gene products using the estimated probability distributions. The resulting network obtained by this method does not give much information about the dynamical behavior of the network. It facilitates the process of building a dynamical model that represents the system to be investigated by giving information about the interaction of genes. Therefore, this method is most suitable if we do not have much idea about the interactions in the system. To give a more detailed description of the reverse engineering method, let $A \in \mathbb{R}^{p \times n}$ represent the gene expression data. Here, the integer n corresponds to the number of features (genes and gene products) and p corresponds to the number of samples. As mentioned earlier, when this approach is considered, it is necessary to be sure that samples have the same context (e.g., each sample should have lung cancer). In the context of specific geno-wide networks, the number of genes under study is in the order of 20000, whereas the number of samples is around 500, so $p \ll n$ as mentioned before. Consider now the expression levels of the genes as random variables $A_1, \ldots, A_n$, so that each column of the matrix A corresponds to independent realizations of the random variable A_i. Since $p \ll n$, the joint distribution of the random variables cannot be reliably inferred from the data. Therefore, the aim of the reverse engineering algorithms is to capture at least the dependence structure in the network. In order to do that, first, the joint distribution of the random variables A_i and A_j is estimated using the matrix X. One such popular reverse engineering algorithm is the so-called ARACNE (Algorithm for the Reconstruction of Accurate Cellular Networks) [19], which uses mutual information in order to deduce the independence structure in the network. Since the mutual information is a symmetric quantity, the network produced by the ARACNE algorithm does not have a direction. In [20], the authors deal with this restriction by using another information theoretic quantity known as the ϕ-mixing coefficient. For two random variables A_i and A_j assuming values in finite sets $\mathbb{A}$ and $\mathbb{B}$, the ϕ-mixing coefficient is defined as follows:

$$\phi(A_i|A_j) := \max_{S \subseteq \mathbb{A}, T \subseteq \mathbb{B}} |\Pr\{A_i \in S|A_j \in T\} - \Pr\{A_i \in S\}|.$$

For discrete random variables, a closed form formula for the ϕ-mixing coefficient is proven in [21]. Therefore, computation of the ϕ-mixing is efficient. The ϕ-mixing coefficient also satisfies the data processing inequality [22], which is used to infer the independence structure of the network. Moreover, it can be easily verified that

$\phi(A_i|A_j) \neq \phi(A_i|A_j)$. Therefore, the resulting network is directed. Furthermore, the resulting graph is also weighted with each weight being equal to the ϕ-mixing coefficient between the corresponding nodes. Reverse engineering methods are generally used for identifying biological pathways that may occur, or to identify genes that have high connectivity so can serve as biomarkers related to a disease.

1.3.3 Continuous-Time Models

In this section, we define the network model which will be the main emphasis of the current monograph. We will investigate a *Continuous-Time Nonlinear Cyclic Model* representing GRNs. Before going into details, we would like to note that there is an inherent stochasticity, which is denoted as noise, in gene regulation. The paper [16] was among the first to observe noise in gene expression; it includes an experimental observation that identical cells grown up in identical conditions have huge differences in their expression levels. The authors claim that this phenomenon is more visible when the biological components (genes) are low in number. In [16], the noise is decomposed into two orthogonal parts. The first part of the noise is called the intrinsic noise, which is inherent in the biochemical process of gene expression. The second part is called as extrinsic noise which is due to the fluctuations in the external environment. Having said that, deterministic models are useful especially when the number of molecules under study is very large or one considers averages of a population of cells. Deterministic models also give information about the structure of the network such as existence of oscillations, and existence of stable equilibrium points. Furthermore, the tools of control theory (see, e.g., [23] for an introduction, and [24] for various methods developed for time delay systems) give information about the stability range of the system. Therefore, deterministic models should be studied along with the stochastic models to have a good idea of the system under consideration. Different ODE modeling approaches for gene regulatory networks have been compared in [25]. Dynamic patterns of gene regulation have been investigated in [26] for the two-gene system. For a review of data integration in dynamic models of gene regulatory networks, see, e.g., [27].

In this manuscript, we investigate global stability of a cyclic dynamical model accounting for the GRN which contains a nonlinear feedback loop and time delays. The dynamical model studied is described in Figure 1.1.

The GRN model shown in Figure 1.1 represents a cyclic connection of subsystems $\Sigma_1, \ldots, \Sigma_m$, where the input of Σ_i is the output of Σ_{i-1} for $i = 2, \ldots, m$, and the feedback connection is established by defining the input of Σ_1 as the output of Σ_m. In the model, each subsystem Σ_i consists of series connections of a stable system Σ_{pi} whose delayed output $p_i(t - \tau_{pi})$ is the input of a stable system Σ_{gi}

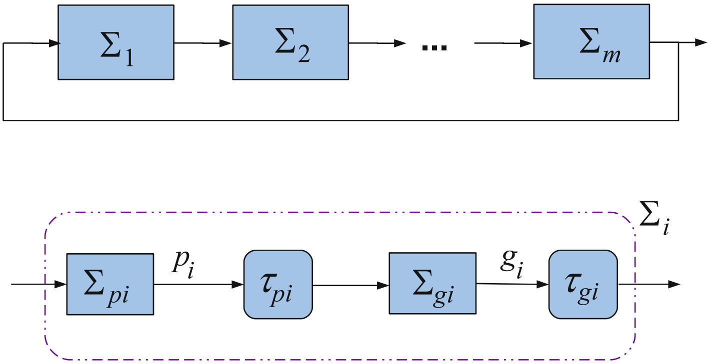


Fig. 1.1 A continuous-time model of GRN.

which generates $g_i(t)$, and the output of Σ_i is $g_i(t-\tau_{gi})$. More precisely, the model is given by a set of differential equations in the following form:

$$
\begin{aligned}
\dot{p}_1(t) &= -k_{p1}p_1(t) + f_{p1}(g_m(t-\tau_{gm})) \\
\dot{g}_1(t) &= -k_{g1}g_1(t) + f_{g1}(p_1(t-\tau_{p1})) \\
&\vdots \\
\dot{p}_m(t) &= -k_{pm}p_m(t) + f_{pm}(g_{m-1}(t-\tau_{g_{m-1}})) \\
\dot{g}_m(t) &= -k_{g1}g_m(t) + f_{gm}(p_m(t-\tau_{pm})),
\end{aligned}
\tag{1.4}
$$

with appropriate initial conditions. The variables $p_i(t)$ and $g_i(t)$ represent the protein and mRNA concentrations, respectively. The parameters k_i represent the degradation rate of the corresponding biological entity.

Models in the general structure of (1.4) are frequently encountered in the modeling of biological processes such as mitogen-activated protein cascades and circadian rhythm generator, see, e.g., [28–30] and [31]. To account for the switch-like phenomena observed in gene regulation, the nonlinear regulation functions are often approximated by Hill functions [32, 33]. In [34], the authors analyze the system (1.4) and prove a local stability result by including explicit information on the value of the time delay. Again, for the local stability of this system, an explicit computation of the upper bound of the delay value is performed in [35]. For double-gene version of the above system with four cascade sub-blocks with four delays, center manifold theory is used in [36] to investigate the existence of Hopf bifurcation. Another challenge for such networks is the estimation of the network parameters from time series data. A work in this direction is [37], where Hill functions are taken as nonlinearities, and the coefficients of specific Hill functions are estimated from experimental data. Apart from the GRN literature, models similar to (1.4) are also found in the neural networks literature. For example, in [30], system (1.4) has been considered with nonlinearities as tangent hyperbolic functions.

In this manuscript, we assume that the functions f_{pi} and f_{gi} are nonlinear and have negative Schwarzian derivatives [38]. For example, Hill functions and tangent hyperbolic function have negative Schwarzian derivatives. A linear model of the repressilator (a special type of GRN) has been analyzed in [39] by using the Schwarzian derivative concept. In [40] a model similar to (1.4) is analyzed; however, the model considered in [40] takes the dynamics for the protein concentration as a linear system, where f_{pi} is the identity operator.

In this study, the nonlinear functions f_{pi} appearing in (1.4) are taken to be general functions with negative Schwarzian derivatives (can be other than Hill functions); so, the results of the present study do not apply to the model studied in [40]. The system (1.4) under single time delay and negative feedback has been studied in [41], where an easy condition for guaranteeing asymptotic stability has been obtained by using the arguments of [42, 43] to embed the original system (1.4) to a discrete-time system. It is worth mentioning that the stochastic behavior of the system does not change the general average behavior of the system, but it may change the equilibrium points of the system quite significantly. Therefore, deterministic models will give us a general behavior of the system, and they are sufficient in most of the cases such as designing synthetic networks.

Chapter 2
Basic Tools from Systems and Control Theory

Abstract This chapter sets up the notation for the rest of the book and introduces basic concepts from control theory for the readers who are not familiar with fundamental feedback stability analysis techniques. Delay-differential equations are considered, and the small gain theorem is given for a delay independent stability condition for linear feedback systems. The Nyquist criterion is given in order to derive the necessary and sufficient conditions for delay dependent stability of such systems.

Keywords Functional differential equations • Equilibrium points • Linear time invariant systems • Systems with time delay • Small gain theorem • Nyquist stability test • Delay margin

2.1 Preliminary Definitions and Notations

In this section, we set up the notations for the rest of the book. Although most of the results presented in this chapter can be generalized to any inner product space, we consider the vector space $\mathbb{R}^n$ equipped with the usual Euclidean norm defined as

$$||x|| = \sqrt{x_1^2 + \ldots + x_n^2}, \quad \text{for} \quad x = (x_1, \ldots, x_n)^T \in \mathbb{R}^n. \tag{2.1}$$

A subset K of the vector space $\mathbb{R}^n$ over the field $\mathbb{R}$ is called a convex cone if for any scalars α , $\beta \in \mathbb{R}_+$ and vectors $x, y \in K$ we have

$$\alpha x + \beta y \in K. \tag{2.2}$$

Since the biological variables, such as the expression levels of genes, enzymes, and mRNA, take positive values, the systems to be considered are analyzed in the cone $\mathbb{R}^n_+$, which is defined as

$$\mathbb{R}^n_+ = \{x \in \mathbb{R}^n : x_i \geq 0 \quad \forall i = 1, 2, \ldots, n\}. \tag{2.3}$$

M.E. Ahsen et al., *Analysis of Deterministic Cyclic Gene Regulatory Network Models with Delays*, SpringerBriefs in Electrical and Computer Engineering,
DOI 10.1007/978-3-319-15606-4_2

For an interval I of the real line, $int(I)$ denotes the interior of I. The symbol $\mathbb{C}$ will denote the set of complex numbers and the set $\overline{\mathbb{C}}_+$ is defined as

$$\overline{\mathbb{C}}_+ = \{s \in \mathbb{C} : Re(s) \geq 0\}. \tag{2.4}$$

For a function

$$f(x) : K \to K, \tag{2.5}$$

where K is any set, $f^n(x)$ will denote the function which is the composition of $f(x)$ with itself n times. Given an interval $I \subseteq \mathbb{R}$, $D^n(I)$ will denote the set of n times continuously differentiable functions defined on the interval I. A function $f(x)$ defined from the normed linear space X to the normed linear space Y is bounded if

$$\text{there exists } M \geq 0 \quad \text{such that} \quad ||f(x)||_Y \leq M||x||_X, \quad \forall x \in X. \tag{2.6}$$

A complex valued function $F(s)$ is said to belong to the set $\mathscr{H}^\infty$ if it is analytic and bounded in $\mathbb{C}_+$. The set $\mathscr{H}^\infty$ is a commutative ring with unity over itself. For a function $F(s) \in \mathscr{H}^\infty$, the infinity norm of F denoted as $||F||_\infty$ is defined as follows:

$$||F||_\infty = \operatorname*{ess\ sup}_{s \in \mathbb{C}_+} |F(s)|. \tag{2.7}$$

Note that this definition makes sense since $F(s)$ is bounded and analytic in $\mathbb{C}_+$.

Let $x(t) \in \mathbb{R}^n$ be a vector function depending on the variable t. The notation $\dot{x}(t)$ stands for $\frac{d}{dt}x(t)$, i.e., the derivative of x with respect to t. A point $y \in \mathbb{R}^n$ is said to be an *omega point* of $x(t)$ if there is an increasing sequence $0 < t_i \to \infty$ and we have

$$\lim_{t_i \to \infty} (x(t_i)) = y.$$

The *omega limit set* of the solution $x(t)$ is the set of omega points of $x(t)$ and will be denoted by $\omega(x(t))$. Similarly, a point $y \in \mathbb{R}^n$ is said to be an *alpha point* of $x(t)$ if there is a decreasing sequence $0 > t_i \to -\infty$ and we have

$$\lim_{t_i \to -\infty} (x(t_i)) = y.$$

The *alpha limit set* of the solution $x(t)$ is the set of alpha points of $x(t)$ and will be denoted by $\alpha(x(t))$.

2.2 Linear Time Invariant Systems

Linear systems can be used to accurately model most of the systems we encounter in engineering, economics, and other fields of science. A retarded linear time invariant (LTI) autonomous system with a single delay has the following state space representation:

$$\dot{x}(t) = A_0 x(t) + A_1 x(t - \tau), \qquad \tau > 0, \tag{2.8}$$

where $A_0,\ A_1 \in \mathbb{R}^{n \times n}$ and $x(t) \in \mathbb{R}^n$. Although the results presented in this section can easily be generalized to multiple delay case, single delay case will be considered here since the mathematical models discussed in Chapters 5 and 6 fit in such a class.

Definition 2.1. The characteristic function $\chi(s)$ associated with the system (2.8) is given by

$$\chi(s) = \det(sI - A_0 - A_1 e^{-\tau s}), \tag{2.9}$$

where $\det(\cdot)$ denotes the determinant of a square matrix.

Definition 2.2. The characteristic function (2.9) is said to be *stable* if

$$\chi(s) \neq 0, \qquad \forall s \in \overline{\mathbb{C}}_+. \tag{2.10}$$

The system (2.8) is said to be stable if its characteristic function is stable. The system (2.8) is said to be stable independent of delay if it is stable for all $\tau \geq 0$.

Assume that in (2.8) the matrix A_1 is in the form: $A_1 = BC$ where the dimensions of the matrices B and C are $n \times 1$ and $1 \times n$, respectively. Then, the dynamical system given in equation (2.8) can be re-written as

$$\begin{aligned} \dot{x}(t) &= A_0 x(t) + Bu(t) \\ y(t) &= Cx(t) \end{aligned}$$

with the time delayed feedback

$$u(t) = y(t - \tau).$$

The characteristic equation of this SISO time delayed feedback system is in the following form:

$$\chi(s) = P_0(s) + P_1(s) e^{-\tau s} = 0, \tag{2.11}$$

where $P_0(s) = det(sI - A_0)$ and $P_1(s) = C\ adj(sI - A_0)\ B$. Note that $P_0(s)$, $P_1(s)$ are polynomials of degree n and $n-1$, respectively. If $P_0(s)$ and $P_1(s)$ in (2.11) do not have a common zero in $\overline{\mathbb{C}}_+$, then for any $s_0 \in \overline{\mathbb{C}}_+$ we have

$$\chi(s_0) = 0 \qquad \Longleftrightarrow \qquad 1 + \frac{P_1(s_0)e^{-\tau s_0}}{P_0(s_0)} = 0. \tag{2.12}$$

In fact, the above condition is equivalent to having

$$1 + G(s)|_{s=s_0} = 0 \quad \text{where} \quad G_0(s) = C(sI - A_0)^{-1}B, \quad G(s) = G_0(s)e^{-hs}. \tag{2.13}$$

The result below is known as the Nyquist stability test (see, e.g., [23]); for us, it will be very useful in deriving local stability conditions.

Proposition 2.1. *Let $G(s)$ and $G_0(s)$ be as defined in (2.13), and assume that A_0 does not have any eigenvalues on the imaginary axis, and let n_u be the number of its eigenvalues in $\mathbb{C}_+$. The characteristic equation $1 + G(s) = 0$ is stable if and only if the Nyquist graph (i.e., the closed path obtained by plotting $G(j\omega)$ as ω increases from $-\infty$ to $+\infty$) encircles -1 exactly n_u times in the counterclockwise direction.*

The proof of the Nyquist test comes from Cauchy's Theorem, and the case where A_0 does have imaginary axis eigenvalues can also be handled by a slight modification, see, e.g., [23]. For the GRN models considered in the next chapters, all the eigenvalues of A_0 are in $\mathbb{C}_-$, i.e. $n_u = 0$; so, we have stability of the characteristic equation if and only if the Nyquist graph does not encircle -1. The next result is known as the Small Gain Theorem, and it can be obtained from Proposition 2.1.

Proposition 2.2. *Let $G(s), H(s) \in \mathscr{H}^\infty$ such that $\|GH\|_\infty < 1$. Then, the characteristic function $\chi(s) = 1 + G(s)H(s)e^{-\tau s}$ is stable for all $\tau \geq 0$.*

Proof. For fixed τ, we know that the characteristic function

$$\chi(s) = 1 + G(s)H(s)e^{-\tau s}$$

is stable if

$$\chi(s) \neq 0, \qquad \forall s \in \mathbb{C}_+.$$

Suppose for some $s_0 \in \mathbb{C}_+$, we have

$$\chi(s_0) = 1 + G(s_0)H(s_0)e^{-\tau s_0} = 0$$

$$\Rightarrow G(s_0)H(s_0)e^{-\tau s_0} = -1$$

$$\Rightarrow |G(s_0)H(s_0)| \geq 1$$
$$\Rightarrow \|GH\|_\infty \geq 1,$$

which contracts the fact that $\|GH\|_\infty < 1$. □

As a corollary of Proposition 2.2, we have the following result.

Lemma 2.1. *Let $G(s) \in \mathscr{H}^\infty$, then for all $|k| < \|G\|_\infty^{-1}$ the characteristic equation*

$$\chi(s) = 1 + kG(s)e^{-\tau s} \tag{2.14}$$

is stable independent of delay.

Proof. Let $H(s) = k$, then we have

$$||GH||_\infty < 1$$

and the result follows from Proposition 2.2. □

Consider a characteristic function of the form (2.14), then $\omega_c > 0$ is called a *gain crossover frequency* of the characteristic equation if it satisfies

$$|kG(j\omega_c)| = 1. \tag{2.15}$$

Similarly, ω_g is a *phase crossover frequency* of the characteristic equation if

$$\angle k + \angle G(j\omega_g) = -\pi\ . \tag{2.16}$$

A characteristic equation of the following form is analyzed next:

$$\chi(s) = 1 + kG(s)e^{-\tau s} = 1 + \frac{ke^{-\tau s}}{(s+a_1)\dots(s+a_n)} = 0\ , \quad k \in \mathbb{R}, \quad a_i \in \mathbb{R}_+. \tag{2.17}$$

Suppose for $k > 0$ and $\tau = 0$ the characteristic function $\chi(s)$ is stable; then, the *phase margin*, PM $:= \pi + \angle G(j\omega_c)$, is positive. Note that time delay τ introduces a phase drop of $\tau\omega$ radians at each frequency $\omega > 0$. So, we define the *delay margin* (abbreviated as DM) as DM $=$ PM$/\omega_c$. For all τ less than the delay margin, the phase margin remains to be positive and stability is preserved. The next result explicitly computes the DM for $k > 0$, and it also determines the values of k for which we have delay independent stability, or instability.

Proposition 2.3. *Consider a characteristic equation in the form* (2.17) *and let*

$$K_l = \prod_{i=1}^{n}(a_i). \tag{2.18}$$

Then, the following statements hold:

1. *If* $0 < k \leq K_l$*, then* $\chi(s)$ *is stable independent of delay.*
2. *If* $K_l < k < K_u$*, then* $\chi(s)$ *is stable for all* $0 \leq \tau < \tau_m$ *and unstable for all* $\tau \geq \tau_m$*, where* τ_m *is the delay margin and it is computed as*

   $$\tau_m = \frac{1}{\omega_c}\left(\pi - \sum_{i=1}^{n} \arctan(\frac{\omega_c}{a_i})\right),$$

   where $\omega_c > 0$ *is the unique gain crossover frequency satisfying*

   $$\prod_{i=1}^{n}(\omega_c^2 + a_i^2) = k^2,$$

   and K_u *is given by the following formula*

   $$K_u = \sqrt{\prod_{i=1}^{n}(\omega_g^2 + a_i^2)},$$

   where $\omega_g > 0$ *is the smallest* ω *satisfying*

   $$\sum_{i=1}^{n} arctan\left(\frac{\omega}{a_i}\right) = -\pi.$$

3. *If* $k \geq K_u$*, then* $\chi(s)$ *is unstable independent of delay.*
4. *If* $-K_l < k < 0$*, then* $\chi(s)$ *is stable independent of delay.*
5. *If* $k \leq -K_l$*, then* $\chi(s)$ *is unstable independent of delay.*

Proof. For fixed $a_i \in \mathbb{R}_+$, let $\chi(s)$ be a characteristic equation in the form (2.17). Simple algebraic manipulations show that

$$1 = \left|\frac{ke^{-\tau j\omega_c}}{(j\omega_c + a_1)\ldots\ldots(j\omega_c + a_n)}\right| \iff \prod_{i=1}^{n}(\omega_c^2 + a_i^2) = k^2 .$$

But $h(\omega) = \prod_{i=1}^{n}(\omega^2 + a_i^2)$ is an increasing function of ω and

$$h(0) = \prod_{i=1}^{n}(a_i^2), \qquad h(\infty) = \infty.$$

Therefore if $k > K_l$, there exists unique $\omega_c > 0$ satisfying

$$\prod_{i=1}^{n}(\omega_c^2 + a_i^2) = k^2.$$

Let $G_\tau(s)$ be defined as

$$G_\tau(s) = \frac{e^{-\tau s}}{\prod_{i=1}^{n}(s + a_i)}.$$

If k satisfies the condition given in parts 1 and 4, then the Nyquist graph of $kG_\tau(j\omega)$ does not encircle -1, for all $\tau \geq 0$; hence, the results follow from Proposition 2.1. For the proof of parts 2 and 3, suppose that

$$k > \prod_{i=1}^{n}(a_i) = K_l$$

and consider the delay free system

$$F(s) = 1 + kG_0(s). \tag{2.19}$$

Since the roots of the characteristic function $F(s)$ depends continuously on the parameter k, it can be concluded that $F(s)$ is stable for all $k < K_u$ where K_u is the smallest positive number such that the characteristic equation

$$1 + K_u G(s) = 0 \tag{2.20}$$

has a root on the imaginary axis [44]. That is, there exists $\omega_g > 0$ such that

$$\begin{aligned} 1 + K_u G_0(j\omega_g) &= 0 \\ &\Rightarrow K_u = -\prod_{i=1}^{n}(j\omega_g + a_i) \\ &\Rightarrow K_u = \sqrt{\prod_{i=1}^{n}(\omega_g^2 + a_i^2)}, \end{aligned}$$

where ω_g is the smallest positive number satisfying

$$\sum_{i=1}^{n} arctan\left(\frac{\omega_g}{a_i}\right) = -\pi.$$

If $k \geq K_u$, then the Nyquist plot of the delay free system encircles the point -1 and the delay free system is unstable by Proposition 2.1. Moreover, if

$$K_l < k < K_u,$$

then the delay free system is stable. By the continuous dependence of the roots with respect to the parameter τ, we know that the system will be stable for all $\tau \in [0\ ,\ \tau_m)$ where τ_m is the smallest positive number such that for $\tau = \tau_m$ the characteristic function $\chi(s)$ has a root on the imaginary axis [44]. That is, there exists $\omega_c > 0$ satisfying

$$1 + \frac{k e^{-j\tau_m \omega_c}}{\prod_{i=1}^{n}(j\omega_c + a_i)} = 0,$$

where ω_c is the unique frequency satisfying

$$\prod_{i=1}^{n}(\omega_c^2 + a_i^2) = k^2$$

and τ_m is given by

$$\tau_m = \frac{1}{\omega_c}\left(\pi - \sum_{i=1}^{n} arctan(\frac{\omega_c}{a_i})\right).$$

Note that τ_m depends on k and we have

$$\tau_m(K_u) = 0, \qquad \tau_m(K_l) = \infty. \tag{2.21}$$

If $\tau \geq \tau_m$, we have

$$\angle(k e^{-\tau j \omega_c} G(j\omega_c)) < -\pi\ , \tag{2.22}$$

because both

$$\tau\omega, \qquad \sum_{i=1}^{n} arctan(\frac{\omega}{a_i}) \tag{2.23}$$

are increasing functions of ω. But (2.22) and (2.23) imply that for $\tau \geq \tau_m$ the Nyquist plot of $kG_\tau(j\omega)$ will encircle the point -1 at least once so $\chi(s)$ is unstable for $\tau \geq \tau_m$. For part 3 of the Proposition, it is shown that if

$$k \geq K_u,$$

then the delay free system is unstable. Using the same arguments as part 2 of the Proposition, the system will remain unstable as delay increases. For the proof of part 5, note that for $k \leq -K_l$ and any positive delay we have

$$\chi(0) \leq 0, \qquad \chi(\infty) = 1 \qquad \forall \tau \geq 0.$$

Intermediate theorem implies that there exists a real number $y \geq 0$ such that

$$\chi(y) = 0 \quad \forall \tau \in \mathbb{R}_+. \tag{2.24}$$

Equation (2.24) proves that the characteristic function is unstable independent of the delay. □

Remark 2.1. A different way to prove the results of Proposition 2.3 can be summarized as follows: (i) derive conditions under which delay free system is stable, (ii) detect crossover frequencies and related critical delay values (that is determining the cases when the characteristic equation has roots on the imaginary-axis), (iii) then by using the continuity argument with respect to the delay parameter the results follow. For further discussions on such an approach see, e.g., [24].

