

ESSAYS ON GENE REGULATORY NETWORK MODELS AND THEIR STABILITY ANALYSIS

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By
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We certify that we have read this dissertation and that in our opinion it is fully adequate, in scope and in quality, as a dissertation for the degree of Doctor of Philosophy.

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ABSTRACT

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Ph.D. in Electrical and Electronics Engineering

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Gene expression is one of the core areas in comprehending and assessing how biological cells work. Gene regulatory networks (GRNs), representing the intricate mechanism between genes and their regulatory modules, are instrumental in controlling gene expression and cell functions. These models shed light on how transcription factors interact with their regulatory modules within a cell. Despite the multitude of studies focusing on the analysis and enhancement of GRNs, there is still room for contributions. This thesis investigates a novel framework inspired by the gene networks constructed using synthetic biology, and presents stability analyses of the nonlinear infinite dimensional dynamical system models arising in this framework.

In the first part of the thesis, we extend a previously studied benchmark GRN model including time delay, and present an analysis of the extended framework. We utilize unmodeled dynamics and possibly ignored interactions, including higher-order dynamics, in our system design. The stability of the extended system is analyzed by considering various nonlinearity functions and design parameters, and the results are compared with those of the benchmark original model.

In the second part, we employ an extension of a gene network model using a multiplicative perturbation of the dynamical system. Each cascaded subsystem in this extended framework has an additional block, including a multiplicative term with a high-pass filter, and the effect of additional parameters on the robustness and delay margin of the system is investigated. Experiments with various design parameters yield that the stability characteristics of GRNs can be improved using the model pertaining to the extension under specific perturbations.

Finally, the third part covers the analysis of nonlinear dynamics and chaos in GRNs, particularly focusing on the two-gene original and extended gene networks. Chaotic dynamics depend strongly on the inclusion of time delays, but the circuit motifs that show chaos differ when both original and extended models are considered. Our results suggest that for a particular higher-order extension of the gene network, it is possible to observe the chaotic dynamics in a two-gene system without adding any self-inhibition. This finding can be explained as a result of the modification of the original benchmark model induced by unmodeled dynamics. We argue that regulatory gene circuit models with additional parameters demonstrate non-periodic dynamics much more easily.

Keywords: Gene regulatory networks, time delay systems, stability analysis, nonlinear dynamics, chaos.

ÖZET

GEN DÜZENLEYİCİ AĞ MODELLERİ VE ONLARIN KARARLILIK ANALİZİ ÜZERİNE DENEMELER

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Gen ifadesi, biyolojik hücrelerin nasıl çalıştığını anlama ve değerlendirme sürecinde öne çıkan alanlardan biridir. Genler ve düzenleyici modüller arasındaki karmaşık mekanizmayı temsil eden gen düzenleyici ağlar, gen ifadesini ve hücre fonksiyonlarını kontrol etmede önemli bir role sahiptir. Bu modeller, transkripsiyon faktörlerinin bir hücre içindeki düzenleyici modüllerle etkileşimlerini açıklar. Gen düzenleyici ağların analizi ve geliştirilmesine odaklanmış birçok çalışma bulunmakla birlikte, hala katkı yapılabilecek alanlar vardır. Bu tez, sentetik biyoloji kullanılarak oluşturulan gen ağlarından ilham alarak geliştirilen yeni bir modeli araştırmakta ve bu modelde ortaya çıkan doğrusal olmayan sonsuz boyutlu dinamiklerin kararlılık analizlerini sunmaktadır.

Tezin ilk bölümünde, daha önce incelenmiş zaman gecikmesi içeren bir referans gen düzenleyici ağ modelinin genişletilmesi ve modelin doğrusallaştırma analizi sunulmuştur. Genişletilmiş modelin tasarımında, modellenmemiş dinamikler ve yüksek dereceli dinamikleri içeren göz ardı edilmesi muhtemel etkileşimler kullanılmıştır. Genişletilmiş sistemin kararlılığı, çeşitli doğrusal olmayan fonksiyonlar ve tasarım değişkenleri göz önünde bulundurularak analiz edilmiş ve sonuçlar referans alınan orijinal model ile karşılaştırılmıştır.

İkinci kısımda, bir gen ağı modelini dinamik sistemin çarpanlı bir pertürbasyonunu kullanarak genişletmekteyiz. Bu genişletilmiş modeldeki her bir kademeli alt sistem, yüksek geçiren filtreye sahip bir çarpanlı terim dahil olmak üzere ek bir blok içermektedir. Ayrıca, sistemin gürbüzlük ve zaman gecikmesi marjı üzerinde ek değişkenlerin etkisi araştırılmıştır. Farklı tasarım değişkenleri ile yapılan deneyler, gen düzenleyici ağların kararlılık özelliklerinin genişletilmiş modelde belirli pertürbasyonlar altında iyileştirilebileceğini göstermektedir.

Üçüncü bölümde iki genli orijinal ve genişletilmiş gen ağlarına odaklanarak, gen düzenleyici ağlardaki doğrusal olmayan dinamiklerin ve kaosun analizi yapılmıştır. Kaotik dinamikler zaman gecikmelerine güçlü bir şekilde bağlıdır, ancak orijinal ve genişletilmiş modellerin her ikisi de dikkate alındığında kaos içeren devre motifleri farklılık göstermektedir. Sonuçlarımız, gen ağına özgü yüksek dereceli bir genişleme için, iki gen içeren bir sistemde herhangi bir öz-engelleme kullanmadan kaotik dinamiklerin gözlemlenebileceğini göstermektedir. Bu bulgu, modellenmemiş dinamikler tarafından tetiklenen orijinal referans modelinin değiştirilmesinin bir sonucu olarak açıklanabilir. Ek parametrelere sahip düzenleyici gen ağı modellerinin periyodik olmayan dinamikleri daha kolay gösterdiğini öne sürmekteyiz.

Anahtar sözcükler: Gen düzenleyici ağlar, zaman gecikmeli sistemler, kararlılık analizleri, doğrusal olmayan dinamikler, kaos.

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to my dear family

Chapter 1

Introduction

Biological gene networks provide insights into the mechanism by which gene expression performs specific functions. Among them, the gene regulatory network (GRN) is established through the interplay between genes that encode transcription factors and regulatory modules they govern within a cell, creating a complex interactive system. The interaction between genes and proteins plays a vital role in various biological processes such as protein synthesis and transcriptions including activation and repression. Numerous investigations have been undertaken to analyze and enhance our understanding of genetic networks. This thesis concerns the stability analysis and nonlinear properties of the dynamical regulatory gene circuit models.

The primary objective in this thesis is to investigate an extension of a previously studied gene regulatory network model, to perform stability and robustness analysis and to study nonlinear dynamics and chaos in gene network models. To this end, we take into account possibly ignored interactions including higher-order dynamics in the earlier models, and consider unmodeled dynamics in the form of neglected biological interactions. This thesis presents the investigation and analysis of an *extended gene regulatory network model* from the perspective of the systems and control theory. In the upcoming sections, we elucidate our motivations based on the findings of existing studies and the proposed methodology.

1.1 Early Studies on Genetic Networks

Understanding and evaluating the functioning of biological cells relies considerably on gene expression [1]. It offers valuable insights into the biological processes by which genes are turned on or off, and how this regulation affects the overall functioning and traits of cells. A series of single molecule events are involved in gene expression, and this can be viewed as a stochastic process because each of these biological reactions is influenced by significant thermal fluctuations [2]. We can learn every aspect of biological processes, including development, illness progression, and reaction to environment stimuli by examining gene expression patterns and mechanisms [3].

A gene regulatory network model is a sophisticated and nonlinear dynamical system representing the interactions between the genes that encode transcription factors and the regulatory protein modules within a cell [4]. These networks are essential for regulating different cellular processes and controlling gene expression [5]. Therefore, the fundamental processes of gene regulation and biological systems can be better understood by modeling these interactions and researching their dynamics. Exploring the activities of certain genes and proteins as well as predicting the dynamics of cellular processes and deciphering their intricacy require an in-depth knowledge of gene networks [6]. In brief, interaction among genes and proteins with one another, defined as a gene network, has a crucial role in various cellular processes such as protein synthesis and transcriptions including activation and repression [7].

Many studies have been dedicated to the analysis and development of genetic networks utilizing a variety of mathematical techniques, ranging from Boolean or Bayesian networks to continuous models represented by differential equations [8]. The study of gene regulation in living organisms greatly enhances our understanding of biological networks and their dynamics [3]. In this regard, patterns of interconnection in the gene network motifs of the bacterium *Escherichia coli* [9], and transcriptional regulatory networks in it have been studied in many different studies [10–14].

A first ever synthetic cyclic network model in *Escherichia coli*, termed the repressilator, has been proposed by Elowitz and Leibler [15]. Studies employing the repressilator model have focused particularly on the dynamical characteristics [16] and the generalized model utilizing the Schwarzian derivate concept [17]. In addition to these studies, the generalized repressilator model with a ring topology has been used to investigate the impact of unstable periodic orbits on the transient dynamics [18]. Furthermore, dynamical aspects including statistical fluctuations and noise evolution of a repressilator model, as well as the phenomena of monostability and limit cycle oscillation, have been examined [19]. In brief, numerous studies utilizing the repressilator can be found in the existing literature, which inspired the development of extended gene regulatory network model proposed in this thesis.

1.2 Methodologies for Modelling and Analyzing Gene Regulatory Networks

Various methodologies have been developed to model and analyze gene regulatory networks, offering different approaches and techniques [20]. The Boolean network model, initially presented by Glass and Kauffman, is a representation of a logical model that qualitatively describes regulatory networks [21]. By taking into account binary states for each gene in the network, it provides a simplified visualization of gene interactions. A more elaborated model compared to the Boolean network model can be considered as the discrete time representation of a gene system [22]. It basically describes the kinetics of gene expression by the help of mathematical equations, considering discrete nature of time. The discrete time approach enables a more accurate understanding of gene regulatory networks and their dynamics by taking explicit time steps into account.

Indeed, functional differential equations or delay differential equations (DDEs) are commonly used to describe gene regulatory network models in the literature [23]. Given the temporal features of gene expression and the impact of feedback

loops, ordinary differential equations (ODEs) and DDEs offer a more accurate representation and provide detailed understanding of the nonlinear dynamical behavior exhibited by biological systems [23]. Thus, we can capture the complex interactions and time-dependent behavior of genes and proteins in regulatory networks using ODEs and DDEs.

Different ODE modelling approaches for gene regulatory networks have been compared and discussed in [24]. Moreover, the viability of employing specific modelling approximations, such as quasi-steady-state mRNA dynamics or piecewise-linear approximations of Hill functions, and their impacts on the overall dynamics have been examined [24]. In a different study, a differential-equation based gene regulatory model that utilizes time series of measurements of a cell has been introduced [25]. Overall, the introduction of differential equations in gene regulatory models enhances our understanding of the complex dynamics underlying gene expression and regulatory processes in a cell.

The dynamics in gene regulatory network are not dictated only by the genes and interactions, the delays need to be considered, since they are ubiquitous and naturally arise [26]. Gene regulatory network models have included time delays in various forms, including discrete time delays [23], time-varying delays [27,28] and mixed time delays combining both discrete and distributed delays [29]. Regardless of the specific structure of the delays, one of the crucial factors affecting the dynamics of gene network models is the presence of time delays [23]. Additionally, as the existence of delays in network models significantly impacts the stability of the overall system, studying a time-delayed network models provides valuable insights into the dynamics of the system. There are many different studies using delay differential equations for describing time delays in genetic regulatory networks [30–32].

1.3 Towards Dynamic Behaviors in Gene Regulatory Networks

It is intuitively clear that the stability of the GRN models is crucial for understanding how systems react when their dynamics are altered. Through the aid of stability analysis, we are able to examine the system's long-term behavior and evaluate if it eventually reaches a steady state or exhibits oscillations or chaotic behavior. Numerous methodologies have been used for the stability analysis of gene regulatory networks with time-delayed feedback, including the secant condition [33], Lyapunov functional and linear matrix inequality [27], Lyapunov-Krasovakii functional and matrix analysis techniques [28]. Each method offers a different approach to examine the stability properties of GRNs with time delays. In addition to stability analysis, there have been studies focusing on bifurcation [29, 31, 34–37] and chaos [37–42] analysis in gene regulatory networks of different types.

Existing studies on the bifurcation analysis of differential equations mostly focus on the Hopf bifurcation theory, which is a fundamental concept in nonlinear dynamics and explains how systems can move from steady state to oscillatory behavior as parameters changes [43]. Apart from biological networks, stability of delayed network models has been explored in various contexts. The stability of functional differential equations under delayed feedback has been examined near a Hopf bifurcation and the results are compared for different delay structures in [44]. Furthermore, a system of delay differential equations representing a model for a pair of neurons is studied, and losing the stability of the fixed point is shown via Hopf bifurcation [45].

Indeed, there is a wide range of studies that utilize Hopf bifurcation in the stability analysis of gene regulatory networks or biological models. A cyclic biochemical system under single time delay and negative feedback has been presented to observe the convergence to the equilibrium, or the existence of periodic solutions by using Hopf bifurcation approach [46]. Using the Hopf bifurcation theorem for functional differential equations, periodic oscillations in gene regulatory networks

by negative cyclic feedback systems have been analyzed in [47]. In this study, a Hopf bifurcation approach has been proposed in the Poincare-Bendixson context.

Typically, biological networks are not chaotic, they operate at the border between order and chaos [48], whereas a two-gene system may show chaotic and hyper-chaotic behavior depending on the cooperativity among the transcription factors [22]. Due to the limited availability of temporal gene expression data, studies exploring the presence of chaos in gene network models are extremely rare, as the conditions required to observe chaotic behavior are highly strict [49]. Some recent studies indicate that even in relatively simple gene circuits consisting of one or two elements, the inclusion of time delays can lead to chaotic dynamics [50,51]. This is because delays have the potential to alter a circuit's dynamical behavior and give rise to chaotic dynamics [52].

1.4 Motivation and Key Contributions

Motivated by the existing literature in this area, this thesis is dedicated to investigate an extension of a previously studied cyclic gene regulatory network model [54] as a generalization of the repressilator [15], and to perform stability analysis of the proposed extended model. This study is inspired by the design of possibly ignored interactions including higher order dynamics in the earlier models and analysis of a novel framework where the subsystems are remodeled with additional parameters.

One of the first key contributions of this thesis is that we present an extension of a nonlinear cyclic GRN model with second order subsystems based on the idea of synthetic gene networks [60] and concern with the analysis of its linearized model. A stability condition for the linearized extended system using the characteristic equation is determined. We further present the stability condition for the extended model considering homogeneous GRNs with Hill functions. We provide numerical analysis to demonstrate the performance of the extended gene regulatory network model and discuss how the additional dynamics affect the stability

characteristics in detail.

In addition, we provide an alternative extended GRN model considering a particular type of multiplicative uncertainty with high pass filter inside the linear time invariant part of each subsystem. We investigate the effect of uncertainty, and show that it may be possible to improve the stability characteristics under specific perturbations. For this extended framework, illustrative examples with simulation results are also provided, and robustness and delay margin analyses are conducted. The main consequences of the proposed extended models are presented in our published works [57–59] during the doctoral studies.

Another key contribution of this thesis is that we determine the two-gene circuit motifs including chaos and observe chaotic dynamics exhibited by original and extended structures. Our approach focuses on a comparison for non-periodic dynamics in the original and extended gene networks. We show that chaotic dynamics occur in the original model when there is a dual feedback in one gene and the delays differ, whereas self-inhibitory delay is not needed to observe the chaos in the extended model. To better illustrate the non-periodic dynamics in the gene regulatory circuits considered, we utilize a method including the phase maps, power spectrum analysis and Lyapunov exponents. In what follows, we make use of the described techniques to illustrate potential chaotic dynamics and thoroughly examine the original and extended models with changes in time delays.

1.5 Organization of the Thesis

This thesis is divided into three main parts, each containing detailed explanations and discussions in separate chapters. The first part focuses on the definition of a cyclic GRN model and some preliminary results. Chapter 2 extends upon a previously proposed benchmark gene network model to second order ODE-based model with time delay and covers the analysis of the extended linearized model. Numerical examples are provided to compare the performance of the gene

regulatory networks, and stability analysis is performed for a second order GRN model with homogeneous nonlinearities.

Chapter 3 covers an extension of a cyclic gene regulatory network with multiplicative perturbation. The objective behind this design is to introduce a novel GRN model that incorporates a specific type of multiplicative uncertainty, along with a high-pass filter within the linear time-invariant part of each subsystem. The impact of the additional parameters on the system's delay margin is investigated, and a robust stability condition analysis is conducted for the extended model. We also observe the impact of specific perturbations and compare the outcomes with the original benchmark gene network model.

Building upon the outcomes of the extended models analyzed in Chapter 2 and Chapter 3, Chapter 4 delves into the exploration of nonlinear dynamics and chaos within gene regulatory network models. We explore the chaotic dynamics of both the original and extended gene network models, highlighting that these models exhibit either regular or chaotic behavior depending on the specific values of the delay parameters. Moreover, the *modified* original benchmark model is examined with respect to the parameter of the nonlinear function to observe the dynamics, specifically to determine whether the model exhibit periodic or chaotic behavior.

Chapter 5 serves as the conclusion of the thesis, where we provide concluding remarks and discuss potential avenues for future research and extensions of the study.

Chapter 2

Extended Gene Regulatory Network Models

The gene regulatory network is formed by the interactive relationship between genes encoding the transcription factors and their regulatory modules within a cell. This network outlines how genes and proteins interact to create biological systems that carry out specific functions. To fully understand the dynamics of genetic regulatory networks, it is crucial to approach it from the perspective of nonlinear system theory, as these networks are complex and nonlinear in nature, with many dimensions to consider. We have developed an extended model design based on synthetic biology principles, building upon a previously analyzed basic gene regulatory network model. The main motivation is to redesign the system with additional parameters, taking into account neglected interactions including higher order and unmodeled dynamics. The work discussed in this chapter has been reported in the preliminary form in [57].

2.1 Introduction

A gene regulatory network model encompasses the interplay between DNA segments and regulatory proteins within a cell, serving as a means for cells to communicate and respond to their surroundings. Cells exhibit a response to environmental stimuli through the activation or expression of specific genes within a gene regulatory network model. For instance, when bacterial cells are cultivated in an environment lacking glucose but abundant in lactose, the genes responsible for lactose digestion are expressed. Consequently, through the process of lactose digestion, the lactose molecules are broken down into glucose. As a result, the cells are able to generate the necessary energy for their metabolic activities from glucose [67].

Recent advancements in microarray technology have made it possible to obtain precise measurements of gene expression levels. Researchers utilize gene expression levels to identify potential biomarkers that may be linked to a particular disease. Thorough analysis of gene expression data has the potential to provide valuable insights into alterations within regulatory networks that can contribute to the development of diseases. As an illustration, the regulatory network components between an individual without cancer and a cancer patient can exhibit notable differences. Performing a differential analysis of the gene regulatory network between a healthy individual and a patient can provide valuable clues that contribute to the understanding and treatment of the specific disease.

In the analysis of gene regulatory network models, various methods can be employed, ranging from Boolean or Bayesian networks to continuous models represented by differential equations. We focus on the continuous time models representing gene regulatory networks, and a comparison has been made among different modeling approaches using ordinary differential equations (ODEs) for GRNs [24].

Fig. 2.1 depicts a cyclic dynamical model that incorporates a gene regulatory network (GRN) with a nonlinear feedback loop and time delays. The model given

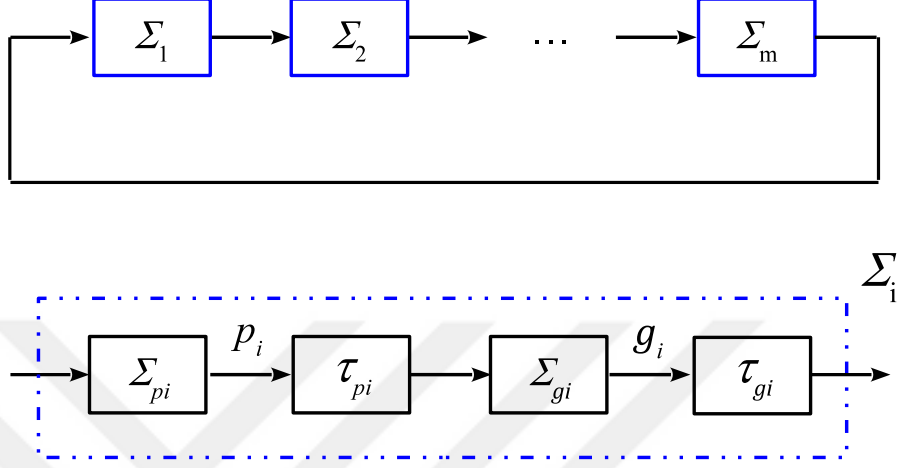


Figure 2.1: A continuous time GRN model

in the Fig. 2.1 illustrates a cyclic connection of subsystems $\Sigma_1, \Sigma_2, \dots, \Sigma_m$, where the input of Σ_i is determined by the output of Σ_{i-1} for $i = 2, \dots, m$. Additionally, a feedback connection is established by setting the input of Σ_1 as the output of Σ_m , [54]. Within the model, the structure of each subsystem is illustrated in Fig. 2.1 and the model can be described by a set of differential equations in the following form:

$$\begin{aligned}
 \dot{p}_1(t) &= -k_{p1}p_1(t) + f_{p1}(g_m(t - \tau_{gm})) \\
 \dot{g}_1(t) &= -k_{g1}g_1(t) + f_{g1}(p_1(t - \tau_{p1})) \\
 &\vdots \\
 \dot{p}_m(t) &= -k_{pm}p_m(t) + f_{pm}(g_{m-1}(t - \tau_{gm-1})) \\
 \dot{g}_m(t) &= -k_{gm}g_m(t) + f_{gm}(p_m(t - \tau_{pm})).
 \end{aligned} \tag{2.1}$$

The variables $p_i(t)$ and $g_i(t)$ correspond to the concentrations of protein and mRNA, respectively, and the parameters k_i represent the degradation rate of corresponding biological entity [54]. Moreover, the functions f_{pi} and f_{gi} are considered as Hill-type nonlinearities.

Our primary objective in this part is to explore an extension of a cyclic gene regulatory network, that has been previously studied [54], as a generalization of

the repressilator [15], and to perform stability analysis of the extended model. This study is motivated by the design of possibly ignored interactions including higher order dynamics in the earlier models and analysis of the new model where the subsystems are remodeled with additional parameters. More specifically, we present the extension of a nonlinear cyclic GRN model with second order subsystems based on the idea of synthetic gene networks and concern with the analysis of its linearized model. A stability condition for the linearized extended system using the characteristic equation is determined. We further present the stability condition for the extended model considering homogeneous GRNs with Hill functions.

2.2 An Earlier Gene Regulatory Network Model

The gene regulatory network model is a cyclic system with delayed feedback as given in Fig. 2.2, where each subsystem has a nonlinearity g_i and linear time invariant part $H_i(s)$. Typically, the simplest model, which is equivalent to (2.1), is given by a set of differential equations:

$$\begin{aligned} \dot{x}_1(t) &= -\lambda_1 x_1(t) + g_1(x_2(t)) \\ \dot{x}_2(t) &= -\lambda_2 x_2(t) + g_2(x_3(t)) \\ &\vdots \\ \dot{x}_n(t) &= -\lambda_n x_n(t) + g_n(x_1(t - \tau)), \end{aligned} \tag{2.2}$$

where τ is the time delay in the network. Here, the variables x_i 's for all $i = 1, 2, \dots, n$, represent mRNA concentrations with degradation rates $\lambda_i > 0$, $i = 1, 2, \dots, n$ [54].

Nonlinearity functions g_i are bounded and defined on $\mathbb{R}_+$. We have either $g'_i(x) < 0$ or $g'_i(x) > 0$, $i = 1, 2, \dots, n$, for all $x \in (0, \infty)$. Typically, g_i 's are Hill type functions, which have the following general form

$$f(x) = \frac{a}{b_0 + b_1 x^m} + c, \quad \text{or} \quad f(x) = \frac{ax^r}{b_0 + b_1 x^m} + c, \tag{2.3}$$

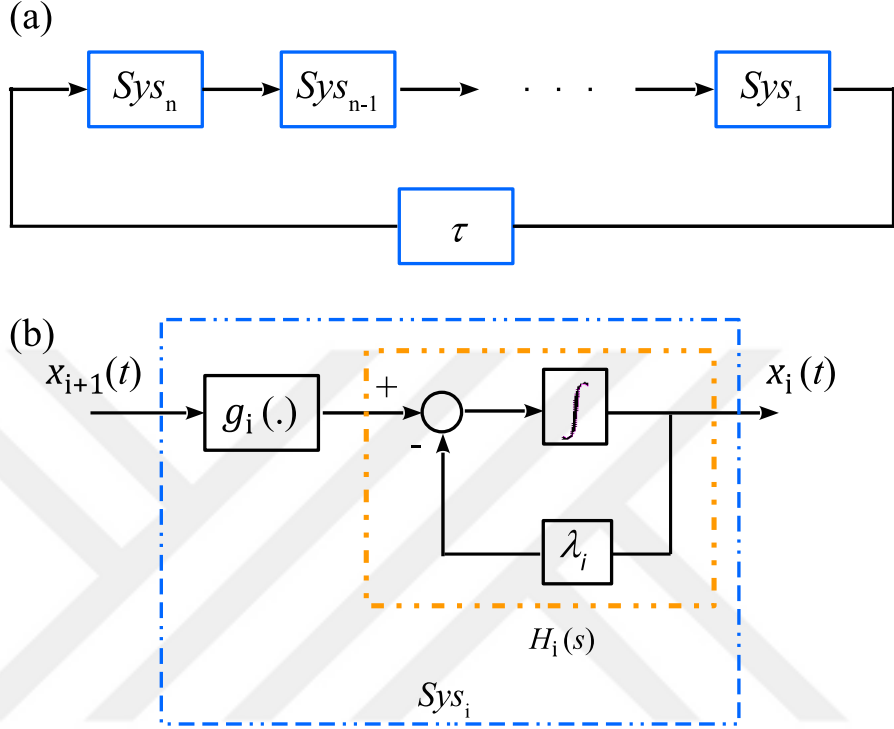


Figure 2.2: (a) GRN model with delayed feedback, (b) Subsystems of the original GRN model

with $a, b_0, b_1 > 0$, $c \geq 0$ and $m, r \in \mathbb{N}$.

The repressilator model proposed by [15] is obtained for $c = 0$, $m \geq 2$, and the linear time invariant part is given by

$$H_i(s) = \frac{1}{(1+s)(1+s/\beta)}. \quad (2.4)$$

Here, β denotes the ratio of the protein decay rate to mRNA decay rate [15]. When $\beta \rightarrow \infty$, the homogeneous system with $\lambda_i = 1$ for $i = 1, 2, \dots, n$, is obtained with the LTI part $H_i(s) = (s+1)^{-1}$.

The GRN in (2.2) is under negative feedback if

$$g'(x) < 0 \quad \forall x \in (0, \infty), \quad (2.5)$$

where the composition function g is given as

$$g(x) = \left(\frac{1}{\lambda_1} g_1 \right) \circ \left(\frac{1}{\lambda_2} g_2 \right) \circ \dots \circ \left(\frac{1}{\lambda_n} g_n \right). \quad (2.6)$$

The system satisfying condition (2.5) has a unique equilibrium point $x_{eq} = [x_1^e, x_2^e, \dots, x_n^e]^\top$ in $\mathbb{R}_+^n$ and the first component, x_1^e , is the unique fixed point of the function $g(x)$ satisfying $g(x_1^e) = x_1^e$. The GRN is stable independent of delay if the sufficient condition $|g'(x_1^e)| < 1$ is satisfied; otherwise it is stable dependent of delay if $|g'(x_1^e)| > 1$, see e.g. [33, 54, 55].

2.3 Extension of the Gene Regulatory Network Model

In this study, cyclic nonlinear time delayed gene regulatory network model described by n cascaded subsystems shown in Fig. 2.2(a) is redesigned by modifying each subsystem with the additional structures as given in Fig. 2.3. The main motivation behind this work is to investigate the following:

- (i) The repressilator [15], a gene network with the LTI part (2.4), can be viewed as a transition to the second order model. On the other hand, in basic homogeneous gene regulatory models studied earlier, the parameter β is ignored and $H_i(s)$ is first order as in Fig. 2.2(b). Therefore, it is important to analyze the effects of neglected dynamics to validate the approximations made in the literature.
- (ii) Furthermore, we design and construct an artificial extended gene regulatory model replacing the system structure with additional subsystems. This idea is generated by the inspiration of gene networks constructed using synthetic biology [61].

To recap, the main idea to extend the gene regulation model has been motivated by the repressilator to consider the unmodeled dynamics in the form of

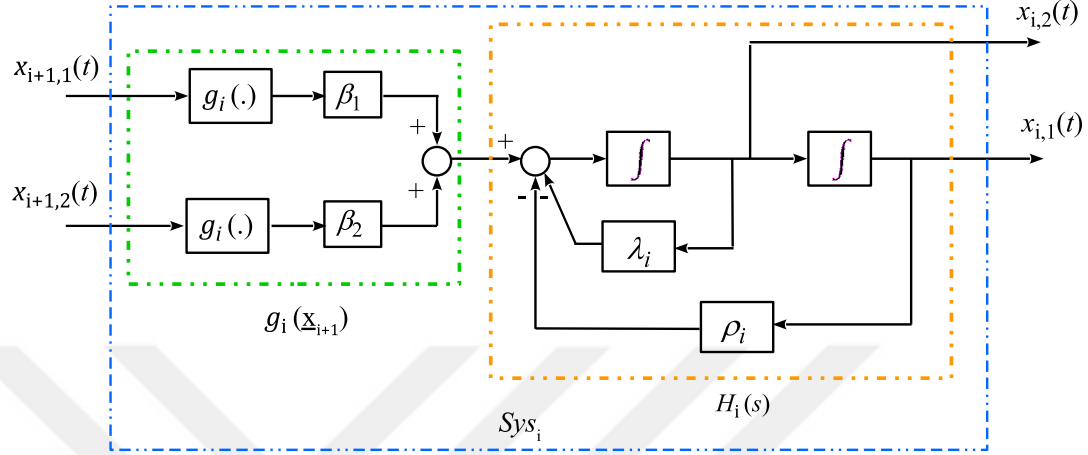


Figure 2.3: Subsystems of the extended GRN model

neglected biological interactions. Recently, we have studied the extension of an earlier gene regulatory model using a multiplicative perturbation [58]. Here, we analyze a more general structure and discuss how the additional dynamics affect the stability characteristics, in particular the delay margin of the system.

To retain the features of gene regulatory network, these additional parts are incorporated into the LTI part by changing subsystem dynamics and considering second order subsystems. Comparing the general structure of the subsystems here with those in the original model given in Fig. 2.2(b), the LTI part is mostly changed but the nonlinearity functions remain the same except for the second terms. Namely, in this extended model, the nonlinearity functions $g_i : \mathbb{R}_+ \rightarrow \mathbb{R}_+$ are similarly bounded and satisfy $g'_i(x) < 0$ or $g'_i(x) > 0$ for all $x \in \mathbb{R}_+$ as in [54].

Considering the extended model shown in Fig. 2.3 each subsystem states are $\underline{x}_i$ and they include two scalar variables such that $\underline{x}_i = [x_{i1}, x_{i2}]^\top$ for $i = 1, \dots, n$. Then, the mathematical model for the extended GRN is given by

$$\begin{aligned}
 \dot{\underline{x}}_1(t) &= A_1 \underline{x}_1(t) + g_1(\underline{x}_2(t)) \\
 \dot{\underline{x}}_2(t) &= A_2 \underline{x}_2(t) + g_2(\underline{x}_3(t)) \\
 &\vdots \\
 \dot{\underline{x}}_n(t) &= A_n \underline{x}_n(t) + g_n(\underline{x}_1(t - \tau)),
 \end{aligned} \tag{2.7}$$

where A_i 's are 2×2 Hurwitz matrices in the form of

$$A_i = \begin{bmatrix} 0 & 1 \\ -\rho_i & -\lambda_i \end{bmatrix}, \quad (2.8)$$

and g_i 's are Hill functions of the form given in (2.3). Here, β_1 and β_2 in Fig. 2.3 are the possible unmodeled interactions of the model, which can be expressed as the additional parameters of the nonlinear part. Moreover, ρ_i is the additional parameter for the extension in the LTI part. Assigning $\beta_1 = 0$, $\beta_2 = 1$ and $\rho_i = 0$, we can capture the input-output behavior of the original model (2.2).

Assumption 1. *In this study, we assume $\lambda_i = 2\zeta_i\omega_0$ and $\rho_i = \omega_0^2$ for all $i = 1, \dots, n$. Both ζ_i and ω_0 are positive, and $0 < \zeta_i \leq 1$.*

Let $\underline{x}_{eq} = [\underline{x}_1^e, \underline{x}_2^e, \dots, \underline{x}_n^e]^\top$ be an equilibrium point of (2.7), where

$$\begin{aligned} \underline{x}_{eq} &= [(x_{11}^e, x_{12}^e), (x_{21}^e, x_{22}^e), \dots, (x_{n1}^e, x_{n2}^e)]^\top \\ &= [(x_{11}^e, 0), (x_{21}^e, 0), \dots, (x_{n1}^e, 0)]^\top \\ &= \left[\left(\frac{g_1(x_{21}^e) + g_1(x_{22}^e)}{\omega_0^2}, 0 \right), \dots, \left(\frac{g_n(x_{11}^e) + g_n(x_{12}^e)}{\omega_0^2}, 0 \right) \right]^\top. \end{aligned} \quad (2.9)$$

As given in (2.9), the second components of each variable of the equilibrium is zero. In order to analyze the Hurwitz case implementation and stability conditions of the extended GRN, characteristic equation has to be computed. We can write the linearized model of (2.7) in a matrix form:

$$\begin{aligned}
\begin{bmatrix} \dot{x}_{11} \\ \dot{x}_{12} \\ \dot{x}_{21} \\ \dot{x}_{22} \\ \vdots \\ \dot{x}_{n1} \\ \dot{x}_{n2} \end{bmatrix} &= \begin{bmatrix} \begin{pmatrix} 0 & 1 \\ -\omega_0^2 & -2\zeta_1\omega_0 \end{pmatrix} & \begin{pmatrix} 0 & 0 \\ g'_1(x_{21}^e) & g'_1(x_{22}^e) \end{pmatrix} & \cdots & 0 \\ 0 & \begin{pmatrix} 0 & 1 \\ -\omega_0^2 & -2\zeta_2\omega_0 \end{pmatrix} & \cdots & 0 \\ \vdots & \ddots & \ddots & \vdots \\ \cdots & \cdots & \begin{pmatrix} 0 & 1 \\ -\omega_0^2 & -2\zeta_n\omega_0 \end{pmatrix} & \end{bmatrix} \begin{bmatrix} x_{11} \\ x_{12} \\ x_{21} \\ x_{22} \\ \vdots \\ x_{n1} \\ x_{n2} \end{bmatrix} \\
&+ \begin{bmatrix} 0 & \cdots & 0 \\ \vdots & \ddots & \vdots \\ \begin{pmatrix} 0 & 0 \\ g'_n(x_{11}^e) & g'_n(x_{12}^e) \end{pmatrix} & \cdots & 0 \end{bmatrix} \begin{bmatrix} x_{11}(t-\tau) \\ x_{12}(t-\tau) \\ x_{21}(t-\tau) \\ x_{22}(t-\tau) \\ \vdots \\ x_{n1}(t-\tau) \\ x_{n2}(t-\tau) \end{bmatrix}, \tag{2.10}
\end{aligned}$$

where $g'_n(x_{11}^e)$ is the partial derivative of the function g_n with respect to x_{11} evaluated at the equilibrium point x_{11}^e . We can represent the result in a more compact form as

$$\dot{\underline{X}} = \begin{bmatrix} A_1 & G_1 & \cdots & \cdots & 0 \\ 0 & A_2 & G_2 & \cdots & 0 \\ \vdots & & \ddots & \ddots & \vdots \\ 0 & \cdots & \cdots & A_n \end{bmatrix} \underline{X}(t) + \begin{bmatrix} 0 & \cdots & 0 \\ \vdots & \ddots & \vdots \\ G_n & \cdots & 0 \end{bmatrix} \underline{X}(t-\tau). \tag{2.11}$$

Thus, we have the following linearization of the system (2.7) around $\underline{x}_{eq}$:

$$\dot{\underline{X}}(t) = \mathbf{A}_0 \underline{X}(t) + \mathbf{A}_1 \underline{X}(t-\tau) \tag{2.12}$$

for $\mathbf{A}_0 = 2n \times 2n$, $\mathbf{A}_1 = 2n \times 2n$ and $\dot{\underline{X}}$, $\underline{X}$, $\underline{X}(t-\tau)$ are $2n \times 1$ matrices. The substructure of these matrices directly follow from the structure of (2.10). In particular, $\mathbf{A}_1$ is in the form

$$\mathbf{A}_1 = \mathbf{B}_1 \mathbf{C}_1$$

with

$$\mathbf{B}_1 = \begin{bmatrix} 0 & \cdots & 0 & 1 \end{bmatrix}^\top, \quad \mathbf{C}_1 = \begin{bmatrix} g'_n(x_{11}^e) & g'_n(x_{12}^e) & 0 & \cdots & 0 \end{bmatrix}$$

for $\mathbf{B}_1 = 2n \times 1$ and $\mathbf{C}_1 = 1 \times 2n$ matrices. Characteristic equation of the extended system (2.7) is given by

$$\det(sI - \mathbf{A}_0 - \mathbf{B}_1 \mathbf{C}_1 e^{-\tau s}) = 0. \quad (2.13)$$

2.3.1 Analysis of the Linearized Model

In order to perform the stability analysis of a linearized extended model, characteristic equation given in (2.13) is examined using Matrix Determinant Lemma [62].

Lemma 1. [62] *If A is an invertible $n \times n$ matrix, and, u and v are two n -dimensional column vectors, then*

$$\det(A + uv^\top) = \det(A) (1 + v^\top A^{-1}u). \quad (2.14)$$

Assume that $A \equiv sI - \mathbf{A}_0$, $u \equiv -\mathbf{B}_1$ and $v^\top \equiv \mathbf{C}_1 e^{-\tau s}$, then the equation (2.13) can be rewritten as follows

$$\det(sI - \mathbf{A}_0 - \mathbf{B}_1 \mathbf{C}_1 e^{-\tau s}) = \det(sI - \mathbf{A}_0) (1 + \mathbf{C}_1 e^{-\tau s} (sI - \mathbf{A}_0)^{-1} (-\mathbf{B}_1)). \quad (2.15)$$

Proposition 1. *Consider the extended GRN system defined by the set of equations (2.7). The characteristic equation of the network is represented as*

$$\Delta(s) = \left(\prod_{i=1}^n (s^2 + 2\zeta_i \omega_0 s + \omega_0^2) \right) - k(s) e^{-\tau s} \quad (2.16)$$

where

$$k(s) = \left(\prod_{i=1}^{n-1} g'_i(x_{i+1,1}^e) + s g'_i(x_{i+1,2}^e) \right) \left(g'_n(x_{11}^e) + s g'_n(x_{12}^e) \right). \quad (2.17)$$

Since all A_i in (2.7) are Hurwitz, their eigenvalues have real parts $-\zeta_i \omega_0 < 0$ for all $i = 1, \dots, n$ and the characteristic equation $\Delta(s)$ of the extended model has all its roots in $\mathbb{C}_-$ if and only if the transfer function

$$T(s) = \frac{G(s)}{1 + G(s)} \quad (2.18)$$

is stable, where

$$G(s) = -\frac{k(s) e^{-\tau s}}{\prod_{i=1}^n (s^2 + 2\zeta_i \omega_0 s + \omega_0^2)}. \quad (2.19)$$

Proof. Using Lemma 1, let us examine the expression given in (2.15). Define the function $Z(s)$

$$Z(s) = \frac{(g'_1(x_{21}^e) + sg'_1(x_{22}^e))(g'_2(x_{31}^e) + sg'_2(x_{32}^e)) \dots (g'_{n-1}(x_{n1}^e) + sg'_{n-1}(x_{n2}^e))}{(s^2 + 2\zeta_1\omega_0s + \omega_0^2)(s^2 + 2\zeta_2\omega_0s + \omega_0^2) \dots (s^2 + 2\zeta_n\omega_0s + \omega_0^2)},$$

and note that

$$\begin{aligned} & C_1 e^{-\tau s} (sI - A_0)^{-1} \\ &= \begin{bmatrix} g'_n(x_{11}^e) e^{-\tau s} & g'_n(x_{12}^e) e^{-\tau s} & 0 & \dots & 0 \end{bmatrix} \begin{bmatrix} \frac{1}{s} & \frac{1}{s^2 + 2\zeta_1\omega_0s + \omega_0^2} & \dots & Z(s) \\ 0 & \frac{s}{s^2 + 2\zeta_1\omega_0s + \omega_0^2} & \vdots & sZ(s) \\ \vdots & \ddots & \ddots & \vdots \\ 0 & 0 & 0 & \frac{s}{s^2 + 2\zeta_n\omega_0s + \omega_0^2} \end{bmatrix} \\ &= \begin{bmatrix} g'_n(x_{11}^e) e^{-\tau s} \frac{1}{s}, & \dots & \dots & g'_n(x_{11}^e) e^{-\tau s} Z(s) + s g'_n(x_{12}^e) e^{-\tau s} Z(s) \end{bmatrix}. \end{aligned}$$

Finally, we have

$$C_1 e^{-\tau s} (sI - A_0)^{-1} B_1 = g'_n(x_{11}^e) e^{-\tau s} Z(s) + s g'_n(x_{12}^e) e^{-\tau s} Z(s),$$

and the equation defined in (2.15) can be represented in the following form:

$$\Delta(s) = \left(\prod_{i=1}^n (s^2 + 2\zeta_i\omega_0s + \omega_0^2) \right) \left(1 - (g'_n(x_{11}^e) e^{-\tau s} Z(s) + s g'_n(x_{12}^e) e^{-\tau s} Z(s)) \right). \quad (2.20)$$

Inserting $Z(s)$ into (2.20), we can formalize the equation as

$$\begin{aligned} \Delta(s) &= \left(\prod_{i=1}^n (s^2 + 2\zeta_i\omega_0s + \omega_0^2) \right) \left(1 - e^{-\tau s} Z(s) (g'_n(x_{11}^e) + s g'_n(x_{12}^e)) \right) \\ &= \left(\prod_{i=1}^n (s^2 + 2\zeta_i\omega_0s + \omega_0^2) \right) \left[1 - e^{-\tau s} \left(\frac{\prod_{i=1}^{n-1} g'_i(x_{i+1,1}^e) + s g'_i(x_{i+1,2}^e)}{\prod_{i=1}^n (s^2 + 2\zeta_i\omega_0s + \omega_0^2)} \right) \dots \right. \\ &\quad \left. (g'_n(x_{11}^e) + s g'_n(x_{12}^e)) \right], \end{aligned}$$

which gives the characteristic equation defined in (2.16). $\square$

Proposition 2. Define open-loop transfer function given in (2.19) for the delay-free case as $G(s) = -k(s)/D_G(s)$, where $-k(s)$ is the numerator polynomial and $D_G(s)$ is the denominator polynomial, respectively. Consider the extended GRN system (2.7) with Hill functions defined in (2.3), and let $\underline{x}_{eq} = [\underline{x}_1^e, \underline{x}_2^e, \dots, \underline{x}_n^e]^\top$ be its equilibrium point. Then, the following statements hold:

- (i) If $\|G\|_\infty < 1$, then the system (2.7) is stable independent of time delay and $\underline{x}_{eq}$ is globally attractive.
- (ii) Suppose $\|G\|_\infty = G(0) > 1$. Then there exists τ_m such that the system (2.7) is locally stable around $\underline{x}_{eq}$ for all $\tau < \tau_m$ and locally unstable for all $\tau \geq \tau_m$.
- (iii) Suppose $G(0) < -1$, then the system (2.7) is locally unstable independent of τ .

Proof. Part (i) is the consequence of the small gain theorem; part (iii) comes from Nyquist stability criterion [54]. For (ii), we calculate the delay margin as follows. For the sake of simplicity of the exposition assume that $|G(j\omega)|$ is decreasing on the interval $\omega \in [0, \infty)$ and hence there is a unique gain crossover frequency ω_c such that $|G(j\omega_c)| = 1$.

Note that for $s = j\omega$, $G(s)$ defined in (2.19) is $G(j\omega) = -\frac{k(j\omega)e^{-\tau\omega j}}{\prod_{i=1}^n(\omega_0^2 - \omega^2 + 2\zeta_i\omega_0\omega j)}$. First we identify the point where $|G(j\omega)| = 1$. Here, the magnitude of $G(j\omega)$ depends on the nonlinearity functions, where the partial derivatives of these functions evaluated at the equilibrium point are the components of $k(s)$ as defined in (2.17).

Let $p(\omega)$ and $q_\tau(\omega)$ be

$$\begin{aligned} p(\omega) &= \left| \frac{-k(j\omega)}{\prod_{i=1}^n(\omega_0^2 - \omega^2 + 2\zeta_i\omega_0\omega j)} \right| \\ q_\tau(\omega) &= \sum_{i=1}^n \arctan\left(\frac{2\zeta_i\omega_0\omega}{\omega_0^2 - \omega^2}\right) + \phi_k + \tau\omega, \end{aligned} \tag{2.21}$$

where ϕ_k is the phase of $k(j\omega)$. Let ω_c be such that $p(\omega_c) = 1$. Consider $k(0) < 0$. Then, by the Nyquist criterion, $T(s)$ in (2.18) is stable if and only if $q_\tau(\omega_c) < \pi$,

more precisely

$$\sum_{i=1}^n \arctan\left(\frac{2\zeta_i\omega_0\omega_c}{\omega_0^2 - \omega_c^2}\right) + \phi_k + \tau\omega_c < \pi. \quad (2.22)$$

Otherwise, it is unstable. Let

$$\tau_m = \frac{\pi - \sum_{i=1}^n \arctan\left(\frac{2\zeta_i\omega_0\omega_c}{\omega_0^2 - \omega_c^2}\right) - \phi_k}{\omega_c}. \quad (2.23)$$

If $\tau > \tau_m$, then $q_\tau(\omega_c) > \pi$ and the system is locally unstable. For any $\tau < \tau_m$, $q_\tau(\omega_c) < \pi$ and the system is locally stable around $\underline{x}_{eq}$. □

Remark 1. For part (ii), if $|G(j\omega)|$ is not decreasing, then there may be multiple crossover frequencies ω_c that give multiple τ_m as defined by (2.23); the delay margin is then the smallest of these τ_m 's.

2.4 Numerical Examples

In this part, we consider the original gene regulatory network (2.2) to be homogeneous where $\lambda_i = 1$ for all $i = 1, 2, \dots, n$ and compare the stability characteristics of the original model with the extended gene regulatory network given in (2.7).

Example 1. Original and extended GRN models in (2.2) and (2.7) under negative feedback with $n = 3$ are examined in this example. Hill functions are chosen considering the delay independent stability of each system:

$$g_1(x) = \frac{1}{1+x^2}, \quad g_2(x) = \frac{3}{1+x}, \quad g_3(x) = \frac{1}{2+x}. \quad (2.24)$$

The stability condition of the original model is directly computed from the function g defined in (2.6) such that

$$g(x) = g_1 \circ g_2 \circ g_3(x) = \frac{x^2 + 6x + 9}{10x^2 + 42x + 45}.$$

The unique equilibrium point of the original model is then computed as

$$\underline{x}_{eq} = [0.1907, 2.0598, 0.4565]^\top,$$

where the gain of the model at the unique fixed point is $|g'(x_1^e)| = |g'(0.1907)| = 0.0442 < 1$. This leads to a system which is stable independently of the time delay as provided in Section 2.2.

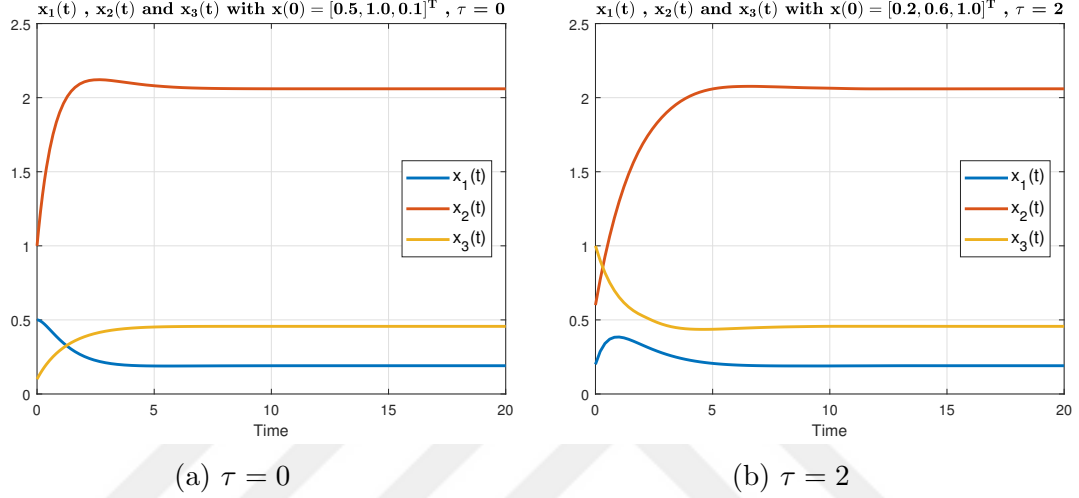


Figure 2.4: $x_1(t)$, $x_2(t)$ and $x_3(t)$ vs t graphs of the original system

Fig. 2.4 illustrates the results of the model both for without time delay and with time delay of 2 seconds. Since we analyze the system stability independent of time delay due to the condition $|g'(x_1^e)| < 1$, the convergence to the equilibrium in both cases are consistent with our expectations.

In the design of the extended model, Hurwitz matrices in (2.7) are considered as $\omega_0 = 1$ and $\zeta_i = 0.5$ for $i = 1, 2, \dots, n$, which lead to the model with an LTI part of

$$H_i(s) = \frac{1}{s^2 + s + 1}$$

in each subsystem. The nonlinear function g of the extended model is analyzed to determine the stability condition of the model. From the equilibrium point analysis, the unique fixed point, x_{11}^e , equals

$$x_{11}^e = \frac{1}{\omega_0^2} g_1 \left(\frac{1}{\omega_0^2} g_2 \cdots \left(\frac{1}{\omega_0^2} g_{n-1} \left(\frac{1}{\omega_0^2} g_n(x_{11}^e) + \frac{1}{\omega_0^2} g_n(x_{12}^e) \right) + \frac{1}{\omega_0^2} g_{n-1}(x_{n2}^e) \right) \cdots \right) + \frac{1}{\omega_0^2} g_1(x_{22}^e).$$

The terms $g_1(x_{22}^e), g_2(x_{32}^e), \dots, g_{n-1}(x_{n2}^e), g_n(x_{12}^e)$ in this equation prevent us for computing the single g function, which should satisfy the condition $x_{11}^e = g(x_{11}^e)$,

to find the unique equilibrium of the extended model. However, the equilibrium point can be determined iteratively with the Hill functions g_i 's in each subsystem. Moreover, the stability of the extended model can be specified by the small gain theorem. In this regard, the equilibrium point is computed as

$$\underline{x}_{eq} = [[1.0444, 0], [4.6407, 0], [0.8285, 0]]^T,$$

considering the Hill functions (2.24) in the design of the extended model with $\omega_0 = 1$ and $\zeta_i = 0.5$ for $i = 1, 2, 3$. To analyze the local stability conditions of the model, the characteristic equation is written using (2.16) and (2.17) as

$$\begin{aligned} X_{extended}(s) &= (s^2 + s + 1)^3 \\ &\quad - (g'_1(x_{21}^e) + sg'_1(x_{22}^e))(g'_2(x_{31}^e) + sg'_2(x_{32}^e))(g'_3(x_{11}^e) + sg'_3(x_{12}^e))e^{-\tau s} \\ &= (s^2 + s + 1)^3 + (0.0137s^2 + 0.01s + 0.0018)e^{-\tau s}, \end{aligned}$$

where it is stable if $X_{extended}(s) \neq 0$ for each $s \in \mathbb{C}_+$. The non-delayed open loop transfer function

$$G_0(s) = \frac{(0.0137s^2 + 0.01s + 0.0018)}{(s^2 + s + 1)^3}$$

has an $\mathcal{H}_\infty$ -norm of $\|G_0\|_\infty = 0.0167$, which is strictly less than 1. This implies that the feedback system is stable independently of the time delay by Proposition 2.

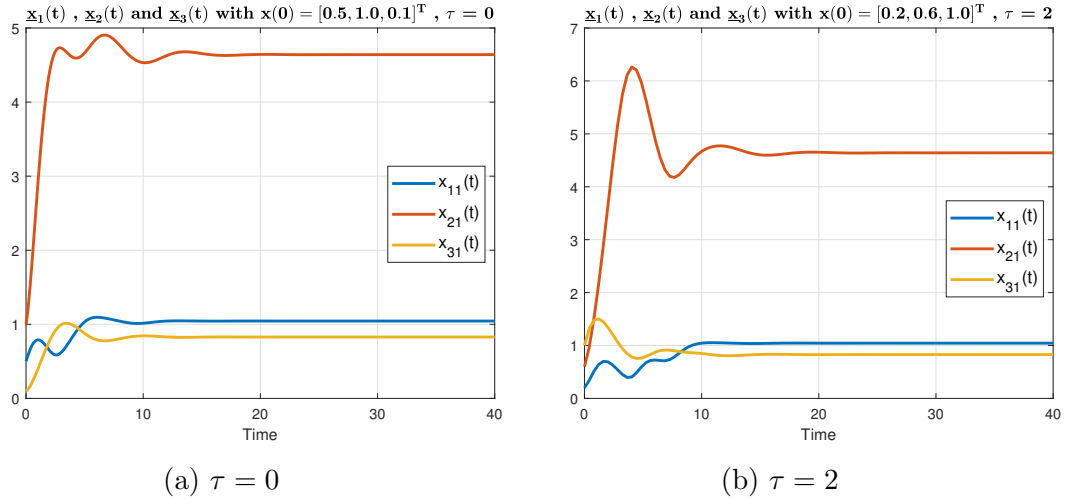


Figure 2.5: $\underline{x}_1(t)$, $\underline{x}_2(t)$ and $\underline{x}_3(t)$ vs t graphs of the extended system

The behavior of the extended model for the delay-free case is given in Fig. 2.5(a) and for the 2 second delay case is given in Fig. 2.5(b). Here, we

similarly observe convergence to the equilibrium in both results since the extended model is also stable independently of the time delay. We see the delay independent stability of the linearized original and extended systems around the equilibrium point in this example.

Example 2. The objective of this example is to analyze the original model for the delay dependent stability case although the extended model is stable independently of the delay. In this regard, the nonlinearity functions are determined as

$$g_1(x) = \frac{1}{1+x^2}, \quad g_2(x) = \frac{4x^2}{0.75+x^2}. \quad (2.25)$$

The composition function g of the original model for the homogeneous case, $\lambda_1 = \lambda_2 = 1$, is computed using (2.6), where

$$g(x) = g_1 \circ g_2(x) = \frac{x^4 + 1.5x^2 + 0.5625}{17x^4 + 1.5x^2 + 0.5625},$$

and the unique equilibrium is

$$x_{eq} = [1/2, 1]^\top.$$

The dc gain of the original model at the unique fixed point equals $|g'(x_1^e)| = |g'(0.5)| = 1.5 > 1$, which gives a system that is stable depending on the delay in the feedback channel. To determine the delay margin of the system, characteristic equation of the linearized model is derived.