Although the GRN model analyzed in this monograph contains nonlinearities, Proposition 2.3 is needed to determine the stability of the linearized systems. It is worth mentioning that the linearized system and nonlinear system have similar local behavior around the vicinity of an equilibrium point of the nonlinear system.

2.3 Functional Differential Equations

Many of the processes we observe in the nature cannot be accurately modeled by means of linear systems. Such processes involve nonlinearities, and many of them involve time delays as well. In this case, the mathematical models can be in the form of functional differential equations. We need to present some results that are widely used in the analysis of functional differential equations. A general model for a nonlinear system is given by

$$\dot{x} = f(t, x(t), x(t-\tau)), \qquad t \in \mathbb{R}_+,\ x(t) \in \mathbb{R}^n,\ f : \mathbb{R} \times \mathbb{R}^n \times \mathbb{R}^n \to \mathbb{R}^n. \tag{2.25}$$

Most of the physical systems that are modeled are casual. Therefore, we assume in (2.25) that

$$\tau \geq 0. \tag{2.26}$$

If the function $f(t, x(t), x(t-\tau))$ in (2.25) does not explicitly depend on t, the system (2.25) is called autonomous. Otherwise, it is called non-autonomous. The

GRN model studied in this work represents an autonomous system. Therefore, in this subsection only autonomous systems are considered. For the rest of this subsection, assume that our system has the following general form:

$$\dot{x} = f(x(t), x(t-\tau)), \qquad x(t) \in \mathbb{R}^n,\ \tau \geq 0,\ f : \mathbb{R}^n \times \mathbb{R}^n \to \mathbb{R}^n. \tag{2.27}$$

To find a solution of a functional differential equation (2.27), we need to know initial values of the states. It is clear that for delay free system, the vector $x(t_0) \in \mathbb{R}^n$, where $t_0 \in \mathbb{R}$ is the initial time, determines $x(t)$, for $t \geq t_0$. On the other hand, for systems with time delay, to find a unique solution, $x(t)$, for $t \geq t_0$, it is necessary to know

$$x(\theta) \qquad \text{for} \qquad t_0 - \tau \leq \theta \leq t_0. \tag{2.28}$$

An excellent book on the analysis of functional differential equations is [45], and it also contains results on the existence, uniqueness, and continuous dependence of the solutions on initial conditions (these topics are beyond the scope of this manuscript). The GRN model to be analyzed here satisfies the technical conditions given in [45] so that it has a unique solution which depends continuously on initial conditions.
Note: *In all the simulation examples given in this book, the initial time instant is taken to be* $t_0 = 0$ *and initial conditions are taken to be constant, i.e.,* $x(\theta) = x(0)$ *for all* $\theta \in [-\tau\ ,\ 0]$. *Hence, for the sake of brevity, only* $x(0)$ *will be explicitly specified in the examples.*

Another concept related to the analysis of functional differential equations is the *equilibrium point*. A constant vector $x_e \in \mathbb{R}^n$ is called an equilibrium point of the system (2.27), if $f(x_e, x_e) = 0$. The linearization of system (2.27) around the equilibrium point x_e leads to the following:

$$\dot{\tilde{x}}(t) = A\tilde{x}(t) + B\tilde{x}(t-\tau), \qquad \tilde{x}(t) = x(t) - x_e, \quad A,\ B\ \in \mathbb{R}^{n \times n}, \tag{2.29}$$

where

$$A_{i,j} = \left.\frac{\partial f_i}{\partial x_j}\right|_{x=x_e}, \ B_{i,j} = \left.\frac{\partial f_i}{\partial x_j(t-\tau)}\right|_{x=x_e}. \tag{2.30}$$

The linearization of a nonlinear system around its equilibrium points plays an important role in the analysis of functional differential equations. In fact, we know that if the characteristic equation of the linearized system (2.29) is stable, then the equilibrium point around which the linearization is done is locally stable. In other words, the solutions with initial conditions in some neighborhood of the equilibrium point converge to the equilibrium point. In some special cases, one can conclude satisfactory information regarding the general behavior of a system by just looking at the linearization of it around its equilibrium points.

2.4 Exercises

Problem 1. Let A_0 and $A_1 = BC$ be given by

$$A_0 = \begin{bmatrix} -1 & 1 & 0 \\ 0 & -2 & 2 \\ 0 & 0 & -3 \end{bmatrix}, \quad B = [0 \ \ 0 \ \ 1]^{\mathrm{T}}, \quad C = [3 \ \ 0 \ \ 0].$$

Determine $G_0(s) = C(sI - A_0)^{-1}B$. Compute $\chi(s) = \det(sI - A_0 - A_1 e^{-\tau s})$, and verify that $\chi(s) = 0$ if and only if $1 + G(s) = 0$ where $G(s) = G_0(s)e^{-\tau s}$.

Hint: You may use the matrix inversion lemma.

Problem 2. Let $G_0(s) = \frac{k}{(s+1)(s+4)}$, with $k \in \mathbb{R}$. Determine the range $k \in (k^- \ , \ k^+)$ such that the characteristic equation $1 + G_0(s)e^{-\tau s} = 0$ is stable independent of delay $\tau \geq 0$.

Problem 3. Let $G_0(s) = \frac{10}{(s+1)(s+4)}$, determine $\tau_{\max} > 0$ such that the characteristic equation $1 + G_0(s)e^{-\tau s} = 0$ is stable for all $\tau \in [0 \ , \tau_{\max})$.

Problem 4. Consider the nonlinear system

$$\begin{aligned} \dot{x}_1(t) &= -x_1(t) + \frac{6}{2 + x_2^2(t)} \\ \dot{x}_2(t) &= -x_2(t) + \frac{4x_1^2(t-\tau)}{1 + x_1^2(t-\tau)}, \end{aligned}$$

where $\tau > 0$. Find the unique equilibrium point of this system, and obtain the linearized system around this equilibrium.

Problem 5. Consider the nonlinear system

$$\begin{aligned} \dot{x}_1(t) &= -x_1(t) + f(x_2(t)) \\ \dot{x}_2(t) &= -x_2(t) + f(x_1(t-\tau)), \end{aligned}$$

where $\tau > 0$ and $f(x) = \frac{2}{0.1+x^2}$. Determine all equilibrium points of this system.

Chapter 3
Functions with Negative Schwarzian Derivatives

Abstract This chapter is devoted to the analysis on functions with NSD (negative Schwarzian derivatives). First, basic properties of functions with NSD are given and a classification result is proven for such functions. Then, an analysis is made on the fixed points for functions with NSD.

Keywords Schwarzian derivatives • Hill functions • Tangent hyperbolic functions • Fixed points

This chapter is devoted to the analysis of functions with NSD (negative Schwarzian derivatives) and consists of two sections. In the first section, basic properties of functions with NSD are given and a classification result is proven for such functions. The second section contains an analysis on the fixed points of functions with NSD.

3.1 Classification of Functions with Negative Schwarzian Derivatives

We start this section with the definition of the Schwarzian derivative. Since the biological entities take only positive values, we will implicitly assume that the functions considered here have their domain and range as positive real numbers. Let a function f be defined from $\mathbb{R}_+$ to $\mathbb{R}_+$. The definition of Schwarzian derivative implicitly requires a function to be at least three times differentiable. For this reason, suppose f is at least three times continuously differentiable, with $f'(x)$, $f''(x)$, $f'''(x)$ representing its first, second, and third derivatives. Then, the *Schwarzian derivative* of the function $f(x)$, see [38], denoted as $Sf(x)$, is defined as:

$$Sf(x) = \begin{cases} -\infty & \text{if } f'(x) = 0 \\ \dfrac{f'''(x)}{f'(x)} - \dfrac{3}{2}\left(\dfrac{f''(x)}{f'(x)}\right)^2 & \text{if } f'(x) \neq 0. \end{cases} \tag{3.1}$$

The following result can be deduced from the definition (3.1).

M.E. Ahsen et al., *Analysis of Deterministic Cyclic Gene Regulatory Network Models with Delays*, SpringerBriefs in Electrical and Computer Engineering,
DOI 10.1007/978-3-319-15606-4_3

Proposition 3.1. *Let $I \subseteq R$ be an interval and suppose $f, g \in D^3(\mathbb{R}_+)$ such that the function $f \circ g(x)$ is well defined. Suppose also that we have*

$$f'(x) \neq 0 \qquad \forall x \in (0, \infty), \tag{3.2}$$

then the following holds:

1. *For any $c \in \mathbb{R}$ and $d \in \mathbb{R} \setminus \{0\}$, $Sf(x) = S(f(x) + c)$ and $Sf(x) = S(df(x))$.*
2. *$S(f \circ g)(x) = Sf(g(x)) \cdot g'(x)^2 + Sg(x)$.*
3. *If $Sf(x) \leq 0$, $Sg(x) < 0$, then $S(f \circ g)(x) < 0$.*
4. *If $Sf(x) < 0 \ \forall x \in int(I)$, then $f'(x)$ cannot have positive local minima nor negative local maxima.*

Proof. 1. Observe that $f'(x) = (f(x) + c)'$ which proves $Sf(x) = S(f(x) + c)$. Furthermore,

$$f'''(x)/f'(x) = (df'''(x))/(df'(x))$$
$$f''(x)/f'(x) = (df''(x))/(df'(x)).$$

Therefore, $Sf(x) = S(df(x))$.

2. The following set of equations lead to the desired result:

$$\begin{aligned}
(f \circ g)'(x) &= f'(g(x))g'(x) \\
(f \circ g)''(x) &= f''(g(x))g'(x)^2 + f'(g(x))g''(x) \\
(f \circ g)'''(x) &= f'''(g(x))(g'(x))^3 + 3f''(g(x))g''(x)g'(x) + f'(g(x))g'''(x) \\
S(f \circ g)(x) &= \frac{(f \circ g)'''}{(f \circ g)'(x)} - \frac{3}{2}\left(\frac{(f \circ g)''(x)}{(f \circ g)'(x)}\right)^2 \\
&= \frac{g'''(x)}{g'(x)} + 3\frac{f''(g(x))g''(x)}{f'(g(x))} + \frac{f'''(g(x))g'(x)^2}{f'(g(x))} \\
&- \frac{3}{2}\left(\frac{f''(g(x))g'(x)}{f'(g(x))} + \frac{g''(x)}{g'(x)}\right)^2 \\
&\Rightarrow S(f \circ g)(x) = Sf(g(x))g'(x)^2 + Sg(x).
\end{aligned}$$

3. Since

$$Sf(x) \leq 0, \quad Sg(x) < 0 \quad \text{and} \quad g'(x)^2 \geq 0 \ \forall x \in int(I), \tag{3.3}$$

part 2 of the Proposition implies that

$$S(f \circ g)(x) = Sf(g(x))g'(x)^2 + Sg(x) < 0. \tag{3.4}$$

4. Suppose f' has a positive local minima at $x \in int(I)$, then

$$\begin{aligned} f'(x) > 0, \quad f''(x) = 0, \quad f'''(x) \geq 0 \\ \Rightarrow Sf(x) > 0 \end{aligned}$$

which is a contradiction. Similarly, suppose that f' have negative local maxima at x, and let

$$h(x) = -f(x).$$

Then, the function h' will have a positive local minima at x and from part 1,

$$Sh(x) = Sf(x) < 0. \tag{3.5}$$

In other words, it was shown that h' cannot have positive local minima, so f' cannot have negative local maxima. ☐

Let us now calculate Schwarzian derivatives of some functions which are commonly used as nonlinearities in the modeling of physical systems. In particular, we will consider Hill functions, which are typical nonlinearities appearing in gene regulatory networks, as illustrated in Section 4.3.

Example 3.1. The exponential function has NSD; more precisely, for any $a \in \mathbb{R}$, we have

$$S(e^{ax}) = -\frac{a^2}{2}, \qquad S(e^{-ax}) = -\frac{5a^2}{2}\,.$$

In real-life problems, we commonly encounter Hill function type nonlinearities. Hill functions have the following general form:

$$f(x) = \frac{a}{b + x^m} + c, \qquad g(x) = \frac{ax^m}{b + x^m} + c \quad a, b > 0 \quad c \geq 0 \quad m \in \mathbb{N}. \tag{3.6}$$

We will now calculate Schwarzian derivatives of Hill functions in the interval $(0, \infty)$. From Proposition 3.1, we know addition and multiplication with a constant does not change the value of the Schwarzian derivative, so we will, without loss of generality, calculate the Schwarzian derivative of the following functions:

$$f(x) = \frac{1}{b + x^m}, \quad g(x) = \frac{x^m}{b + x^m} \qquad b > 0. \tag{3.7}$$

Notice that

$$f(x) = \frac{1}{b + x^m} = -\frac{1}{b}\left(\frac{x^m}{b + x^m} - 1\right) = -\frac{1}{b}\left(g(x) - 1\right).$$

Then from Proposition 3.1 it follows that

$$Sf(x) = Sg(x) = S\left(\frac{1}{b + x^m}\right).$$

Therefore, without loss of generality, we will only calculate $Sf(x)$. For this purpose, let

$$h_1(x) = b + x^m, \quad h_2(x) = \frac{1}{x}.$$

Then,

$$\begin{aligned} f(x) &= h_2 \circ h_1(x), \\ &\Rightarrow Sf(x) = S(h_2 \circ h_1)(x) = Sh_2(h_1(x))h_1'(x)^2 + Sh_1(x) \\ &\quad Sh_1(x) = -\frac{(m^2-1)}{x^2}, \quad Sh_2(x) = 0 \\ &\Rightarrow Sf(x) = -\frac{(m^2-1)}{x^2}. \end{aligned}$$

Lastly, let us calculate the Schwarzian derivative of the tangent hyperbolic function defined as

$$f(x) = a\ \tanh(bx) = a\left(\frac{e^{2bx}-1}{e^{2bx}+1}\right) \qquad a, b \in \mathbb{R}_+.$$

Let

$$g(x) = e^{2bx}, \qquad h(x) = a\frac{x-1}{x+1}, \tag{3.8}$$

then $f(x) = h \circ g(x)$ and

$$\begin{aligned} Sg(x) &= -2b^2 \\ Sh(x) &= 0 \\ &\Rightarrow Sf(x) = S(h \circ g)(x) = -2b^2 < 0. \end{aligned}$$

As a corollary of the above, we have the following result.

Corollary 3.1. *Let* a, $b > 0$, $c \geq 0$ *and* $m \in \mathbb{N}$ *be constants. Suppose* f *and* g *are Hill functions of the form* (3.6). *Then, one of the following holds:*

1. *If* $m = 1$, *then* $Sf(x) = Sg(x) = 0$.
2. *If* $m > 1$, *then* $Sf(x) = Sg(x) < 0$.
3. *If* $h(x) = a\,tanh(bx)$, *then S(h(x))*< 0.

In the sequel, we try to classify functions with negative Schwarzian derivatives. Next result helps this endeavor.

Lemma 3.1. *Let h be a three times differentiable function from* $\mathbb{R}_+$ *to* $Y \subseteq \mathbb{R}_+$ *and suppose that we have*

$$-\infty < Sh(x) < 0, \qquad \forall x \in (0,\infty). \tag{3.9}$$

Then h' *cannot be constant for any* $[a,b] \subseteq (0,\infty)$ *with* $a < b$. *Moreover, suppose that*

$$h'(x) > 0 \quad \forall \ x \in (0,\infty), \tag{3.10}$$

and there exists $c \in \mathbb{R}_+$ *such that* $h''(c) < 0$, *then we have*

$$h''(d) \leq 0, \quad \forall \ d \geq c. \tag{3.11}$$

Proof. For the first part of Lemma, suppose on the contrary that there exist positive constants $a < b$ such that h' is constant in $[a,b]$. Let $c \in (a,b)$, then $h''(c) = 0 = h'''(c)$, but this implies that

$$Sh(c) = 0,$$

which is a contradiction. Therefore, h' cannot be constant in any subinterval of $\mathbb{R}_+$. For the second part of Lemma, suppose that there exist positive real numbers $c < d$ such that $h''(c) < 0$ and $h''(d) > 0$. Let I be defined as

$$I = [c,d]. \tag{3.12}$$

Since h' is a continuous function and I is a compact set, there exist $x_1, x_2 \in I$ such that $h'(x_1) \leq h'(x) \leq h'(x_2)$ for all $x \in I$. But since $h''(c) < 0$, there exists $y \geq c$ satisfying

$$h'(y) < h'(c). \tag{3.13}$$

Similarly, since $h''(d) > 0$ there exists $z \leq d$ satisfying

$$h'(z) < h'(d). \tag{3.14}$$

Equations (3.13) and (3.14) imply that $x_1 \neq c$ and $x_1 \neq d$ and we have $h'(x_1) \leq h'(x)$, $\forall x \in I$. Hence, by definition, x_1 is a positive local minima of the function h'. But since $Sh(x) < 0$, h' cannot have a positive local minima. Therefore, we have $h''(d) \leq 0$, $\forall d \geq c$. □

Having in mind the technical assumptions of Lemma 3.1, suppose that a function $h(x)$ satisfies

$$h''(y) = 0, \qquad h'(y) > 0 \tag{3.15}$$

for some $y \in (0, \infty)$. Then, it follows

$$Sh(y) = \frac{h'''(y)}{h'(y)} - \frac{3}{2}\left(\frac{h''(y)}{h'(y)}\right)^2 \tag{3.16}$$

$$= \frac{h'''(y)}{h'(y)} < 0 \tag{3.17}$$

$$\Rightarrow h'''(y) < 0, \tag{3.18}$$

which implies that the point y is a positive local maxima of the function h'. Combining Lemmas 3.1 and equation (3.18), we conclude that if $h'(x)$ is decreasing in some interval $[a, b]$ then it must be decreasing in $[b, \infty]$. In particular, if $h''(0) < 0$, then $h''(x) \leq 0$ for all $x \geq 0$ which implies that $h'(x)$ is a decreasing function. Combining this fact with Lemma 3.1, the result below is obtained.

Corollary 3.2. *Let h be a three times differentiable function defined from $\mathbb{R}_+$ to $Y \subseteq \mathbb{R}_+$ and suppose that we have*

$$Sh(x) < 0 \qquad \text{and} \qquad h'(x) > 0, \qquad \forall x \in (0, \infty)$$

Then h' is a function from $\mathbb{R}_+$ to $Y \subseteq \mathbb{R}_+$ satisfying one of the following properties:

1. *h' is a strictly increasing function on $[0, \infty)$.*
2. *h' is a strictly decreasing function on $[0, \infty)$.*
3. *There exists $a \geq 0$ such that $h'(x)$ is strictly increasing in $(0, a)$ and strictly decreasing in (a, ∞).* □

Note that Lemma 3.1 implies that h' cannot be constant in any interval, so the strictly increasing or decreasing function assumptions in the statement of Corollary 3.2 are without loss of generality. Corollary 3.2 is a general statement also covering unbounded functions, though the functions we are particularly interested in are bounded.

Remark 3.1. Let h be a function satisfying the assumptions of Corollary 3.2. Moreover, suppose that h is bounded. Then h' cannot be a strictly increasing function, otherwise h cannot be bounded. Therefore, for a bounded function h with a negative Schwarzian derivative, either h' is a strictly decreasing function in $[0, \infty]$ or there exists $a \geq 0$ such that h' is strictly increasing in $(0, a)$ and strictly decreasing in (a, ∞). □

Remark 3.1 leads us to the following definition:

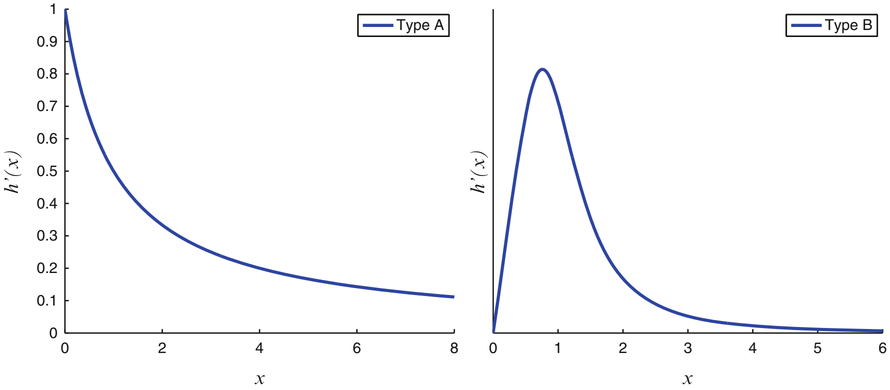


Fig. 3.1 Typical h' vs x graphs for type A and B functions.

Definition 3.1. For a bounded function h with a negative Schwarzian derivative, we will say h is of type A if h' is a strictly decreasing function, and of type B otherwise. The two types of such functions, satisfying $h'(x) > 0$, are illustrated in Figure 3.1. ☐

Also note that whether the function h is of type A or B, the following property holds:

$$\lim_{x\to\infty} h'(x) = 0. \tag{3.19}$$

Remark 3.2. It is easy to determine whether a function h is of type A or B. If $h'(0) = 0$, then it is clear that the function h is of type B. If

$$h'(0) > 0, \tag{3.20}$$

and $h''(0) > 0$, then h is of type B. If (3.20) is satisfied and

$$h''(0) \leq 0, \tag{3.21}$$

then h is of type A.

3.2 Fixed Points

In this section, we analyze fixed points of functions with NSD. In this manuscript, we assume that the nonlinearity functions are monotonic, bounded functions, which take positive values. Therefore, a prototype function r is defined from $\mathbb{R}_+$ to $X \subseteq \mathbb{R}_+$ such that

$$r'(x) > 0 \quad \text{or} \quad r'(x) < 0, \quad \forall x > 0.$$

We allow the derivative of r to be zero at $x = 0$ as this does not violate the monotonicity assumption of r. Moreover, we will assume that r has negative Schwarzian derivative. The point x_0 is said to be a fixed point of r if we have

$$r(x_0) = x_0.$$

Our first result is concerned with the total number of fixed points of the prototype function r.

Proposition 3.2. *Let r be a bounded function from $\mathbb{R}_+$ to $X \subseteq \mathbb{R}_+$. Moreover, assume that r has negative Schwarzian derivative. Then, one of the following properties holds:*

1. *If $r'(x) < 0$ for all $x > 0$, then r has a unique fixed point.*
2. *If $r'(x) > 0$ for all $x > 0$, then r has at most three fixed points.*

Proof. For the first part of the proof, assume $r'(x) < 0$ for all $x > 0$, which implies that $r(x)$ is a strictly decreasing function. Moreover, since $r(x)$ has NSD, from Lemma 3.1, it cannot be constant on any interval. Therefore, we have $r(0) > 0$ and since r is bounded, it follows that

$$0 < r(x) < B, \ \forall x \in \mathbb{R}_+.$$

Let $f(x) = r(x) - x$, then $f(0) > 0$, $f(B) < 0$ and f is continuous being the summation of two continuous functions. Therefore, by the intermediate value theorem, there exists some real number $x_0 \in [0, B]$ such that $f(x_0) = 0$, which implies that $r(x_0) = x_0$. Hence, r has at least one fixed point. To prove the uniqueness of the fixed point, assume $x_1 < x_2$ are two different fixed points of the function r. Then, we have $r(x_1) = x_1$ and $r(x_2) = x_2$. But from mean value theorem, there exists $c \in (x_1, x_2)$ such that

$$r'(c) = \frac{r(x_2) - r(x_1)}{x_2 - x_1} = 1,$$

which is a contradiction to the assumption that $r'(x) < 0$ for all positive x. Therefore, $r(x)$ can have a unique fixed point, which completes the first part of the proof. For the second part, assume that r has four fixed points $x_1 < x_2 < x_3 < x_4$. Since $r'(x)$ cannot be constant in any interval, there must exist $y_1 \in (x_1, x_2)$ and $y_3 \in (x_3, x_4)$ such that $r'(y_1) > 1$ and $r'(y_3) > 1$. Again from the mean value theorem, there exists $y_2 \in (x_2, x_3)$ such that $r'(y_2) = 1$. Hence, we have $y_2 \in (y_1, y_3)$ and $r'(y_2) < \min\{r'(y_1), r'(y_3)\}$. Now, since r' is continuous and the set $[y_1, y_3]$ is compact, so r' attains its infimum at some $y_{min} \in (y_1, y_3)$, which is a positive local minima. But this contradicts Proposition 3.1, which states that a function with NSD cannot have a positive local minima. Therefore, r cannot have more than three fixed points. □

The proposition above shows that if r is strictly decreasing, it has a unique fixed point. On the other hand, if r is strictly increasing, it proves that r has at most three fixed points. Next, we analyze the values of r' at its fixed points. We start with the strictly decreasing case.

Proposition 3.3. *Let $r(x) : \mathbb{R}_+ \to X \subseteq \mathbb{R}_+$ be a function with a negative Schwarzian derivative satisfying*

$$r'(x) < 0, \qquad \forall x \in (0, \infty).$$

Then r has a unique fixed point x_0 and

$$r'(x_0) \neq -1. \tag{3.22}$$

Proof. First of all, it is assumed that r is a non-constant, positive, and strictly decreasing function. Therefore, it must satisfy $r(0) > 0$. Since the function r is monotonic, we have

$$0 < r(x) < r(0) \qquad \forall x \in (0, \infty),$$

which implies that r is bounded. The uniqueness of the fixed point of the function r follows from Proposition 3.2. Let x_0 be the unique fixed point of r. Since $r(0) > 0$, we have $x_0 > 0$. Let us define

$$f(x) = r \circ r(x). \tag{3.23}$$

Since r is bounded, the function f is also bounded. By assumption the function r has NSD, then from Lemma 3.1, it follows that the function f also has negative Schwarzian derivative. Moreover, by the chain rule, we have $f' = r' \cdot r' > 0$. From Section 3.1, we know that the function f is either of type A or B. Let f be of type A and suppose that

$$r'(x_0) = -1, \tag{3.24}$$

then

$$f'(x_0) = (r'(x_0))^2 = 1.$$

We know $0 < x_0$. Therefore, we have

$$f'(x) > 1 \qquad \forall\ x\ \in [0, x_0).$$

Let us define h as

$$h(x) = f(x) - x.$$

Notice the following facts:

$$h(0) = f(0) > 0, \qquad h'(x) = f'(x) - 1 > 0 \quad \forall x \in [0, x_0).$$

Then, by fundamental theorem of calculus, we have the following inequality:

$$h(x_0) = 0 = h(0) + \int_0^{x_0} h'(x)dx > h(0) > 0\ ,$$

leading to a contradiction. Therefore, when the function f is of type A, it follows

$$f'(x_0) < 1 \Rightarrow r'(x_0) \neq -1.$$

Suppose now the function f is of type B, and

$$f'(x_0) = 1.$$

We know that f can have either a unique fixed point or three fixed points. Since f is of type B, we have either

$$f'(x) < 1, \quad \forall x \in [0, x_0) \quad \text{or} \quad f'(x) < 1, \quad \forall x \in (x_0, \infty).$$

If $f'(x) < 1$ in $[0, x_0)$, we have

$$h(x_0) = 0 = h(0) + \int_0^{x_0} h'(x)dx > h(0) > 0,$$

leading to a contradiction. For the other case assume that $f'(x) < 1$ in (x_0, ∞). Then, for any $x > x_0$ we have

$$h(x) = h(x_0) + \int_{x_0}^{x} h'(x)dx < 0 + x - x_0 < x$$

which implies that the function f cannot have a fixed point x greater than x_0. Also if

$$f'(x) < 1, \qquad \forall x \in [0, x_0),$$

the following equality follows:

$$h(x_0) = 0 = h(0) + \int_0^{x_0} h'(x)dx > h(0) > 0,$$

which is again a contradiction. Therefore, considering the shape of type B function f, we see that there exists some $t < x_0$ such that

$$f'(x) > 1, \qquad \forall x \in (t, x_0).$$

As a result, we have the following

$$\begin{aligned} h(x_0) &= 0 = h(t) + \int_t^{x_0} h'(x)dx = h(t) - a \\ &\Rightarrow h(t) = -\int_t^{x_0} h'(x)dx < 0. \end{aligned}$$

Since we have

$$h(0) > 0 \qquad \text{and} \qquad h(t) < 0,$$

intermediate value theorem implies that there exists some real number $k \in (0, t)$ such that

$$h(k) = 0.$$

Therefore, the point $k < x_0$ is a fixed point of f. Then, we should have another fixed point of the function f which is larger than x_0. But we showed that f cannot have a fixed point larger than x_0 which gives us another contradiction. Therefore, we get the desired result, that is $f(x_0) = (r'(x_0))^2 \neq 1$. □

Next, we analyze the situation when r is strictly increasing. We have three different results characterizing such a case.

Proposition 3.4. *Let $r(x) : \mathbb{R}_+ \to X \subseteq \mathbb{R}_+$ be a function with a negative Schwarzian derivative such that $r'(x) > 0$ for all $x \in \mathbb{R}_+$. Suppose that r is bounded and at least three times continuously differentiable. Let x_f denote the greatest positive fixed point of g. Then,*

$$r'(x_f) \leq 1. \tag{3.25}$$

Proof. Assume that $r'(x_f) > 1$. Then, since the function r' is continuous, there exists $\epsilon > 0$ such that $r'(x) > 1$ for any $x \in (x_f, x_f + \epsilon)$. Using the fundamental theorem of calculus, it follows that

$$r(x_f + \epsilon) = r(x_f) + \int_{x_f}^{x_f+\epsilon} r'(x)dx > r(x_f) + \epsilon = x_f + \epsilon,$$

since $r'(x) > 1$ for any $x \in (x_f, x_f + \epsilon)$. Hence, we have $r(x_f + \epsilon) > x_f + \epsilon$. Since the function r is bounded and continuous, there should exist a fixed point x_0

of r such that $x_0 > x_f + \epsilon$. This is contradiction to the fact that x_f represents the greatest fixed point of r. Therefore, $r'(x_f) \leq 1$. □

The second result shows that r has at most two fixed points if r is of type A.

Proposition 3.5. *Let $r(x) : \mathbb{R}_+ \to X \subseteq \mathbb{R}_+$ be a type A function. Suppose that r is bounded and continuously differentiable. Then, r has at most two fixed points. In particular, r has two fixed points if and only if $r'(0) > 1$ and $r(0) = 0$, i.e. the origin is a fixed point. Moreover, if $x_0 > 0$ is a fixed point of r, then*

$$r'(x_0) < 1. \tag{3.26}$$

Proof. Assume that r is of type A. Then r' is strictly decreasing in $\mathbb{R}_+$. Let $x_0 > 0$ be any fixed point of r. Suppose $r'(x_0) \geq 1$. Then

$$r'(x) > 1 \qquad \forall x \in [0, x_0].$$

From the mean value theorem, for some $y \in (0, x_0)$, the following inequality holds:

$$r'(y) = \frac{r(x_0) - r(0)}{x_0} \leq \frac{x_0 - r(0)}{x_0} \leq 1.$$

On the other hand,

$$r'(x) > 1, \qquad \forall x \in [0, x_0],$$

leads to a contradiction. Therefore, $r'(x_0) < 1$. Next, suppose $r'(0) > 1$ and $r(0) = 0$. If $r(x)$ has no other fixed points, we have either $r(x) > x$ or $r(x) < x$ for all $x \in (0, \infty)$. Since r is bounded, we have $r(x) < x$ for all $x \in (0, \infty)$. But since r' is continuous and strictly decreasing $\exists \epsilon > 0$ such that $r'(x) > 1$ for $x \in (0, \epsilon)$. Then, the fundamental theorem of calculus implies that

$$r(\epsilon) = r(0) + \int_0^{\epsilon} r'(x)dx > 0 + \epsilon,$$

which is a contradiction to the assumption that $r(x) < x$ for all $x \in (0, \infty)$. Hence, $r(x)$ has another fixed point greater than 0. Assume that $0 < x_1 < x_2$ are two fixed points of $r(x)$. Then from the first part of the proof, it is easy to see that $r'(x) < 1$ for $x > x_1$. Moreover, from the mean value theorem, we can find $y \in (x_1, x_2)$ such that:

$$r'(y) = \frac{r(x_2) - r(x_1)}{x_2 - x_1} = 1,$$

which is a contradiction to the assumption that $r'(x) < 1$ for $x > x_1$. Therefore, r can only have a unique fixed point greater than 0. ☐

Our final result deals with the case when r is of type B and has exactly three fixed points. This result is especially useful for studying the bistable behavior discussed in Chapter 6.

Proposition 3.6. *Let $r(x)$ be a type B function. Then r has at most three fixed points and at most two of them satisfy $r'(x) \leq 1$. Moreover, if the function r has exactly three fixed points $x_1 < x_2 < x_3$, then*

$$r'(x_1) < 1,\ r'(x_2) > 1,\ r'(x_3) < 1.$$

Before starting the proof, it is worth noting that for r to have three fixed points, it should be a type B function.

Proof. We have already proved that the function r has at most three fixed points. If r has two or less fixed points, then the proposition is proven automatically. Therefore, assume that r has three fixed points $x_1 < x_2 < x_3$, and assume that $r'(x_i) \leq 1$ for all $i = 1, 2, 3$. Now since $r(x_1) = x_1$ and $r(x_2) = x_2$ there exist $y_1 \in (x_1, x_2)$ such that $r'(y_1) > 1$. Otherwise, if $r'(y) \leq 1$ for all $y \in (x_1, x_2)$, using the fundamental theorem of calculus, it follows that

$$r(x_2) = r(x_1) + \int_{x_1}^{x_2} r'(x)dx < r(x_2),$$

where we used the fact that r' cannot be constant in (x_1, x_2). This is contradiction to the fact that x_2 is a fixed point of r. Similarly, there exists $y_2 \in (x_2, x_3)$ such that $r'(y_2) > 1$. Since $x_2 \in (y_1, y_2)$ and $r'(x_2) \leq 1$, r' has a positive local minima in (y_1, y_2), which is a contradiction to Proposition 3.1.

Let us continue with the second part of the proof. Assume that $x_1 < x_2 < x_3$ are three fixed points of the function r. Using the same argument from the first part of the proof, we can find $y_1 \in (x_1, x_2)$ and $y_2 \in (x_2, x_3)$ such that $r'(y_1) > 1$ and $r'(y_2) > 1$. Hence, using exactly the same argument as the first part we can conclude that $r'(x_2) > 1$. Next, suppose that $r'(x_3) = 1$, but from Corollary 3.2, we conclude that $r'(x) > 1$ for all $x \in [x_2, x_3)$. Then, from the fundamental theorem of calculus, it follows that:

$$r(x_3) = r(x_2) + \int_{x_2}^{x_3} r'(x)dx > x_2 + x_3 - x_2,$$

which is a contradiction to the fact that x_3 is a fixed point of $g(x)$. Therefore, we conclude that $r'(x_3) < 1$. Finally, assume that $r'(x_1) \geq 1$. Then, we should have $r'(x) > 1$ for all $x \in (x_1, x_2)$, since otherwise $r'(x)$ has a local minima in (x_1, x_2), which is contradiction. But since $r'(x_2) > 1$ and $r\prime(x)$ is continuous, there exists

$\epsilon > 0$ such that $r'(x) > 1$ in $[x_2 - \epsilon, x_2]$. Then, again from the fundamental theorem of calculus, it follows that

$$r(x_2) = r(x_1) + \int_{x_1}^{x_2} r'(x)dx > x_1 + x_2 - x_1,$$

which again is a contradiction to the fact that x_2 is a fixed point of r. Therefore, $r'(x_1) < 1$, which completes the proof. □

Next, we deal with the case where $r(x)$ has a special form.

Remark 3.3. Suppose the function r is defined as follows:

$$r(x) = f \circ f(x), \tag{3.27}$$

where f is a function defined from $\mathbb{R}_+$ to $X \subseteq \mathbb{R}_+$ such that

$$Sf(x) < 0 \quad \text{and} \quad f'(x) < 0, \quad \forall x \in (0, \infty). \tag{3.28}$$

Then, by the convolution property of the Schwarzian derivative we have

$$Sr(x) < 0, \quad \forall x \in (0, \infty). \tag{3.29}$$

Moreover, if x_0 is a fixed point of r, then one of the following holds:

1. x_0 is a fixed point of f.
2. $x_0 < f(x_0)$, so $r(f(x_0)) = f(f(f(x_0))) = f(x_0)$ and r has another fixed point $x_1 > x_0$.
3. $f(x_0) < x_0$, so $r(f(x_0)) = f(f(f(x_0))) = f(x_0)$ and r has another fixed point $x_1 < x_0$. □

Proposition 3.7. *Let r be a function of the form given in (3.27), where f satisfies (3.28). Let x_0 be the unique fixed point of the function f. Then, the following properties hold:*

1. *If $|r'(x_0)| < 1$, then r has the unique fixed point x_0.*
2. *If $r(x)$ is of type A, then r has the unique fixed point x_0 satisfying $r'(x_0) < 1$.*
3. *If $r(x)$ is of type B and*
   - ***(i)*** *If $r'(x_0) < 1$, then r has the unique fixed point x_0*
   - ***(ii)*** *$r'(x_0) > 1$, then r has exactly three fixed points.*

Proof. First note that since f is a strictly decreasing function, the following inequality holds:

$$f(0) > f(x) > 0 \qquad \forall x > 0. \tag{3.30}$$

So, f is a bounded function, which implies that the function r is bounded. From Proposition 3.3, we conclude that f has a unique fixed point $x_0 > 0$ such that $f'(x_0) \neq -1$. Observe that

$$r'(x) = f'(f(x))f'(x).$$

Since

$$f'(x) < 0 \qquad \forall x \in (0, \infty),$$

we have

$$r'(x) > 0 \qquad \forall x \in (0, \infty).$$

At the unique fixed point x_0 of f, we have the following equality:

$$r'(x_0) = f'(f(x_0))f'(x_0) = (f'(x_0))^2.$$

Therefore, the following equivalences are verified:

$$|f'(x_0)| < 1 \Leftrightarrow g'(x_0) < 1$$
$$|f'(x_0)| > 1 \Leftrightarrow g'(x_0) > 1.$$

Having a negative Schwarzian derivative, r is either of type A or type B. Therefore, if we prove second and third part of the Proposition, then the first part will follow. Second part follows directly from Proposition 3.5 by noting that 0 cannot be a fixed point of r. For the third part, we assume that r is of type B. Introduce a new function in the following way:

$$h(x) = x - r(x). \tag{3.31}$$

Then, clearly,

$$h(0) < 0 \quad \text{and} \quad h'(x) = 1 - r'(x). \tag{3.32}$$

Note that the zero crossings of h and the fixed points of r are the same. Suppose that

$$f'(x_0) < 1 \implies r'(x_0) < 1. \tag{3.33}$$

Also assume that the function r has a fixed point y which is different from x_0. From Remark 3.3, we can safely assume that

$$y < x_0. \tag{3.34}$$

Again from Remark 3.3 we have another fixed point of h which is denoted by z and is greater than x_0. For type B functions, we have either

$$r'(x) < r'(x_0) < 1 \qquad \forall x \in [0, x_0] \tag{3.35}$$

or

$$r'(x) < r'(x_0) < 1 \qquad \forall x \in [x_0, \infty]. \tag{3.36}$$

If the condition (3.35) is satisfied, then we have $h(0) < 0$, $h(x_0) = 0$ and

$$h'(x) > 0 \quad x \in [0, x_0]. \tag{3.37}$$

Then, it is clear that $h(y) < 0$; so,

$$r(y) \neq 0 \tag{3.38}$$

which is a contradiction. For the case (3.36), using a similar argument, we can show that $r(z) \neq 0$. Hence, if (3.33) is satisfied, then h has the unique fixed point x_0. Now, assume that

$$f'(x_0) > 1. \tag{3.39}$$

But for a type B function r, we can have at most two different values such that t_1 and t_2 such that

$$r'(t_i) = 1 \qquad \text{for } i = 1, 2. \tag{3.40}$$

Hence h can have at most three zero crossings which implies that the function r has at most three fixed points. From (3.39) we can deduce the following

$$\exists x_1 > x_0 \quad \text{such that} \quad h(x_1) < 0, \tag{3.41}$$

but since the function r is bounded we have

$$\lim_{t\to\infty}(h(x)) = \lim_{t\to\infty}(x - r(x)) = \infty. \tag{3.42}$$

Therefore, h has a zero crossing greater than x_0, thus r has a fixed point greater than x_0. But, from Proposition 3.6, we know that the function r has at most three fixed points. From Remark 3.3, we can conclude that g has exactly three fixed points. □

Final result of this section reduces the process of finding the fixed points of some multidimensional functions defined on the cone $\mathbb{R}^n_+$ to finding the fixed points of a function defined on $\mathbb{R}_+$.

Proposition 3.8. *Let* $h(x) : \mathbb{R}_+^n \to Y \subseteq \mathbb{R}_+^n$ *be defined as*

$$h(x_1, x_2, \ldots, x_n) = \begin{pmatrix} h_1(x_2) \\ \vdots \\ h_{n-1}(x_n) \\ h_n(x_1) \end{pmatrix},$$

where

$$h_i(x_i) : \mathbb{R}_+ \to Y_i \subseteq \mathbb{R}_+ \qquad \forall i = 1, 2, \ldots, n.$$

Let the function $q(x)$ *from* $\mathbb{R}_+$ *to* $Y_1 \subseteq \mathbb{R}_+$ *be defined as*

$$q(x) = h_1 \circ h_2 \circ \ldots \circ h_n(x). \tag{3.43}$$

The number of fixed points of the functions h *and* q *have the same cardinality.*

Proof. Let $x = (x_1, x_2, \ldots, x_n)$ be a fixed point of h. Then, the following holds:

$$x_1 = h_1(x_2)\ , \quad x_2 = h_2(x_3)\ , \cdots\ , \ x_n = h_n(x_1)$$

i.e., $x_1 = h_1(x_2) = h_1 \circ h_2(x_3) = \ldots = h_1 \circ h_2 \circ \ldots \circ h_n(x_1) = q(x_1)$. Hence, x_1 is a fixed point of q. Conversely, assume that $q(x_1) = x_1$, and let

$$u = (x_1, h_2 \circ \ldots \circ h_n(x_1), h_3 \circ \ldots \circ h_n(x_1), \ldots, h_n(x_1)).$$

It is easy to check that this special u satisfies $h(u) = u$. Note that if x, y are fixed points of h such that $x_1 = y_1$, then it follows that

$$\begin{aligned} x_n &= h_n(x_1) = h_n(y_1) = y_n \\ x_{n-1} &= h_{n-1}(x_n) = h_{n-1}(y_n) = y_{n-1} \\ &\vdots \\ x_2 &= h_2(x_3) = h_2(y_3) = y_2, \end{aligned}$$

which implies that

$$x = y.$$

So for any fixed point of q, we can find a unique fixed point of h. Therefore, the number of fixed points of h and q can be bijectively mapped to each other so they have the same cardinality. □

3.3 Exercises

Problem 1. Consider the following functions

$$h_1(x) = \frac{e^{4x} - 1}{e^{4x} + 1}\ , \qquad h_2(x) = \frac{1}{2 + x^3}, \qquad x \geq 0.$$

(a) Verify that both of these functions have NSD, and they satisfy $h_i'(x) > 0$ for all $x > 0, i = 1, 2$.