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

The feedback system formed around the non-delayed open-loop transfer function $\frac{3/2}{(s+1)^2}$ has a crossover frequency $\omega_c = 0.7071$ rad/sec. The phase margin of the closed-loop system is $PM = 109.47^\circ = 0.6082\pi$ rad. Hence, the delay margin of the original model is given as $DM_{original} = PM/\omega_c = 2.7020$ sec, which means that the system is stable for $0 \leq \tau < 2.7020$ sec and unstable for $\tau \geq 2.7020$ sec.

The results of the original model is shown in Fig. 2.6, where the result of the delay-free system is provided in Fig. 2.6(a) and of the system with $\tau = 3$ is given in Fig. 2.6(b). The unstability results in an oscillatory behavior when there is a 3 second delay greater than the delay margin of the model, $DM_{original}$.

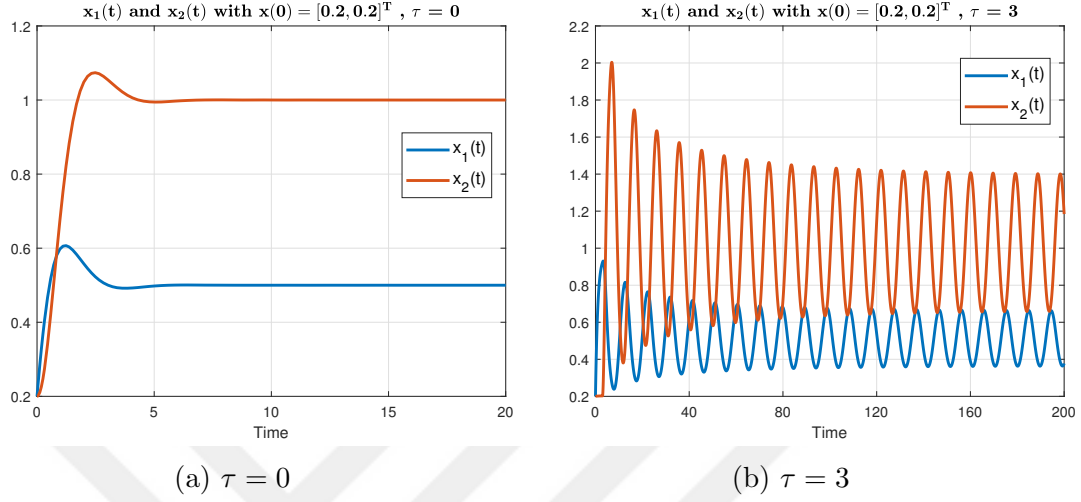


Figure 2.6: $x_1(t)$ and $x_2(t)$ vs t graphs of the original system

The extended model is now designed with the parameters $\omega_0 = 1$ and $\zeta_1 = \zeta_2 = 1$ for the Hill functions given in (2.25). The equilibrium point of the extended model is computed as

$$\underline{x}_{eq} = [[1.1353, 0], [2.5285, 0]]^T,$$

and the characteristic equation is derived from (2.16) and (2.17):

$$\begin{aligned} X_{extended}(s) &= (s^2 + 2s + 1)^2 - (g'_1(x_{21}^e) + sg'_1(x_{22}^e))(g'_2(x_{11}^e) + sg'_2(x_{12}^e))e^{-\tau s} \\ &= (s^2 + 2s + 1)^2 + 0.1516e^{-\tau s}, \end{aligned}$$

where the $\mathcal{H}_\infty$ -norm of the non-delayed open-loop transfer function $G_0(s) = \frac{0.1516}{(s^2 + 2s + 1)^2}$ equals $\|G_0\|_\infty = 0.1516 < 1$. This leads to a feedback system that is stable independently of the delay. Fig. 2.7 shows the behavior of the extended model for both without time delay and with 3 second delay, where the solutions converge to the equilibrium. This result is consistent with Proposition 2. The extension yields a stability independent of time delay for the GRN model, which is a clear improvement.

Example 3. Here, we observe stability dependent of time delay case for the extended GRN model while the original model is stable independent of delay. We consider the extended model with $\omega_0 = 1$ and $\zeta_i = 1$ for $i = 1, 2$ with the

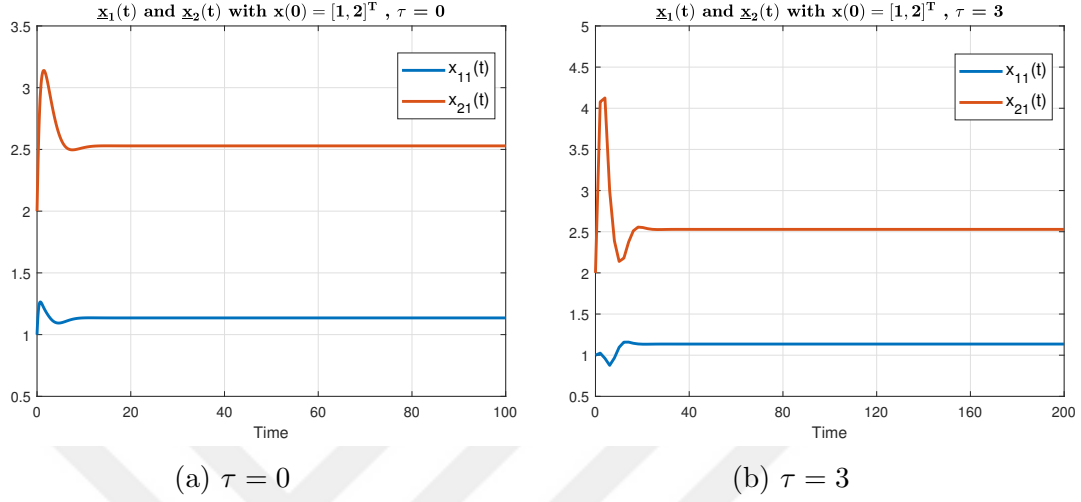


Figure 2.7: $\underline{x}_1(t)$ and $\underline{x}_2(t)$ vs t graphs of the extended system

nonlinearity functions

$$g_1(x) = \frac{1}{2 + x^4}, \quad g_2(x) = \frac{15x^2}{5 + x^2}. \quad (2.26)$$

Using the nonlinearity functions, we can calculate the unique equilibrium point of the extended system as

$$\underline{x}_{eq} = [[0.6964, 0], [1.3261, 0]]^\top,$$

and derive the characteristic equation of the linearized system using (2.16) and (2.17) such that

$$\begin{aligned} X_{extended}(s) &= (s^2 + 2s + 1)^2 - (g'_1(x_{21}^e) + sg'_1(x_{22}^e))(g'_2(x_{11}^e) + sg'_2(x_{12}^e))e^{-\tau s} \\ &= (s^2 + 2s + 1)^2 - (-1.249)e^{-\tau s}. \end{aligned}$$

The $\mathcal{H}_\infty$ -norm of the open-loop transfer function $G_0(s) = \frac{1.249}{(s^2 + 2s + 1)^2}$ is $\|G_0\|_\infty = 1.249 > 1$. Then, by Proposition 2, we expect that the solution converges to the equilibrium for $\tau < \tau_m$. Closed-loop system formed around $G_0(s)$ has a phase margin of $PM = 104.32^\circ$ with a crossover frequency $\omega_c = 0.343$ rad/sec. Hence, the delay margin of the extended model is given as $DM_{extended} = PM/\omega_c = 5.31$ sec, which means that the system is stable for $0 \leq \tau < 5.31$ sec and unstable for $\tau \geq 5.31$ sec.

The results are given below in Fig. 2.8. Here, the solution of the system depends on the time delay in the model. Fig. 2.8(a) shows the behavior of the

system with no delay. As expected, the solution converges to $\underline{x}_{eq}$. Fig. 2.8(b) shows the behavior of the system with $\tau = 6$ sec, where $\tau > \tau_m = DM_{extended}$. We observe oscillatory behavior in this case, which is consistent with Proposition 2.

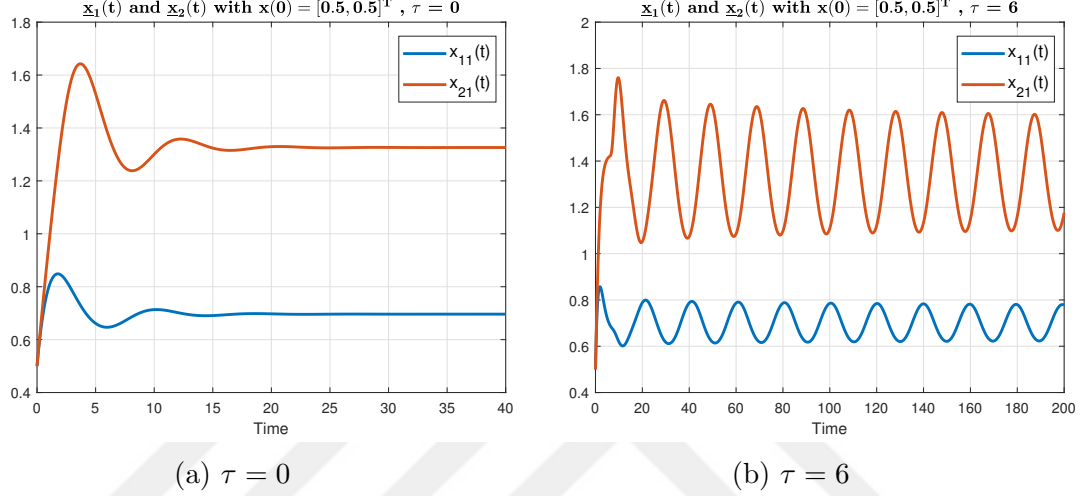


Figure 2.8: $\underline{x}_1(t)$ and $\underline{x}_2(t)$ vs t graphs of the extended system

The original model is considered to be homogeneous with $\lambda_1 = \lambda_2 = 1$. The composition function g of the model for the Hill functions (2.26) is computed using (2.6), where

$$g(x) = g_1 \circ g_2(x) = \frac{(x^2 + 5)^4}{2(x^2 + 5)^4 + (15x^2)^4},$$

and the unique equilibrium is

$$\underline{x}_{eq} = [0.4652, 0.6222]^T.$$

Then, the dc gain of the original model at the unique fixed point is computed. $|g'(x_1^e)| = 0.5345 < 1$, which means that the model is stable independent of delay in the feedback channel. This example illustrates that additional ignored dynamics in the system may reduce the delay margin; this is in contrast to the previous example where additional dynamics have improved the stability properties.

Example 4. In this example, we now examine the stability of the original and extended models for the delay dependent case. Hill functions are determined considering delay dependent stability:

$$g_1(x) = \frac{1}{2 + x^4}, \quad g_2(x) = \frac{14x^2}{3 + x^2}. \quad (2.27)$$

The original model is considered with $\lambda_i = 1$ for $i = 1, 2$, and the composition function g is computed for the nonlinearity functions (2.27) such that

$$g(x) = g_1 \circ g_2(x) = \frac{(x^2 + 3)^4}{2(x^2 + 3)^4 + (14x^2)^4},$$

Then, the unique equilibrium of the original model is

$$x_{eq} = [0.4213, 0.7819]^\top,$$

where the value $|g'(x_1^e)|$ is $1.189 > 1$. This leads to a system which is stable depending on the delay. For the delay margin computation, characteristic equation of the model is derived.

$$X_{original}(s) = (s + 1)^2 + 1.189e^{-\tau s},$$

and the closed loop system formed around the non-delayed open-loop transfer function $\frac{1.189}{(s+1)^2}$ has a crossover frequency $\omega_c = 0.435$ rad/sec. The corresponding phase margin of the feedback system is $PM = 132.96^\circ$. So, the delay margin of the original model is computed as $DM_{original} = PM/\omega_c = 5.33$ sec. The behavior of the model is given in Fig. 2.9, where the response of the delay free system is given in Fig. 2.9(a) and of the system with $\tau = 6$ sec is given in Fig. 2.9(b). Since $\tau = 6 > DM_{original} = 5.33$, oscillations around the equilibrium are observed, as expected.

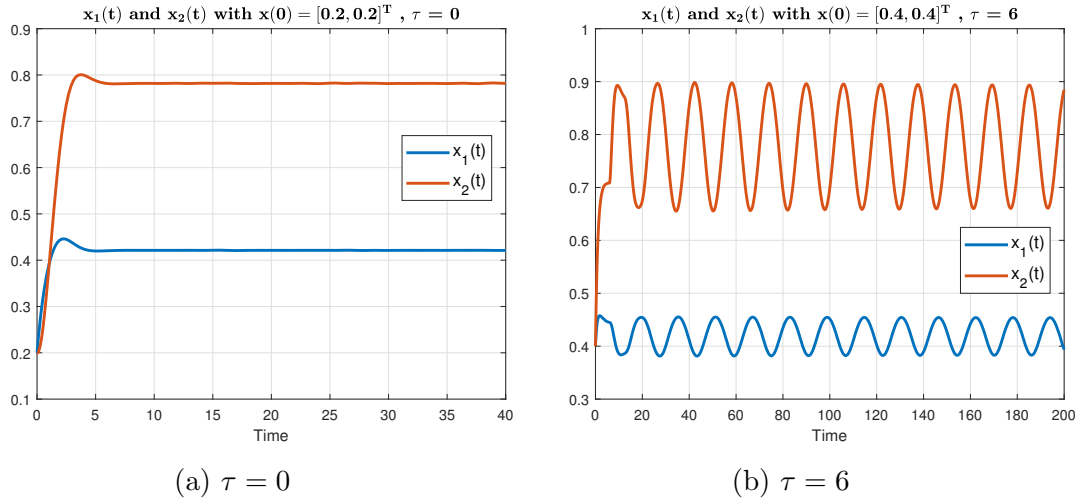


Figure 2.9: $x_1(t)$ and $x_2(t)$ vs t graphs of the original system

Then, the extended model is designed with $\omega_0 = 1$ and $\zeta_1 = \zeta_2 = 1$. The unique equilibrium point of the system with these parameters is computed as

$$\underline{x}_{eq} = [[0.6196, 0], [1.5882, 0]]^\top.$$

The characteristic equation of the linearized extended model is then derived:

$$\begin{aligned} X_{extended}(s) &= (s^2 + 2s + 1)^2 - (g'_1(x_{21}^e) + sg'_1(x_{22}^e))(g'_2(x_{11}^e) + sg'_2(x_{12}^e))e^{-\tau s} \\ &= (s^2 + 2s + 1)^2 - (-1.041)e^{-\tau s}. \end{aligned}$$

The $\mathcal{H}_\infty$ -norm of the non-delayed open-loop transfer function $G_0(s) = \frac{1.041}{(s^2 + 2s + 1)^2}$ is $\|G_0\|_\infty = 1.041 > 1$. From Proposition 2, we expect that the solution converges to the equilibrium for $\tau < \tau_m$. The feedback system formed around $G_0(s)$ has a phase margin $PM = 147.38^\circ$ and a crossover frequency $\omega_c = 0.143$ rad/sec. Then, the delay margin of the extended model is computed as $DM_{extended} = PM/\omega_c = 17.953$ sec.

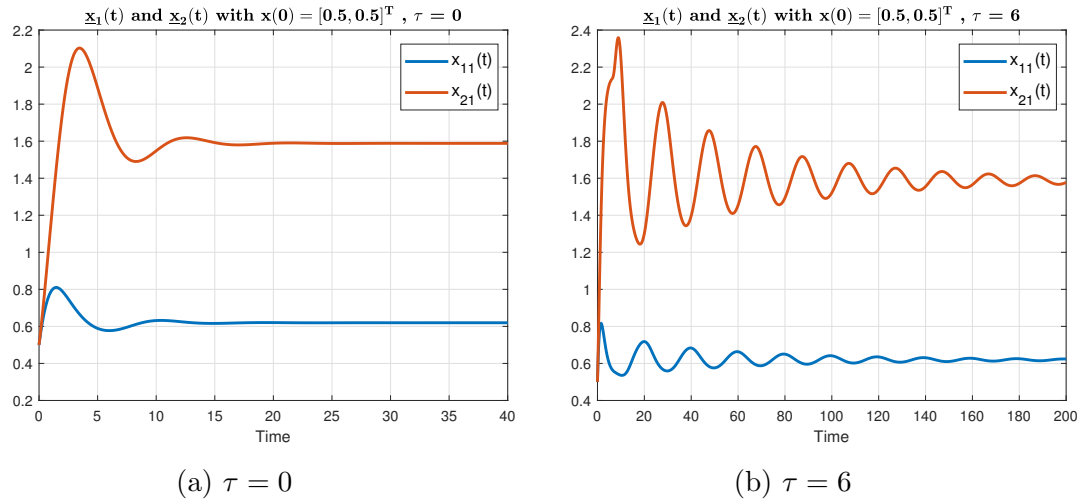


Figure 2.10: $\underline{x}_1(t)$ and $\underline{x}_2(t)$ vs t graphs of the extended system

The behavior of the extended model in this example is given in Fig. 2.10. There exists a convergence for the delay free case. Similarly when $\tau = 6$, the model can now overcome the oscillations after a while and tolerate 6 sec delay without violating stability since delay margin of the extended model is very large. So, the additional dynamics have improved the delay margin, but not in an unbounded fashion.

Example 5. This example covers a different case for the extended model, where the conditions in Proposition 2 are not satisfied. We consider the extended model with $\omega_0 = 1$ and $\zeta_i = 1$ for $i = 1, 2$ with the nonlinearity functions

$$g_1(x) = \frac{1}{1+x}, \quad g_2(x) = \frac{9x^2}{2+x^2}. \quad (2.28)$$

The unique equilibrium point of the system is calculated as

$$\underline{x}_{eq} = [[1.2084, 0], [3.7981, 0]]^\top,$$

and the characteristic equation of the linearized extended model using the Hill functions (2.28) is derived as

$$\begin{aligned} X_{extended}(s) &= (s^2 + 2s + 1)^2 - (g'_1(x_{21}^e) + sg'_1(x_{22}^e))(g'_2(x_{11}^e) + sg'_2(x_{12}^e))e^{-\tau s} \\ &= (s^2 + 2s + 1)^2 - (-3.633s - 0.1578)e^{-\tau s}. \end{aligned}$$

Then, the $\mathcal{H}_\infty$ -norm of the open-loop transfer function $G_0(s) = \frac{3.633s+0.1578}{(s^2+2s+1)^2}$ is computed: $\|G_0\|_\infty = 1.1833 > 1$. However, there are multiple crossover frequencies where $|G_0(j\omega)| = 1$.

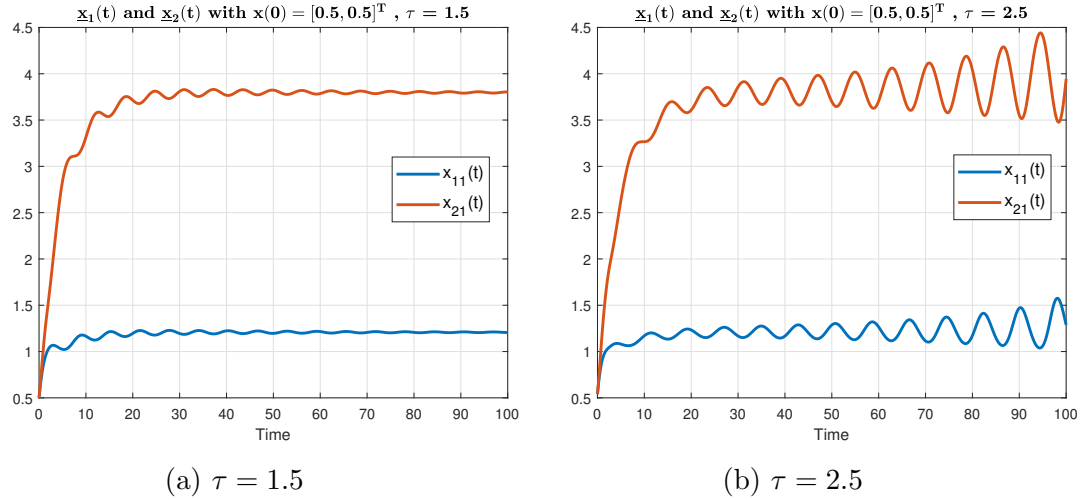


Figure 2.11: $\underline{x}_1(t)$ and $\underline{x}_2(t)$ vs t graphs of the extended system

We compute the delay margins of this model in order to analyze the system. The feedback system formed around $G_0(s)$ has a crossover frequency $\omega_c = 0.89$ rad/sec and a phase margin of $PM = 99.4^\circ$. Then, $PM/\omega_c = 1.93$ sec. In addition, there is another crossover with a frequency of $\omega_c = 0.34$ rad/sec and a

phase margin of $PM = 187.68^\circ$, which gives $PM/\omega_c = 9.64$ sec. So the real DM is the smaller of these two values, 1.93 sec.

The behavior of the extended model in this example is given in Fig. 2.11. The response of the system for $\tau = 2.5$ is oscillatory as seen in Fig. 2.11(b), while the response for $\tau = 1.5$ converges to the equilibrium. The conditions of Proposition 2 are not met in this example as provided above, however, the stability of the extended model can be analyzed numerically in this case.

2.5 Stability Analysis for Homogeneous GRNs with Specific Hill Functions

Consider the original GRN system (2.2) to be homogeneous with $\lambda_i = 1$ for all $i = 1, \dots, n$, and each $g_i(x)$ to be a Hill function of the form $g_i(x) = f(x) = \frac{a}{b+x^m}$, $f : \mathbb{R}_+ \rightarrow \mathbb{R}_+$, for $a > 0, b > 0$ and $m \in \mathbb{N}$ with $m \geq 2$. The small gain condition previously analyzed in [54] said that the condition $|f'(x_1^e)| < 1$ corresponds to the delay independent stability of the linearized system where x_1^e is the unique fixed point of the function g given in (2.6). Based on the equilibrium point analysis of the original GRN model in [53, 54], the unique fixed point satisfies the condition

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

The delay-independent stability condition gives that if

$$|f'(x_1^e)| < 1 \iff \left| \frac{ma(x_1^e)^{m-1}}{(b + (x_1^e)^m)^2} \right| < 1 \iff \left| m \left(1 - \frac{bx_1^e}{a} \right) \right| < 1 \quad (2.29)$$

then the homogeneous GRN is locally stable independently of the time delay around its unique equilibrium point.

2.5.1 Conditions for Stability Independent of Delay for the Extended GRN Model

Now we are considering the extended GRN model (2.7) with $\zeta_i = \zeta$ for $i = 1, 2, \dots, n$, and we develop a stability condition for the homogeneous case. Similarly, g_i 's have the form:

$$g_i(x) := f(x) = \frac{a}{b + x^m}, \quad f : \mathbb{R}_+ \rightarrow \mathbb{R}_+ \quad (2.30)$$

where $a > 0, b > 0$ and $m \in \mathbb{N}$ with $m \geq 2$.

Proposition 3. *Consider the extended system (2.7) as a homogeneous model with Hill functions defined in (2.30). The unique equilibrium point of the system is $\underline{x}_{eq} = [\underline{x}_1^e, \underline{x}_1^e, \dots, \underline{x}_1^e]^\top = [(x_{11}^e, 0), \dots, (x_{11}^e, 0)]^\top$, where*

$$x_{11}^e = \frac{1}{\omega_0^2} (f(x_{11}^e) + f(0)) = \frac{1}{\omega_0^2} \left(\frac{a}{b + (x_{11}^e)^m} + \frac{a}{b} \right). \quad (2.31)$$

Proof. The equilibrium conditions can be written from the delay differential equations as

$$\begin{aligned} 0 &= \dot{x}_{11} = x_{12}^e \\ 0 &= \dot{x}_{12} = -\omega_0^2 x_{11}^e - 2\zeta\omega_0 x_{12}^e + f(x_{21}^e) + f(x_{22}^e) \\ 0 &= \dot{x}_{21} = x_{22}^e \\ 0 &= \dot{x}_{22} = -\omega_0^2 x_{21}^e - 2\zeta\omega_0 x_{22}^e + f(x_{31}^e) + f(x_{32}^e) \\ &\vdots \\ 0 &= \dot{x}_{n1} = x_{n2}^e \\ 0 &= \dot{x}_{n2} = -\omega_0^2 x_{n1}^e - 2\zeta\omega_0 x_{n2}^e + f(x_{11}^e) + f(x_{12}^e), \end{aligned}$$

which gives

$$\begin{aligned} x_{12}^e &= x_{22}^e = \dots = x_{n2}^e = 0, \\ x_{11}^e &= \frac{1}{\omega_0^2} (f(x_{21}^e) + f(x_{22}^e)), \\ x_{21}^e &= \frac{1}{\omega_0^2} (f(x_{31}^e) + f(x_{32}^e)), \\ &\vdots \\ x_{n1}^e &= \frac{1}{\omega_0^2} (f(x_{11}^e) + f(x_{12}^e)). \end{aligned} \quad (2.32)$$

Note that

$$x_{11}^e = \frac{1}{\omega_0^2} \left[f \left(\frac{1}{\omega_0^2} \left[f \cdots \left(\frac{1}{\omega_0^2} \left[f \left(\frac{1}{\omega_0^2} (f(x_{11}^e) + f(x_{12}^e)) \right) + f(x_{n2}^e) \right] \right) \cdots \right] \right) + f(x_{22}^e) \right].$$

Then, using $f(x_{12}^e) = f(x_{22}^e) = \dots = f(x_{n2}^e) = f(0) = a/b$, we can rewrite x_{11}^e as

$$x_{11}^e = \frac{1}{\omega_0^2} \left[f \left(\frac{1}{\omega_0^2} \left[f \cdots \left(\frac{1}{\omega_0^2} \left[f \left(\frac{1}{\omega_0^2} \left(f(x_{11}^e) + \frac{a}{b} \right) \right) + \frac{a}{b} \right] \right) \cdots \right] \right) + \frac{a}{b} \right].$$

Moreover, $x_{n1}^e, \dots, x_{21}^e$ satisfy the same equation, e.g.,

$$x_{n1}^e = \frac{1}{\omega_0^2} \left[f \left(\frac{1}{\omega_0^2} \left[f \cdots \left(\frac{1}{\omega_0^2} \left[f \left(\frac{1}{\omega_0^2} \left(f(x_{n1}^e) + \frac{a}{b} \right) \right) + \frac{a}{b} \right] \right) \cdots \right] \right) + \frac{a}{b} \right]$$

which gives that $x_{11}^e = x_{21}^e = \dots = x_{n1}^e$. Then, the unique equilibrium of the extended homogeneous model can be expressed as $\underline{x}_{eq} = [(x_{11}^e, 0), \dots, (x_{11}^e, 0)]^\top$.