(b) Check that h_1 and h_2 satisfy the properties stated in Proposition 3.1.

(c) Determine if h_i is Type A or Type B, for $i = 1, 2$.

(d) Find the fixed points of h_1 and h_2.

Problem 2. Let $f(x) = \frac{3}{1+2x^2}$ and $h = f \circ f$. Compute the fixed points of h. What are the values of h' at these fixed points?

Problem 3. Give an example of a function $r(x)$ illustrating the results of Proposition 3.5.

Chapter 4
Deterministic ODE-Based Model with Time Delay

Abstract This chapter is devoted to the derivation of the ODE-based model with time delay that is to be analyzed in the forthcoming chapters. In particular, an equivalent simplified mathematical model of the GRN model is proposed through some interpretations of the interconnection scheme. Next, the stability conditions for the linearized system around an equilibrium point are discussed. Finally, a specific example (the repressilator) is given to illustrate the motivation behind the model considered.

Keywords Gene regulatory networks • Cyclic systems • Delayed feedback • Stability independent of delay • Repressilator

This chapter is devoted to the derivation of the ODE-based model with time delay that is to be analyzed in the forthcoming chapters. In particular, an equivalent simplified mathematical model of the GRN model (1.4) is proposed through some interpretations of the interconnection scheme. Next, the stability of the linearized system around an equilibrium point is discussed in detail. As mentioned in the previous chapters, such stability results will be useful in performing the analysis for the nonlinear system under consideration. Finally, a specific example (the repressilator) is given to illustrate the motivation behind the model considered.

4.1 Model Transformation and Simplification

Recall that the model (1.4) is in the form:

$$\begin{aligned}
\dot{z}_1(t) &= -\lambda_1 z_1(t) + g_1(z_2(t-\tau_1)) \\
\dot{z}_2(t) &= -\lambda_2 z_2(t) + g_2(z_3(t-\tau_2)) \\
&\vdots \\
\dot{z}_n(t) &= -\lambda_n z_n(t) + g_n(z_1(t-\tau_n)).
\end{aligned} \tag{4.1}$$

M.E. Ahsen et al., *Analysis of Deterministic Cyclic Gene Regulatory Network Models with Delays*, SpringerBriefs in Electrical and Computer Engineering,
DOI 10.1007/978-3-319-15606-4_4

By a change of variables: $x_1(t) = z_1(t)$,

$$x_i(t) := z_i(t - h_i), \qquad h_i = \sum_{k=1}^{i-1}(\tau_k), \qquad i = 2, \ldots, n \tag{4.2}$$

we obtain the simplified mathematical model (4.3), which is equivalent to (4.1):

$$\begin{aligned}\dot{x}_1(t) &= -\lambda_1 x_1(t) + g_1(x_2(t))\\ \dot{x}_2(t) &= -\lambda_2 x_2(t) + g_2(x_3(t))\\ &\vdots\\ \dot{x}_n(t) &= -\lambda_n x_n(t) + g_n(x_1(t - \tau)),\end{aligned} \tag{4.3}$$

where

$$\tau = \sum_{k=1}^{n} \tau_k,$$

the scalar variables x_j, $j = 1, \ldots, n$ represent the protein and mRNA concentrations on the feedforward path; and λ_j's $j = 1, \ldots, n$, are either k_{gi} or k_{pi}, $i = 1, \ldots, m$; similarly, g_j's represent either f_{gi} or f_{pi}. In summary, the simplified model to be considered in the book can be represented by the dynamical system shown in Figure 4.1. Since each subsystem Sys_j in Figure 4.1 is stable, the following assumption holds:

Assumption 1: For all $j = 1, 2, \ldots, n$, we have $\lambda_j > 0$.

As far as the nonlinear terms $g_j(\cdot)$ are concerned, the following assumption will be in effect for the rest of this book.

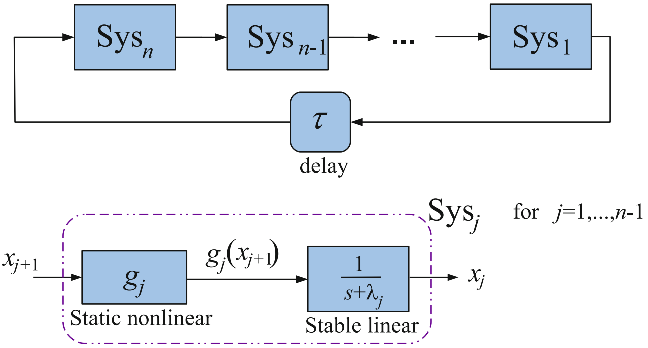


Fig. 4.1 Simplified cyclic nonlinear model with delayed feedback.

Assumption 2: For all $j = 1, 2, \dots, n$, the nonlinearity functions g_j satisfy:

(i) g_j is a bounded function defined on $\mathbb{R}_+$;
(ii) we have either

$$g_j'(x) < 0 \qquad \text{or} \qquad g_j'(x) > 0 \qquad \forall x \in (0, \infty), \tag{4.4}$$

(iii) the nonlinearity functions g_j satisfy $Sg_j(x) \le 0$, with at least one of them satisfying $Sg_j(x) < 0$.

Assumption 2 means that each g_j is a monotone function and takes positive values. The nonlinearity functions have $\mathbb{R}_+$ as their domain since their domain represents biological variables which take positive values. The nonlinearity functions in the proposed model are either Hill functions or tangent hyperbolic function; hence, they are bounded and monotonic. Therefore, these assumptions do not impose any constraints on our analysis of the GRN, and they are compatible with various modeling studies, e.g., [37]. Also note that $g_j'(0) = 0$ is allowed, since it does not violate the monotonicity of g_j.

Definition 4.1. With the function g defined as

$$g = \left(\frac{1}{\lambda_1} g_1\right) \circ \left(\frac{1}{\lambda_2} g_2\right) \circ \dots \circ \left(\frac{1}{\lambda_n} g_n\right) \tag{4.5}$$

the gene regulatory network is said to be under *negative feedback* if

$$g'(x) < 0 \qquad \forall x \in (0, \infty);$$

conversely, the gene regulatory network is under *positive feedback* if

$$g'(x) > 0 \qquad \forall x \in (0, \infty).$$

In the next two chapters, we will investigate the GRN under both negative and positive feedback conditions.

At this point, it should be re-emphasized that the system (4.3) is infinite dimensional. We will assume that the initial condition required to solve this system of functional differential equations is from the set

$$\mathscr{X} = \{\phi(a) = [\phi_1(a) \dots \phi_n(a)]^{\mathrm{T}} \;:\; \phi_i(\cdot) \in \mathscr{C}([-\tau\ ,\ 0], \mathbb{R}) \;\; \phi(a) \ge 0\},$$

where $\mathscr{C}([-\tau\ ,\ 0], \mathbb{R})$ denotes the set of real valued continuous functions defined on the interval $[-\tau, 0]$. In other words, we consider continuous functions taking positive values as initial conditions for the states $x_i(a)$, for $a \in [-\tau, 0]$.

The function g defined in (4.5) plays a crucial role in the analysis of the GRN model (4.3). For example, if $x^e = [x_1^e, \dots, x_n^e]^{\mathrm{T}}$ is an equilibrium point, then the following must be satisfied

$$x_1^e = \frac{1}{\lambda_1} g_1(x_2^e) \tag{4.6}$$

$$\vdots$$

$$x_{n-1}^e = \frac{1}{\lambda_{n-1}} g_{n-1}(x_n^e) \tag{4.7}$$

$$x_n^e = \frac{1}{\lambda_n} g_n(x_1^e). \tag{4.8}$$

Therefore, all equilibrium points are determined from the values $x_1^e \in \mathbb{R}_+$ satisfying

$$x_1^e = g(x_1^e). \tag{4.9}$$

Once $x_1^e > 0$ is determined, remaining coordinates $x_2^e, \dots, x_n^e$ are computed from the set of equations (4.6)–(4.8). The questions of whether we have a unique or multiple equilibrium points, and whether these points are stable or not, are the subject of the remaining chapters.

4.2 Analysis of the Linearized Model

Let $x_{eq} = [x_1, \dots, x_n]^{\mathrm{T}}$ be an equilibrium point of the system. Then, we have:

$$x_i = \frac{g(x_{i+1})}{\lambda_i}, \text{ for } i = 1, \dots, n-1, \; x_n = \frac{g(x_1)}{\lambda_n}.$$

Using Proposition 3.8, we obtain the following result.

Proposition 4.1. *The system* (4.3) *has as many equilibrium as the number of fixed points of* $g(x)$ *given in* (4.5).

Proof. Let

$$h(x_1, x_2, \dots, x_n) = \begin{pmatrix} \frac{g_1(x_2)}{\lambda_1} \\ \vdots \\ \frac{g_{n-1}(x_n)}{\lambda_{n-1}} \\ \frac{g_n(x_1)}{\lambda_n} \end{pmatrix},$$

and g be as in (4.5). Then, by using Proposition 3.8, we conclude that h has as many fixed point as g. But each fixed point of h is an equilibrium point of the system, which concludes the proof. □

If we analyze the equation in depth, we see that for any equilibrium point of the system in the form $x_{eq} = [x_1, \dots, x_n]^{\mathrm{T}}$, the first component x_1 is a fixed point of the function $g(x)$. To continue our analysis, let $x_{eq} = [x_1, \dots, x_n]^{\mathrm{T}}$ be an arbitrary equilibrium point of (4.3). Then, we have the following linearization of system (4.3) around x_{eq}:

$$\dot{x}(t) = A_0 x(t) + A_1 x(t-\tau) \tag{4.10}$$

where

$$A_0 = \begin{bmatrix} -\lambda_1 & g_1'(x_2) & 0 & \dots & 0 \\ 0 & -\lambda_2 & g_2'(x_3) & \dots & 0 \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ 0 & 0 & \dots & \dots & -\lambda_n \end{bmatrix} \quad \text{and} \quad A_1 = B_1 C_1$$

with

$$\begin{aligned} B_1 &= [0 \ \cdots \ 0 \ \ 1]^{\mathrm{T}} \\ C_1 &= [g_n'(x_1) \ \ 0 \ \cdots \ 0] \, . \end{aligned}$$

The characteristic equation of the system (4.10) is

$$det(sI - A_0 - B_1 C_1 e^{-\tau s}) = 0$$

which is equivalent to $\chi(s) = 0$, where

$$\chi(s) = \left(\prod_{i=1}^{n} (s + \lambda_i) \right) - k e^{-\tau s} \tag{4.11}$$

$$k = \left(\prod_{i=1}^{n-1} g_i'(x_{i+1}) \right) g_n'(x_1). \tag{4.12}$$

Since we have $\lambda_i > 0$, the characteristic function $\chi(s)$ defined in (4.11) has all its roots in $\mathbb{C}_-$ if and only if the transfer function

$$T(s) := \frac{G(s)}{1 + G(s)} \quad \text{where} \quad G(s) = -\frac{k e^{-\tau s}}{\prod_{i=1}^{n} (s + \lambda_i)} \tag{4.13}$$

is stable. Note that

$$k \left(\prod_{i=1}^{n} \frac{1}{\lambda_i} \right) = g'(x_1) ,$$

so,

$$1 - T(s) = \left(1 - \frac{g'(x_1)e^{-\tau s}}{\prod_{i=1}^{n}(1 + s/\lambda_i)} \right)^{-1} . \tag{4.14}$$

We will use the transfer functions given in (4.13) and (4.14) frequently for the analysis of the GRN. In fact, the local stability analysis of an equilibrium point will provide us useful insights in the general behavior of the system. In particular, in Chapter 5, it is shown that if the unique equilibrium point of the GRN is locally unstable, then the system exhibits periodic solutions for large enough delay. We complete this section with a result on delay independent stability of the transfer function $T(s)$.

Proposition 4.2. *Consider the transfer function* $T(s)$ *given in* (4.13) *where* $\lambda_i > 0$ *for all* $i = 1, \dots n$*. If* $|g'(x_1)| < 1$*, then* $T(s)$ *is stable independent of delay.*

Proof. It is straightforward to derive the result from the small gain theorem (Proposition 2.2 and Lemma 2.1). □

4.3 A Synthetic Circuit: The Repressilator

In this section, we derive a dynamic model of repressilators from mass action law and Michelis–Menten kinetics. The detailed derivation of the chemical reaction equations is beyond the scope of this book. Also, the assumptions made in this section are standard in biochemistry. An interested reader may refer to a popular biochemistry book such as [46].

The repressilator is a synthetic genetic regulatory network first suggested in [47], where the authors used three transcriptional repressors in cascade to build an oscillating network in *Escherichia coli*. The synthetic network periodically induces the synthesis of green fluorescent protein. In this section, we first derive the dynamics of a 1-repressilator from mass action law. Then, using this 1-repressilator model, we derive a dynamical model for the n-repressilator.

The 1-repressilator denotes a circuit that represses itself by binding to some operator in the bacterial DNA. The kinetic reactions describing this model is as follows:

$$\left.\begin{aligned} G &\xrightarrow{k_1} G + P \quad \text{transcription+translation} \\ P + G &\underset{k_2^-}{\overset{k_2^+}{\rightleftharpoons}} PG \quad \text{inhibition} \\ P &\xrightarrow{k_3} \emptyset \quad \text{degradation} \end{aligned}\right\} \tag{4.15}$$

where G, P, and PG represent the Gene, Protein, and Protein-Gene complex, which provides self-inhibition. The above model (4.15) is given in [48]. Although we can introduce delays in each reaction in (4.15), for the sake of simplicity we assume that those delays are negligible. Using mass action law, we can write the dynamics of the gene regulation given in (4.15) as follows:

$$\left.\begin{aligned} \tfrac{d}{dt}G(t) &= k_{-2}PG(t) - k_2G(t)P(t) \\ \tfrac{d}{dt}PG(t) &= -k_{-2}PG(t) + k_2G(t)P(t) \\ \tfrac{d}{dt}P(t) &= k_{-2}PG(t) - k_2G(t)P(t) + k_1G(t) - k_3P(t) \end{aligned}\right\} \tag{4.16}$$

where the number of total genes is assumed to be constant, which implies

$$G(t) + PG(t) = G(0), \quad \forall t \geq 0. \tag{4.17}$$

Above, $G(0)$ denotes the initial state of the gene. If we apply the conservation law in (4.17), with the steady state assumption $\frac{dG(t)}{dt} = 0$, the protein dynamics in model (4.15) becomes

$$\frac{dP(t)}{dt} = -k_3P(t) + \frac{k_1G(0)}{1 + \frac{k_2}{k_{-2}}P(t)}, \tag{4.18}$$

where the term $\frac{k_1G(0)}{1+\frac{k_2}{k_{-2}}P(t)}$ is a Hill function that denotes inhibition, i.e., negative feedback.

Using the 1-repressilator model, one can define the n repressilator model illustrated in Figure 4.2. The model contains n proteins P_i, $i = 1, \ldots, n$, which

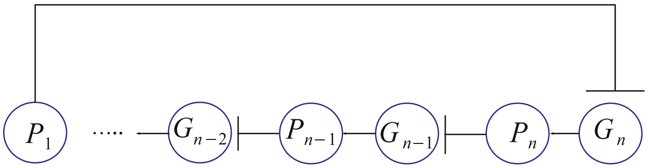


Fig. 4.2 The n-repressilator model.

are produced from the respective gene G_i, which in turn is inhibited by P_{i+1}. The respective chemical kinematics can be described as:

$$\left.\begin{array}{l} G_i \xrightarrow{k_{3i-2}} G_i + P_i \quad \text{transcription+translation} \\[2ex] P_{i+1} + G_i \underset{k_{-3i+1}}{\overset{k_{3i-1}}{\rightleftharpoons}} PG \quad \text{inhibition} \\[2ex] P_i \xrightarrow{k_{3i}} \emptyset \quad \text{degradation.} \end{array}\right\} \tag{4.19}$$

For each i, from conservation law, we have

$$G_i(0) = G_i(t) + PG_i(t), \quad \forall t \geq 0.$$

The steady state assumption, $\frac{dG_i}{dt} = 0$, leads to

$$\frac{d}{dt}P_i(t) = -\lambda_i P_i(t) + \frac{a_i G_i(0)}{1 + b_i P_{i+1}(t)}, \quad i = 1, \ldots, n, \tag{4.20}$$

where λ_i, a_i and b_i are positive constants and we implicitly assumed the cyclic structure so that $P_{n+1} = P_1$, $G_{n+1} = G_1$, and neglected the time delay. The Hill function representing inhibition has negative derivative. If n is odd, the overall system will be under negative feedback. On the other hand, if n is even, the system will be under positive feedback. We will perform the analysis for both the negative and positive feedback in the next two chapters.

4.4 Exercises

Problem 1. Consider the system

$$\begin{aligned} \dot{x}_1(t) &= -x_1(t) + f(x_2(t)) \\ \dot{x}_2(t) &= -x_2(t) + f(x_1(t-\tau)), \end{aligned}$$

where $\tau > 0$ and $f(x) = \frac{2}{0.1+x^2}$. For the computation of the equilibrium points of this system, the reader is referred to Section 2.4. Now compute the fixed points of $g = f \circ f$, and show how we can compute the equilibrium points from the fixed points of g.

Problem 2. Find the linearization of the system in Problem 1 at each of the equilibrium points, and determine whether the linearized system is locally stable or unstable independent of delay.

Problem 3. Consider the system

$$\dot{x}_1(t) = -x_1(t) + \frac{6}{2 + x_2^2(t)}$$

$$\dot{x}_2(t) = -x_2(t) + \frac{4x_1^2(t-\tau)}{1 + x_1^2(t-\tau)},$$

where $\tau > 0$. For the computation of the equilibrium points of this system, the reader is referred to Section 2.4. Show that the system is under negative feedback, and the characteristic equation of the linear system is in the form $1 + G(s) = 0$, where $G(s) = \frac{4e^{-\tau s}}{3(s+1)^2}$. By drawing the Nyquist graph $G(j\omega)$ for $\tau = 3.5$ sec and $\tau = 3.7$ sec, prove that the linear system is stable for $\tau < \tau_c$ and unstable for $\tau \geq \tau_c$, for some $\tau_c \in (3.5\ ,\ 3.7)$. Determine the exact value of τ_c.

Chapter 5
Gene Regulatory Networks Under Negative Feedback

Abstract In this chapter, we consider the simplified GRN model, with the assumption that it is under delayed negative feedback. By analyzing the fixed points of a single function determined from the nonlinear connections, we show that in this case the system has a unique equilibrium point in the positive cone. Then, delay independent global stability, and instability, conditions are derived. For a delay dependent stability condition the secant condition is extended to cover systems with time delays. Special stability conditions are derived for homogenous GRNs where nonlinearities are Hill functions.

Keywords Gene regulatory networks • Cyclic systems • Delayed feedback Negative feedback • Secant condition • Delay dependent local stability • Delay independent global stability • Hill functions

In this chapter, we consider the simplified GRN model (4.3) with the assumption that it is under delayed negative feedback. More precisely, recall from Chapter 4 that the dynamical system equations are:

$$\left.\begin{aligned}\dot{x}_1(t) &= -\lambda_1 x_1(t) + g_1(x_2(t))\\ \dot{x}_2(t) &= -\lambda_2 x_2(t) + g_2(x_3(t))\\ &\vdots\\ \dot{x}_n(t) &= -\lambda_n x_n(t) + g_n(x_1(t-\tau))\,,\end{aligned}\right\} \tag{5.1}$$

where $\tau > 0$ is the delay in the feedback loop. By Assumption 1 of Chapter 4, $\lambda_i > 0$ for all $i = 1, \ldots, n$. Moreover, by Assumption 2 of Chapter 4, each g_i is a bounded monotone function taking positive values and having negative Schwarzian derivatives, $i = 1, \ldots, n$. Recall that the GRN is under *negative feedback* if

$$g'(x) < 0 \qquad \forall x \in (0, \infty), \tag{5.2}$$

M.E. Ahsen et al., *Analysis of Deterministic Cyclic Gene Regulatory Network Models with Delays*, SpringerBriefs in Electrical and Computer Engineering,
DOI 10.1007/978-3-319-15606-4_5

where

$$g = (\frac{1}{\lambda_1} g_1) \circ (\frac{1}{\lambda_2} g_2) \circ \cdots \circ (\frac{1}{\lambda_n} g_n). \tag{5.3}$$

We start by first showing that g has a unique fixed point, which implies that the system has a unique equilibrium point.

Proposition 5.1. *Consider the system in* (5.1) *under negative feedback. Then, the system has a unique equilibrium point in* $\mathbb{R}^n_+$.

Proof. When the system is under negative feedback, by using Proposition 3.3 it is easy to see that g has a unique fixed point. Then, Proposition 4.1 implies that the system (5.1) has as many equilibrium points as the fixed points of g. Since g has a unique fixed point, the system has a unique equilibrium point. □

When the system is under negative feedback, from Proposition 3.3 we see that the system has a unique fixed point x_1 such that $g(x_1) \neq -1$. Therefore, we have two possibilities: either $g(x_1) < -1$ or $g(x_1) > -1$. In the sequel, both situations will be investigated separately.

5.1 Stability Conditions for GRNs Under Negative Feedback

In this chapter, we need a generalized version of the well-known Poincaré–Bendixson Theorem [49]. That particular theorem requires that any solution of the system (5.1) to be bounded. Our next result establishes such a property.

Proposition 5.2. *For the system* (5.1)*,* $\mathbb{R}^n_+$ *is a positively invariant set and, for any set of initial conditions, the corresponding solution of the system remains bounded.*

Proof. To prove positive invariance, we only need to check the direction of the vectors on the boundaries of the region $\mathbb{R}^n_+ = \{[x_1, x_2, \ldots, x_n]^{\mathrm{T}} \in \mathbb{R}^n : x_i \geq 0 \quad \forall i = 1, 2, \ldots, n\}$. The boundaries of the region $\mathbb{R}^n_+$ are just the planes $x_i = 0$. If $x_i = 0$ for some $i = 1, \ldots, n$, then we have $\dot{x}_i = f(x_{i-1}) \geq 0$, so if the derivative is zero, then x_i stays on the boundary, otherwise x_i moves inside the region $\mathbb{R}^n_+$ showing that the region $\mathbb{R}^n_+$ is an invariant set of the system (5.1). For the second part of the Proposition, note that if $x_i(t)$ becomes greater than

$$\frac{1}{\lambda_i} \sup_{a \geq 0} |g_i(a)|,$$

then we have $\dot{x}_i(t) < 0$, which means that $x_i(t)$ is decreasing. Hence, the solutions remain bounded for any positive initial condition. □

Next, a generalized version of the Poincaré–Bendixson Theorem is presented.