Combining (2.30) and (2.32), we have

$$x_{11}^e = \frac{1}{\omega_0^2} \left(\frac{a}{b + (x_{11}^e)^m} + \frac{a}{b} \right).$$

□

The characteristic equation of the homogeneous extended GRN model using Proposition 1 is

$$\begin{aligned} \Delta(s) = & \left(\prod_{i=1}^n (s^2 + 2\zeta\omega_0 s + \omega_0^2) \right) \\ & - \left(\prod_{i=1}^{n-1} f'(x_{i+1,1}^e) + s f'(x_{i+1,2}^e) \right) (f'(x_{11}^e) + s f'(x_{12}^e)) e^{-\tau s}, \end{aligned} \quad (2.33)$$

which can be expressed as

$$\Delta(s) = (s^2 + 2\zeta\omega_0 s + \omega_0^2)^n - (f'(x_{11}^e) + s f'(x_{12}^e))^n e^{-\tau s}. \quad (2.34)$$

Note that

$$f'(x) = -\frac{ma(x)^{m-1}}{(b + (x)^m)^2}$$

and $f'(0) = 0$. Using this, (2.34) becomes

$$\Delta(s) = (s^2 + 2\zeta\omega_0 s + \omega_0^2)^n - (f'(x_{11}^e))^n e^{-\tau s}.$$

Then, the extended homogeneous GRN model is stable independent of time delay if

$$\|G_0(s)\|_\infty < 1 \iff \left\| \frac{(f'(x_{11}^e))^n}{(s^2 + 2\zeta\omega_0 s + \omega_0^2)^n} \right\|_\infty < 1. \quad (2.35)$$

Define that

$$k = |f'(x_{11}^e)| = \frac{ma(x_{11}^e)^{m-1}}{(b + (x_{11}^e)^m)^2} \quad (2.36)$$

then, (2.35) is equivalent to

$$\left\| \frac{k}{(s^2 + 2\zeta\omega_0 s + \omega_0^2)} \right\|_\infty^n < 1 \iff \left\| \frac{k}{(s^2 + 2\zeta\omega_0 s + \omega_0^2)} \right\|_\infty < 1.$$

For $s = j\omega$, we have

$$\begin{aligned} \left| \frac{\frac{k}{\omega_0^2}}{\left(1 - \frac{\omega^2}{\omega_0^2} + j2\zeta\frac{\omega}{\omega_0}\right)} \right| < 1 &\iff \left| \frac{\frac{k}{\omega_0^2}}{(1 - \hat{\omega}^2 + j2\zeta\hat{\omega})} \right| < 1 \\ &\iff \frac{\frac{k}{\omega_0^2}}{\sqrt{(1 - \hat{\omega}^2)^2 + (2\zeta\hat{\omega})^2}} < 1 \\ &\iff \frac{k^2}{\omega_0^4} < (1 - \hat{\omega}^2)^2 + (2\zeta\hat{\omega})^2 \end{aligned}$$

where $\hat{\omega} = \omega/\omega_0$ and k is given in (2.36). Here, we have two different conditions depending on the value of ζ , and the following result is established.

Proposition 4. *Consider the extended system (2.7) with Hill functions defined in (2.30). Let $\underline{x}_{eq} = [\underline{x}_1^e, \underline{x}_1^e, \dots, \underline{x}_1^e]^\top$ be the unique equilibrium point of the system. Then, the following statements hold:*

(i) *If $\zeta < 1/\sqrt{2}$ and a, b, m satisfy*

$$(\omega_0^2 x_{11}^e)^2 - \frac{a}{b} \left(3 - \frac{2\zeta\sqrt{(1 - \zeta^2)}}{m} \right) (\omega_0^2 x_{11}^e) + \frac{2a^2}{b} > 0, \quad (2.37)$$

then the extended GRN is locally stable around $\underline{x}_{eq}$, independent of delay.

(ii) If $\zeta \geq 1/\sqrt{2}$ and a, b, m satisfy

$$(\omega_0^2 x_{11}^e)^2 - \frac{a}{b} \left(3 - \frac{1}{m} \right) (\omega_0^2 x_{11}^e) + \frac{2a^2}{b} > 0, \quad (2.38)$$

then the extended GRN is locally stable around $\underline{x}_{eq}$, independent of delay.

Proof. For part (i), when $\zeta < 1/\sqrt{2}$, we can use the critical value for $\hat{\omega}^2 = (1 - 2\zeta^2)$, [63]:

$$\begin{aligned} \frac{k^2}{\omega_0^4} < (1 - \hat{\omega}^2)^2 + (2\zeta\hat{\omega})^2 &\iff \frac{k^2}{\omega_0^4} < 4\zeta^2(1 - \zeta^2) \\ &\iff \frac{k}{\omega_0^2} < 2\zeta\sqrt{(1 - \zeta^2)}. \end{aligned}$$

Using the definition of k given in (2.36) and inserting (2.31) into the derivative, we can obtain the following inequalities.

$$\begin{aligned} \frac{k}{\omega_0^2} < 2\zeta\sqrt{(1 - \zeta^2)} &\iff \frac{1}{\omega_0^2} \left(\frac{ma(x_{11}^e)^{m-1}}{(b + (x_{11}^e)^m)^2} \right) < 2\zeta\sqrt{(1 - \zeta^2)} \\ &\iff ma(x_{11}^e)^{m-1} < \omega_0^2 \left(2\zeta\sqrt{(1 - \zeta^2)} \right) \left(\frac{a^2}{(\omega_0^2 x_{11}^e - a/b)^2} \right) \\ &\iff ma \left(\frac{a/x_{11}^e}{\omega_0^2 x_{11}^e - a/b} - \frac{b}{x_{11}^e} \right) (\omega_0^2 x_{11}^e - a/b)^2 < \omega_0^2 \left(2\zeta\sqrt{(1 - \zeta^2)} \right) a^2 \\ &\iff ma (2a - b\omega_0^2 x_{11}^e) (\omega_0^2 x_{11}^e - a/b) < a\omega_0^2 \left(2\zeta\sqrt{(1 - \zeta^2)} \right) (x_{11}^e) \\ &\iff -bm (\omega_0^2 x_{11}^e)^2 + 3am(\omega_0^2 x_{11}^e) - \frac{2a^2}{b}m - a \left(2\zeta\sqrt{(1 - \zeta^2)} \right) (\omega_0^2 x_{11}^e) < 0 \\ &\iff (\omega_0^2 x_{11}^e)^2 - \frac{a}{b} \left(3 - \frac{2\zeta\sqrt{(1 - \zeta^2)}}{m} \right) (\omega_0^2 x_{11}^e) + \frac{2a^2}{b} > 0. \end{aligned}$$

Moreover, when $\zeta \geq 1/\sqrt{2}$, the stability of the GRN model is satisfied if

$$\begin{aligned} \frac{k}{\omega_0^2} < 1 &\iff \frac{1}{\omega_0^2} \left(\frac{ma(x_{11}^e)^{m-1}}{(b + (x_{11}^e)^m)^2} \right) < 1 \\ &\iff ma(x_{11}^e)^{m-1} < \omega_0^2 \left(\frac{a^2}{(\omega_0^2 x_{11}^e - \frac{a}{b})^2} \right) \\ &\iff ma \left(\frac{a/x_{11}^e}{\omega_0^2 x_{11}^e - a/b} - \frac{b}{x_{11}^e} \right) \left(\omega_0^2 x_{11}^e - \frac{a}{b} \right)^2 < \omega_0^2 a^2 \\ &\iff ma (2a - b\omega_0^2 x_{11}^e) \left(\omega_0^2 x_{11}^e - \frac{a}{b} \right) < a\omega_0^2 (x_{11}^e) \\ &\iff (\omega_0^2 x_{11}^e)^2 - \frac{a}{b} \left(3 - \frac{1}{m} \right) (\omega_0^2 x_{11}^e) + \frac{2a^2}{b} > 0. \end{aligned}$$

□

2.5.2 A Numerical Example

This example covers the stability analysis of the original and extended gene regulatory network models with Hill-type nonlinearities for the homogeneous case. Original model is designed using $\lambda_i = 1$ for all i , and extended model is designed by choosing $\omega_0 = 1$. Let one of the parameters of the Hill function, b , is fixed as 1, and a takes values between the interval $a \in [0.5, 2]$. Moreover, $m \in [2, 3, 4, 5, 6, 7]$. Assume the condition for the stability of the original model given in (2.29) is defined as:

$$R_{or}(a) < 1 \quad \text{where} \quad R_{or}(a) = \left| m \left(1 - \frac{bx_1^e}{a} \right) \right|.$$

Then, using (2.38) in Proposition 4, we have

$$\begin{aligned} \frac{b}{a} m \omega_0^4 x_{11}^e - 3m \omega_0^2 + \omega_0^2 + \frac{2a}{x_{11}^e} m &> 0 \\ m \left(-\frac{b}{a} \omega_0^4 x_{11}^e + 3\omega_0^2 - \frac{2a}{x_{11}^e} \right) &< \omega_0^2 \\ m \left(3 - \frac{b}{a} \omega_0^2 x_{11}^e - \frac{2a}{\omega_0^2 x_{11}^e} \right) &< 1 \end{aligned}$$

for the stability of the extended model when $\zeta \geq 1/\sqrt{2}$. Similarly, for $\zeta < 1/\sqrt{2}$, using (2.37) in Proposition 4, we get

$$m \left(\frac{1}{2\zeta\sqrt{1-\zeta^2}} \right) \left(3 - \frac{b}{a} \omega_0^2 x_{11}^e - \frac{2a}{\omega_0^2 x_{11}^e} \right) < 1.$$

Let us define the stability condition of the extended GRN as

$$\begin{aligned} R_{ext}(a) &< 1 \quad \text{where} \\ R_{ext}(a) &= \begin{cases} m \left(\frac{1}{2\zeta\sqrt{1-\zeta^2}} \right) \left(3 - \frac{b}{a} \omega_0^2 x_{11}^e - \frac{2a}{\omega_0^2 x_{11}^e} \right) & \text{if } \zeta < 1/\sqrt{2} \\ m \left(3 - \frac{b}{a} \omega_0^2 x_{11}^e - \frac{2a}{\omega_0^2 x_{11}^e} \right) & \text{if } \zeta \geq 1/\sqrt{2}. \end{cases} \end{aligned} \quad (2.39)$$

Fig. 2.12(a) shows the change of $R_{or}(a)$ for different m values. As m increasing, it becomes hard to satisfy the condition $R_{or}(a) < 1$. For example, when $m = 2$,

all values of a in the interval $[0.5, 2)$ satisfy the stability condition, however, when $m = 7$, only the values of a between $[0.5, 0.9)$ satisfy the condition. Fig. 2.12(b) illustrates the results of the extended model when $\zeta \geq 1/\sqrt{2}$ for different m values. Here, only for $m = 6$ and $m = 7$, there are small intervals not satisfying $R_{ext}(a) < 1$. When $a \in [0.65, 0.85]$ and when $a \in [0.57, 0.96]$, the stability condition is not met for $m = 6$ and $m = 7$, respectively.

Fig. 2.13 gives the results of $R_{ext}(a)$ for $\zeta < 1/\sqrt{2}$. On the left, $R_{ext}(a)$ given in (2.39) is evaluated when ζ value is 0.5, and on the right, $R_{ext}(a)$ is calculated when $\zeta = 0.25$. The responses in Fig. 2.13(a) are similar to Fig. 2.12(b), when $a \in [0.57, 1]$ and when $a \in [0.52, 1.06]$, the condition is not satisfied for $m = 6$ and $m = 7$, respectively. However, decreasing ζ value gives a worse result; as m increasing, the condition $R_{ext}(a) < 1$ is satisfied only for some a values. For example, all values of a in the given interval meet the stability condition for $m = 2$, but $R_{ext}(a) < 1$ is satisfied only for $a > 1.3$ when $m = 7$. Note that the responses when $\zeta < 1/\sqrt{2}$ given in Fig. 2.13 are similar, there is a scaling by a factor of $1/2\zeta\sqrt{1-\zeta^2}$.

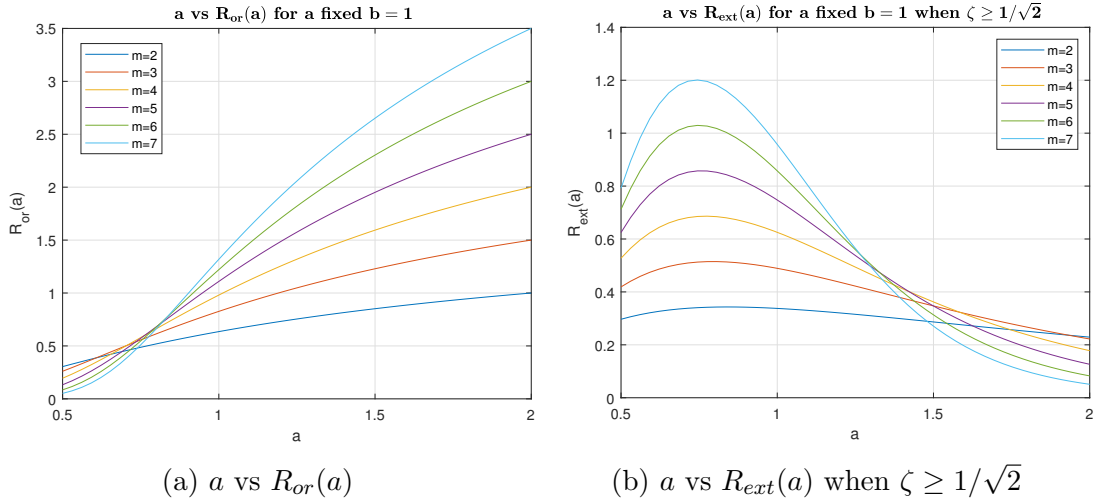
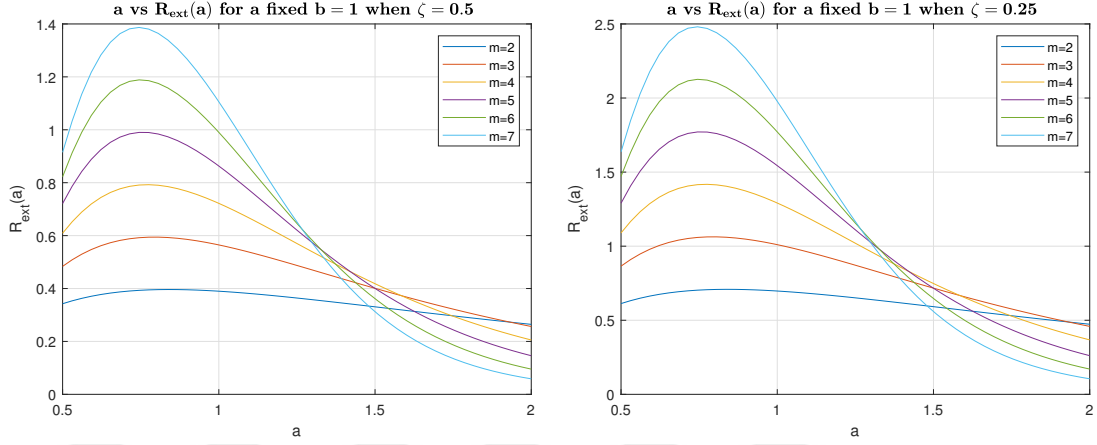


Figure 2.12: a vs $R(a)$ for a fixed b



(a) a vs $R_{ext}(a)$ when $\zeta < 1/\sqrt{2}$

(b) a vs $R_{ext}(a)$ when $\zeta < 1/\sqrt{2}$

Figure 2.13: a vs $R_{ext}(a)$ for a fixed b when $\zeta < 1/\sqrt{2}$

2.6 Conclusion

In this chapter, the gene regulatory network model derived earlier for cyclic systems with time delay is revisited for the case where each subsystem is considered as second order. Additional parameters taking account of unmodeled dynamics are included in the design of the extended model. We have provided detailed analysis of the extended GRN and derived the characteristic equation of the linearized model. Stability of the extended system is investigated considering different design parameters. Additionally, the condition for stability independent of delay, developed before, is revisited for the extended model with time delay. Our analysis provide guidelines on how synthetic biological extensions should be designed for stability improvement in GRNs.

Here we considered pointwise constant delay in the network structure. It may be possible to consider time-varying delays in the model. There exist studies where time delays are assumed to be time-varying [27, 28]. In that case, the linearization analysis of the extended model and determining the stability conditions become slightly more involved. This is an important point worth investigating for the extended models considered here.

Chapter 3

Robustness Analysis of a Gene Regulatory Network Model

Motivated by the work on gene regulatory network models, we will analyze an extension with multiplicative perturbation, involving higher order dynamics, of a cyclic gene regulatory network under negative feedback using the model studied in [54]. Because the earlier models are simple, even the second order extension like the repressilator model is valuable. The objective behind this design is to present a novel GRN model considering a particular type of multiplicative uncertainty with high pass filter inside the linear time invariant part of each subsystem. Robustness and delay margin analysis have been performed for this extended model. The main body of this work presented in this chapter appears in [58], in preliminary form.

3.1 Introduction

There exists an inherent tradeoff in modeling between fidelity and simplicity [66]. The approximations and simplifications in the dynamics are involved in obtaining a mathematical model, as they facilitates the analysis of systems. However, this

makes it difficult to have models that accurately represent reality. This tradeoff becomes particularly relevant when modeling uncertainty.

Mainly two types of uncertainty exist, unmodeled dynamics and parametric uncertainty [56]. Unmodeled dynamics may refer to the neglected biological interactions or unaccounted system dynamics that can significantly affect the system's behavior. On the other hand, parametric uncertainty encompasses uncertainties related to the values of system parameters, such as degradation rates or reaction rates. A class of uncertain nonlinear systems with unmodeled dynamics and disturbances is widely studied in literature. By investigating these uncertainties, researchers aim to enhance our understanding of complex systems and devise robust solutions that can handle the associated challenges.

Our approach in this study is somewhat different: we consider the possible unmodeled dynamics in the existing gene regulatory network model using a multiplicative uncertainty with high pass filter. Rather than introducing Hurwitz matrices with second order dynamics proposed and analyzed in Chapter 2, we focus on the design of subsystems using multiplicative perturbation. The goal with such an approach is to complement, not to replace, subsystems inspired by the repressilator to incorporate the unmodeled dynamics arising from neglected biological interactions.

3.2 Extension of an Earlier GRN Model Using Multiplicative Perturbation

In this section, we extend the gene regulatory network structure given in (2.2) using a multiplicative term with high pass filter as illustrated in Fig. 3.1 for the LTI part ($H_i(s)$) in each subsystem. With this extension, we investigate two questions:

- i. Whether we can improve the stability characteristics by modifying the model where it is physically possible to replace the system structure with

additional subsystems (for example GRNs constructed using synthetic biology).

- ii. Effect of the unmodeled dynamics (in the form of neglected biological interactions) on the system's delay margin.

An interesting special case is the repressilator model: for $\delta = -1$ and $W(s) = s/(s + \beta)$ in Fig. 3.1, we obtain H_i given by (2.4).

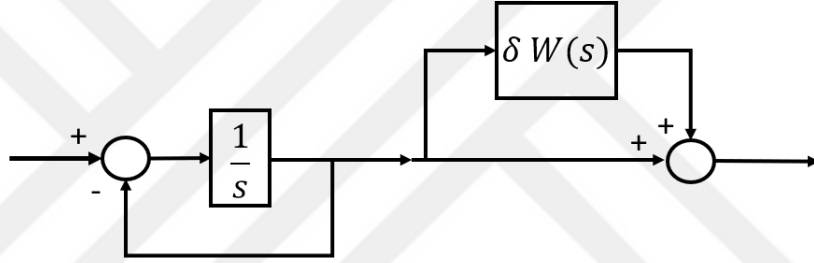


Figure 3.1: Multiplicative uncertainty with high-pass filter W , $\delta \neq 0$.

The additional block $\delta W(s)$ captures the unmodeled dynamics in the system. It may be possible to consider $W \in H_\infty$, with $\|W\|_\infty \leq 1$, as a normalized uncertainty bound, as in classical robust control; in this case δ is scaling the uncertainty magnitude. In this study, we explore what happens if we specifically choose

$$W(s) = \frac{\hat{\tau}s}{1 + \hat{\tau}s} \quad (3.1)$$

with $\hat{\tau} > 0$ and $\delta \neq 0$.

Fig. 3.2 shows the structure of each subsystem in this extended model. The LTI part, $H_{i,ext}(s)$, is

$$H_{i,ext}(s) = \left(\frac{1}{s+1} \right) \left(\frac{1 + (1+\delta)\hat{\tau}s}{1 + \hat{\tau}s} \right). \quad (3.2)$$

In the state space, the extended GRN model is now given by the following set of

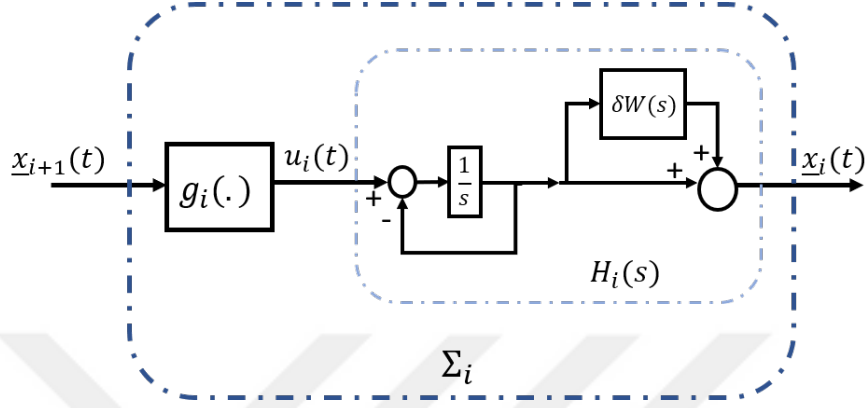


Figure 3.2: Subsystems of the extended GRN model.

differential equations

$$\begin{aligned}
 \dot{\underline{x}}_1(t) &= \begin{bmatrix} 0 & 1 \\ -\gamma & -(1+\gamma) \end{bmatrix} \underline{x}_1(t) + \begin{bmatrix} 0 \\ 1 \end{bmatrix} g_1 \left(\begin{bmatrix} \gamma & (1+\delta) \end{bmatrix} \underline{x}_2(t) \right) \\
 \dot{\underline{x}}_2(t) &= \begin{bmatrix} 0 & 1 \\ -\gamma & -(1+\gamma) \end{bmatrix} \underline{x}_2(t) + \begin{bmatrix} 0 \\ 1 \end{bmatrix} g_2 \left(\begin{bmatrix} \gamma & (1+\delta) \end{bmatrix} \underline{x}_3(t) \right) \\
 &\vdots \\
 \dot{\underline{x}}_n(t) &= \begin{bmatrix} 0 & 1 \\ -\gamma & -(1+\gamma) \end{bmatrix} \underline{x}_n(t) + \begin{bmatrix} 0 \\ 1 \end{bmatrix} g_n \left(\begin{bmatrix} \gamma & (1+\delta) \end{bmatrix} \underline{x}_1(t-\tau) \right),
 \end{aligned} \tag{3.3}$$

where $\gamma = \frac{1}{\tau}$. Note that each $\underline{x}_i(t)$ includes two variables in this structure such that $\underline{x}_i(t) = [x_{i1}(t), x_{i2}(t)]^\top$ for $i = 1, \dots, n$. Based on the equilibrium point analysis of (3.3), the function $g(x)$ in the extended model with multiplicative perturbation is defined as

$$g(x) = g_1 \circ g_2 \circ \dots \circ g_n \tag{3.4}$$

and the equilibrium point of the network from the definition of g function is

$$\begin{aligned}
 \underline{x}_{eq} &= \left[\frac{1}{\tau} \underline{x}_1^e, \frac{1}{\tau} \underline{x}_2^e, \dots, \frac{1}{\tau} \underline{x}_n^e \right]^\top = \left[\hat{x}_1^e, \hat{x}_2^e, \dots, \hat{x}_n^e \right]^\top \\
 &= \left[[\hat{x}_{11}^e, 0], [\hat{x}_{21}^e, 0], \dots, [\hat{x}_{n1}^e, 0] \right]^\top,
 \end{aligned} \tag{3.5}$$

where all the second components are zero. At the unique fixed point $\hat{x}_{11}^e = \frac{1}{\tau} x_{11}^e$,

the equation

$$\hat{x}_{11}^e = g(\hat{x}_{11}^e) \quad (3.6)$$

is satisfied.

3.2.1 Effects of $(\delta, \hat{\tau})$ on the Delay Margin

Here we illustrate the effects of $(\delta, \hat{\tau})$ on the delay margin of the system for a specific GRN model with two subsystems. We consider the biological systems in (2.2) and (3.3) under negative feedback. The nonlinearity functions are given by

$$g_1(x) = \frac{6}{2+x^2}, \quad g_2(x) = \frac{4x^2}{1+x^2}. \quad (3.7)$$

In the original model, $g(x)$ is computed using (2.6) for the case $\lambda_1 = \lambda_2 = 1$:

$$g(x) = g_1 \circ g_2(x) = \frac{6x^4 + 12x^2 + 6}{18x^4 + 4x^2 + 2}. \quad (3.8)$$

The unique equilibrium point of the system is $x_{eq} = [1, 2]^\top$ and at the fixed point of g , $|g'(x_1^e)| = |g'(1)| = 4/3 > 1$, which means that stability of the system depends on the time delay in the network.

In the extended model with multiplicative uncertainty, $g(x)$ is computed from (3.4) directly. It is same as the g function of the original model given in (3.8) since we consider $\lambda_i = 1$, $i = 1, 2$. The unique equilibrium point is calculated as $\underline{x}_{eq} = \left[[1, 0], [2, 0] \right]^\top$ for the extended case with these nonlinearity functions. At the fixed point of g , $\hat{x}_{11}^e = 1$, the gain is $|g'(1)| = 4/3 > 1$, same as the original model.

Then, the characteristic equations of the linearized system for both original and extended models are derived. For the original GRN model, characteristic equation is given as

$$X_{or}(s) = (s+1)^2 + \frac{4}{3}e^{-\tau s} \quad (3.9)$$

and the feedback system formed around the non-delayed open loop transfer function $G_{or}(s) = \frac{4/3}{(s+1)^2}$ has the gain crossover frequency $\omega_{c,or} = 0.5774$ rad/sec with

a phase margin of $PM_{or} = 2\pi/3 \text{ rad} = 120^\circ$. The linearized system is stable for all $0 \leq \tau < DM_{or}$ and unstable for $\tau \geq DM_{or}$ where $DM_{or} = PM_{or}/\omega_{c,or} = 3.6276$ sec.

For the extended GRN model, the characteristic equation is derived as

$$X_{ext}(s) = (s+1)^2 \left(\frac{1 + \hat{\tau}s}{1 + (1+\delta)\hat{\tau}s} \right)^2 + \frac{4}{3}e^{-\tau s} \quad (3.10)$$

and for each $\delta \in (-1, 1)$ and $\hat{\tau} \in (10^{-3}, 10^{0.7})$, the delay margin of the extended GRN model, DM_{ext} , is computed from the open-loop transfer function. The ratio $DM_{ext}/DM_{or} = DM_{ext}/3.6276$ is illustrated in Fig. 3.3.

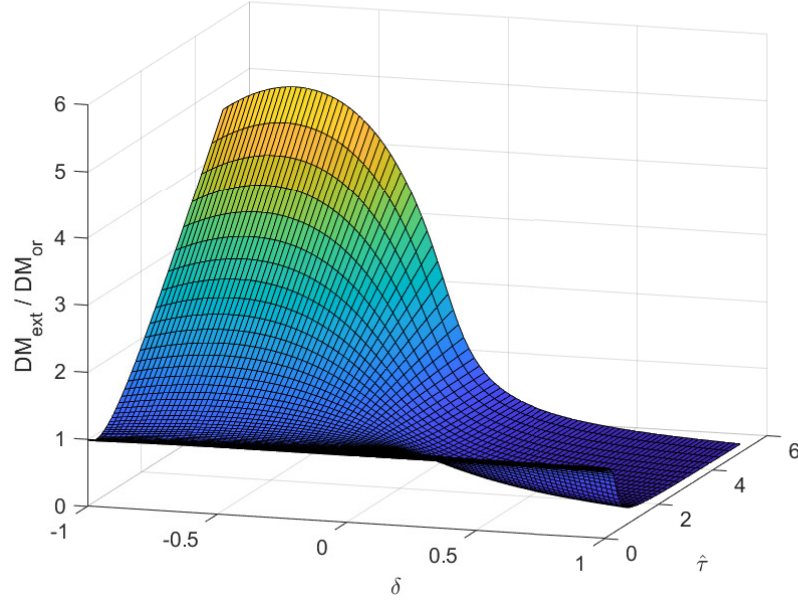


Figure 3.3: Delay margin ratio of the original and extended model of GRNs.

Note that for the negative δ values, the ratio is mostly greater than 1, which is an improvement of the delay margin with this additional block. However, as understood from Fig. 3.3, the delay margin cannot be improved for arbitrary $(\delta, \hat{\tau})$ pairs.