Theorem 5.1 ([49]). *Consider the system* (5.1)*. Assume that* $\lambda_i > 0$*, and* g_i *is a bounded monotone function taking positive values and having negative Schwarzian derivatives, for all* $i = 1, \ldots, n$*. Let the system be under negative feedback, so that it has the unique equilibrium point* $x_{eq} = [x_1, \ldots, x_n]^{\mathrm{T}}$*. Let* $x(t)$ *be a solution of the system* (5.1) *which is bounded in* $\mathbb{R}^n_+$*. Let* $\omega(x)$ *denote the omega-limit set of the solution* $x(t)$*. Then, either (i)* $\omega(x)$ *is a single non-constant periodic orbit; or (ii) for each solution* $u(t)$ *of* (5.1) *in* $\omega(x)$*, we have*

$$\alpha(u(t)) \cap \omega(u(t)) \subseteq \{x_{eq}\}.$$

The above theorem implies that (5.1) cannot have a chaotic behavior. In particular, the condition (ii) of the theorem says that the omega-limit set of $x(\cdot)$ consists of the equilibrium point x_{eq} and a set of orbits homoclinic to x_{eq} (note that, since the equilibrium point is *unique* under negative feedback, the existence of heteroclinic orbits are automatically ruled out). Moreover, if the equilibrium is locally unstable, then according to Theorem 5.1, the system will have either periodic orbit or an orbit homoclinic to x_{eq}.

Remark 5.1. In the recent work [50], the authors refer to both non-constant periodic orbits and homoclinic orbits as *oscillations*, and state that

> “In practice, however, the possibility of homoclinic orbits is negligibly small,…”

which is consistent with [41], where it is observed that simulations do not lead to homoclinic orbits. Similarly, in our extensive simulations we have not observed homoclinic orbits. However, we cannot theoretically rule out homoclinic orbits. In order to simplify the notation, similar to [50], in this work, we denote both non-constant periodic orbits and homoclinic orbits as *oscillations*. With this notation, if the unique equilibrium point of the system is locally unstable, then according to Theorem 5.1, the system exhibits oscillations. Therefore, remaining parts of this chapter will be devoted to finding conditions for (a) global stability and (b) local instability around the equilibrium.

In order to derive the main result of this section, Theorem 1 of [51] will be used. The ODE model studied in [51] is given as:

$$\dot{z}_1(t) = -k_1(z_1) + h_1(z_n(t - T_n)) \text{ for } t > T_n$$
$$\dot{z}_j(t) = -k_j(z_j) + h_{j-1}(z_{j-1}(t - T_{j-1})) \text{ for } t > T_n,\ j = 2, 3, \ldots, n-1, \quad (5.4)$$

where $z_1(t), \ldots, z_n(t)$ are continuous non-negative functions of time $t \geq 0$. For each j, k_j is a continuous strictly increasing function satisfying

- $k_j(0) = 0$, and $k_j(z_j) \to \infty$ as $z_j \to \infty$.
- T_j is a non-negative constant.
- h_j is a continuous monotonic function with $h_j(z_j) \geq 0$ for $z_j \geq 0$.

Under these assumptions, define

$$\Phi(u) = k_n^{-1} \circ h_{n-1} \circ \ldots \circ h_1 \circ k_1^{-1} h_n(u), \ u \geq 0. \tag{5.5}$$

Then the system is defined under negative feedback if the function Φ is decreasing. Using a similar argument as in Proposition 5.1, we can show that the system (5.4) has a unique equilibrium point, which is denoted as $a = (a_1, a_2, \ldots, a_n)$. With these definitions we are ready to state Theorem 1 of [51].

Theorem 5.2 ([51, Theorem 1]). *Consider the system* (5.4) *under negative feedback so that the function* Φ *defined in* (5.5) *is decreasing.*

- *If the function* $\Phi \circ \Phi(u)$ *has the unique fixed point* $u = a_n$*, then* $z(t) \to a$ *as* $t \to \infty$ *for arbitrary (non-negative) initial conditions.*
- *If the function* $\Phi \circ \Phi(u)$ *has any number of non-negative fixed points, but if* ℓ *and* L *are the lower and upper bounds of these fixed points, then for any non-negative solution* $z(t) = [z_1(t), z_2(t), \ldots, z_n(t)]^{\mathrm{T}}$ *of the system* (5.4)*, we have*

$$\ell < \underline{\lim_{t\to\infty} z_i(t)} \leq \lim_{t\to\infty} \overline{z_i(t)} < L \qquad \forall i = 1, 2, \ldots, n. \tag{5.6}$$

Next, Theorem 5.2 will be applied to the network (5.1) under negative feedback. In particular, we have the following result.

Theorem 5.3. *Consider the system* (5.1) *under negative feedback so that the function* g *defined in* (5.3) *is decreasing. Let* $x_{eq} = [x_1, \ldots, x_n]$ *denote the unique equilibrium point of the system* (5.1).

- *If the function* $g \circ g(u)$ *has the unique fixed point* $u = x_1$*, then* $x(t) \to x_{eq}$ *as* $t \to \infty$ *for arbitrary (non-negative) initial conditions.*
- *If the function* $g \circ g(u)$ *has any number of non-negative fixed points, but if* ℓ *and* L *are the lower and upper bounds of these fixed points, then for any non-negative solution* $x(t) = [x_1(t), x_2(t), \ldots, x_n(t)]^{\mathrm{T}}$ *of the system* (5.1)*, we have*

$$\ell < \underline{\lim_{t\to\infty} x_i(t)} \leq \lim_{t\to\infty} \overline{x_i(t)} < L \qquad \forall i = 1, 2, \ldots, n. \tag{5.7}$$

Proof. If we let $x_n(t) := z_1(t)$, $x_{n-1}(t) := z_2(t), \ldots, x_1(t) := z_n(t)$, the ODE model (5.1) becomes the same as (5.4) with $k_j(z_j) = \lambda_j z_j$, $h_j = g_j$, $T_1, \ldots, T_{n-1} = 0$, and $T_n = \tau$. Moreover, the function Φ defined in (5.5) becomes the function g defined in (5.3). Then, the result follows from Theorem 5.2. Note that the non-negativity of the solutions follow from Proposition 5.2 and monotonicity of the functions g_i come from the assumptions made in the problem setup. □

Theorem 5.3 leads to the following result.

Proposition 5.3. *Consider the system* (5.1)*. Assume that* $\lambda_i >$*, and* g_i *is a bounded monotone function taking positive values and having negative Schwarzian derivatives, for all* $i = 1, \ldots, n$*. Let the system be under negative feedback, so that* *it has the unique equilibrium point* $x_{eq} = [x_1, \ldots, x_n]^{\mathrm{T}}$. *In this case, by Lemma 3.8, g defined in* (4.5) *has the unique fixed point* x_1. *If*

$$|g'(x_1)| < 1, \tag{5.8}$$

then for any non-negative initial condition the solution satisfies

$$\lim_{t\to\infty} x(t) = x_{eq}. \tag{5.9}$$

Proof. By Theorem 5.3, it is easy to see that we get the desired result if we can show that the function

$$f(u) = g(g(u))$$

has a unique fixed point. Since the nonlinearity functions g_i have negative Schwarzian derivatives, by Lemma 3.1 the functions g and f have negative Schwarzian derivatives. Hence the function f is in the form of Proposition 3.7. Thus, if we have

$$|g'(x_1)| < 1,$$

then, by Proposition 3.7, we conclude that the function f has a unique fixed point which is at the same time the unique fixed point of the function g. Since the function $f(u)$ has a unique fixed point, the desired result follows from Theorem 5.3. □

Now, the above result is illustrated with an example.

Example 5.1. Consider the system (5.1) with $n = 3$, $\lambda_1 = \lambda_2 = \lambda_3 = 1$, and the nonlinearity functions are given by

$$g_1(x) = \frac{1}{2+x^2}, \qquad g_2(x) = \frac{2}{1+x}, \qquad g_3(x) = \frac{1}{2+x}. \tag{5.10}$$

Then, $g(x) = g_1 \circ g_2 \circ g_3(x)$ is given as

$$g(x) = \frac{x^2 + 6x + 9}{6x^2 + 28x + 34}.$$

The unique fixed point of g can be found as 0.2551, and the unique equilibrium point of the system can be found as $x_{eq} = [0.2551, 1.3856, 0.4434]^{\mathrm{T}}$. At the unique fixed point of g, we have

$$|g'(0.2551)| = 0.034 < 1.$$

Fig. 5.1 $x_1(t)$, $x_2(t)$, and $x_3(t)$ vs t graphs of the system with $x(0) = [0.5, 1.0, 0.1]^{\mathrm{T}},\ \tau = 0$.

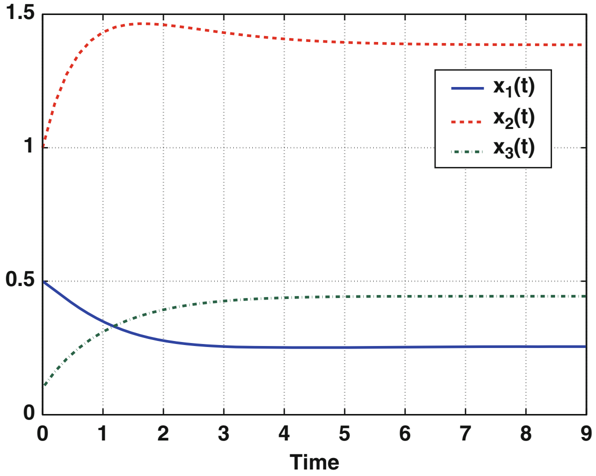
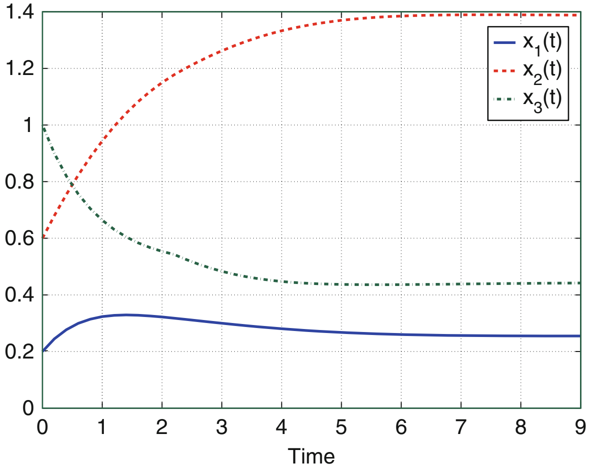


Fig. 5.2 $x_1(t)$, $x_2(t)$, and $x_3(t)$ vs t graphs of the system with $x(0) = [0.2, 0.6, 1.0]^{\mathrm{T}},\ \tau = 2$.



Therefore, by Proposition 5.3, we expect that the solution converges to x_{eq} independent of delay. Figure 5.1 shows the solution of the system with $x(0) = [0.5, 1.0, 0.1]^{\mathrm{T}},\ \tau = 0$. As expected, the solution converges to x_{eq}. Figure 5.2 is the solution of the same system with $x(0) = [0.2, 0.6, 1.0]^{\mathrm{T}},\ \tau = 2$. The solution again converges to x_{eq}.

Using Proposition 4.2, we see that the condition:

$$|g'(x_1)| < 1$$

also corresponds to the delay independent stability of the linearized system around its unique equilibrium point. Therefore, Proposition 5.3 is consistent with the result of Proposition 4.2. Most of the nonlinearity functions considered in biological systems have negative Schwarzian derivatives, including the Hill functions. Thus, the results stated in Proposition 5.3 are useful not only for the analysis of gene regulatory networks but also for other biological processes, e.g. hematopoiesis [52].

Next, we deal with the case when $|g'(x_1)| > 1$. First, by using Proposition 3.7, we conclude that the function $f(x) = g \circ g(x)$ has three fixed points, one of which is x_1, where x_1 is the unique fixed point of g. Our next results provide upper and lower bounds for any solution of the system including the case $|g'(x_1)| > 1$.

Proposition 5.4. *Consider the system (5.1). Assume that $\lambda_i >$, and g_i is a bounded monotone function taking positive values and having negative Schwarzian derivatives, for all $i = 1, \ldots, n$. Let the system be under negative feedback, so that it has the unique equilibrium point $x_{eq} = [x_1, \ldots, x_n]^{\mathrm{T}}$. In this case, by Lemma 3.8, g defined in (4.5) has the unique fixed point x_1. If*

$$|g'(x_1)| > 1, \tag{5.11}$$

then $f(x) = g \circ g(x)$ has exactly three fixed points, one of which is x_1. Let $x_1^- < x_1$ and $x_1^+ > x_1$ denote its other two fixed points. Then, if $x(t) = [x_1(t), \ldots, x_n(t)]^{\mathrm{T}}$ is the solution of the system (5.1) with any positive initial condition, we have

$$x_1^- < \underline{\lim_{t\to\infty}}\, x_i(t) \le \overline{\lim_{t\to\infty}}\, x_i(t) < x_1^+ \qquad \forall i = 1, 2, \ldots, n. \tag{5.12}$$

Proof. The fact that f has exactly three fixed points follows from Proposition 3.7. Then, the two bounds in (5.12) follow immediately from Theorem 5.2. □

We now analyze the stability of the linearized system, which we then use to show the existence of oscillations, in the spirit of [50]. In particular, if the unique equilibrium point x_{eq} is locally unstable, then we have oscillations. Recall that the stability of the linear system is equivalent to having $T \in \mathscr{H}^\infty$, where

$$T(s) = G(s)(1 + G(s))^{-1}, \quad G(s) = -\frac{g'(x_1)e^{-\tau s}}{\prod_{i=1}^{n}(1 + s/\lambda_i)}.$$

Let again $x_{eq} = [x_1, \ldots, x_n]^{\mathrm{T}}$ denote the unique equilibrium point of system (5.1) under negative feedback, so that x_1 is the unique fixed point of g. We have shown in Proposition 4.2 that if $|g'(x_1)| < 1$, then the system is locally stable around x_{eq} independent of delay. But the small gain theorem is conservative, so it only gives us some sufficient condition. A less conservative local stability condition for the *delay free* system is the so-called *secant condition*, [53]. Accordingly, the delay free system is locally stable if

$$|g'(x_1)| < \left(\sec\frac{\pi}{n}\right)^n. \tag{5.13}$$

Note that the right-hand side of the above equation is always greater than 1, so the secant condition is less conservative than the small gain condition.

The condition (5.13) is related to "diagonal stability" of cyclic systems, see, e.g., [54–56] and their references for further discussion. The next result is a generalization of the secant condition for systems with time delay. In particular, it provides a bound for τ while still maintaining the system as locally stable.

Proposition 5.5. *Let $x_{eq} = [x_1, \ldots, x_n]^{\mathrm{T}}$ be the unique equilibrium point of the system (5.1) under negative feedback. Let $\lambda = \max_i \lambda_i$ and suppose that τ is fixed. If*

$$|g'(x_1)| < \left(\sec \frac{\pi}{n}\right)^n, \tag{5.14}$$

and

$$\tau < \frac{\pi - n \arccos\left(\left(\frac{1}{|g'(x_1)|}\right)^{1/n}\right)}{\omega_m}, \tag{5.15}$$

where $\omega_m = \lambda \sqrt{|g'(x_1)|^{\frac{2}{n}} - 1}$, then the system is locally stable around x_{eq}.

Proof. Recall that $g'(x_1) < 0$; for the sake of clarity, let us re-define the closed loop transfer function of the linearized system

$$T(s) = \frac{G(s)}{1 + G(s)}, \quad \text{where} \quad G(s) = \frac{|g'(x_1)| e^{-\tau s}}{\prod_{i=1}^{n} (1 + s/\lambda_i)} \tag{5.16}$$

The linearized system is stable if and only if $T \in \mathscr{H}^\infty$. Let $p(\omega)$ and $q_\tau(\omega)$ be

$$p(\omega) = \prod_{i=1}^{n} \sqrt{\left(\frac{\omega}{\lambda_i}\right)^2 + 1},$$

$$q_\tau(\omega) = \sum_{i=1}^{n} \arctan\left(\frac{\omega}{\lambda_i}\right) + \tau\omega. \tag{5.17}$$

Note that both p and q_τ are increasing functions of ω. The equilibrium point x_{eq} is locally stable if and only if all the poles of $T(s)$ have negative real parts. Let ω_c be such that $p(\omega_c) = |g'(x_1)|$. By using the Nyquist criterion, we conclude that $T(s)$ is stable if and only if

$$q_\tau(\omega_c) < \pi. \tag{5.18}$$

Now we assume that (5.14) holds, so the delay free system is locally stable around x_{eq} by the secant condition. Let

$$\gamma_i = \arctan\left(\frac{\omega_c}{\lambda_i}\right).$$

Since each γ_i is positive, by the definition of tangent inverse function they must be in the interval $\gamma_i \in (0, \pi/2)$ for each i. Thus, under nonzero time delay, $T(s)$ is stable if and only if

$$\tau\omega_c \leq \pi - \sum_{i=1}^{n} \gamma_i \ .$$

Note that

$$\cos(\gamma_i) = \sqrt{\frac{\lambda_i^2}{\lambda_i^2 + \omega_c^2}},$$

so it follows

$$\frac{1}{\prod_{i=1}^{n} \cos(\gamma_i)} = |g'(x_1)|.$$

Similar to [53], we use the fact that

$$\prod_{i=1}^{n} \cos(\gamma_i) < \left(\cos\left(\frac{\sum_{i=1}^{n} \gamma_i}{n}\right)\right)^n,$$

so we have

$$|g'(x_1)| = \frac{1}{\prod_{i=1}^{n} \cos(\gamma_i)} > \frac{1}{\left(\cos\left(\frac{\sum_{i=1}^{n} \gamma_i}{n}\right)\right)^n}.$$

The above equation implies that

$$\sum_{i=1}^{n} \gamma_i < n \arccos\left(\left(\frac{1}{|g'(x_1)|}\right)^{1/n}\right). \tag{5.19}$$

Therefore,

$$\pi - n \arccos\left(\left(\frac{1}{|g'(x_1)|}\right)^{1/n}\right) < \pi - \sum_{i=1}^{n} \gamma_i .$$

Hence, if $\tau\omega_c < \pi - n \arccos\left(\left(\frac{1}{|g'(x_1)|}\right)^{1/n}\right)$, then the system is locally stable around x_{eq}. Let $\omega_m = \lambda\sqrt{|g'(x_1)|^{\frac{2}{n}} - 1}$, note that $\omega_c \leq \omega_m$, so $\tau\omega_c \leq \tau\omega_m$. Therefore, if

$$\tau < \frac{\pi - n \arccos\left(\left(\frac{1}{|g'(x_1)|}\right)^{1/n}\right)}{\omega_m},$$

then the system is locally stable around x_{eq}, which concludes the proof. □

Note that for the particular case $\lambda_1 = \cdots = \lambda_n$ we have $\omega_m = \omega_c$.

The next result shows that if $|g'(x_1)| > 1$, then there exists τ_c such that for any $\tau > \tau_c$ the system is locally unstable around x_{eq}.

Proposition 5.6. *Let $x_{eq} = [x_1, \ldots, x_n]^{\mathrm{T}}$ be the unique equilibrium point of the system (5.1) under negative feedback. Assume that*

$$|g'(x_1)| > 1,$$

then there exists τ_c such that for any $\tau > \tau_c$ the system (5.1) is locally unstable around x_{eq}.

Proof. We will again proceed as in the Proposition 5.5. Hence, let ω_c be such that $p(\omega_c) = 1$, where $p(\omega)$ is given in (5.17). Note that existence of such a unique ω_c is guaranteed by the assumption that $1 < |g'(x_1)|$. Then by the Nyquist criterion, the system is locally unstable around x_{eq} if $q_\tau(\omega_c) > \pi$, where $q_\tau(\omega)$ is defined in (5.5). Note that $q_\tau(\omega)$ is an increasing function of τ. Now let

$$\tau_c = \frac{\pi - \sum_{i=1}^{n} \arctan \frac{\omega_c}{\lambda_i}}{\omega_c}.$$

It is easy to observe that if $\tau > \tau_c$ then $q_\tau(\omega_c) > \pi$, so the system is locally unstable around x_{eq}. □

Proposition 5.6 shows that if $|g'(x_1)| > 1$ then for sufficiently high values of the delay the system is locally unstable. Moreover, using the tools in the proof of Proposition 5.6, we can actually calculate the threshold τ_c such that the system is locally unstable for all $\tau > \tau_c$. Our final result in this section gives necessary conditions for existence of periodic oscillations of the system.

Proposition 5.7. *Let $x_{eq} = [x_1, \ldots, x_n]^{\mathrm{T}}$ be the unique equilibrium point of the system (5.1) under negative feedback. Let*

$$|g'(x_1)| > 1.$$

Then, there exists τ_c such that for any $\tau > \tau_c$ the system (5.1) *has oscillatory response for all initial conditions, except for the equilibrium itself. Note that oscillations may include convergence towards a homoclinic orbit.*

Proof. Using Proposition 5.6, we establish the existence of τ_c such that the system is locally unstable around x_{eq} for $\tau > \tau_c$. Then, using Theorem 5.1, we conclude that the system has oscillations for $\tau > \tau_c$. □

Let us illustrate these results with an example.

Example 5.2. Consider the following biological system under negative feedback:

$$\begin{aligned}\dot{x}_1(t) &= -x_1(t) + \frac{6}{2 + x_2^2(t)}\\ \dot{x}_2(t) &= -x_2(t) + \frac{4x_1^2(t-\tau)}{1 + x_1^2(t-\tau)}.\end{aligned} \tag{5.20}$$

We can calculate the unique equilibrium point of the system (5.20) as $x_{eq} = [1, 2]^{\mathrm{T}}$, from which we obtain the linearization of the system around x_{eq} as:

$$\begin{aligned}\dot{x}_1(t) &= -x_1(t) - \frac{2}{3}x_2(t),\\ \dot{x}_2(t) &= -x_2(t) + 2x_1(t-\tau).\end{aligned} \tag{5.21}$$

We can easily verify that the system (5.21) is under negative feedback by examining the linearization of it. Moreover, in this case the function $g(x)$ is given as

$$g(x) = g_1 \circ g_2(x) = g_1\left(\frac{4x^2}{1+x^2}\right) = \frac{6x^4 + 12x^2 + 6}{18x^4 + 4x^2 + 2},$$

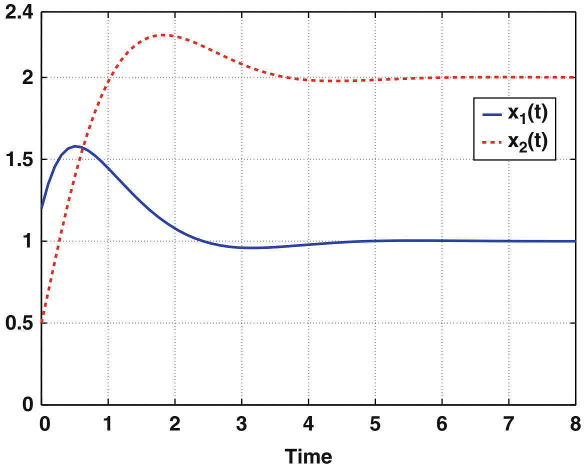
which has the unique fixed point $x_f = 1$ and $g'(x_f) = -4/3$. Then the characteristic equation of the linearized system is given as

$$\chi(s) = (s+1)^2 + \frac{4}{3}e^{-\tau s}.$$

It can be easily verified that when $\tau = 0$, the roots of $\chi(s)$ are given as $-1 \pm j2/\sqrt{3}$, which implies that the delay free system is locally stable. Figure 5.3 shows the behavior of the delay free system. As expected, the solution converges to x_{eq}.