3.2.2 Analysis for the Largest Allowable Uncertainty Gain

Assuming δ unknown, it is also possible to perform an analysis for $|\delta|_{\max}$, the largest allowable uncertainty gain, depending on fixed parameters $\hat{\tau}$ and τ . For $n = 2$, when *only one subsystem* has the perturbation, the characteristic equation is

$$1 + (1 + \Delta(s)) \frac{ke^{-\tau s}}{(s+1)^2} = 0, \quad (3.11)$$

where $\Delta(s) = \delta \left(\frac{\hat{\tau}s}{1+\hat{\tau}s} \right) = \delta W(s)$ and $k = 4/3$ for our example using the nonlinear functions given in (3.7). Below we examine this case.

Define

$$G_\tau(s) = \frac{ke^{-\tau s}}{(s+1)^2}.$$

Then, the characteristic equation of the system with one perturbed subsystem has the following form:

$$1 + \Delta(s) \frac{G_\tau(s)}{1 + G_\tau(s)} = 0,$$

which can be re-written as

$$1 + \delta W(s) T_\tau(s) = 0$$

for $T_\tau(s) = G_\tau(s)/(1 + G_\tau(s))$. Here we assume $\tau < DM_{or}$, so that $T_\tau(s)$ is stable. Then, robust stability condition of the system is

$$\|WT_\tau\|_\infty < \frac{1}{\delta}.$$

Hence, the maximum allowable value of $|\delta|$ is determined as

$$|\delta|_{\max} = \frac{1}{\|WT_\tau\|_\infty}. \quad (3.12)$$

For each $\hat{\tau} \in (10^{-1}, 10)$ and time delay $\tau \in (0, DM_{or})$, $|\delta|_{\max}$ is depicted in Fig. 3.4. Except for the smallest values of $\hat{\tau}$, the maximum value is almost between 0 and 1, meaning that $|\delta|$ should be smaller than or equal to 1 in the implementation.

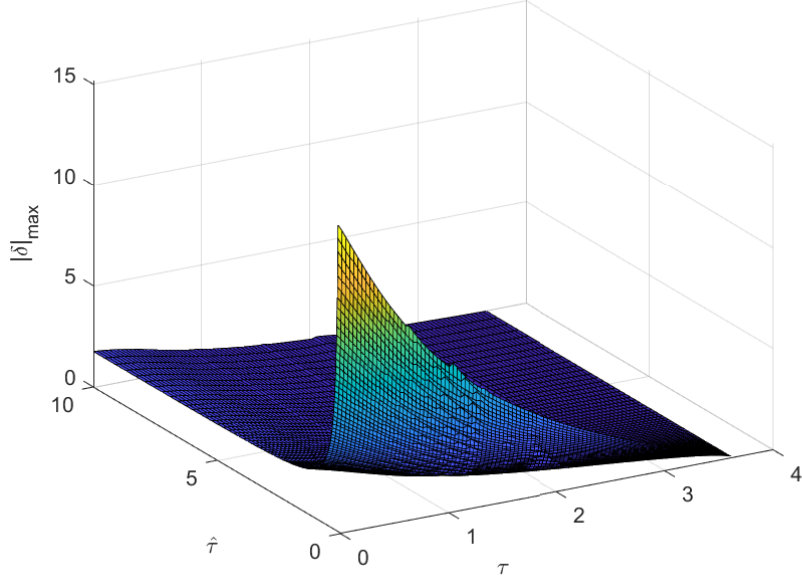


Figure 3.4: $|\delta|_{\max}$ with respect to different values of $\hat{\tau}$ and time delay $\tau < 3.6276$.

3.2.3 Analysis for the Robust Stability Condition

The characteristic equation given in (3.11) when *only one subsystem* has the perturbation can be also written as

$$1 + (1 + \Delta(s)) \frac{k}{(s+1)^2} + (1 + \Delta(s)) \frac{k(e^{-\tau s} - 1)}{(s+1)^2} = 0.$$

With the following definition of G_{Δ}

$$G_{\Delta}(s) = \frac{k(1 + \Delta(s))}{(s+1)^2},$$

the characteristic equation has the form

$$1 + \left(\frac{G_{\Delta}(s)}{1 + G_{\Delta}(s)} \right) (e^{-\tau s} - 1) = 0.$$

Then, the system is robustly stable if

$$\|T_{\Delta}W_{\tau}\|_{\infty} < 1 \tag{3.13}$$

provided that $T_\Delta \in \mathcal{H}_\infty$ for $T_\Delta(s) = G_\Delta(s)/(1 + G_\Delta(s))$ and $W_\tau(s) = (e^{-\tau s} - 1)$. Using the definitions of $G_\Delta(s)$ and $\Delta(s)$, $T_\Delta(s)$ can be simply described in the following form:

$$T_\Delta(s) = \frac{T_k(s) + \delta \left(\frac{\hat{\tau}s}{1+\hat{\tau}s} \right) T_k(s)}{1 + \delta \left(\frac{\hat{\tau}s}{1+\hat{\tau}s} \right) T_k(s)},$$

where $T_k(s) = k / ((s+1)^2 + k)$.

Note that $W_\tau(s)$ is an infinite-dimensional transfer function; a finite-dimensional uncertainty bound $\widetilde{W}_\tau(s)$ can be introduced as $|\widetilde{W}_\tau(j\omega)| \geq |W_\tau(j\omega)|$ for all ω , see e.g., [64]. Here we use the bound given in [65]:

$$\widetilde{W}_\tau(s) = \frac{(2 + \epsilon_1)(\tau s + \epsilon_2)(\tau s + 2.5)}{(\tau s)^2 + (2 + \sqrt{3})\tau s + 5 + 2\epsilon_1} \quad (3.14)$$

with $\epsilon_1 = 0.028$ and $\epsilon_2 = 2 \times 10^{-6}$. Fig. 3.5 gives the magnitude of the bound described in (3.14).

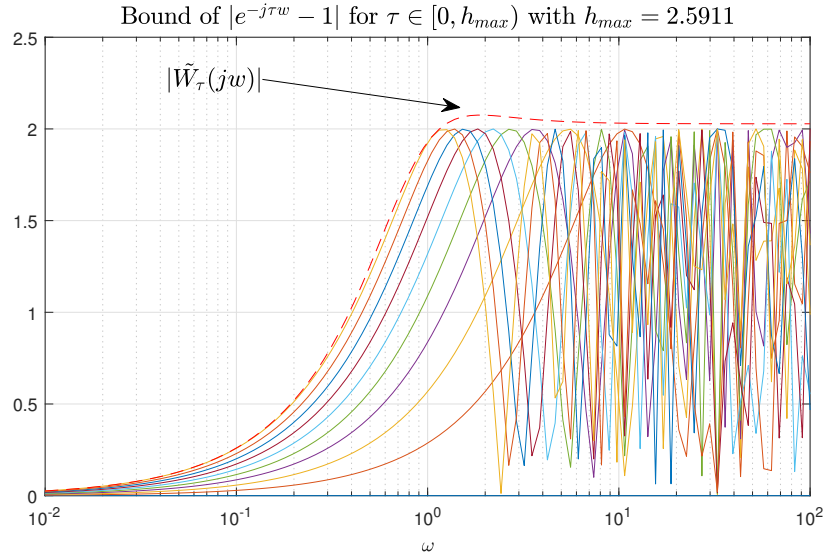


Figure 3.5: Bound of $|e^{-j\tau\omega} - 1|$ for $\tau \in [0, h_{max})$ with $h_{max} = 2.59$.

Now, the stability condition in (3.13) is tested using $\widetilde{W}_\tau(s)$ for each $\hat{\tau} \in (10^{-1}, 10)$ and time delay $\tau \in (0, DM_{or})$. For a fixed $\hat{\tau}$, we first compute

$$|\delta|_{\max} = \frac{1}{\| \left(\frac{\hat{\tau}s}{\hat{\tau}s+1} \right) T_k(s) \|_\infty}. \quad (3.15)$$

Then for δ values in $(0, |\delta|_{\max})$ we find the largest τ , denoted by $\tau_{\max}$, satisfying (3.13). The process is continued for several feasible $\hat{\tau}$ values. Algorithm 1 describes the procedure.

Algorithm 1 Computation of maximum τ

```

Let  $\hat{\tau} = \{10^{-1}, \dots, 10\}$ 
Let  $\tau = \{0, \dots, DM_{or}\}$ 
 $k \leftarrow 4/3$ 
 $m \leftarrow 1$ 
Calculate  $T_k(s)$ , check stability of  $T_{\Delta}(s)$ 
for  $i \leftarrow 1$  to  $length(\hat{\tau})$  do
    Calculate  $W(s)$ 
     $|\delta|_{\max} \leftarrow 1/||W(s)T_k(s)||_{\infty}$ 
     $\delta \leftarrow \{0, \dots, |\delta|_{\max}\}$ 
    for  $j \leftarrow 1$  to  $length(\delta)$  do
        Calculate  $T_{\Delta}(s)$ 
         $l \leftarrow 1$ 
        repeat
            Calculate  $\widetilde{W}_{\tau}(s)$ 
             $\tau_{feasible}(m) \leftarrow \tau(l)$ 
             $m \leftarrow m + 1$ 
             $l \leftarrow l + 1$ 
        until  $||T_{\Delta}(s)\widetilde{W}_{\tau}(s)||_{\infty} \geq 1$ 
         $\tau_{\max}(i, j) \leftarrow max(\tau_{feasible})$ 
         $m \leftarrow 1$ 
    end for
end for

```

3.3 Impact of Specific Perturbations

Our goal in this section is to observe the performance of the extended model using multiplicative perturbation. Simulations are conducted to compare the outcomes of an extended GRN model incorporating multiplicative uncertainty, using specific parameter choices, with the results of the original GRN model outlined in Section 2.2.

Example 1. We consider the biological systems in (2.2) and (3.3) under negative feedback with $n = 2$. The nonlinearity functions are given by (3.7). Similarly, the original model is considered to be homogeneous hence all the analysis in the Section 3.2.1 apply for the earlier original case.

In the extended model with multiplicative uncertainty, $g(x)$ is same as (3.8). Then, by choosing the parameters in Fig. 3.1 as $\delta = -0.5$ and $\hat{\tau} = 2$, the unique equilibrium point is computed as $\underline{x}_{eq} = \begin{bmatrix} [1, 0], & [2, 0] \end{bmatrix}^\top$. At the fixed point of g , $\hat{x}_{11}^e = 1$, the critical value is $|g'(1)| = 4/3 > 1$, meaning that the system is stable depending on the time delay in the model.

The characteristic equation of the extended model is

$$X_{ext}(s) = (s + 1)^2 \left(\frac{1 + 2s}{1 + s} \right)^2 + \frac{4}{3} e^{-\tau s} \quad (3.16)$$

and the feedback system formed around the non-delayed open loop transfer function $G_{ext}(s) = \frac{4/3}{(1+2s)^2}$ has the gain crossover frequency $\omega_{c,ext} = 0.2887$ rad/sec with a phase margin of $PM_{ext} = 2\pi/3$ rad = 120° . In this extended structure, delay margin of the linearized system is $DM_{ext} = PM_{ext}/\omega_{c,ext} = 7.2552$ sec, which means that the extended linearized system is stable for all $0 \leq \tau < 7.2552$ sec. Hence with the parameters $(\delta, \hat{\tau}) = (-0.5, 2)$, the delay margin of the extended gene regulatory network model is increased. Note that in this particular “extended” model with multiplicative perturbation, subsystems are first-order too, namely $H_i(s) = \frac{1}{1+2s}$, $i = 1, 2$.

Fig. 3.6(a) illustrates the behavior of the delay-free original system, the solution converges to the unique equilibrium as expected. On the other hand, the

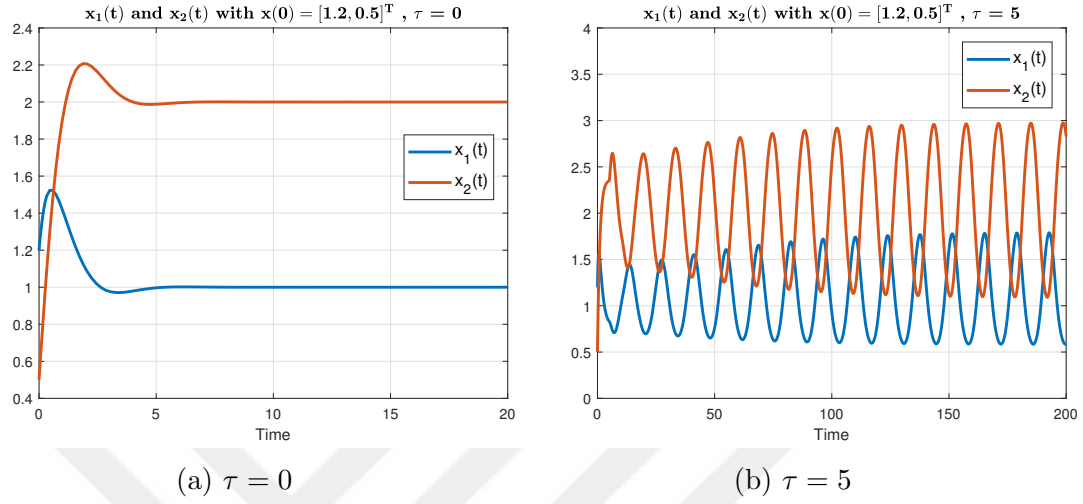


Figure 3.6: $x_1(t), x_2(t)$ vs t graph of the original system with $x(0) = [1.2, 0.5]^T$, $\tau = 0$ and $\tau = 5$ sec.

behavior of the original system with $\tau = 5$ sec, which is greater than DM_{or} , is depicted in Fig. 3.6(b) and we observe oscillatory behavior in this case consistent with our expectations.

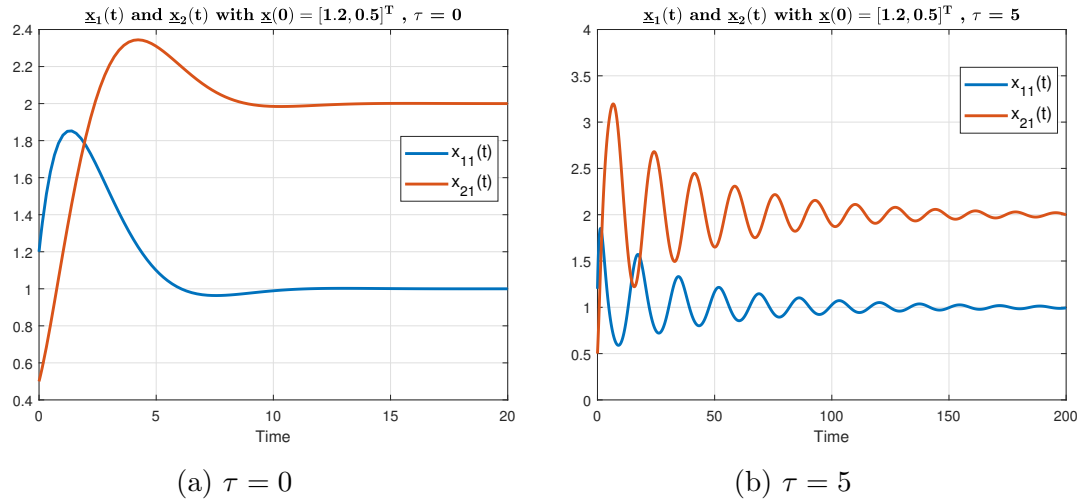


Figure 3.7: $\underline{x}_1(t), \underline{x}_2(t)$ vs t graph of the extended system with $\underline{x}(0) = [[1.2, 1.2], [0.5, 0.5]]^T$, $\tau = 0$ and $\tau = 5$ sec.

Fig. 3.7(a) shows the solution of the delay-free extended system. As expected, the solution converges to $\underline{x}_{eq}$. Fig. 3.7(b) illustrates the behavior of the same system with $\tau = 5$ sec, which is less than DM_{ext} . In this case, the extended model with multiplicative uncertainty can overcome the oscillations and reach

the equilibrium under a time delay of 5 seconds. Clearly, this is a significant improvement.

Example 2. We consider the biological systems in (2.2) and (3.3) under negative feedback with $n = 2$ and the nonlinearity functions are same as the previous example. Hence, the previous analysis of the original GRN model also applies to this example. The difference is in the parameter choices of the extended structure: δ and $\hat{\tau}$ are now chosen as 0.5 and 2, respectively.

For the extended GRN model with multiplicative perturbation, the characteristic equation is given as

$$X_{ext}(s) = (s + 1)^2 \left(\frac{1 + 2s}{1 + 3s} \right)^2 + \frac{4}{3} e^{-\tau s} \quad (3.17)$$

and the non-delayed open loop transfer function is computed as $G_{ext}(s) = \frac{4/3(1+3s)^2}{(s+1)^2(1+2s)^2}$. The feedback system formed around $G_{ext}(s)$ has a gain crossover frequency $\omega_{c,ext} = 1.3403$ rad/sec and a phase margin of $PM_{ext} = 0.4802\pi$ rad = 86.4375° , which is less than the phase margin of the extended structure in *Example 1*. Hence, the delay margin of the linearized system decreases to $DM_{ext} = PM_{ext}/\omega_{c,ext} = 1.1256$ sec.

The solution of the original model under 2 second time delay is illustrated in Fig. 3.8; since $\tau = 2 < DM_{or} = 3.6276$ sec, the model can overcome the oscillations and converge to the unique equilibrium. Fig. 3.9 shows the behavior of the extended model with parameters $(\delta, \hat{\tau}) = (0.5, 2)$ under a time delay of 2 seconds. Since τ is greater than the delay margin, DM_{ext} , in this case, we observe oscillatory behavior as expected. This illustrates that certain specific perturbations in the subsystems may reduce the delay margin.

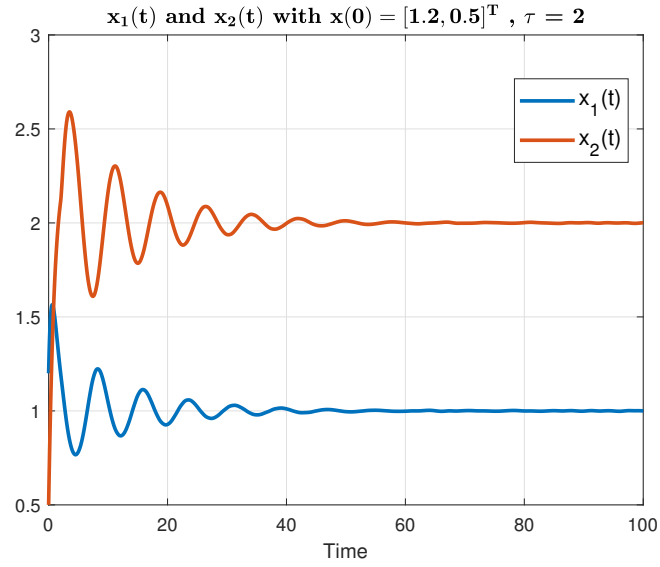


Figure 3.8: $x_1(t), x_2(t)$ vs t graph of the original system with $x(0) = [1.2, 0.5]^T$, $\tau = 2$ sec.

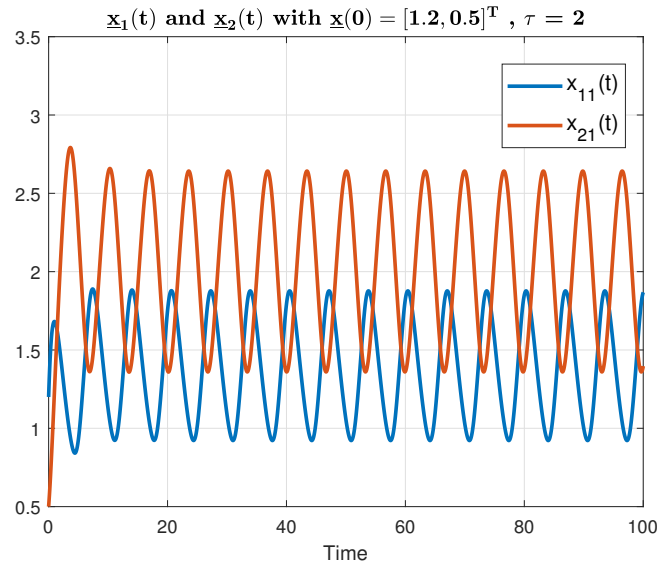


Figure 3.9: $\underline{x}_1(t), \underline{x}_2(t)$ vs t graph of the extended system with $\underline{x}(0) = [[1.2, 1.2], [0.5, 0.5]]^T$, $\tau = 2$ sec.

3.4 Conclusion

In this chapter, we considered an extension of a gene regulatory network model with a cyclic nonlinear time delayed feedback system by utilizing multiplicative uncertainty with high-pass filter. Additional block appears in each cascaded subsystem in the network structure. We analyzed stability robustness and delay margin of this extended GRN model.

The present work demonstrates that it may be possible to improve the stability characteristics under specific perturbations. On the other hand, for arbitrary parameter choices in the perturbed model, the delay margin of the system is typically less than that of the original system.

Here we considered first-order multiplicative perturbation for each first-order nominal subsystem. It may be possible to consider arbitrary order perturbations and treat $\delta|W(j\omega)|$ as the upper bound of the uncertainty. In that case, the equilibrium analysis and delay margin computations become slightly more involved, at least notation-wise.

Chapter 4

Nonlinear Dynamics and Chaos in Gene Regulatory Network Models

In this chapter, we focus on studying the nonlinear and chaotic dynamics in the original and extended model of a gene network, with a specific emphasis on analyzing the chaos in a two-gene system. Our approach involves comparing the presence of non-periodic dynamics and the structure of the original and extended gene network models existing chaos. To better visualize and analyze the non-periodic dynamics in the gene regulatory circuits under study, we employ a methodology that involves examining phase maps, conducting power spectrum analysis and calculating Lyapunov exponents. Utilizing these techniques, we illustrate potential chaotic dynamics and conduct a thorough investigation of the original and extended models while considering variations in time delays.

4.1 Introduction

Genetic regulatory networks are inherently complex and nonlinear, comprising multiple interconnected dimensions that need to be taken into account for a thorough analysis. Hence, it is essential to approach them from the perspective of nonlinear system theory to gain a comprehensive understanding of gene regulatory networks. These models, although seemingly simple, have the potential to exhibit complex phenomena, such as chaos, based on the nonlinear dynamics and time delays involved. However, there is currently limited research that has explored these issues in detail.

Inspired by Lorenz's strange attractor and the chaotic behavior it represents, it can be said that even a simple deterministic system can exhibit highly unpredictable dynamics. Across various parameter ranges, the solutions of the system display irregular oscillations that never repeat exactly, yet remain confined within a bounded region of phase space. The behavior of the system on the attractor displays a high sensitivity to initial conditions, mostly referred to as sensitive dependence [72]. In other words, even small variations in the initial conditions can lead to significantly different trajectories and outcomes over time. This sensitivity highlights the chaotic nature of the system and the difficulty in predicting its long-term behavior with precision.

Sensitive dependence on initial conditions means that two trajectories starting very close together will move further apart from each other, eventually resulting in completely different future paths [72]. To visualize this idea, we assume that $y(t)$ is a point on the attractor and we imagine a nearby point represented as $y(t) + \delta(t)$, where δ is a very small separation vector with an initial length of $\|\delta_0\| = 10^{-15}$ [72]. The representation is illustrated in Fig. 4.1. This tiny separation $\delta(t)$ grows with $\|\delta(t)\| \sim \|\delta_0\|e^{\lambda t}$, where $\lambda \approx 0.9$ in numerical studies of the Lorenz attractor [73]. Therefore, it can be claimed that neighboring trajectories diverge exponentially fast, and the number λ is commonly referred to as the Lyapunov exponent [72]. Among the n different Lyapunov exponents associated with an n -dimensional system, this particular value represents the largest

Lyapunov exponent. Moreover, the value of λ can vary depending on the specific trajectory being studied, hence it is necessary to average the calculations over multiple points on the same trajectory to obtain an accurate estimate.

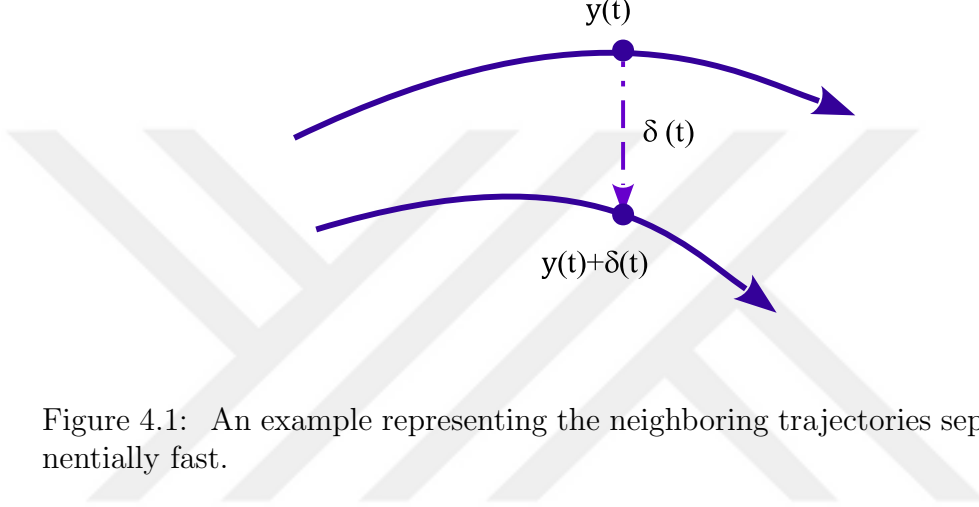


Figure 4.1: An example representing the neighboring trajectories separate exponentially fast.

In the broadest sense, when a system exhibits a positive Lyapunov exponent, it implies that the system is sensitive to initial conditions and has a limited predictability horizon. Beyond a certain time frame, the accuracy of predictions for the system's behavior deteriorates due to the exponential divergence of nearby trajectories. From this perspective, chaos can be defined as the presence of aperiodic and unpredictable long-term behavior in a deterministic system, which arises due to the system's sensitivity to small changes in initial conditions, leading to significant divergence of trajectories over time [72].

Our main focus in this chapter is to explore the periodic and chaotic behavior of the original and extended gene regulatory network models. We demonstrate that chaotic dynamics occur in the original model when a dual feedback is present in one gene and the delays differ. Interestingly, the extended model does not require a self-inhibitory delay to exhibit chaotic behavior. The literature extensively explores diverse methods and criteria to analyze nonlinear dynamics and chaotic phenomena. These include techniques such as phase-space representation of the system dynamics [74], the use of Lorenz maps [73], the computation of Lyapunov exponents [71], Poincare map [75], and Grassberger-Procaccia algorithm [76].

These tools and approaches offer valuable insights into understanding and characterizing chaotic behaviors in complex systems. To provide a comprehensive understanding of the non-periodic dynamics observed in the gene regulatory circuits under investigation, we employ a method that includes the utilization of phase maps, power spectrum analysis, time trajectories and Lyapunov exponents. By employing these techniques, we can visually represent the phase-space dynamics, examine the frequency content of the system's behavior, and quantify the presence of chaos through the computation of Lyapunov exponents.

4.2 Equilibrium and Stability Analysis

This section covers equilibrium and stability analysis, and linearization of original and extended two-gene network models exhibiting chaotic behaviors.

4.2.1 Modified Original Two-Gene Model

A standard model of the original gene network given in (2.2) for a two-gene system with one delayed feedback and two coupled feedback loops with equal and different delays does not show any chaotic behavior.

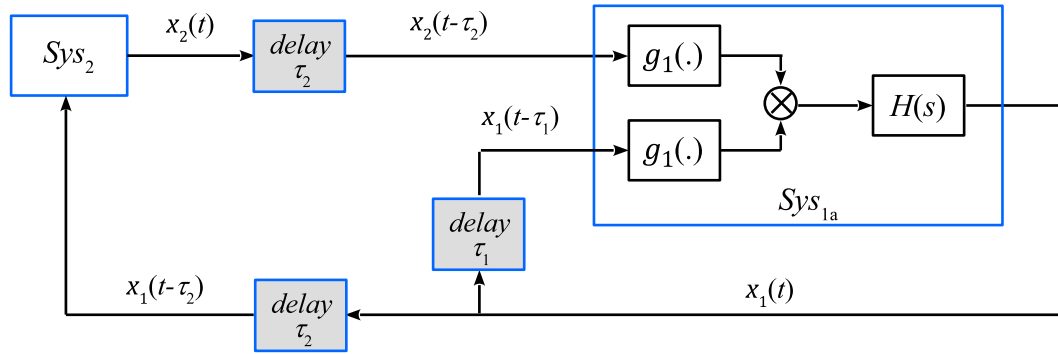


Figure 4.2: Modified original GRN model with two genes.

Therefore, we make modifications to the original model, as depicted in Fig. 4.2,

which illustrates a gene network motif featuring two negative feedback loops within a single gene. Here, we add a self-inhibitory delay for gene x_1 . Biological representation for a modified two-gene model is shown in Fig. 4.3, where x_1 and x_2 represent the corresponding two genes.

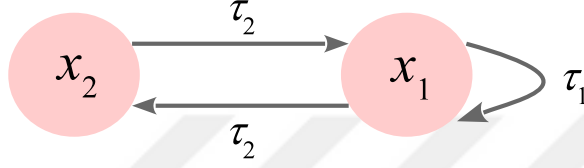


Figure 4.3: Circuit motif of the modified original GRN model with two genes.

The subsystem Sys_2 has a structure given in Fig. 2.2(b) with first-order LTI part, whereas subsystem Sys_1 is modified as illustrated in Fig. 4.2 (described as Sys_{1a}). The model is considered homogeneous with $\lambda_i = 1$ for $i = 1, 2$, and given by a set of differential equations:

$$\begin{aligned}\dot{x}_1(t) &= -x_1(t) + g_1(x_2(t - \tau_2))g_1(x_1(t - \tau_1)) \\ \dot{x}_2(t) &= -x_2(t) + g_2(x_1(t - \tau_2)).\end{aligned}\tag{4.1}$$

The time delays τ_1 and τ_2 are not necessarily the same in this configuration. Let $x_{eq} = [x_1^e, x_2^e]^\top$ be an equilibrium point of (4.1), where

$$\begin{aligned}x_1^e &= g_1(x_2^e)g_1(x_1^e) \\ x_2^e &= g_2(x_1^e).\end{aligned}\tag{4.2}$$

In this structure, nonlinear functions previously defined in (2.3) are chosen in the form of

$$g_i(x) = \frac{a_i}{1 + x^{m_i}}, \quad i = 1, 2\tag{4.3}$$

by considering $r = 0$ and $b_0 = b_1 = 1$. Then the equilibrium of a two-gene modified original model is represented as follows:

$$\begin{aligned}x_1^e &= \left(\frac{a_1}{1 + \left(\frac{a_2}{1 + (x_1^e)^{m_2}} \right)^{m_1}} \right) \left(\frac{a_1}{1 + (x_1^e)^{m_1}} \right) \\ x_2^e &= \frac{a_2}{1 + (x_1^e)^{m_2}}.\end{aligned}\tag{4.4}$$

The linearization of the modified two-gene original gene network model (4.1) can be analyzed around x_{eq} , by setting $\delta x_1(t) = x_1(t) - x_1^e$ and $\delta x_2(t) = x_2(t) - x_2^e$:

$$\begin{aligned}\dot{\delta x}_1(t) &= -\delta x_1(t) + \frac{\partial}{\partial x_1} (g_1(x_2)g_1(x_1)) \delta x_1(t - \tau_1) + \frac{\partial}{\partial x_2} (g_1(x_2)g_1(x_1)) \delta x_2(t - \tau_2) \\ \dot{\delta x}_2(t) &= -\delta x_2(t) + \frac{\partial}{\partial x_1} (g_2(x_1)) \delta x_1(t - \tau_2).\end{aligned}\tag{4.5}$$

Then the characteristic equation of the network is represented as

$$(s + 1)^2 + \gamma_1(s + 1)e^{-\tau_1 s} - \gamma_2\gamma_3e^{-2\tau_2 s} = 0\tag{4.6}$$

where

$$\begin{aligned}\gamma_1 &= -g_1(x_2^e) \frac{\partial}{\partial x_1} g_1(x_1^e) = -\frac{a_1}{1 + (x_2^e)^{m_1}} \left(\frac{-a_1 m_1 (x_1^e)^{m_1-1}}{(1 + (x_1^e)^{m_1})^2} \right) \\ \gamma_2 &= -g_1(x_1^e) \frac{\partial}{\partial x_2} g_1(x_2^e) = -\frac{a_1}{1 + (x_1^e)^{m_1}} \left(\frac{-a_1 m_1 (x_2^e)^{m_1-1}}{(1 + (x_2^e)^{m_1})^2} \right) \\ \gamma_3 &= -\frac{\partial}{\partial x_1} g_2(x_1^e) = \frac{a_2 m_2 (x_1^e)^{m_2-1}}{(1 + (x_1^e)^{m_2})^2}\end{aligned}\tag{4.7}$$

which are evaluated at the equilibrium given in (4.4). Since the model is homogeneous with $\lambda_i = 1$ for $i = 1, 2$, the characteristic equation defined in (4.6) has all its roots in $\mathbb{C}_-$ if and only if the transfer function

$$T(s) := \frac{G(s)}{1 + G(s)}\tag{4.8}$$

with

$$G(s) = \frac{\gamma_1(s + 1)e^{-\tau_1 s} - \gamma_2\gamma_3e^{-2\tau_2 s}}{(s + 1)^2}\tag{4.9}$$

is stable.

4.2.2 Extended Two-Gene Model

In order to comprehend the mechanism behind chaotic dynamics within the extended gene network, our focus is directed towards the motif that exhibits chaotic behavior. In a two-gene structure, the model with one delayed feedback, and two coupled feedback loops with equal and different delays show non-periodic behavior. Here, in the equilibrium and stability analysis, we focus on two delayed model

where delays differ as given in Fig. 4.4, since this structure is more inclusive. In this scenario, we observe non-periodic oscillatory dynamics without adding any self-inhibitory feedback loop in one of the genes, as observed in the two-gene original model proposed in Fig. 4.2. Note that each subsystem states, Sys_1 and Sys_2 , in Fig. 4.4 have the structure given in Fig. 2.3.

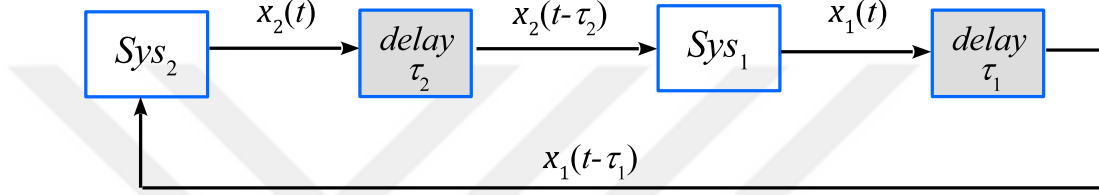


Figure 4.4: Extended GRN model with two genes.

Biological representation for a two-gene extended model with different delays is also shown in Fig. 4.5, where $\underline{x}_1$ and $\underline{x}_2$ represent the corresponding two genes.

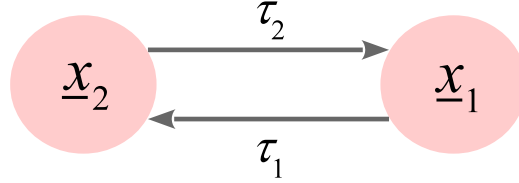


Figure 4.5: Circuit motif of the extended GRN model with two genes.

The mathematical model of the extended structure demonstrated in Fig. 4.4 is given by

$$\begin{aligned}\dot{\underline{x}}_1(t) &= A_1 \underline{x}_1(t) + g_1(\underline{x}_2(t - \tau_2)) \\ \dot{\underline{x}}_2(t) &= A_2 \underline{x}_2(t) + g_2(\underline{x}_1(t - \tau_1)),\end{aligned}\tag{4.10}$$

where A_i 's are 2×2 Hurwitz matrices defined in (2.8). Here, we use the form of

$$A_i = \begin{bmatrix} 0 & 1 \\ -\rho_i & -\lambda_i \end{bmatrix} = \begin{bmatrix} 0 & 1 \\ -\omega_0^2 & -2\zeta_i\omega_0 \end{bmatrix}\tag{4.11}$$

for $i = 1, 2$. In the implementation, the parameters are specified as $\omega_0 = 1$ and

$\zeta_i = 1$ for $i = 1, 2$. Let $\underline{x}_{eq} = [\underline{x}_1^e, \underline{x}_2^e]^\top$ be an equilibrium point of (4.10), where

$$\begin{aligned}\underline{x}_1^e &= (x_{11}^e, x_{12}^e) = \left(\frac{g_1(x_{21}^e) + g_1(x_{22}^e)}{\omega_0^2}, 0 \right) \\ \underline{x}_2^e &= (x_{21}^e, x_{22}^e) = \left(\frac{g_2(x_{11}^e) + g_2(x_{12}^e)}{\omega_0^2}, 0 \right).\end{aligned}\tag{4.12}$$

Using $x_{12}^e = x_{22}^e = 0$, $\omega_0 = 1$ and Hill functions in the form of (4.3), the equilibrium is given as

$$\begin{aligned}\underline{x}_1^e &= \left(\frac{a_1}{1 + (x_{21}^e)^{m_1}} + a_1, 0 \right) \\ \underline{x}_2^e &= \left(\frac{a_2}{1 + (x_{11}^e)^{m_2}} + a_2, 0 \right).\end{aligned}\tag{4.13}$$

We have the following linearization of the two-gene extended model (4.10) around $\underline{x}_{eq}$:

$$\dot{\underline{x}}(t) = A\underline{x}(t) + B\underline{x}(t - \tau_1) + C\underline{x}(t - \tau_2)\tag{4.14}$$

where

$$\begin{aligned}A &= \begin{bmatrix} A_1 & 0 \\ 0 & A_2 \end{bmatrix} \quad \text{with} \quad A_i = \begin{pmatrix} 0 & 1 \\ -\omega_0^2 & -2\zeta\omega_0 \end{pmatrix}, \quad \zeta_i = \zeta, \\ B &= \begin{bmatrix} 0 & 0 \\ G_2 & 0 \end{bmatrix} \quad \text{with} \quad G_2 = \begin{pmatrix} 0 & 0 \\ g_2'(x_{11}^e) & g_2'(x_{12}^e) \end{pmatrix}, \\ C &= \begin{bmatrix} 0 & G_1 \\ 0 & 0 \end{bmatrix} \quad \text{with} \quad G_1 = \begin{pmatrix} 0 & 0 \\ g_1'(x_{21}^e) & g_1'(x_{22}^e) \end{pmatrix}.\end{aligned}$$

The characteristic equation of the system is

$$\det(sI - A - Be^{-\tau_1 s} - Ce^{-\tau_2 s}) = 0,\tag{4.15}$$

which is equivalent to

$$(s^2 + 2\zeta\omega_0 s + \omega_0^2)^2 - (g_1'(x_{21}^e) + sg_1'(x_{22}^e))(g_2'(x_{11}^e) + sg_2'(x_{12}^e))e^{-(\tau_1 + \tau_2)s} = 0.\tag{4.16}$$

Since A_i for $i = 1, 2$ is Hurwitz, its eigenvalues have real parts $-\zeta\omega_0 < 0$ and the characteristic equation of the extended model (4.16) has all its roots in $\mathbb{C}_-$ if and only if the transfer function

$$T(s) := \frac{G(s)}{1 + G(s)}\tag{4.17}$$

is stable, where

$$G(s) = -\frac{(g'_1(x_{21}^e) + sg'_1(x_{22}^e))(g'_2(x_{11}^e) + sg'_2(x_{12}^e))e^{-(\tau_1+\tau_2)s}}{(s^2 + 2\zeta\omega_0s + \omega_0^2)^2}. \quad (4.18)$$

4.3 Periodic Behavior

This section covers the analysis of periodic behavior in the modified original two-gene model described and given in Section 4.2.1. We analyze the behavior of the model in Fig. 4.2 choosing $g_1(x) = g_2(x)$, using the definition given in (4.3), i.e.,

$$g_1(x) = g_2(x) = \frac{10}{1+x^2}, \quad (4.19)$$

where the Hill coefficient, m , is 2. The equilibrium point of the model (4.1) with these Hill functions is

$$x_{eq} = [x_1^e, x_2^e]^\top = [4.1832, 0.5406]^\top,$$

and the characteristic equation of the model defined in (4.6) is computed

$$\Delta(s) = (s+1)^2 + 1.8921(s+1)e^{-\tau_1s} - 0.8556e^{-2\tau_2s} = 0 \quad (4.20)$$

where $\gamma_1 = 1.8921$, $\gamma_2 = 3.5$ and $\gamma_3 = 2.445$. Open loop transfer function of the two-gene modified model is then given as

$$G(s) = \frac{1.8921(s+1)e^{-\tau_1s} - 0.8556e^{-2\tau_2s}}{(s+1)^2}. \quad (4.21)$$

In order to analyze the stability of the model, we apply geometric characterization by considering the characteristic equation in the form of

$$\Delta(s) = 1 + a_1(s)e^{-\tau_1s} + a_2(s)e^{-2\tau_2s} = 0, \quad (4.22)$$

where

$$a_1(s) = \frac{1.8921(s+1)}{(s+1)^2}, \quad a_2(s) = -\frac{0.8556}{(s+1)^2}. \quad (4.23)$$

For $s = j\omega$, the terms of $\Delta(s)$ defined in (4.22) are vectors in the complex plane, the magnitudes of which are independent of time delays τ_1 and τ_2 [68].

The vectors depicted in Fig. 4.6 sum to a resultant vector of zero. Here, we can establish conditions known as triangle inequalities in the complex plane for a triangle to be formed, as the norms of the vectors involved are unaffected by the delays τ_1 and τ_2 . These conditions are derived from the fundamental principle that the length of each side of a triangle cannot exceed the sum of the lengths of the other two sides such that

$$\begin{aligned} |a_1(j\omega)| + |a_2(j\omega)| &\geq 1 \\ -1 &\leq |a_1(j\omega)| - |a_2(j\omega)| \leq 1. \end{aligned} \quad (4.24)$$

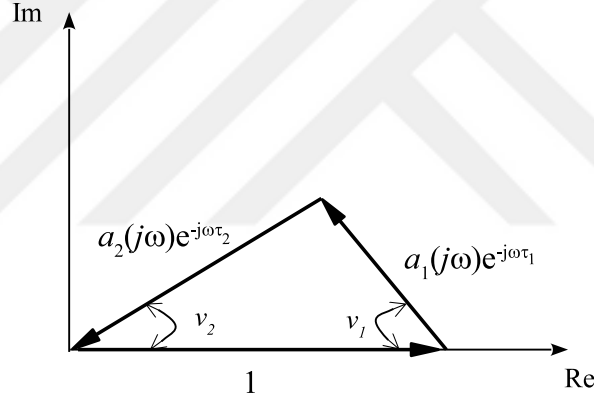


Figure 4.6: The triangle formed by vectors $|a_1(j\omega)|$, $|a_2(j\omega)|$ and 1, given in (4.22). In the complex plane, the equation (4.22) is expressed as the sum of three vectors. If these vectors form a triangle in the complex domain, it indicates that the characteristic equation possesses a solution at $s = j\omega$.

After determining all values of ω , the crossing frequency set Ω , that satisfy the triangle conditions, the delays τ_1 and τ_2 can be calculated by utilizing these values of ω along with the orientation of the vectors involved. By varying the frequency ω , the norms $|a_1(j\omega)| + |a_2(j\omega)|$ and $|a_1(j\omega)| - |a_2(j\omega)|$ are plotted and visualized as a function of ω in Fig. 4.7 for the system (4.23). The frequency ranges determined in Fig. 4.7 generate the delay solutions τ_1 and τ_2 , where the crossing frequency set Ω is identified as

$$\Omega = [0.5758, 2.0606]. \quad (4.25)$$

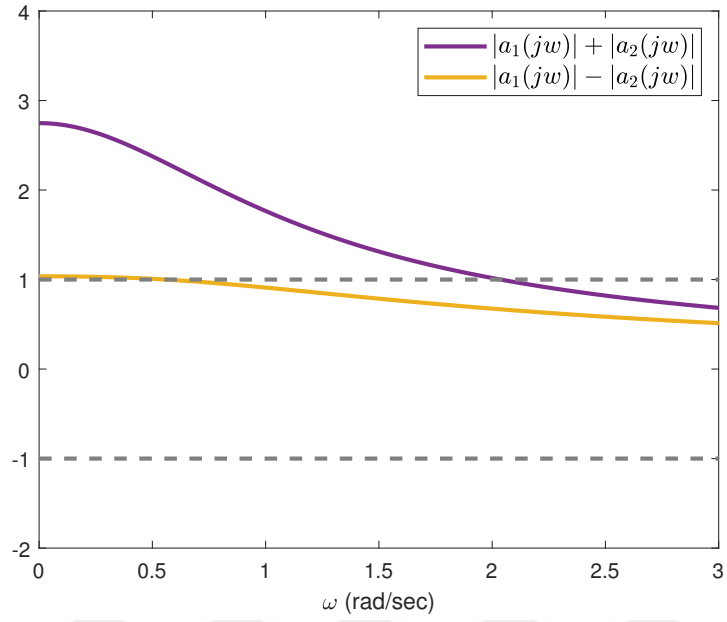


Figure 4.7: The range of frequencies over which the triangle conditions are satisfied.

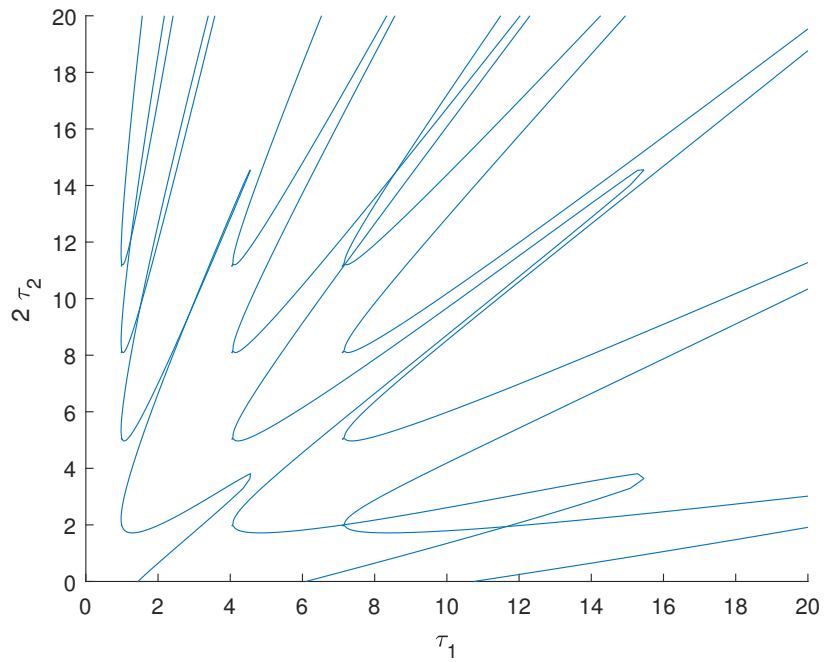


Figure 4.8: Delay solutions on open-ended curves.

The stability crossing curves, C , corresponding to Ω can encompass closed curves, spiral-like curves with axes oriented either horizontally, vertically or diagonally, and open-ended curves with both ends approaching ∞ [69]. Delay solutions on open-ended spirals for the system analyzed in this subsection is given Fig. 4.8. In this example, the curves C observed in Fig. 4.8 are referred to as stability-switching curves. These curves represent the values of delay for which the characteristic equation exhibits a pair of roots located on the imaginary axis [68, 69].

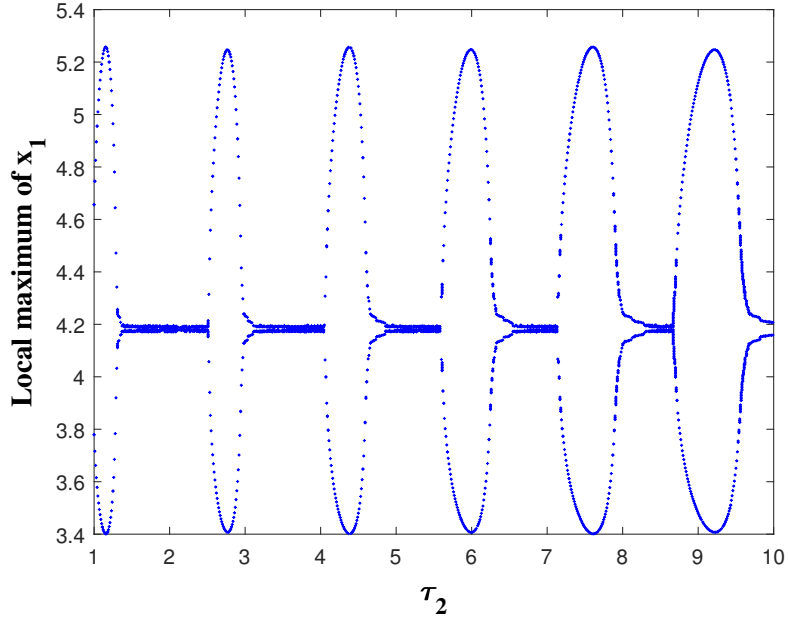


Figure 4.9: Bifurcation diagram with local maxima of x_1 with respect to τ_2 , by setting $\tau_1 = 1$ sec.

Varying τ_2 relative to $\tau_1 = 1$ sec, local maximum concentration of variable x_1 is computed and given in Fig. 4.9 to determine and observe the critical bifurcations. Hopf bifurcation points, where there is a pair of complex conjugate eigenvalues at $s = \pm i\omega$, can be also seen in Fig. 4.9. Note that Fig. 4.8 shows the delay solutions in $\tau_1 - 2\tau_2$ plane considering the delays in the characteristic equation (4.22), whereas the Hopf bifurcation points illustrated in Fig. 4.9 is computed considering τ_2 .

4.3.1 Delay Margin Analysis

In this part, we observe the delay margin of the modified two-gene original model with nonlinear functions (4.19). Characteristic equation of the linearized system is computed and given in (4.20). Note that the delay-free system is stable with roots at $s_1 = -3.26$ and $s_2 = -0.624$, and delay margin for the delay-free open loop transfer function is $DM = 1.9582$ sec.

In order to investigate the effects of τ_1 and τ_2 on the delay margin, the open loop transfer function of the two-gene modified model (4.21) is written in the form of

$$G(s) = e^{-\tau_1 s} \underbrace{\left(\frac{1.8921(s+1) - 0.8556e^{-(2\tau_2-\tau_1)s}}{(s+1)^2} \right)}_{G_{OL}(s)}. \quad (4.26)$$

First, we assume $\tau_1 < 2\tau_2$ and define $\Delta_\tau = 2\tau_2 - \tau_1$. By using different values for Δ_τ , we observe the delay margin of the model based on the delay parameter τ_1 . In the analysis, we utilize the bode diagram of $G_{OL}(s)$ for fixed values of Δ_τ , and compare the result with YALTA toolbox dedicated to the stability analysis of multiple delay systems [70]. As an example, the bode diagram of $G_{OL}(s)$ defined in (4.26) for $\Delta_\tau = 5$ sec is given in Fig. 4.10. The delay margin is computed at each of the three different frequencies where the gain reaches 0 dB, and the smallest delay margin, $DM = 1.0071$ sec, is chosen. For this system, the delay margin is also computed using YALTA Matlab toolbox with $\tau_1 = 1$ sec and $\tau_2 = 3$ sec, where $DM_{Yalta} = 1.0218$ sec.

This procedure is repeated for multiple Δ_τ values, and an estimated stability region for $\tau_1 < 2\tau_2$ is derived based on the obtained delay margin values. In this case, the system is stable if the delay margin, DM , is greater than the delay value τ_1 . Therefore, to determine the stability region, we consider $\tau_1 = DM$ and find the stable range for each Δ_τ value. Ultimately, the stable region for the case $\tau_1 < 2\tau_2$ is determined by combining all the individual sub-areas together as illustrated in Fig. 4.11.

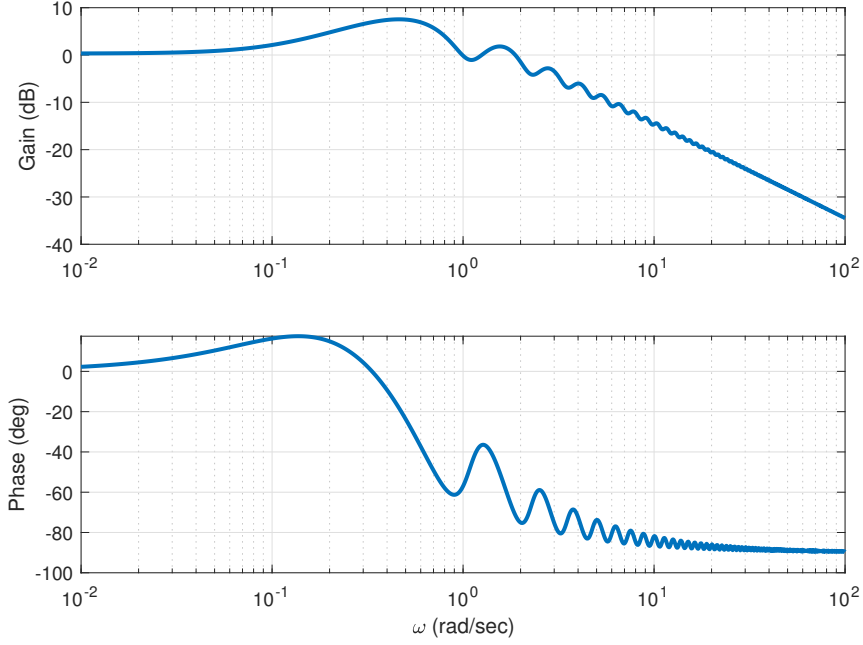


Figure 4.10: Bode plots of $G_{OL}(s)$ when $\tau_1 < 2\tau_2$ for $\Delta_\tau = 5$ sec.

Then, we assume that $\tau_1 > 2\tau_2$ and consider the open loop transfer function of the two-gene modified model (4.21) in the form of

$$G(s) = e^{-2\tau_2 s} \underbrace{\left(\frac{1.8921(s+1)e^{-(\tau_1-2\tau_2)s} - 0.8556}{(s+1)^2} \right)}_{G_{OL}(s)}. \quad (4.27)$$

By introducing $\Delta_\tau = \tau_1 - 2\tau_2$ as the delay parameter in $G_{OL}(s)$, we have followed the same methodology. By employing different values for Δ_τ , we observe the delay margin of the model both using the bode plots of $G_{OL}(s)$ and YALTA Matlab toolbox, and continue this iterative process to gain a comprehensive understanding of the stable region. Here, the system is stable if the delay margin is greater than the delay value $2\tau_2$, hence we define $2\tau_2 = DM$ to specify the stable area for each Δ_τ value. The final stability region for the case $\tau_1 > 2\tau_2$ is obtained and depicted in Fig. 4.12.

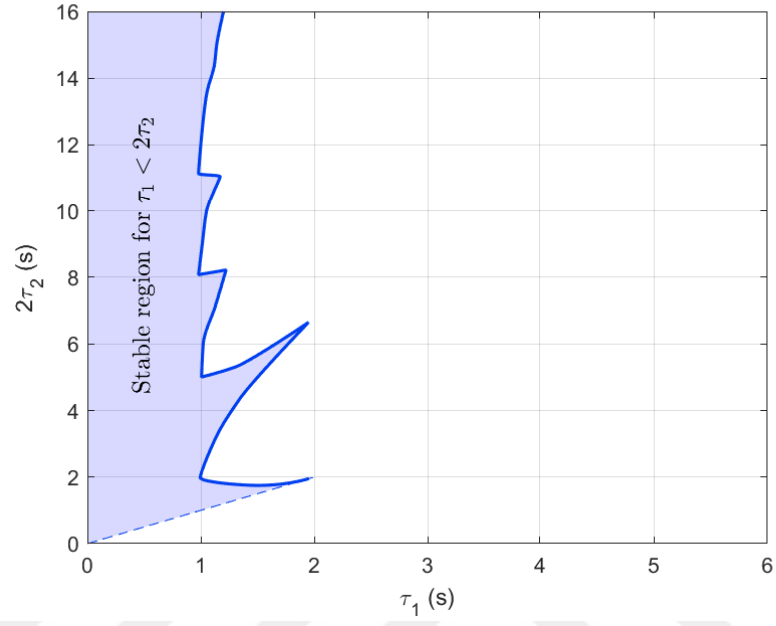


Figure 4.11: Stability region for $\tau_1 < 2\tau_2$, with different Δ_τ values.