Further note that the feedback system formed around the non-delayed open loop transfer function $G_0(s) = \frac{4/3}{(s+1)^2}$ has the gain crossover frequency $\omega_c = 1/\sqrt{3}$ rad/sec, with a phase margin of $PM = 2\pi/3$ rad. So, the delay margin of the linearized system (5.21) is $DM = PM/\omega_c = 2\pi/\sqrt{3}$ sec, see [23]. This means that the linearized system (5.21) is stable for all $0 \leq \tau < 2\pi/\sqrt{3}$ sec, and unstable for all $\tau \geq \tau_c = 2\pi/\sqrt{3} \approx 3.6276$ sec. This result is consistent with

Fig. 5.3 $x_1(t)$, $x_2(t)$ vs t graph of the system with $x(0) = [1.2, 0.5]^{\mathrm{T}}, \tau = 0.$



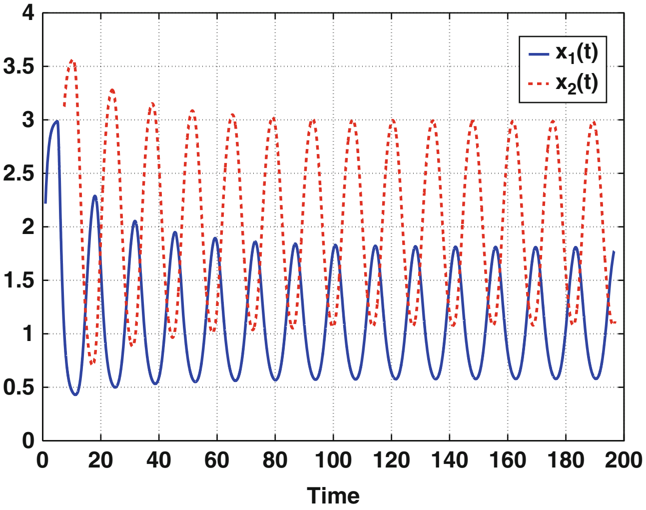


Fig. 5.4 $x_1(t)$, $x_2(t)$ vs t graph of the system with $x(0) = [1.2, 0.5]^{\mathrm{T}}, \tau = 5$ sec.

Proposition 5.6. Figure 5.4 shows the behavior of the system with $\tau = 5$ sec. In this case, we observe oscillatory behavior which is consistent with Proposition 5.7.

From the propositions proved so far, it follows that if $|g'(x_1)| < 1$, the solution converges to the unique equilibrium point of the system, independent of τ. If $|g'(x_1)| > 1$ and $\tau > \tau_c$, it is proven that the solution is oscillatory (see also [57] for a similar result). The only missing part in the analysis is when $|g'(x_1)| > 1$ and $\tau < \tau_c$, in which case the system is locally stable around x_{eq}. Hence, the system is stable around some neighborhood of the equilibrium x_{eq}. Finding the region of attraction is an interesting open problem. In fact, one cannot rule out periodic solutions in this case using the arguments given in this manuscript. On the other hand, our simulations suggest that the solutions still converge to the equilibrium provided that the local stability condition derived here is satisfied. Further research is required to establish this observation. In fact, in our opinion, this is one of the most important open problems in nonlinear system theory.

5.2 Homogeneous Gene Regulatory Networks with Hill Functions

In this section, we consider the homogenous gene regulatory network under negative feedback with Hill function type nonlinearities. More precisely, consider the following system:

$$\begin{aligned} \dot{x}_i(t) &= -x_i(t) + f(x_{i+1}(t)) \qquad i = 1, 2, \ldots, n-1 \\ \dot{x}_n(t) &= -x_n(t) + f(x_1(t-\tau)), \end{aligned} \tag{5.22}$$

where the function $f(x)$ is arbitrary such that

$$f'(x) < 0, \qquad \forall x \in (0, \infty), \tag{5.23}$$

and f has negative Schwarzian derivative. Note that equation (5.23) combined with Proposition 5.1 implies that the function f has a unique fixed point, say x_1. Also notice that to have negative feedback, n should be an odd number. From Proposition 5.1, it is known that under negative feedback the system has a unique fixed point, which is in the form $x_{eq} = [x_1, \ldots, x_1]^{\mathrm{T}}$.

Proposition 5.8. *Consider the homogenous GRN model given in* (5.22) *where n is an odd integer and f satisfies* (5.23) *and $Sf(x) < 0$ for all $x > 0$. Let $x_1 > 0$ denote the unique fixed point of $f(x)$. Then, $x_{eq} = [x_1, \ldots, x_1]^{\mathrm{T}}$ is the unique equilibrium point of the system* (5.22)*, and the following statements hold:*

- *If $|f'(x_1)| < 1$, then any solution of* (5.22) *converges to x_{eq} independent of delay.*
- *If $|f'(x_1)| > 1$, the function $f \circ f$ has exactly 3 fixed points, including x_1. Let $x_1^- < x_1$ and $x_1^+ > x_1$ denote the other two fixed points of $f \circ f$. Then, any solution $x(t) = [x_1(t), \ldots, x_n(t)]^{\mathrm{T}}$ of* (5.22) *satisfies*

$$x_1^- < \underline{\lim_{t\to\infty}}\, x_i(t) \le \overline{\lim_{t\to\infty}}\, x_i(t) < x_1^+ \qquad \forall i = 1, 2, \ldots, n.$$

Moreover, there exists $\tau_c \ge 0$ such that for every value of the delay $\tau > \tau_c$ the system has an oscillatory behavior.

Proof. Note that in the homogenous network $g(x)$ is given as

$$g(x) = f^n(x),$$

and since n is an odd number

$$g'(x) < 0, \ \forall x > 0, \quad \text{and } g'(x_1) = (f'(x_1))^n.$$

But the above inequality implies that

$$|g'(x_1)| < 1 \ \Leftrightarrow \ |f'(x_1)| < 1, \quad |g'(x_1)| > 1 \ \Leftrightarrow \ |f'(x_1)| > 1. \tag{5.24}$$

If $|f'(x_1)| < 1$, then by using (5.24) it is easy to see that $|g'(x_1)| < 1$. Then, part (i) of the proof follows from Proposition 5.3. If $|f'(x_1)| > 1$, then again by using (5.24) it can be shown that $|g'(x_1)| > 1$. Parts (ii) and (iii) follow from Propositions 5.4 and 5.7, respectively. The explicit calculation of τ_c can be found in the proof of Proposition 5.6. □

Next, assume f given in (5.22) has the following form:

$$f(x) = \frac{a}{b + x^m}, \qquad a, b > 0, \quad m \in \{1, 2, 3, \dots\}. \tag{5.25}$$

Also note that to have negative feedback, we should have odd number of interactions between genes. If n is even, the system is under positive feedback, which is studied in Chapter 6. Accordingly, assume that n is an odd integer. Since $f(0) > 0$ and f is decreasing, f has a unique fixed point, x_1, satisfying

$$x_1 = f(x_1) = \frac{a}{b + x_1^m}. \tag{5.26}$$

Then the unique equilibrium point of system (5.22) is $x_{eq} = [x_1, \dots, x_1]^{\mathrm{T}}$. Note that

$$f'(x_1) = -\frac{m\, a\, x_1^{m-1}}{(b + x_1^m)^2} = -\frac{m\, x_1^{m+1}}{a}. \tag{5.27}$$

From (5.26), it is straightforward to get the following identity:

$$x_1^{m+1} = a - b x_1. \tag{5.28}$$

Then, $g(x)$ can be written as

$$g(x) = f^n(x). \tag{5.29}$$

From Proposition 5.8, the system (5.22) is stable if we have

$$|g'(x_1)| < 1 \quad \Leftrightarrow \quad |f'(x_1)|^n < 1. \tag{5.30}$$

Combining (5.26) and (5.27), the following set of inequalities are obtained

$$\begin{aligned} |f'(x_1)|^n < 1 \Rightarrow |f'(x_1)| &< 1. \\ \Rightarrow \frac{mx_1^{m+1}}{a} &< 1 \\ \Rightarrow mx_1^{m+1} &< a \\ \Rightarrow m(a - bx_1) &< a \\ \Rightarrow \frac{(m-1)a}{mb} &< x_1. \end{aligned}$$

Let $p : \mathbb{R} \to \mathbb{R}$ be given by

$$p(x) = x^{m+1} + bx - a. \tag{5.31}$$

Clearly, $p(x_1) = 0$ and

$$p'(x) = (m+1)x^m + b > 0 \quad \forall x \in (0, \infty) \quad \text{and} \quad p(0) = -a < 0. \tag{5.32}$$

Since $p(x_1) = 0$ and p is strictly increasing, one gets

$$p\left(\frac{(m-1)a}{mb}\right) < 0 \Leftrightarrow \frac{(m-1)a}{mb} < x_1. \tag{5.33}$$

Finally, the following identities hold:

$$\begin{aligned} p\left(\frac{(m-1)a}{mb}\right) &= \left(\frac{m-1}{m}\right)^{m+1} \left(\frac{a}{b}\right)^{m+1} + \frac{m-1}{m}a - a \\ &= \left(\frac{m-1}{m}\right)^{m+1} \left(\frac{a}{b}\right)^{m+1} - \frac{a}{m}. \end{aligned} \tag{5.34}$$

Combining (5.33) and (5.34), we arrive at the following set of inequalities:

$$\begin{aligned} p\left(\frac{(m-1)a}{mb}\right) &< 0 \\ &\Leftrightarrow \left(\frac{m-1}{m}\right)^{m+1}\left(\frac{a}{b}\right)^{m+1} < \frac{a}{m} \\ &\Leftrightarrow \left(\frac{a}{m}\right)^{m} < \left(\frac{b}{m-1}\right)^{m+1}. \end{aligned} \tag{5.35}$$

If the constants a, b, and m satisfy the inequality (5.35), then from Proposition 5.3 the unique equilibrium point of system (5.22) is globally attractive. The arguments we had so far are valid for $m > 1$ case. For $m = 1$, the Hill functions do not have negative Schwarzian derivative. Now let $m = 1$ and a and b arbitrary positive real numbers. Then, the system (5.22) has the unique equilibrium point $x_{eq} = [x_1, \ldots, x_1]^{\mathrm{T}}$ satisfying

$$\begin{aligned} x_1 &= \frac{a}{b + x_1} \\ &\Rightarrow x_1^2 = a - bx_1 \end{aligned}$$

From Proposition 5.3, the unique equilibrium point of (5.22) is globally attractive if

$$\begin{aligned} g'(x_1) &= \frac{a}{(b + x_1)^2} = \frac{x_1^2}{a} < 1 \\ &\Rightarrow a - bx_1 < a \\ &\Rightarrow 0 < x_1. \end{aligned} \tag{5.36}$$

Equation (5.36) shows that for $m = 1$ the unique equilibrium point of (5.22) is globally attractive regardless of the values of the positive constants a and b. Thus the following result has been established.

Proposition 5.9. *Consider system (5.22) with f defined in (5.25), and let $x_{eq} = [x_1, \ldots, x_1]^{\mathrm{T}}$ be its equilibrium point. Then, the following statements hold:*

(i) If $m = 1$, then x_{eq} is globally attractive for all positive constants a, b.
(ii) If $m = 2, 3, \ldots$ and a, b, m satisfy

$$\left(\frac{a}{m}\right)^{m} < \left(\frac{b}{m-1}\right)^{m+1},$$

then x_{eq} is globally attractive.

(iii) For cases other than (i) and (ii), the system (5.22) has oscillatory behavior for sufficiently large values of delay.

Proof. From the above arguments, if (i) or (ii) holds, then at the unique fixed point x_1 of $g(x)$, we have

$$|g'(x_1)| < 1.$$

Hence the result follows from Proposition 5.3. If the conditions in (i) and (ii) are not satisfied, then

$$|g'(x_1)| > 1.$$

Then, (iii) follows from Proposition 5.8. Note that in this case we can calculate τ_c such that for $\tau > \tau_c$ the system has oscillatory behavior. For details, see the proof of Proposition 5.6. ☐

Example 5.3 (Homogenous Negative Feedback Case). In this example, we will try to highlight the results of Proposition 5.9. Consider the homogenous negative feedback case with $a = 1.5$, $b = 0.5$, $m = 2$, $n = 3$ and f is given by

$$f(x) = \frac{1.5}{0.5 + x^2}. \tag{5.37}$$

Note that

$$\left(\frac{a}{m}\right)^m = 0.5625 > \left(\frac{b}{m-1}\right)^{m+1} = 0.125. \tag{5.38}$$

Then, the function

$$h(x) = f \circ f(x)$$

has three fixed points given by $y_1 = 0.1771$, $y_2 = 1$ and $y_3 = 2.8229$. Moreover, the system has the unique equilibrium point $x_{eq} = [1, 1, 1]^{\mathrm{T}}$. The linearization of the system around x_{eq} is given as

$$\begin{aligned}
\dot{x}_1(t) &= -x_1(t) - \frac{4}{3}x_2(t) \\
\dot{x}_2(t) &= -x_2(t) - \frac{4}{3}x_3(t) \\
\dot{x}_3(t) &= -x_3(t) - \frac{4}{3}x_1(t-\tau).
\end{aligned}$$

For sufficiently large values of the delay, from Propositions 5.7 and 5.9, oscillatory solutions of the system are expected to satisfy

$$0.1171 = y_1 < \lim_{t\to\infty} \underline{x_i(t)} \le \lim_{t\to\infty} \overline{x_i(t)} < y_3 = 2.8229 \qquad \forall i = 1, 2, 3. \tag{5.39}$$

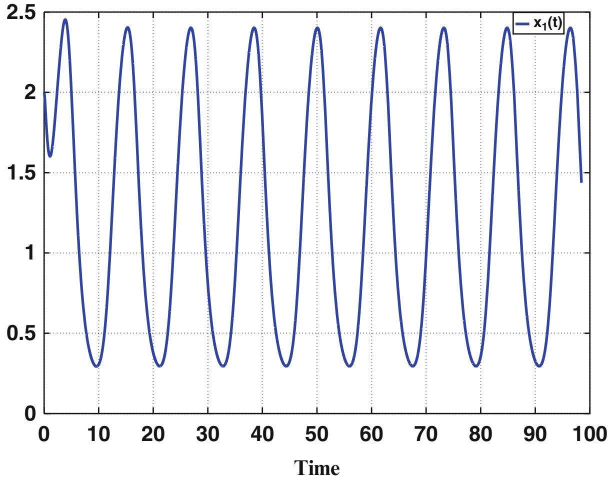


Fig. 5.5 $x_1(t)$ vs t graph of the system with $x(0) = [2.0, 0.5, 0.2]^{\mathrm{T}}$, $\tau = 0$.

The simulation results corresponding to $x_1(t)$, with initial conditions $x_1(0) = 2.0$, $x_2(0) = 0.5$, $x_3(0) = 0.2$, and $\tau = 0$, are shown in Figure 5.5. The other two coordinates behave similarly. We have a periodic solution as expected and the inequality in (5.39) is satisfied.

Consider the same problem with $a = 2$, $b = 3$, $m = 2$, $n = 3$. Then,

$$f(x) = \frac{2}{3 + x^2} \quad \text{which implies} \quad \left(\frac{a}{m}\right)^m = 1 < \left(\frac{b}{m-1}\right)^{m+1} = 27. \tag{5.40}$$

The unique equilibrium point of the system can be calculated as

$$x_{eq} = [0.5961, 0.5961, 0.5961]^{\mathrm{T}}. \tag{5.41}$$

From Proposition 5.9, we expect the solution of the system to converge to x_{eq} regardless of the initial condition and the time delay. The simulation results are shown in Figures 5.6 and 5.7 for two different sets of initial conditions and time delays. The simulation results are consistent with the theory: $x(t)$ converges to x_{eq} independent of delay.

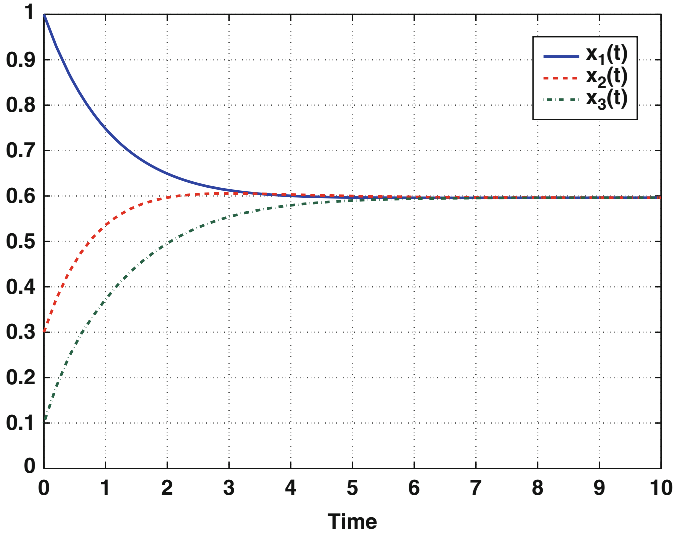


Fig. 5.6 $x_1(t)$, $x_2(t)$, and $x_3(t)$ vs t graphs of the system with $x(0) = [1.0, 0.3, 0.1]^{\mathrm{T}}$, $\tau = 0.5$.

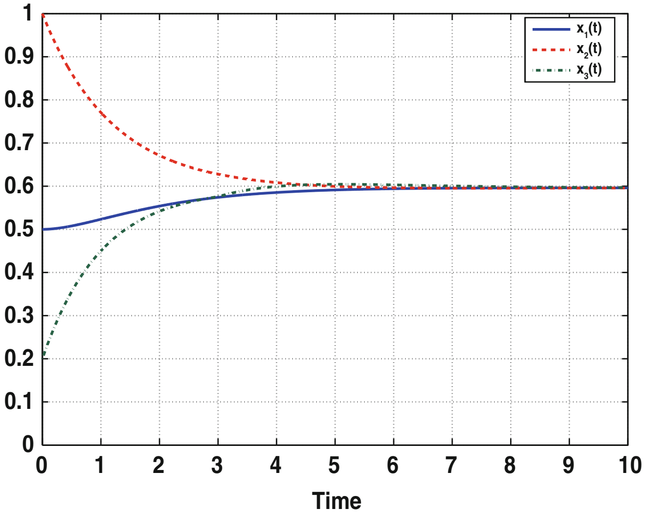


Fig. 5.7 $x_1(t)$, $x_2(t)$, and $x_3(t)$ vs t graphs of the system with $x(0) = [0.5, 1.0, 0.2]^{\mathrm{T}}$, $\tau = 3$.

5.3 Exercises

Problem 1. Consider the nonlinear system:

$$\dot{x}_1(t) = -\lambda_1 x_1(t) + g_1(x_2(t)) \tag{5.42}$$

$$\dot{x}_2(t) = -\lambda_2 x_2(t) + g_2(x_1(t-\tau)), \tag{5.43}$$

where $g_1(x) = \frac{6}{2+x^2}$, $g_2(x) = \frac{4x^2}{1+x^2}$, $\lambda_1 > 0$, $\lambda_2 > 0$, and $\tau > 0$. As in (5.3) define $g = (\frac{1}{\lambda_1} g_1) \circ (\frac{1}{\lambda_2} g_2)$.

(*a*) For $\lambda_1 = \lambda_2 = 1$, the unique equilibrium point is computed above from the unique positive fixed point $x_f = 1$ of g, as $x_{eq} = [1 \quad 2]^{\mathrm{T}}$. Clearly, since $n = 2$, in this case, the condition (5.14) is automatically satisfied. Find the largest τ satisfying (5.15), compare this result with τ_c found above (recall that $\tau_c = 2\pi/\sqrt{3}$). What is the level of conservatism of Proposition 5.5?

(*b*) Now let $\lambda_1 = 2$ and $\lambda_2 = 0.5$. Determine the equilibrium point of (5.42) and obtain the characteristic equation of linearized system around this equilibrium in the form $1 + G_0(s)e^{-\tau s} = 0$, where $G_0(s) = \frac{K}{(1+s/\lambda_1)(1+s/\lambda_2)}$ for $K = |g'(x_f)|$ and x_f is the fixed point of g. Now compute ω_c such that $|G_0(j\omega_c)| = 1$. Determine the $\tau_c = PM/\omega_c$ where the phase margin is $PM = \pi + \angle G_0(j\omega_c)$ (phase of $G_0(j\omega_c)$ is in the interval $(-\pi\ ,\ 0]$). Verify that in this case $\omega_c < \omega_m$. Find the largest delay value τ satisfying (5.15), and compare it with τ_c. What is the level of conservatism of Proposition 5.5 in this case?

Problem 2. For the system defined in Problem 1, part *b*, show that Proposition 5.5 becomes less conservative if we change the definition of ω_m to

$$\tilde{\omega}_m = \tilde{\lambda}\ \left(|g'(x_f)|^2 - 1\right)^{1/2n}, \qquad \text{where} \quad \tilde{\lambda} = \left(\prod_{i=1}^{n} \lambda_i\right)^{1/n}.$$

Prove that $\omega_c < \tilde{\omega}_m < \omega_m$ in this case. Repeat the exercise for $\lambda_1 = 1$, $\lambda_2 = 0.25$. Examine the stability conditions given in Section III of [58] and compare them to the sufficient condition obtained in Proposition 5.5.

Problem 3. Consider the nonlinear system

$$\dot{x}_1(t) = -\lambda_1 x_1(t) + f(x_2(t)) \tag{5.44}$$

$$\dot{x}_2(t) = -\lambda_2 x_2(t) + f(x_1(t-\tau)), \tag{5.45}$$

where $\lambda_1 > 0$, $\lambda_2 > 0$, $\tau > 0$ and f is in the form (5.25). Try to find an extension of Proposition 5.9 for the case $\lambda_1 \neq \lambda_2$, i.e. the stability/instability conditions are expressed in terms of the parameters a, b, m, λ_1, and λ_2. Give numerical examples and simulation results illustrating this result.

Chapter 6

Gene Regulatory Networks Under Positive Feedback

Abstract In this chapter, we consider the simplified GRN model, with the assumption that it is under delayed positive feedback. By analyzing the fixed points of a single function determined from the nonlinear connections, we show that the system may have three equilibrium points in the positive cone. When the system has a unique equilibrium, generically all solutions converge to this point. When there are three equilibrium points, the system shows a bistable behavior. Homogenous GRNs under delayed positive feedback are analyzed, and their stability and bistability are determined from the parameters of the Hill function used in the nonlinear coupling.