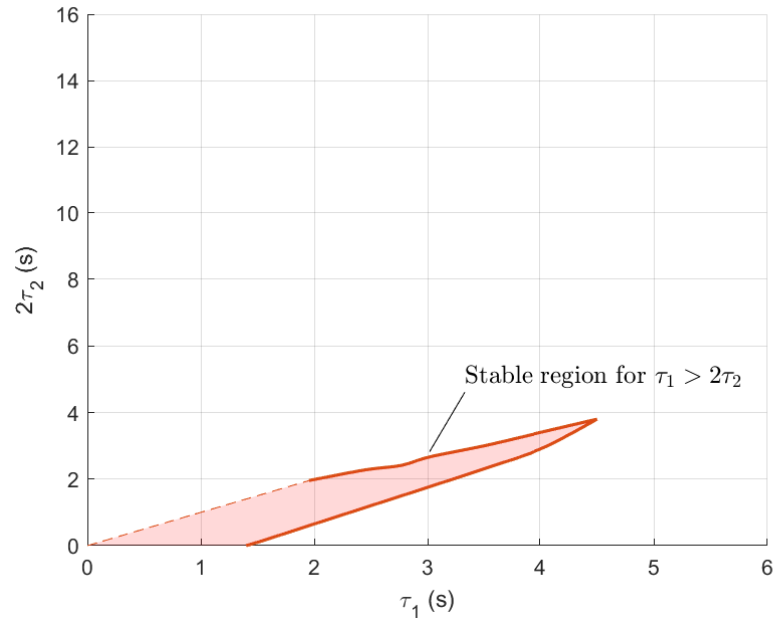


Figure 4.12: Stability region for $\tau_1 > 2\tau_2$, with different Δ_τ values.

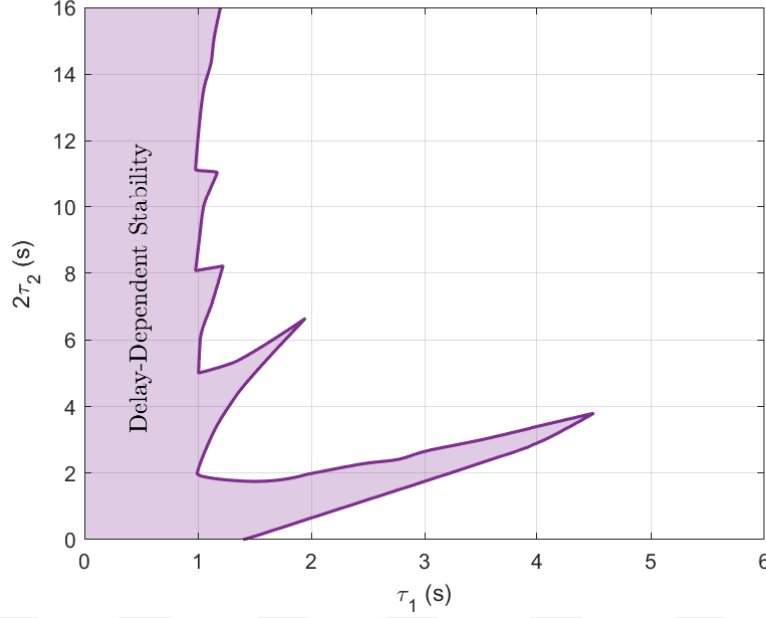


Figure 4.13: First stability crossing boundary. The shaded regions in the $\tau_1 - 2\tau_2$ parameter space represent the stability regions of the two-gene modified original model (4.1) with nonlinear functions (4.19).

Using all the approaches explained above leads to the stability boundaries shown in $\tau_1 - 2\tau_2$ plane in Fig. 4.13. The analysis reveals that the dynamics are stable within the shaded region for the two-gene modified model (4.1) with a Hill parameter $m = 2$ and nonlinear functions defined in (4.19), and unstable otherwise. As anticipated, this stability curve almost aligns with the first switching curve depicted in Fig. 4.8. Besides, the first curve is given in Fig. 4.14, where the left-side of the curve represents the stable region. In particular, the stable region for $\tau_1 > 2\tau_2$ given in Fig. 4.12 closely aligns with the relevant part of the Fig. 4.14. Here, the small circles represent the computed delay margin boundaries with the grid search method.

The stability region obtained in Fig. 4.13 is determined by checking stability condition for discretely selected points in $\tau_1 - 2\tau_2$ plane. Whereas, the stability crossing boundary illustrated in Fig. 4.14 is obtained by implementing the exact analytical methods described in [69]. Therefore, some narrow stability regions

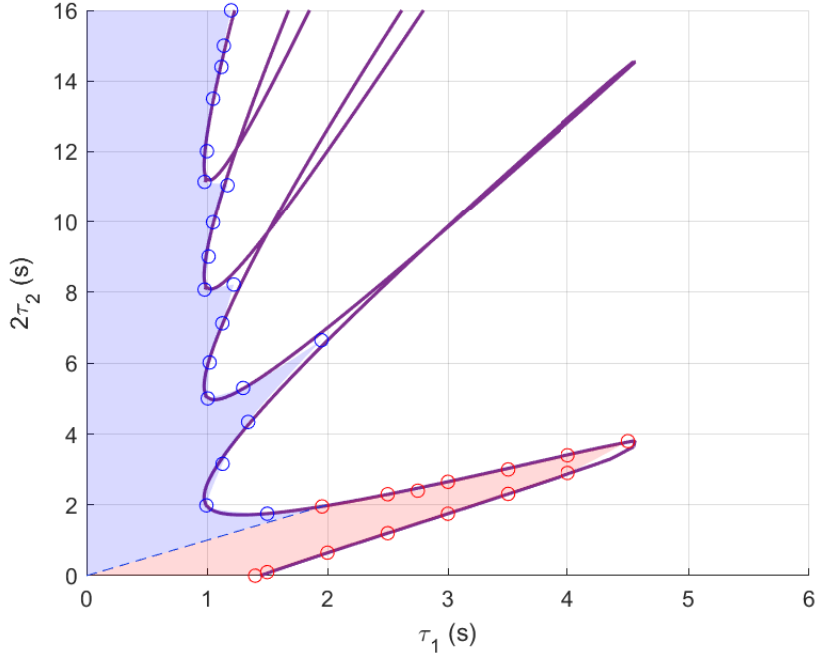


Figure 4.14: Delay solutions on open-ended curves, representing the first stability-switching curve.

are missed in the grid search method used in Fig. 4.13.

4.4 Chaotic Behavior

This section describes and compares the analysis of chaotic dynamics both in the original and extended gene networks. Chaotic behavior may arise in gene expression when each subsystem contains a minimum of two interconnected feedback loops with disparate time delays [51]. Essentially, the minimal motif necessary to observe chaos in gene networks is the inclusion of two-delay feedback [50]. Nevertheless, it is possible to prevent chaotic dynamics in dual feedback gene expression with appropriate parameter selections. As reported, in the double feedback motif involving a single gene, chaotic solutions manifest when there is a significant disparity in the delays within the two arms of the gene [50]. In this section, we

investigate the possible chaotic dynamics in the original and extended gene network models (systems described in Section 4.2.1 and Section 4.2.2, respectively), and present the analysis in detail.

4.4.1 Chaotic Dynamics in the Original Gene Network Model

In this section, we examine the original model to assess the presence of any chaotic behavior. As previously stated in Section 4.2, a standard model provided in (2.2) for a two-gene system does not exhibit any chaotic behavior; hence, we have proposed a modified original structure (4.1) for the chaos analysis, where the structure is given in Fig. 4.2 and Fig. 4.3. Here, we first show that the model with one delayed feedback loop and two feedback loops with equal delays do not have any chaos. Then, we analyze the modified model given in Section 4.2.1 with different Hill functions and delay parameters.

4.4.1.1 The model with one delayed feedback

A biological representation of the two-gene original model with one delayed feedback is depicted in Fig. 4.15. The mathematical model for this structure is analyzed in Section 2.2 in detail, and for two-gene system, we have

$$\begin{aligned}\dot{x}_1(t) &= -x_1(t) + g_1(x_2(t)) \\ \dot{x}_2(t) &= -x_2(t) + g_2(x_1(t - \tau)),\end{aligned}\tag{4.28}$$

where the degradation rates are $\lambda_1 = \lambda_2 = 1$.

In the analysis, we consider the nonlinear functions

$$g_1(x) = \frac{1}{2 + x^4}, \quad g_2(x) = \frac{14x^2}{3 + x^2},\tag{4.29}$$

and observe the bifurcation diagram by changing the delay parameter. Fig. 4.16 shows the bifurcation diagram of the fixed point (steady-state line), the maximum

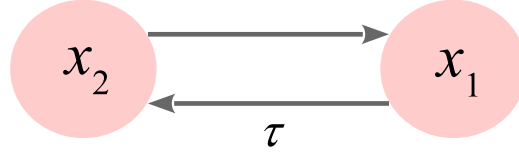


Figure 4.15: Original GRN model with two genes including one delayed feedback.

level (upper line), and minimum level (lower line) of protein x_1 with respect to different values of the time delay τ .

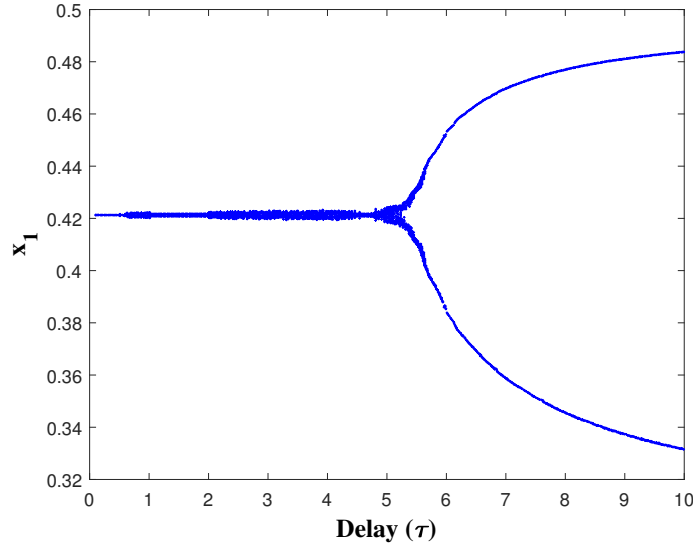


Figure 4.16: Bifurcation diagram of the fixed point, maximum level and minimum level of protein x_1 with respect to different values of τ .

The characteristic equation of the model, $X(s) = (s + 1)^2 + 1.189e^{-\tau s}$, and corresponding open-loop transfer function $\frac{1.189}{(s+1)^2}$ give that the delay margin of the system is $DM = 5.33$ sec. Moreover, the unique equilibrium of the model is $x_{eq} = [0.4213, 0.7819]^\top$. From the bifurcation diagram illustrated in Fig. 4.16, it can be claimed that when τ is less than the Hopf bifurcation point, $\tau_h = 5.33$ sec, the circuit is in a steady state and converges to the equilibrium $x_1^e = 0.4213$. Otherwise, when τ is larger than τ_h , the circuit has dynamics of periodic oscillations.

The model is observed under 6 sec time delay, where the periodic oscillations can be seen in Fig. 4.17. Moreover, Fig. 4.18 illustrates the power spectrum analysis for the concentration levels x_1 and x_2 , there is one dominant peak since the dynamics are periodic.

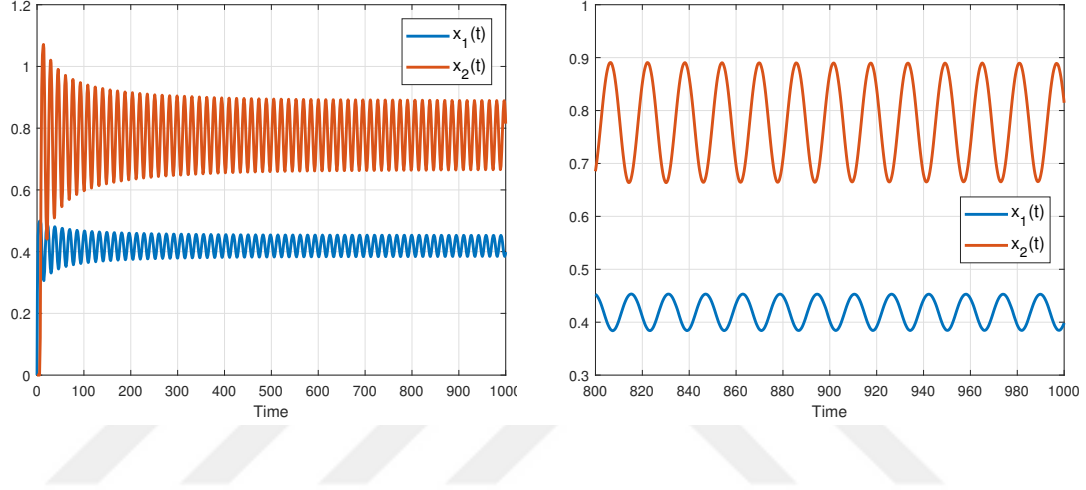


Figure 4.17: $x_1(t), x_2(t)$ vs t graph of the original one delayed system with $\tau = 6$ sec.

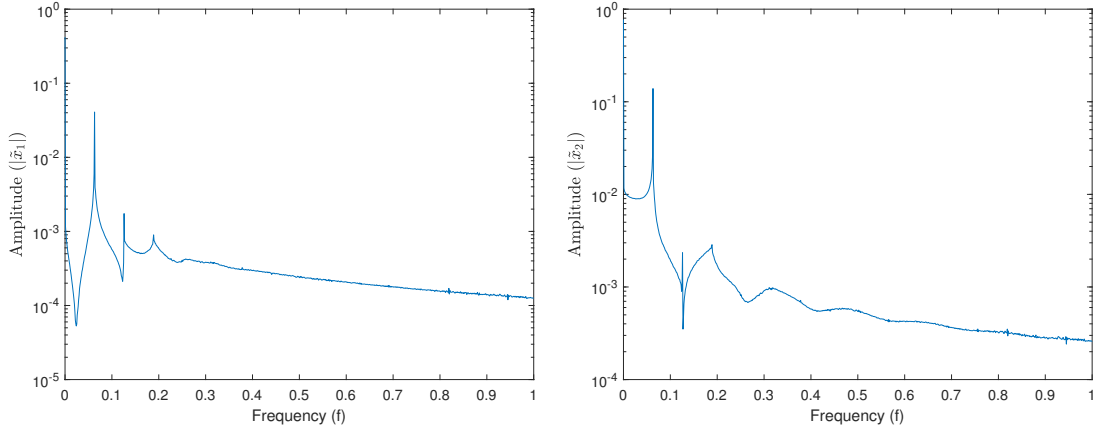


Figure 4.18: Power spectrum for $x_1(t)$ and $x_2(t)$ of the original one delayed system with $\tau = 6$ sec.

Increasing the delay parameter, τ , does not cause any chaotic behavior in the original two-gene system with one delayed feedback, beyond the Hopf bifurcation

point ($\tau_h = 5.33$ sec in the given example), we observe the emergence of oscillatory dynamics.

4.4.1.2 The model with two delayed feedback

This part covers the analysis for the two-gene original model with two coupled feedback loops, where the biological representation is shown in Fig. 4.19.

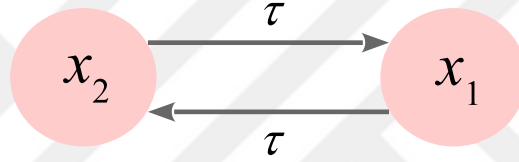


Figure 4.19: Original GRN model with two genes including two delayed feedback.

Similar to the analysis provided in the one delayed case, we examine the mathematical model

$$\begin{aligned} \dot{x}_1(t) &= -x_1(t) + g_1(x_2(t - \tau)) \\ \dot{x}_2(t) &= -x_2(t) + g_2(x_1(t - \tau)), \end{aligned} \tag{4.30}$$

with the degradation rates $\lambda_1 = \lambda_2 = 1$ choosing the same Hill functions (4.29). Introducing an additional delay as in Fig. 4.19 yields comparable outcomes to the previous scenario. Increasing the delay value gives oscillatory dynamics, there is also no chaotic behavior for this model.

Bifurcation diagram of the protein level x_1 with respect to the values of delay parameter τ is demonstrated in Fig. 4.20, in this case we observe that the Hopf bifurcation point, τ_h , is decreased to almost 1.5 sec. Likewise, when τ exceeds this value, oscillatory dynamics become apparent.

The behavior of the model for $\tau = 5$ sec is analyzed, and the corresponding oscillatory time trajectories are depicted in Fig. 4.21. Power spectra of the whole time trajectory for $x_1(t)$ and $x_2(t)$ are given in Fig. 4.22, here due to the periodic nature of the dynamics, we observe a single dominant peak.

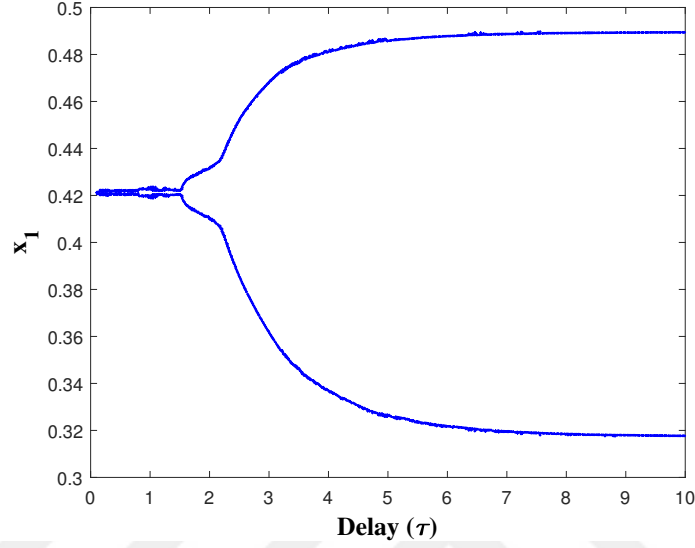


Figure 4.20: Bifurcation diagram of the fixed point, maximum level and minimum level of protein x_1 with respect to different values of τ .

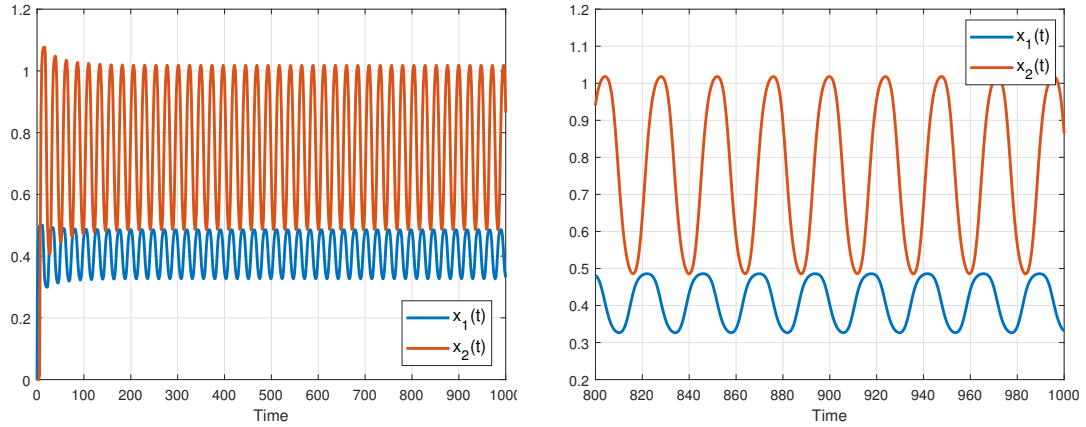


Figure 4.21: $x_1(t), x_2(t)$ vs t graph of the original two delayed system with $\tau = 5$ sec.

Furthermore, the structure in Fig. 4.19 is examined by selecting different delay values, yet consistent results are obtained, indicating the absence of any chaotic behavior.

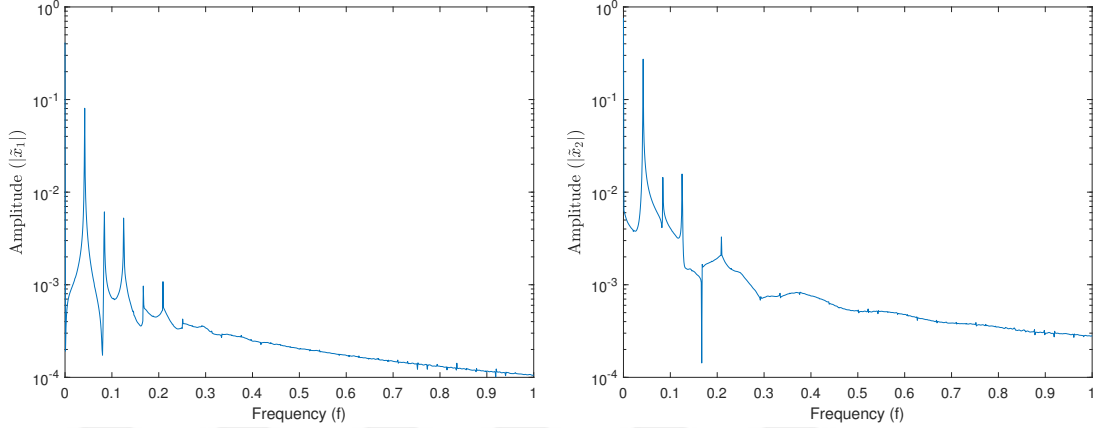


Figure 4.22: Power spectrum for $x_1(t)$ and $x_2(t)$ of the original two delayed system with $\tau = 5$ sec.

4.4.1.3 The modified original two-gene model

As stated and analyzed, the utilization of the model given in (2.2) for a two-gene system does not directly manifest any chaotic behavior. Therefore, we have proposed a modified original two-gene model in Fig. 4.2 and have provided the equilibrium and stability analysis, the linearization and characteristic equation in detail. In this section, we investigate the chaotic dynamics within the proposed modified two-gene model (dynamical system defined in Section 4.2.1).

Initially, the nonlinear functions $g_1(x)$ and $g_2(x)$ in Fig. 4.2 are selected to be equal, using the definition in (4.3), i.e.,

$$g_1(x) = g_2(x) = \frac{10}{1 + x^4}, \quad (4.31)$$

where the Hill parameter, $m = 4$. The LTI system of the model, $H(s)$, is determined first-order homogeneous such that $H_i(s) = H(s) = 1/(s + 1)$ for $i = 1, 2$.

In order to explore the non-trivial dynamics, we focus on the potential chaotic dynamics and examine the local maximum values of variable x_1 . Fig. 4.23 illustrates the local maximum of x_1 when time delay τ_1 is set to be 15 seconds while varying the time delay τ_2 . The unique equilibrium point of the model (4.1) with nonlinear functions (4.31) is calculated as $x_{eq} = [x_1^e, x_2^e]^\top = [0.01, 10]^\top$. The

system exhibits steady-state dynamics when $\tau_2 < 3.6$ sec and $6.4 \text{ sec} < \tau_2 < 7.6$ sec. In Fig. 4.23, it appears to be very close to zero due to the proximity of the equilibrium x_1^e to zero.

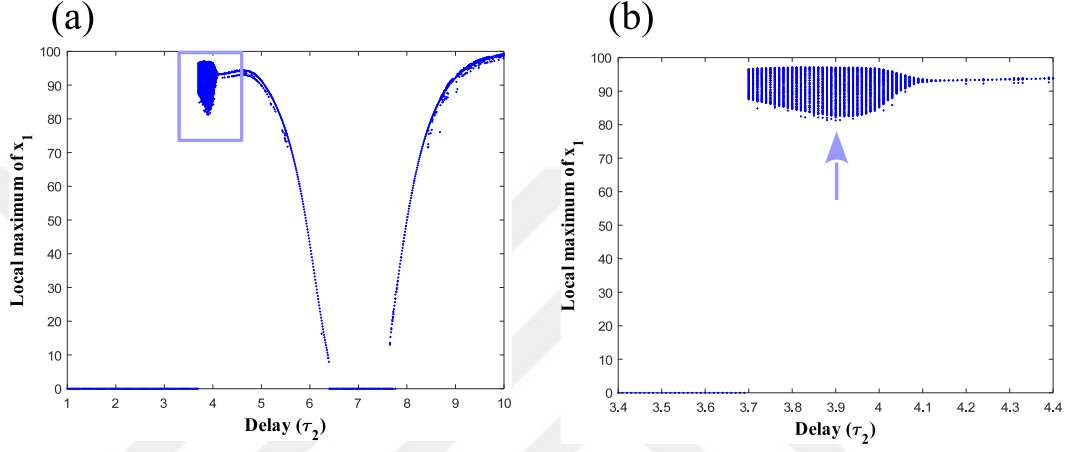


Figure 4.23: Chaotic dynamics for the two-gene modified original model with identical g_i 's. (a) Bifurcation diagram with local maxima of x_1 with respect to τ_2 , by setting $\tau_1 = 15$ sec. The non-periodic dynamics are shown when $3.7 \text{ sec} < \tau_2 < 4.1 \text{ sec}$. (b) The detailed diagram for the specified region with a square, where the circuit exhibits chaotic dynamics for $\tau_2 = 3.9$ sec (demonstrated with an arrow).

Fig. 4.23(a) indicates a transition between steady-state phase, chaotic or quasi periodic phase, and the oscillatory phase. Chaotic dynamics may appear when $3.7 \text{ sec} < \tau_2 < 4.1 \text{ sec}$, as shown in Fig. 4.23(b), and periodic or steady-state dynamics may be observed otherwise. As an example, we pick the value of time delay $\tau_2 = 3.9$ sec, illustrated with an arrow in Fig. 4.23(b), and examine the behavior of the model. The phase-space maps of concentration x_1 are presented in Fig. 4.24, where the map with derivative on the vertical axis is given in Fig. 4.24(a) and the map with $x_1(t - \tau_2)$ on the vertical axis is provided in Fig. 4.24(b). In contrast to a simple oscillator with a periodic solution, where the trajectory would trace a loop or multiple sub-loops in the case of a complex waveform, the chaotic dynamics exhibit a fractal attractor in the phase-space map [50].

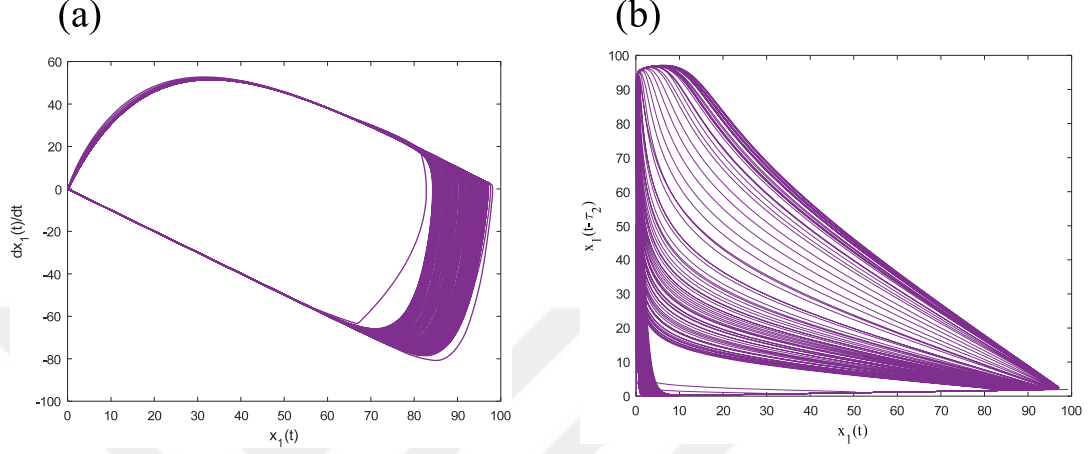


Figure 4.24: Chaotic dynamics for the two-gene modified original model with identical g_i 's. (a) The phase-space map of $x_1(t)$ vs. its time derivative $dx_1(t)/dt$. (b) Trace of dynamics in phase space with $x_1(t - \tau_2)$ on the vertical axis, where $\tau_2 = 3.9$ sec.