Keywords Gene regulatory networks • Cyclic systems • Delayed feedback Positive feedback • Bistability • Hill functions

In this chapter we consider the simplified GRN model (4.3) with the assumption that it is under delayed positive feedback. For the sake of completeness, the ODE-based model defined in Chapter 4 is re-written

$$\begin{aligned} \dot{x}_1(t) &= -\lambda_1 x_1(t) + g_1(x_2(t)) \\ &\vdots \\ \dot{x}_{n-1}(t) &= -\lambda_{n-1} x_{n-1}(t) + g_{n-1}(x_n(t)) \\ \dot{x}_n(t) &= -\lambda_n x_n(t) + g_n(x_1(t-\tau)), \end{aligned} \tag{6.1}$$

where $\tau > 0$ is the delay in the feedback loop, $\lambda_i > 0$ and each g_i is a bounded monotone function taking positive values and having negative Schwarzian derivatives, $i = 1, \ldots, n$. Recall that the GRN is under *positive feedback* if

$$g'(x) > 0, \qquad \forall x \in (0, \infty), \tag{6.2}$$

where

$$g = (\frac{1}{\lambda_1} g_1) \circ (\frac{1}{\lambda_2} g_2) \circ \cdots \circ (\frac{1}{\lambda_n} g_n). \tag{6.3}$$

M.E. Ahsen et al., *Analysis of Deterministic Cyclic Gene Regulatory Network Models with Delays*, SpringerBriefs in Electrical and Computer Engineering,
DOI 10.1007/978-3-319-15606-4_6

It is easier to determine the behavior of the system (6.1) under positive feedback compared to the negative feedback case; in the sense that, as we shall see, generically the solution converges to one of the equilibrium points of the system. Hence, if the system has a unique equilibrium point, then the solution converges to the equilibrium point for almost every non-negative initial condition and any delay value $\tau \geq 0$. The most interesting phenomena under positive feedback is *bistability*. Basically, a bistable system has two stable states and an unstable state and the system can converge to one of the stable states depending on the initial conditions and the value of the delay. In biology, bistability is a key concept for understanding the cellular functioning. It is involved in critical processes such as cellular differentiation and apoptosis, see, e.g., [59]. In this chapter, we give conditions that will lead to the bistability of the system.

6.1 General Conditions for Global Stability

We start with a result from [60], which states that, generically, the solution of (6.1) converges to one of the equilibrium points.

Theorem 6.1 ([60, 61]). *Consider system* (6.1) *under positive feedback with initial conditions from*

$$\mathscr{X} = \{\phi(a) = [\phi_1(a)\ldots\phi_n(a)]^{\mathrm{T}} \ : \ \phi_i(\cdot) \in \mathscr{C}([-\tau\ ,\ 0],\mathbb{R}),\ \ \phi(a) \geq 0\}.$$

Then, almost every solution of system (6.1) *converges to one of its equilibrium points.* □

The above result is a consequence of Theorem 4.12 of [60]; for its application to (6.1) under positive feedback, see Theorem 3 of [61] and its references for technical arguments of the proof. A similar result also appears in Section 7.2 of [62]. What is meant by "*almost every solution converge*" is that there is a dense open subset of $\mathscr{X}$ such that any initial condition from this subset leads to a convergent solution; see [60] and also [63–65] for measure theoretic details on the definition of a *dense subset* on infinite dimensional spaces.

Next, let us turn back to the analysis of the *linearized system under positive feedback*. Note that different from the negative feedback case, in the positive feedback case, we may have more than one equilibrium point, which implies that $g(x)$ may have more than one fixed point. Observe that for any fixed point x_1 of g, the corresponding equilibrium point of the system is given as $x_{eq} = [x_1, \ldots, x_n]^{\mathrm{T}}$, where

$$x_n = \frac{g_n(x_1)}{\lambda_n},\ x_{n-1} = \frac{g_{n-1}(x_n)}{\lambda_{n-1}}, \ldots, x_2 = \frac{g_2(x_3)}{\lambda_2},\ x_1 = \frac{g_1(x_2)}{\lambda_2} = g(x_1).$$

Let $x_{eq} = [x_1, \ldots, x_n]^{\mathrm{T}}$ be an equilibrium point of the system. Then, as in the negative feedback case x_1 is a fixed point of g. The stability of the linear system around x_{eq} is determined from the locations of the poles of the transfer function

$$T(s) = \frac{G(s)}{1 - G(s)}, \quad \text{where} \quad G(s) = \frac{g'(x_1)e^{-\tau s}}{\prod_{i=1}^{n} \left(\frac{s}{\lambda_i} + 1 \right)}. \tag{6.4}$$

Since $g'(x) > 0$, from Proposition 2.3, we get the following result regarding the stability of the linearized system.

Proposition 6.1. *Consider the system* (6.1) *under positive feedback. Let* $x_{eq} = [x_1, \ldots, x_n]^{\mathrm{T}}$ *be an equilibrium point of the system. If*

$$g'(x_1) < 1, \tag{6.5}$$

then the system is locally stable around x_{eq} *independent of the delay. On the other hand, if*

$$g'(x_1) \geq 1, \tag{6.6}$$

the system is locally unstable independent of delay.

Proof. If $g'(x_1) = 1$, the rightmost pole of $T(s)$ is at zero, so the system violates our definition of local stability (we are interested in local asymptotic stability). For the cases $g'(x_1) > 1$ and $g'(x_1) < 1$, the results are obtained directly from Proposition 2.3. ☐

According to Theorem 6.1, generically, the system converges to one of its equilibrium points independent of delay. Proposition 3.5 deals exactly with that case. It implies that the largest fixed point x_f of g satisfies

$$g'(x_f) < 1.$$

Then, by Proposition 6.1, the system is locally stable independent of delay around the equilibrium point $x_{eq} = [x_1, \ldots, x_n]^{\mathrm{T}}$, where

$$x_1 = x_f, \quad x_n = g_n(x_f)/\lambda_n, \quad \ldots, \quad x_2 = g_2(x_3)/\lambda_2.$$

We now elaborate our analysis of the positive feedback case by using the results from Chapter 3. The result below gives a general condition on the existence of *unique equilibrium point* for (6.1).

Proposition 6.2. *Consider the system* (6.1) *under positive feedback. If* $g'(x) < 1$ *for all* $x \geq 0$*, then* $g(x)$ *has a unique fixed point. In this case, the system* (6.1) *has a unique equilibrium point* x_{eq} *which is globally attracting, for almost every initial condition from* $\mathscr{X}$.

Proof. Suppose $g'(x) < 1$ for all $x \geq 0$, but g has two fixed points $x_1 < x_2$. Then, a routine application of the mean value theorem implies that there exists $c \in (x_1, x_2)$ such that

$$g'(c) = \frac{g(x_2) - g(x_1)}{x_2 - x_1} = 1,$$

which is a contradiction. Hence, g has a unique fixed point. Convergence to the unique equilibrium, for almost every initial condition from $\mathscr{X}$, follows from Theorem 6.1. □

The condition given in the above theorem is by no means necessary. Simply, it provides a sufficient condition for the existence of a unique fixed point of the function $g(x)$.

Proposition 6.3. *Consider the system (6.1) under positive feedback. If $g'(0) > 1$, then the system (6.1) has a unique equilibrium point x_{eq} which is locally stable independent of delay. Moreover, if $g(0) > 0$, then g has a unique fixed point.*

Proof. Recall that the assumptions in the problem setup imply that g has negative Schwarzian derivative. Then, by Remark 3.1, g is either of Type A or Type B. First suppose that g is of type A. Then, the result follows from Proposition 3.5, which states that g has at most two fixed points including 0. If $g(0) > 0$, then from Proposition 3.5, we conclude that g has unique fixed point $x_1 > 0$ such that the corresponding equilibrium point of the system is locally stable. If $g(0) = 0$, again Proposition 3.5 shows that g has two fixed points 0 and x_1 such that $g'(x_1) < 1$. The fixed point of the system corresponding to the fixed point 0 of g is locally unstable independent of delay by our assumption that $g'(0) > 1$ (see Proposition 6.1). Other fixed point of the system, corresponding to the fixed point x_1 of $g(x)$, is locally stable independent of delay as we have $g'(x_1) < 1$. Next assume $g(x)$ is of type B. Combining our assumption $g'(0) > 1$ with Remark 3.1, we conclude that there exists $x_0 > 0$ such that

$$g'(x) > 1, \ \ \forall x \in [0, x_0), \text{ and } g'(x) \leq 1, \ \forall x \geq x_0.$$

Note since $g(0) > 0$, for any $y \in [0, x_0)$, we have

$$g(y) = g(0) + \int_0^y g'(x)dx > g(0) + y,$$

which implies that g cannot have any fixed points in $[0, x_0]$ apart from 0. When $x > x_0$, since $g'(x) \leq 1$, g has a unique fixed point $x_1 > x_0$ such that $g'(x_1) \leq 1$. Whether 0 is a fixed point or not, our assumption $g'(0) > 1$ implies that the equilibrium point of the system corresponding to 0 is unstable independent of delay by Proposition 6.1. On the other hand, the fixed point corresponding to x_1 is locally stable independent of delay again by Proposition 6.1. □

Next, we investigate the bistability of the system by using Proposition 3.6. The bistability condition depends only on the number of fixed points of g.

Proposition 6.4. *Consider the system* (6.1) *under positive feedback. Moreover, suppose that* $g(x)$ *has three fixed points* $x_1^- < x_1 < x_1^+$. *Then, the system exhibits bistable behavior.*

Proof. Suppose g has three fixed points $x_1^- < x_1 < x_1^+$. Then, Proposition 3.6 implies that

$$g'(x_1^-) < 1, \quad g'(x_1) > 1, \quad g'(x_1^+) < 1.$$

From Proposition 6.1, it can be concluded that the two equilibrium points corresponding to x_1^- and x_1^+ are locally stable independent of delay, whereas the equilibrium point of the system corresponding to x_1 is locally unstable independent of delay. But since the system has two locally stable equilibria, the solution of the system will converge to one of these equilibrium points depending on the initial value. Hence, the system exhibits a bistable behavior. ☐

6.2 Analysis of Homogenous Gene Regulatory Networks

In this section, *homogenous* gene regulatory networks of the form (6.1) are studied; i.e., it is assumed that $\lambda_i = 1$ and there exists a function f such that

$$g_i(x) = f(x), \qquad \forall i = 1, 2, \ldots, n.$$

For the sake of clarity, let us rewrite the homogenous network as:

$$\begin{aligned} \dot{x}_i(t) &= -x_i(t) + f(x_{i+1}(t)) \qquad i = 1, 2, \ldots, n-1 \\ \dot{x}_n(t) &= -x_n(t) + f(x_1(t-\tau)). \end{aligned} \tag{6.7}$$

Note that no special structure is assumed for f yet. But, due to the monotonicity assumption, we have either

$$f'(x) > 0 \ \forall x \in (0, \infty) \quad \text{or} \quad f'(x) < 0 \ \forall x \in (0, \infty).$$

The following result plays a crucial role in the remainder of this section.

Lemma 6.1. *Let* $k(x) : \mathbb{R}_+ \to I \subseteq \mathbb{R}_+$ *be a three times continuously differentiable function satisfying* $k'(x) > 0$ *for all* $x \in (0, \infty)$. *Let* $h(x)$ *be defined on* $\mathbb{R}_+$ *as* $h(x) = k^m(x)$, *for some positive integer* m. *Then, any fixed point of* h *is a fixed point of* k.

Proof. Suppose that $h(0) = 0$ and $k(0) > 0$. Then we have

$$h(0) = k^n(0) > \ldots > k(k(0)) > k(0) > 0,$$

which is a contradiction. Therefore, $k(0) = 0$ and 0 is a fixed point of the function k. Let $x > 0$ be a fixed point of the function h and suppose $k(x) \neq x$. Then, either $x < k(x)$ or $k(x) < x$. If $x < k(x)$, since k is a strictly increasing function, it follows that

$$h(x) = k^n(x) > \ldots > k(x) > x,$$

leading to a contradiction. Similarly, if $k(x) < x$, then

$$h(x) = k^n(x) < \ldots < k(x) < x,$$

which is again a contradiction. Therefore, we should have $k(x) = x$. Also, it is easy to see that any fixed point x of k is a fixed point of h. Thus we conclude that the functions k and h have the same fixed points. □

Remark 6.1. The homogenous system is under positive feedback if

(i) $f'(x) > 0$ for all $x \in (0, \infty)$, or
(ii) $f'(x) < 0$ for all $x \in (0, \infty)$ and n is a positive even integer. □

First, consider the case *(ii)* of Remark 6.1.

Proposition 6.5. *Consider the homogenous gene regulatory network (6.7) under positive feedback with $f'(x) < 0$. Then, $g(x) = f^n(x)$ and f has the unique fixed point x_1. If*

$$|f'(x_1)| < 1,$$

then the system has the unique fixed point $x_{eq} = [x_1, \ldots, x_1]^{\mathrm{T}}$ which is globally attractive, independent of delay, for almost every initial condition from $\mathscr{X}$. Otherwise, if

$$|f'(x_1)| > 1,$$

the system exhibits bistable behavior.

Proof. When $f'(x) < 0$, in order for the system to be under positive feedback n should be an even number. Let $n = 2 \times m_1$ and let $k(x) = f^2(x)$. Then, we have $g(x) = k^{m_1}(x)$. Moreover, from chain rule

$$k'(x) = f'(f(x))f'(x) > 0, \quad \forall x > 0.$$

Also note that, from Lemma 6.1, g has the same fixed points as k. If

$$|f'(x_1)| < 1,$$

then from Proposition 3.7, the function k has the unique fixed point x_1. In this case, the homogenous GRN has the unique equilibrium point $x_{eq} = [x_1, \ldots, x_1]^{\mathrm{T}}$. Hence, Theorem 6.1 implies that almost all the solutions converge to x_{eq} independent of delay. Next assume that

$$f'(x_1) > 1.$$

Then, again from Proposition 3.7, k has three fixed points $x_1^- < x_1 < x_1^+$. Hence, g has the same three fixed points. Therefore, Proposition 3.6 implies that the system exhibits bistable behavior. □

Next, assume a special form for the nonlinearity function $f(x)$, which is given as follows:

$$f(x) = \frac{a}{b + x^k}, \qquad a, b > 0, \quad k \in \{1,\ 2,\ 3, \ldots\}. \tag{6.8}$$

Note that $f'(x) < 0$. In this case, the following proposition holds.

Proposition 6.6. *Consider the homogenous gene regulatory network* (6.7) *under positive feedback with f given as in* (6.8)*. In this case, if $k = 1$ or a, b, k satisfy*

$$\left(\frac{a}{k}\right)^k < \left(\frac{b}{k-1}\right)^{k+1}, \tag{6.9}$$

then the system has a unique equilibrium point which is globally attractive for almost every initial condition from $\mathscr{X}$. Otherwise, the system exhibits bistable behavior.

Proof. Since $f(x)$ has negative derivative, it has a unique fixed point. Let x_1 be the unique fixed point of $f(x)$. If $k = 1$ or (6.9) is satisfied, by using the same argument as in the proof of Proposition 5.9, it follows that $|f'(x_1)| < 1$. Otherwise, $|f'(x_1)| > 1$. The rest of the proof follows from Proposition 6.5. □

Now consider the case *(i)* of Remark 6.1, where $f(x)$ satisfies

$$f'(x) > 0, \quad \forall x \in (0, \infty). \tag{6.10}$$

Lemma 6.2. *Consider the homogenous gene regulatory network* (6.7) *under positive feedback with the nonlinearity function $f(x)$ satisfying* (6.10)*. Then, the function $g(x) = f^n(x)$, where n is even, has as many fixed points as f. In particular, if f has a unique fixed point, then the system* (6.7) *has a unique equilibrium which is globally attractive for almost all initial conditions from $\mathscr{X}$.*

Proof. Lemma 6.1 and Theorem 6.1 lead to the desired result. □

For the case (6.10), the above Lemma reduces the whole analysis to the investigation of the fixed points of f. If f has a negative Schwarzian derivative, then we have theoretically shown that it has one, two, or three fixed points. As an example, consider the following Hill type of functions and try to find some conditions regarding its fixed points:

$$f(x) = \frac{ax^m}{b + x^m} + c, \quad a,\ b,\ c > 0. \tag{6.11}$$

Note that zero is ruled out as a fixed point by taking the constant c strictly positive. Then $x > 0$ is a fixed point of the function defined in (6.11) if x is a root of the following polynomial:

$$h(x) = x^{m+1} - (a + c)x^m + bx - bc. \tag{6.12}$$

Some interesting cases regarding the function (6.12) may occur. For example, consider the following numerical values: $a = 3.6$, $b = 5$, $m = 2$, and $c = 0.4$. Then

$$h(x) = x^{m+1} - (a + c)x^m + bx - bc = (x - 1)^2(x - 2)$$

which implies that the function f has exactly two fixed points, both of which are different than 0.

Let us now try to find a sufficient condition depending on the parameters a, b, c, and m so that the function f defined in (6.11) has a unique equilibrium point. First note that, for arbitrary positive constants a, b, c, and m, the following holds:
$h(0) = -bc < 0$. Therefore, if we have

$$h'(x) \geq 0 \qquad \forall x \in \mathbb{R}_+, \tag{6.13}$$

then $h(x)$ can have at most one positive root, so f has a unique fixed point. For $m > 1$,

$$\begin{aligned} h'(x) &= (m + 1)x^m - (m)(a + c)x^{m-1} + b \\ &= x^{m-1}((m + 1)x - m(a + c)) + b \ = \ h_1(x) + b. \end{aligned}$$

In order to guarantee (6.13), we should have $h_1(x) \geq -b$ for all $x \in \mathbb{R}_+$. But h_1 takes its minimum at the point y where

$$h_1'(y) = 0. \tag{6.14}$$

As a result of (6.14), we get the following relations:

$$\begin{aligned} h_1'(x) &= (m+1)(m)x^{m-1} - (m)(m-1)(a+c)x^{m-2} \\ &= x^{m-2}(m)(m+1)(x - \frac{m-1}{m+1}(a+c)) \; ; \\ &\Rightarrow h_1'(y) = 0 \Leftrightarrow y = \frac{m-1}{m+1}(a+c) \; ; \\ &\Rightarrow \min_{x \geq 0} h_1(x) = h_1\left(\frac{m-1}{m+1}(a+c)\right) \; = \; -\left(\frac{m-1}{m+1}\right)^{m-1} (a+c)^m . \end{aligned}$$

Combining this with (6.13) and (6.14), the following result is obtained:

$$\left(\frac{m-1}{m+1}\right)^{m-1} (a+c)^m \leq b \Rightarrow h_1(x) \geq -b \Rightarrow h'(x) \geq 0.$$

Proposition 6.7. *Let f be given as a Hill function in the form* (6.11). *Then, the following properties hold:*

(i) *If $m = 1$, then for any positive a, b, and c, the function f has a unique fixed point.*

(ii) *If $m = 2, 3, \ldots$ and the positive constants a, b, and c satisfy*

$$\left(\frac{m-1}{m+1}\right)^{m-1} (a+c)^m \leq b,$$

then f has a unique fixed point.

In both of the cases above the homogenous GRN (6.7) *has a unique equilibrium point, which is globally attractive for almost all initial conditions from $\mathscr{X}$.*

Proof. We already proved the case *(ii)*. For the case where $m = 1$, let a, b, and c be arbitrary positive constants. If y is a fixed point of the function f, we have

$$h(y) = y^2 + (b - a - c)y - bc = 0.$$

But h can have at most two roots. Since $h(0) < 0$ and $h(-\infty) = \infty$, the function h has only one positive root; so, f has a unique fixed point. In both of the above cases, the function f has a unique fixed point, which implies that the homogenous network (6.7) has a unique equilibrium. On the other hand, by Theorem 6.1, when the system has a unique equilibrium point almost every solution of (6.7) converges to this equilibrium. □

We will now illustrate the results of this section with two numerical examples. One of the first examples of positive feedback in biology is the bistable toggle switch studied in [66]. As in [66], we assume that two proteins mutually repress the expression of each other. The system can be modeled by the following equations:

$$\begin{aligned}\dot{x}_1(t) &= -x_1(t) + f(x_2(t))\\ \dot{x}_2(t) &= -x_2(t) + f(x_1(t-\tau)),\end{aligned} \tag{6.15}$$

where the nonlinear function f is given as follows:

$$f(x) = \frac{a}{b + x^k}, \qquad a, b > 0, \quad k \in \{1,\ 2,\ 3, \ldots\}. \tag{6.16}$$

Example 6.1. Let the nonlinear function f be given by

$$f(x) = \frac{1}{1 + x^2}.$$

Applying the inequality in Proposition 6.6, one sees that

$$\left(\frac{a}{k}\right)^k = 0.25 < (\frac{b}{k-1})^{k+1} = 1.$$

The unique fixed point of the function f is calculated as $x_1 = 0.68$, which gives the unique equilibrium point of the system as $x_{eq} = [0.68, 0.68]^{\mathrm{T}}$. From Proposition 6.6, we expect that the solution will converge to its unique equilibrium point independent of delay. Simulation results in Figures 6.1 and 6.2 show that the solutions of the system converge to x_{eq} under different initial conditions for different delay values.

Example 6.2. In order to illustrate the bistable behavior, now let us take

$$f(x) = \frac{2}{0.1 + x^2}.$$

Again applying the inequality in Proposition 6.6, it is easily observed that

$$\left(\frac{a}{k}\right)^k = 1 > \left(\frac{b}{k-1}\right)^{k+1} = 10^{-3}.$$

One can find the unique fixed point of f as $x_1 = 1.2335$ and the three equilibrium points of the system can be found as $x_{eq1} = [0.005, 20]^{\mathrm{T}}$, $x_{eq2} = [1.2335, 1.2335]^{\mathrm{T}}$ and $x_{eq3} = [20, 0.005]^{\mathrm{T}}$. Note that $|f'(x_1)| = 1.8767 > 1$. From Proposition 6.6,

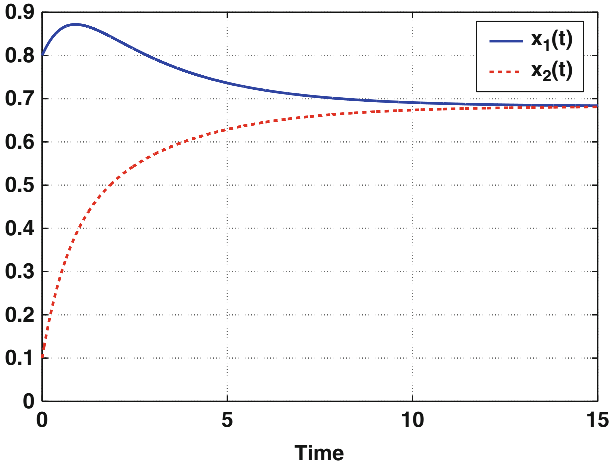
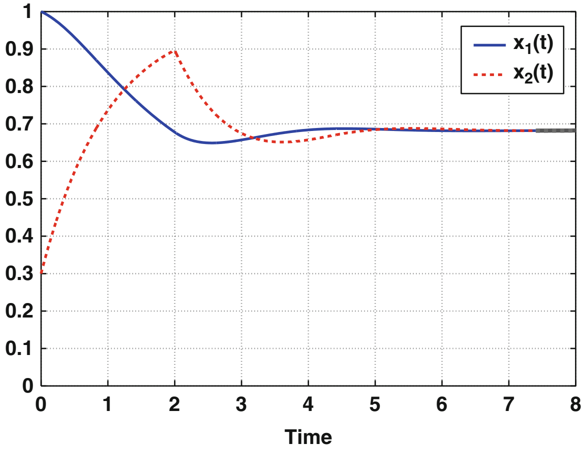


Fig. 6.1 Simulation of the system in Example 6.1 with initial conditions $x(0) = [0.8, 0.1]^{\mathrm{T}}$, $\tau = 0$.

Fig. 6.2 Simulation of the system in Example 6.1 with initial conditions $x(0) = [1, 0.3]^{\mathrm{T}}$, $\tau = 2$.



we expect the system to show a bistable behavior. Simulation results shown in Figures 6.3 and 6.4 illustrate this behavior: depending on the initial conditions and the value of the time delay, $x(t)$ converges to either x_{1eq} or x_{3eq}.

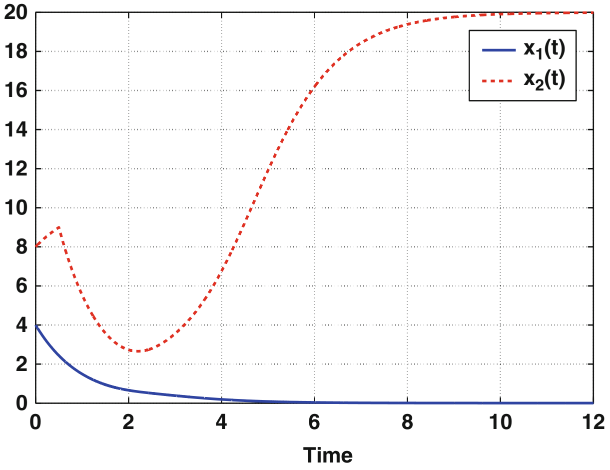


Fig. 6.3 Simulation of the system in Example 6.2 with initial conditions $x(0) = [4, 8]^\mathrm{T}$, $\tau = 0.5$.

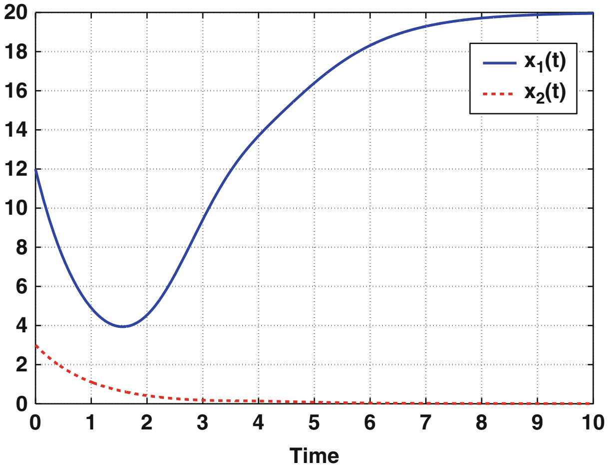


Fig. 6.4 Simulation of the system in Example 6.2 with initial conditions $x(0) = [12, 3]^\mathrm{T}$, $\tau = 2$.

6.3 Exercises

Problem 1. Consider the nonlinear system

$$\dot{x}_1(t) = -\lambda_1 x_1(t) + g_1(x_2(t)) \tag{6.17}$$

$$\dot{x}_2(t) = -\lambda_2 x_2(t) + g_2(x_1(t-\tau)), \tag{6.18}$$

where $g_1(x) = \frac{4}{b+x^4}$, $g_2(x) = \frac{2}{0.2+x^2}$, $\lambda_1 = 2$, $\lambda_2 = 1$, $b > 0$, and $\tau > 0$. As before, define $g = (\frac{1}{\lambda_1} g_1) \circ (\frac{1}{\lambda_1} g_2)$.

(*a*) Determine the equilibrium point(s) from the fixed point(s) of g for two cases $b = 0.1$ and $b = 1.1$. Is the system stable independent of delay, or shows bi-stable behavior, for these two values of b?
(*b*) Run a time domain simulation illustrating the results of part (*a*).
(*c*) What is the largest allowable value of b so that the solution converges to the unique equilibrium?

Problem 2. Consider the nonlinear system

$$\dot{x}_1(t) = -\lambda_1 x_1(t) + g_1(x_2(t)) \tag{6.19}$$

$$\dot{x}_2(t) = -\lambda_2 x_2(t) + g_2(x_1(t-\tau)), \tag{6.20}$$

where $g_1(x) = \frac{2}{0.5+x^4}$, $g_2(x) = \frac{2}{0.1+x^2}$, $\lambda_1 > 0$, $\lambda_2 = 1$, and $\tau > 0$. As before, define $g = (\frac{1}{\lambda_1} g_1) \circ (\frac{1}{\lambda_1} g_2)$.

(*a*) Determine the equilibrium point(s) from the fixed point(s) of g for two cases $\lambda_1 = 2$ and $\lambda_1 = 0.5$. Is the system stable independent of delay, or shows bi-stable behavior, for these two values of λ_1?
(*b*) Run a time domain simulation illustrating the results of part (*a*).
(*c*) What is the smallest allowable value of λ_1 such that the solution converges to unique equilibrium?

Chapter 7
Summary and Concluding Remarks

Abstract In this chapter, the analysis results for GRNs under negative and positive feedback are discussed and concluding remarks are made. Some possible future research directions are also pointed out.

Keywords Gene regulatory networks • Time delay • Negative feedback • Positive feedback • Global stability • Local stability • Region of attraction • Bistability

In this brief, we have studied the dynamical behavior of the cyclic network (7.1), which represents a deterministic model of the gene regulatory networks under delayed feedback:

$$\left.\begin{aligned}\dot{x}_i(t) &= -\lambda_i x_i(t) + g_i(x_{i+1}(t)), \quad i = 1, \ldots, n-1,\\ \dot{x}_n(t) &= -\lambda_n x_n(t) + g_n(x_1(t-\tau)), \quad \tau \geq 0.\end{aligned}\right\} \tag{7.1}$$

It is assumed that in (7.1) each $\lambda_i > 0$, and the functions g_i are monotonically increasing, or decreasing, with a negative Schwarzian derivative on $\mathbb{R}_+$. Typical examples of GRNs have g_i as Hill functions, satisfying these properties. The equilibrium points of the system (7.1) are determined from the fixed points of the function

$$g := \left(\frac{1}{\lambda_1} g_1\right) \circ \ \ldots \ \circ \left(\frac{1}{\lambda_n} g_n\right).$$

7.1 GRNs Under Negative Feedback

We have seen that in the negative feedback case, where $g'(x) < 0$ for all $x > 0$, the function g has unique fixed point x_1 satisfying $g(x_1) = x_1$. Then, the system (7.1) has an equilibrium point x_{eq}, which is uniquely determined from

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DOI 10.1007/978-3-319-15606-4_7

$$x_{eq} = [x_1, \ \dots \ , x_n]^{\mathrm{T}} \ : \quad x_n = \frac{g_n(x_1)}{\lambda_n}, \ x_{n-1} = \frac{g_{n-1}(x_n)}{\lambda_{n-1}} \ , \ \dots \ , \ x_2 = \frac{g_2(x_3)}{\lambda_2}.$$

In this case, a simple global stability condition is determined as $|g'(x_1)| < 1$, which implies that all solutions $x(t)$ converge to x_{eq} as $t \to \infty$. We have also proven that, under the assumptions made on g_i, $i = 1, \dots, n$, the value of $g'(x_1)$ cannot be equal to -1. Furthermore, when $|g'(x_1)| > 1$ and the delay value τ is greater than a critical value τ_c the equilibrium point x_{eq} is locally unstable, which simply means that the system (7.1) has an oscillatory response. The value of τ_c is determined from the stability analysis of the linearized system, and computed from the delay margin of the non-delayed system by using the Nyquist criterion. In any case, we know that $x_i(t) \geq 0$ and the solution remains bounded. By analyzing the fixed points of $g(x)$ for the case $|g'(x_1)| > 1$ we were able to determine the upper and lower bounds of $x_i(t)$ as $t \to \infty$.

In order to simplify the exposition, we have denoted both solutions with a non-constant periodic orbit and solutions that converge to a homoclinic orbit as oscillations. However, up-to-date there is no theoretical evidence that can rule out the existence of homoclinic orbits. In our extensive simulations, we observed that all locally unstable solutions converge to a non-constant periodic orbit. It is still an open question to prove this observed behavior and find conditions on the functions g_i and the initial conditions so that homoclinic orbits are ruled out.

The most interesting situation for the GRN under negative feedback is when $|g'(x_1)| > 1$ and $\tau < \tau_c$. In this case, x_{eq} is locally stable. In other words, if the initial condition is sufficiently close to x_{eq}, then the solution $x(t)$ converges to the equilibrium. However, at this point, finding the largest region of attraction is an open research problem.

7.2 GRNs Under Positive Feedback

In Chapter 6 we have studied the positive feedback case where $g'(x) > 0$ for all $x \geq 0$. In this case $g(x)$ may have several fixed points (at most three). We have examined conditions under which (7.1) admits unique equilibrium which is globally attractive. Also we have seen that if g has three fixed points, then the system (7.1) exhibits a bistable behavior.

Some interesting open problems, related to the positive feedback case, arise when $g'(x_1) = 1$ where x_1 is a fixed point of g. First open question is whether $g'(x_1) = 1$ is possible under standing assumptions made in this work. Second, if this happens, then except the single pole at the origin, all poles of $T(s)$ defined in (6.4) are in $\mathbb{C}_-$. This means that there exist initial conditions (albeit a set of measure zero) for which the local behavior near the corresponding equilibrium is stable (in the sense of Lyapunov) but *not asymptotically stable*. Finding such initial conditions, and determining the response of the original nonlinear system under these conditions is still an interesting open problem.

References

1. M. Ahsen, H. Özbay, and S.-I. Niculescu, “On the analysis of a dynamical model representing gene regulatory networks under negative feedback,” *International Journal of Robust and Nonlinear Control*, vol. 24, pp. 1609–1627, 2014.
2. M. E. Ahsen, “Analysis of two types of cyclic biological system models with time delays,” Master’s thesis, Bilkent University, 2011.
3. M. E. Ahsen, H. Özbay, and S.-I. Niculescu, “Analysis of gene regulatory networks under positive feedback,” in *Delay Systems: From Theory to Numerics and Applications*. T. Vyhlidal, J-F. Lafay, R. Sipahi (Eds.), Springer, 2014, pp. 127–140.
4. J. D. Watson and F. H. Crick, “Molecular structure of nucleic acids,” *Nature*, vol. 171, pp. 737–738, 1953.
5. International Human Genome Sequencing Consortium, “Finishing the euchromatic sequence of the human genome,” *Nature*, vol. 431, no. 7011, pp. 931–945, 2004.
6. The Cancer Genome Atlas, http://cancergenome.nih.gov/, 2013.
7. A. Tozeren and S. W. Byers, *New Biology for Engineers and Computer Scientists*. Prentice Hall, 2003.
8. L. Ma, J. Wagner, J. J. Rice, W. Hu, A. J. Levine, and G. A. Stolovitzky, “A plausible model for the digital response of p53 to DNA damage,” *Proceedings of the National Academy of Sciences of the USA (PNAS)*, vol. 102, no. 40, pp. 14266–14271, 2005.
9. R. Tibshirani, “Regression shrinkage and selection via the Lasso,” *Journal of the Royal Statistical Society. Series B (Methodological)*, pp. 267–288, 1996.
10. H. Zou and T. Hastie, “Regularization and variable selection via the elastic net,” *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, vol. 67, no. 2, pp. 301–320, 2005.
11. C. Cortes and V. Vapnik, “Support-vector networks,” *Machine Learning*, vol. 20, no. 3, pp. 273–297, 1995.
12. T. Hastie, R. Tibshirani, and J. Friedman, *The Elements of Statistical Learning*. Springer, 2009.
13. I. Guyon, J. Weston, S. Barnhill, and V. Vapnik, “Gene selection for cancer classification using support vector machines,” *Machine Learning*, vol. 46, no. 1–3, pp. 389–422, 2002.
14. P. S. Bradley and O. L. Mangasarian, “Feature selection via concave minimization and support vector machines.” in *15th International Conference on Machine Learning (ICML ’98)*, 1998, pp. 82–90.
15. M. E. Ahsen, N. K. Singh, T. Boren, M. Vidyasagar, and M. A. White, “A new feature selection algorithm for two-class classification problems and application to endometrial cancer,” in *51st IEEE Conference on Decision and Control (CDC 2012)*, December 2012, pp. 2976–2982.

M.E. Ahsen et al., *Analysis of Deterministic Cyclic Gene Regulatory Network Models with Delays*, SpringerBriefs in Electrical and Computer Engineering,
DOI 10.1007/978-3-319-15606-4

16. M. B. Elowitz, A. J. Levine, E. D. Siggia, and P. S. Swain, “Stochastic gene expression in a single cell,” *Science*, vol. 297, no. 5584, pp. 1183–1186, 2002.
17. P. E. Purnick and R. Weiss, “The second wave of synthetic biology: from modules to systems,” *Nature Reviews Molecular Cell Biology*, vol. 10, no. 6, pp. 410–422, 2009.
18. C. Troein, “Gene regulatory networks: Dynamics and stability,” Ph.D. dissertation, Lund University, 2007.
19. A. A. Margolin, I. Nemenman, K. Basso, C. Wiggins, G. Stolovitzky, R. D. Favera, and A. Califano, “Aracne: an algorithm for the reconstruction of gene regulatory networks in a mammalian cellular context,” *BMC Bioinformatics*, vol. 7, no. Suppl 1, p. S7, 2006.
20. N. Singh, M. E. Ahsen, S. Mankala, M. Vidyasagar, and M. White, “Inferring weighted and directed gene interaction networks from gene expression data using the phi-mixing coefficient,” in *2012 IEEE International Workshop on Genomic Signal Processing and Statistics (GENSIPS 2012)*. IEEE, 2012, pp. 168–171.
21. M. E. Ahsen and M. Vidyasagar, “On the computation of mixing coefficients between discrete-valued random variables,” in *9th Asian Control Conference (ASCC 2013), Istanbul, Turkey*, 2013.
22. M. E. Ahsen and M. Vidyasagar, “Mixing coefficients between discrete and real random variables: Computation and properties.” *IEEE Transactions on Automatic Control*, vol. 59, no. 1, pp. 34–47, 2014.
23. H. Özbay, *Introduction to Feedback Control Theory*. CRC Press, 1999.
24. W. Michiels and S.-I. Niculescu, *Stability and stabilization of time-delay systems: an eigenvalue-based approach*. SIAM, 2007.
25. A. Polynikis, S. Hogan, and M. di Bernardo, “Comparing different ode modelling approaches for gene regulatory networks,” *Journal of Theoretical Biology*, vol. 261, no. 4, pp. 511–530, 2009.
26. S. Widder, J. Schicho, and P. Schuster, “Dynamic patterns of gene regulation I: simple two-gene systems,” *Journal of Theoretical Biology*, vol. 246, no. 3, pp. 395–419, 2007.
27. M. Hecker, S. Lambeck, S. Toepfer, E. Van Someren, and R. Guthke, “Gene regulatory network inference: data integration in dynamic models – a review,” *Biosystems*, vol. 96, no. 1, pp. 86–103, 2009.
28. A. Goldbeter, *Biochemical Oscillations and Cellular Rhythms*. Cambridge University Press, 1997.
29. M. R. Roussel, “The use of delay differential equations in chemical kinetics,” *The Journal of Physical Chemistry*, vol. 100, no. 20, pp. 8323–8330, 1996.
30. T. olde Scheper, D. Klinkenberg, C. Pennartz, and J. Van Pelt, “A mathematical model for the intracellular circadian rhythm generator,” *The Journal of Neuroscience*, vol. 19, no. 1, pp. 40–47, 1999.
31. E. D. Sontag, “Asymptotic amplitudes and cauchy gains: a small-gain principle and an application to inhibitory biological feedback,” *Systems & Control Letters*, vol. 47, no. 2, pp. 167–179, 2002.
32. D. Chu, N. R. Zabet, and B. Mitavskiy, “Models of transcription factor binding: sensitivity of activation functions to model assumptions,” *Journal of Theoretical Biology*, vol. 257, no. 3, pp. 419–429, 2009.
33. H. De Jong, “Modeling and simulation of genetic regulatory systems: a literature review,” *Journal of Computational Biology*, vol. 9, no. 1, pp. 67–103, 2002.
34. L. Chen and K. Aihara, “Stability of genetic regulatory networks with time delay,” *IEEE Transactions on Circuits and Systems I: Fundamental Theory and Applications*, vol. 49, no. 5, pp. 602–608, 2002.
35. C.-I. Morarescu and S.-I. Niculescu, “Some remarks on the delay effects on the stability of biochemical networks,” in *16th Mediterranean Conference on Control and Automation*. IEEE, 2008, pp. 801–805.
36. K. Wang, L. Wang, Z. Teng, and H. Jiang, “Stability and bifurcation of genetic regulatory networks with delays,” *Neurocomputing*, vol. 73, no. 16, pp. 2882–2892, 2010.

37. J. Gebert, N. Radde, and G.-W. Weber, “Modeling gene regulatory networks with piecewise linear differential equations,” *European Journal of Operational Research*, vol. 181, no. 3, pp. 1148–1165, 2007.
38. H. Sedaghat, *Nonlinear Difference Equations: theory with applications to social science models*. Springer, 2003.
39. S. Müller, J. Hofbauer, L. Endler, C. Flamm, S. Widder, and P. Schuster, “A generalized model of the repressilator,” *Journal of Mathematical Biology*, vol. 53, no. 6, pp. 905–937, 2006.
40. A. Verdugo, “Dynamics of gene networks with time delays,” Ph.D. dissertation, Cornell University, 2009.
41. G. A. Enciso, “On the asymptotic behavior of a cyclic biochemical system with delay,” in *45th IEEE Conference on Decision and Control*, pp. 2388–2393, 2006.
42. D. Angeli and E. D. Sontag, “Multi-stability in monotone input/output systems,” *Systems & Control Letters*, vol. 51, no. 3, pp. 185–202, 2004.
43. E. Liz, M. Pinto, G. Robledo, S. Trofimchuk, and V. Tkachenko, “Wright type delay differential equations with negative Schwarzian,” *Dynamical Systems*, vol. 9, no. 2, pp. 309–321, 2003.
44. K. Gu, J. Chen, and V. L. Kharitonov, *Stability of Time-Delay Systems*. Birkhauser, 2003.
45. J. K. Hale, and S. M. Verduyn Lunel, *Introduction to Functional Differential Equations*. Springer, 1993, vol. 99.
46. J. M. Berg, J. L. Tymoczko, and L. Stryer, *Biochemistry*. W. H. Freeman and Company: New York, 2002.
47. M. B. Elowitz and S. Leibler, “A synthetic oscillatory network of transcriptional regulators,” *Nature*, vol. 403, no. 6767, pp. 335–338, 2000.
48. F. Alves and R. Dilão, “A simple framework to describe the regulation of gene expression in prokaryotes,” *Comptes Rendus Biologies*, vol. 328, no. 5, pp. 429–444, 2005.
49. J. Mallet-Paret and G. R. Sell, “The Poincaré–Bendixson theorem for monotone cyclic feedback systems with delay,” *Journal of Differential Equations*, vol. 125, no. 2, pp. 441–489, 1996.
50. Y. Hori, M. Takada, and S. Hara, “Biochemical oscillations in delayed negative cyclic feedback: Existence and profiles,” *Automatica*, vol. 49, no. 9, pp. 2581–2590, 2013.
51. D. Allwright, “A global stability criterion for simple control loops,” *Journal of Mathematical Biology*, vol. 4, no. 4, pp. 363–373, 1977.
52. M. Mackey, “Unified hypothesis for the origin of aplastic anemia and periodic hematopoiesis,” *Blood*, vol. 51, no. 5, p. 941, 1978.
53. E. D. Sontag, “Passivity gains and the secant condition for stability,” *Systems & Control Letters*, vol. 55, no. 3, pp. 177–183, 2006.
54. M. Arcak and E. D. Sontag, “Diagonal stability of a class of cyclic systems and its connection with the secant criterion,” *Automatica*, vol. 42, no. 9, pp. 1531–1537, 2006.
55. H. K. Wimmer, “Diagonal stability of matrices with cyclic structure and the secant condition,” *Systems & Control Letters*, vol. 58, no. 5, pp. 309–313, 2009.
56. T.-H. Kim, Y. Hori, and S. Hara, “Robust stability analysis of gene–protein regulatory networks with cyclic activation–repression interconnections,” *Systems & Control Letters*, vol. 60, no. 6, pp. 373–382, 2011.
57. R. Wang, Z. Jing, and L. Chen, “Modelling periodic oscillation in gene regulatory networks by cyclic feedback systems,” *Bulletin of Mathematical Biology*, vol. 67, no. 2, pp. 339–367, 2005.
58. J. Wagner and G. Stolovitzky, “Stability and time-delay modeling of negative feedback loops,” *Proceedings of the IEEE*, vol. 96, no. 8, pp. 1398–1410, 2008.
59. A. Verdugo, P. Vinod, J. J. Tyson, and B. Novak, “Molecular mechanisms creating bistable switches at cell cycle transitions,” *Open Biology*, vol. 3, no. 3, p. 120179, 2013.
60. M. W. Hirsch and H. Smith, “Monotone dynamical systems,” in *Handbook of Differential Equations, Ordinary Differential Equations, vol. 2*, D. P. Cañada, A. and A. Fonda, Eds., pp. 239–357, Elsevier, 2005.
61. G. A. Enciso, “A dichotomy for a class of cyclic delay systems,” *Mathematical Biosciences*, vol. 208, pp. 63–75, 2007.

62. G. A. Enciso and E. D. Sontag, “Monotone bifurcation graphs,” *Journal of Biological Dynamics*, vol. 2, no. 2, pp. 121–139, 2008.
63. H. L. Smith, *Monotone Dynamical Systems: an introduction to the theory of competitive and cooperative systems*. American Mathematical Soc., 1995, vol. 41.
64. T. Hunt, B. R. Sauer and J. A. Yorke, “Prevalence: A translation-invariant “almost every” on infinite-dimensional spaces,” *Bulletin of the American Mathematical Society*, vol. 27, no. 2, pp. 217–237, 1992.
65. W. Ott and J. A. Yorke, “Prevalence,” *Bulletin of the American Mathematical Society*, vol. 42, no. 3, pp. 263–290, 2005.
66. T. S. Gardner, C. R. Cantor, and J. J. Collins, “Construction of a genetic toggle switch in escherichia coli,” *Nature*, vol. 403, no. 6767, pp. 339–342, 2000.

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M.E. Ahsen et al., *Analysis of Deterministic Cyclic Gene Regulatory Network Models with Delays*, SpringerBriefs in Electrical and Computer Engineering,
DOI 10.1007/978-3-319-15606-4