The time response of $x_1(t)$ after initial transients for $\tau_1 = 15$ sec and $\tau_2 = 3.9$ sec is depicted in Fig. 4.25(a), we can clearly observe the variable maximums. Fig. 4.25(b) shows the power spectrum estimate of variable x_1 , many sharp peaks are present at distinct frequencies.

We can conclude that the circuit depicted in Fig. 4.2 with identical nonlinearity functions can display chaotic dynamics, which are contingent upon the specific values of the time delays. In order to validate this, we further compute the dominant Lyapunov exponent of this model using the method proposed by Wolf *et al* [71]. By employing a phase space reconstruction delay of 10 and an embedding dimension of 3, we have analyzed a dataset comprising 4096 samples taken at intervals of 0.3 seconds. The positive dominant Lyapunov exponent is computed as 0.0134 bits/sec, further confirming the presence of chaotic dynamics in the system.

Moreover, we also analyze the two-gene modified model by selecting the non-linear functions $g_1(x)$ and $g_2(x)$ in Fig. 4.2 different, using the definition in (4.3),

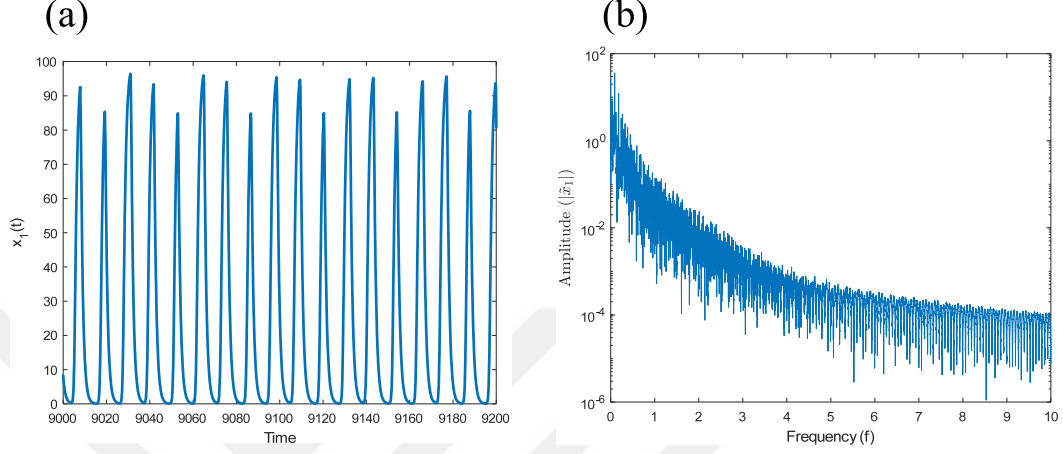


Figure 4.25: Chaotic dynamics for the two-gene modified original model with identical g_i 's. (a) Time trajectory of concentration $x_1(t)$ after initial transients. (b) Power spectra of the concentration x_1 for the case when $\tau_1 = 15$ sec and $\tau_2 = 3.9$ sec.

i.e.,

$$g_1(x) = \frac{10}{1+x^4}, \quad g_2(x) = \frac{5}{1+x^6}, \quad (4.32)$$

where the Hill parameter of the first nonlinear function is $m_1 = 4$, and the second one is $m_2 = 1$. Similarly, the LTI system of the model, $H(s)$, is determined first-order homogeneous such that $H_i(s) = H(s) = 1/(s+1)$ for $i = 1, 2$. Here our objective is to investigate the behavior of the model by increasing the Hill parameter and to examine the resulting chaotic dynamics when the Hill parameter is higher.

The possible regions of non-periodic oscillatory dynamics in this case are also determined based on the local maximum concentration of variable x_1 by varying τ_2 relative to $\tau_1 = 15$ sec. The bifurcation diagram of the local maximum of x_1 is presented in Fig. 4.26(a). In this structure, we identify two potential intervals where chaotic behavior may arise such that the dynamics may be non-periodic when $4.05 \text{ sec} < \tau_2 < 4.6 \text{ sec}$ and $6.7 \text{ sec} < \tau_2 < 6.95 \text{ sec}$. We initially examine the dynamics within the first region by choosing $\tau_2 = 4.1$ sec, illustrated with an

arrow in Fig. 4.26(b), to gain insights into the system's behavior.

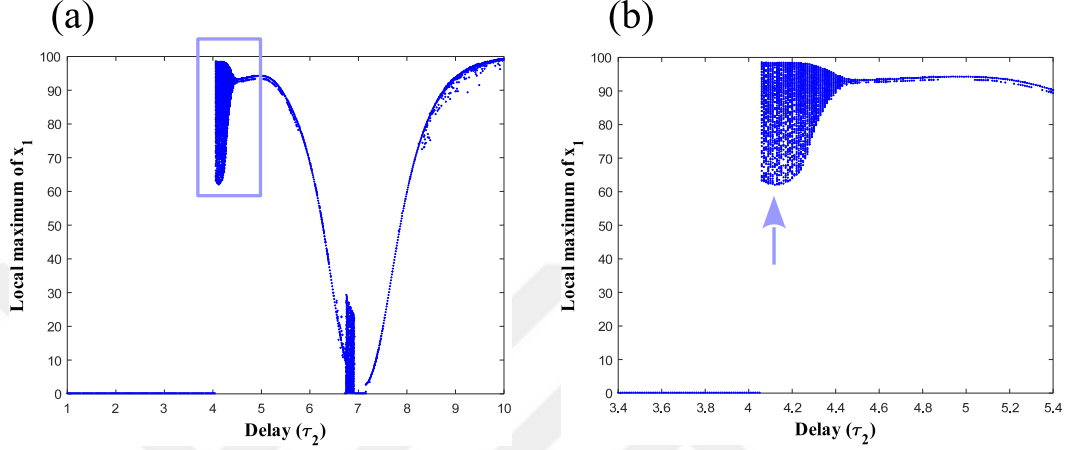


Figure 4.26: Chaotic dynamics for the two-gene modified original model with different g_i 's. (a) Bifurcation diagram with local maxima of x_1 with respect to τ_2 , by setting $\tau_1 = 15$ sec. The non-periodic dynamics are shown when $4.05 \text{ sec} < \tau_2 < 4.6 \text{ sec}$ and $6.7 \text{ sec} < \tau_2 < 6.95 \text{ sec}$. (b) The detailed diagram for the specified region with a square, where the circuit exhibits chaotic dynamics for $\tau_2 = 4.1 \text{ sec}$ (demonstrated with an arrow).

The phase-space maps of $x_1(t)$ are depicted in Fig. 4.27(a) with the derivative on the vertical axis and in Fig. 4.27(b) with $x_1(t - \tau_2)$ on the vertical axis. Here the maps exhibit nearly a fractal region, indicating complex and intricate dynamics within the system. Therefore, it can be concluded that a simple oscillator with a periodic solution is not present in this system. Fig. 4.28(a) demonstrates the time trace of dynamics when $\tau_1 = 15 \text{ sec}$ and $\tau_2 = 4.1 \text{ sec}$. The variable maximum values in the time response can be seen clearly. Moreover, the Fourier spectrum of x_1 is given in Fig. 4.28(b). In the power spectrum estimate, numerous peaks are observed across the entire frequency range.

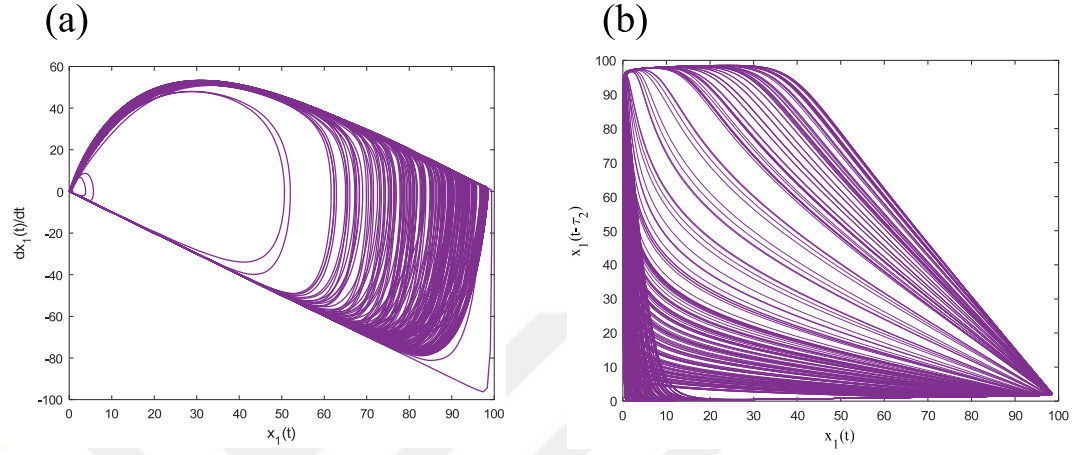


Figure 4.27: Chaotic dynamics for the two-gene modified original model with different g_i 's. (a) The phase-space map of $x_1(t)$ vs. its time derivative $dx_1(t)/dt$. (b) Trace of dynamics in phase space with $x_1(t - \tau_2)$ on the vertical axis, where $\tau_2 = 4.1$ sec.

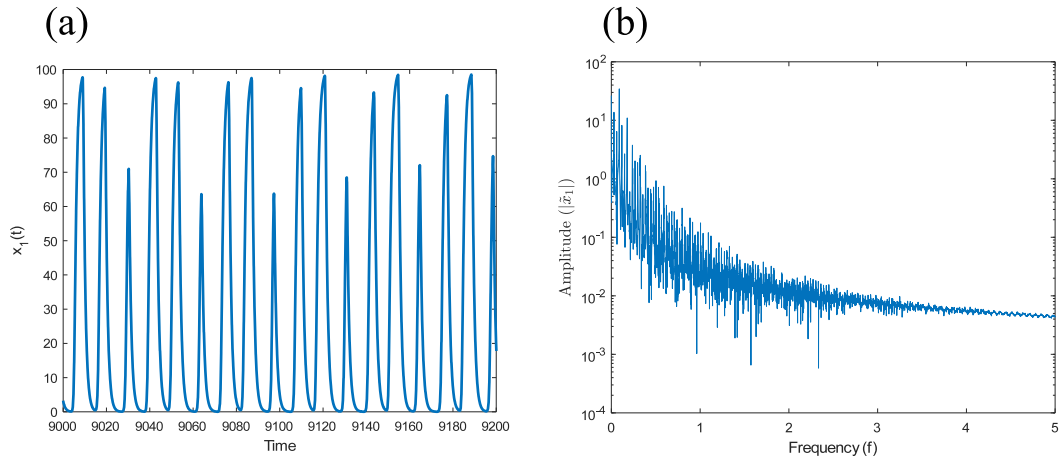


Figure 4.28: Chaotic dynamics for the two-gene modified original model with different g_i 's. (a) Time trajectory of concentration $x_1(t)$ after initial transients. (b) Power spectra of the concentration x_1 for the case when $\tau_1 = 15$ sec and $\tau_2 = 4.1$ sec.

To definitively ascertain the chaotic nature of the dynamics in this case, the dominant Lyapunov exponent [71] is computed using a dataset comprising 8192 samples collected at every 0.05 seconds. We determined that the system exhibits a positive dominant Lyapunov exponent of 0.4701 bits/sec with an embedding dimension of 4 and a reconstruction delay of 5. This calculation provides clear evidence of the chaotic behavior exhibited by the system.

We then examine the dynamics of the model with different Hill functions given in (4.32) by choosing $\tau_2 = 6.8$ sec. The local maximum of x_1 is depicted in Fig. 4.29(a), and the small interval showing the presumably chaotic dynamics is provided in Fig. 4.29(b), where the specified delay value $\tau_2 = 6.8$ sec is shown with an arrow. There is a transition between oscillatory phase and chaotic phase, periodic dynamics are observed outside of the interval $6.7 \text{ sec} < \tau_2 < 6.95 \text{ sec}$.

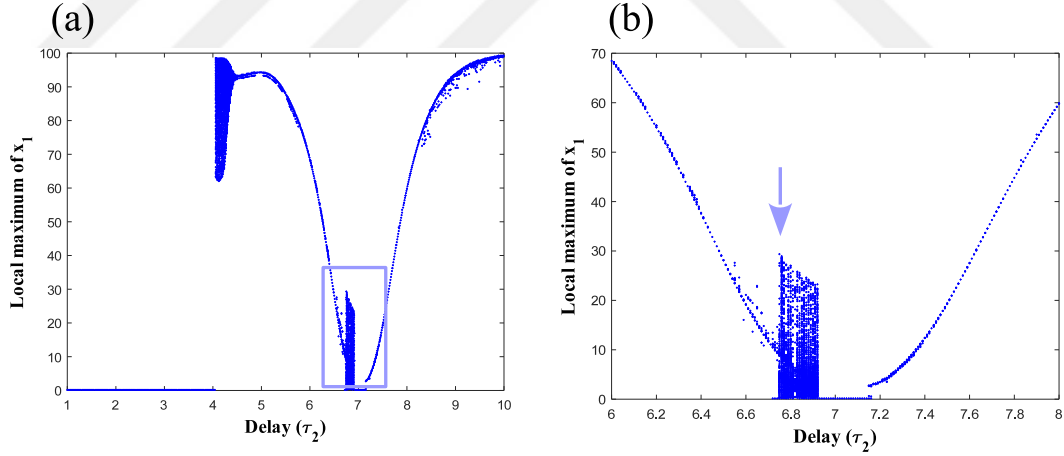


Figure 4.29: Chaotic dynamics for the two-gene modified original model with different g_i 's. (a) Bifurcation diagram with local maxima of x_1 with respect to τ_2 , by setting $\tau_1 = 15$ sec. The non-periodic dynamics are shown when $4.05 \text{ sec} < \tau_2 < 4.6 \text{ sec}$ and $6.7 \text{ sec} < \tau_2 < 6.95 \text{ sec}$. (b) The detailed diagram for the specified region with a square, where the circuit exhibits chaotic dynamics for $\tau_2 = 6.8 \text{ sec}$ (demonstrated with an arrow).

The analysis reveals that the phase-space maps of the variable x_1 trace out a fractal region, as visually observed in Fig. 4.30. Furthermore, it can be asserted that the phase-maps are more irregular compared to the results given in Fig. 4.24 and Fig. 4.27, especially the phase-map with derivative on the vertical axis shown in Fig. 4.30(a). The time response of $x_1(t)$ after initial transients for $\tau_1 = 15$ sec and $\tau_2 = 6.8$ sec is demonstrated in Fig. 4.31(a). In this case, the time dynamics are more pulsatile and non-periodic dynamics are clearly visible. Moreover, Fig. 4.31(b) illustrates the power spectra of x_1 , which have numerous spikes across the whole frequency range, indicating the complex and non-periodic dynamics.

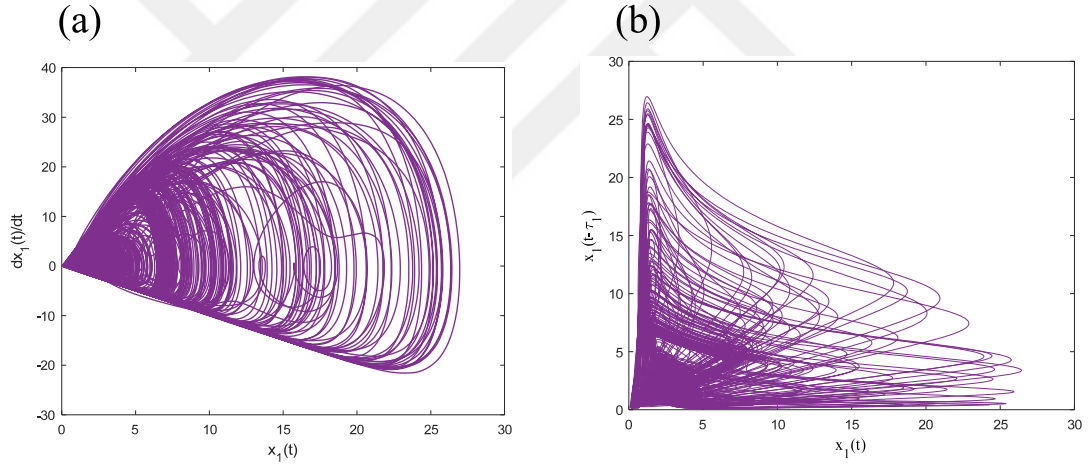


Figure 4.30: Chaotic dynamics for the two-gene modified original model with different g_i 's. (a) The phase-space map of $x_1(t)$ vs. its time derivative $dx_1(t)/dt$. (b) Trace of dynamics in phase space with $x_1(t - \tau_1)$ on the vertical axis, where $\tau_2 = 6.8$ sec.

Likewise, the Wolf method [71] is applied to the dataset with 9182 samples taken every 0.05 seconds using an embedding dimension of 4 and a phase space reconstruction delay of 3, resulting in the computation of a positive dominant Lyapunov exponent of 0.5732 bits/sec. It is important to note that the dominant Lyapunov exponent computed in this case surpasses the Lyapunov exponents obtained in the previous analysis. In other words, increasing the delay parameter τ_2 and using different nonlinear functions give greater positive Lyapunov exponent.

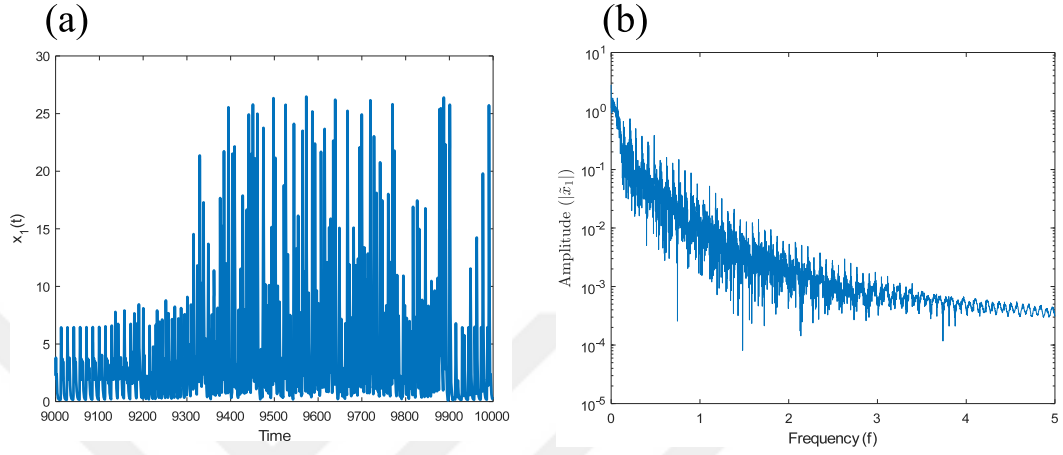


Figure 4.31: Chaotic dynamics for the two-gene modified original model with different g_i 's. (a) Time trajectory of concentration $x_1(t)$ after initial transients. (b) Power spectra of the concentration x_1 for the case when $\tau_1 = 15$ sec and $\tau_2 = 6.8$ sec.

Through the utilization of the Lyapunov exponents and the analysis, we can confirm that the modified original model consisting of two genes can demonstrate chaos, depending on the values of τ_1 and τ_2 . Moreover, Hill functions play a crucial role in observing chaotic behavior in the original model.

4.4.2 Chaotic Dynamics in the Extended Gene Network Model

In this section, our objective is to analyze the extended gene network and determine if the model exhibits any chaotic behavior. Initially, we examine the extended model (2.7) with one delayed feedback, and observe the dynamics in this case by varying the delay parameter. Then, the extended model with two coupled feedback loops including equal and different delay values is studied, and non-periodic oscillatory dynamics are observed without adding any self-inhibitory feedback loop to one of the variables as in the two-gene modified original model.

4.4.2.1 The model with one delayed feedback

A biological representation of the two-gene extended model with one delayed feedback is shown in Fig. 4.32. The mathematical model for this structure is examined in Section 2.3 in detail, and for two-gene system, we have

$$\begin{aligned}\dot{\underline{x}}_1(t) &= A_1 \underline{x}_1(t) + g_1(\underline{x}_2(t)) \\ \dot{\underline{x}}_2(t) &= A_2 \underline{x}_2(t) + g_2(\underline{x}_1(t - \tau))\end{aligned}\tag{4.33}$$

where A_i 's are 2×2 Hurwitz matrices in the form of

$$A_i = \begin{bmatrix} 0 & 1 \\ -\rho_i & -\lambda_i \end{bmatrix} = \begin{bmatrix} 0 & 1 \\ -1 & -2 \end{bmatrix}\tag{4.34}$$

for $i = 1, 2$. In the analysis, we consider the nonlinear functions

$$g_1(x) = \frac{10}{1 + x^4}, \quad g_2(x) = \frac{5}{1 + x^4},\tag{4.35}$$

and determine the equilibrium of the model using (4.12), i.e.,

$$\underline{x}_{eq} = [\underline{x}_1^e, \underline{x}_2^e]^\top = [(10, 0), (5, 0)]^\top.$$

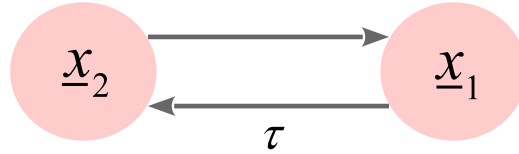


Figure 4.32: Extended GRN model with 2 genes including one delayed feedback

Fig. 4.33 illustrates the bifurcation diagram of the first component of the variable x_1 , namely x_{11} , by changing the delay parameter τ . There is a transition between steady-state dynamics and non-periodic dynamics, where the model converges to the equilibrium when $\tau < 6.7$ sec, and shows non-periodic behavior otherwise. For instance, the time responses of the variable x_{11} are depicted in Fig. 4.34, where Fig. 4.34(a) shows the behavior of the model with $\tau = 6.7$ sec and Fig. 4.34(b) shows the behavior with $\tau = 18$ sec. As we increase the value of delay parameter, we observe that the model exhibits more complex and non-periodic dynamics.

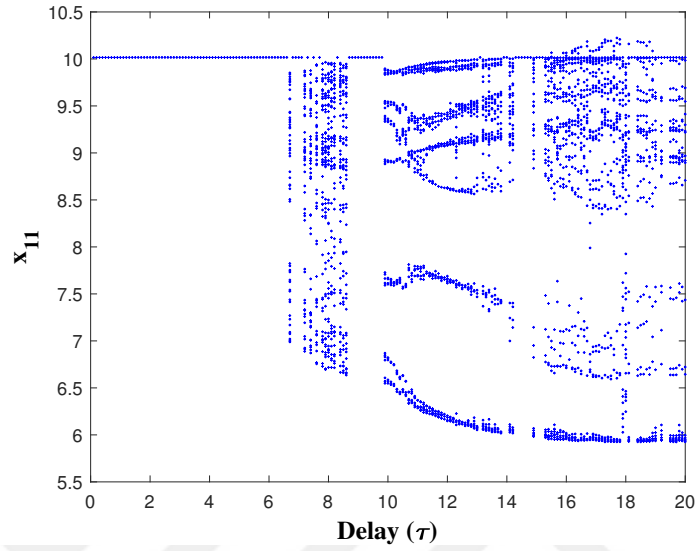


Figure 4.33: Bifurcation diagram of the variable x_{11} with respect to different values of τ .

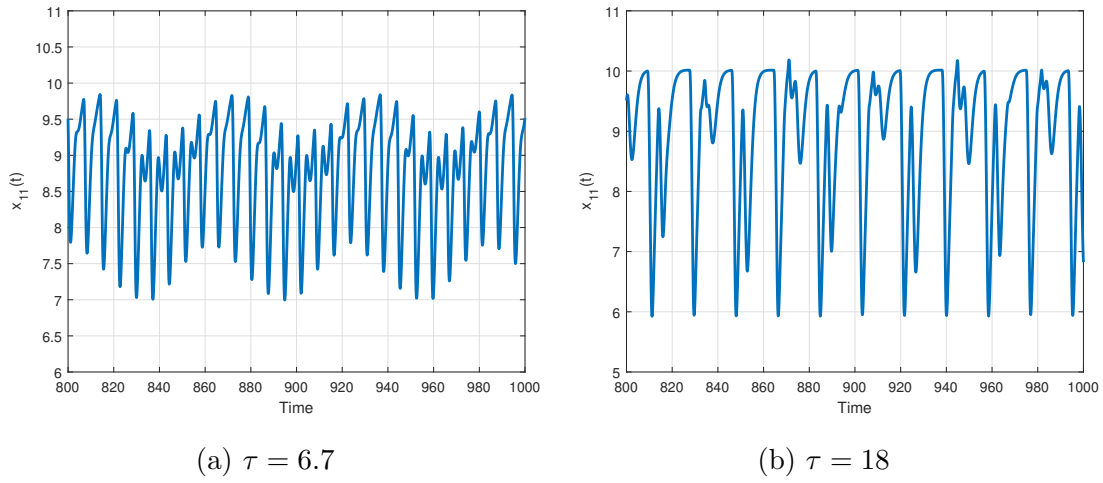


Figure 4.34: Time trajectory of $x_{11}(t)$ of the extended one delayed system after initial transients.

4.4.2.2 The model with two equal delayed feedback

This part covers the analysis for the two-gene extended model with two coupled feedback loops, where the biological representation with equal delays is shown in Fig. 4.35.

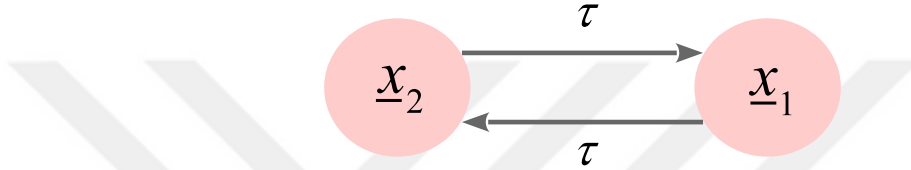


Figure 4.35: Extended GRN model with 2 genes including two equal delayed feedback

Similar to the analysis mentioned in the one delayed case, we examine the mathematical model for a two-gene system with two equal delays

$$\begin{aligned}\dot{x}_1(t) &= A_1 x_1(t) + g_1(x_2(t - \tau)) \\ \dot{x}_2(t) &= A_2 x_2(t) + g_2(x_1(t - \tau))\end{aligned}\tag{4.36}$$

with the parameters given in (4.34) and using the same Hill functions defined in (4.35). The introduction of an additional equal delay leads to similar outcomes as in the previous scenario. Fig. 4.36 depicts the bifurcation diagram of the first component, x_{11} , of the variable x_1 , showing the changes in its behavior as the delay parameter τ is varied. When the delay parameter is divided into two equal delays, the dynamics exhibit remarkable similarities to the case with a single delay given in Fig. 4.33. Likewise, we observe a transition between steady-state dynamics and non-periodic dynamics in a similar manner, i.e., when $\tau < 3$ sec, the model converges to the equilibrium $x_{11}^e = 10$.

Fig. 4.37 illustrates the time trajectories of $x_{11}(t)$ for different delay values: $\tau = 3$ sec and $\tau = 9$ sec. We examine the response of the model at the critical delay value, which is when the system transitions from steady-state to aperiodic behavior at $\tau = 3$ sec, as illustrated in Fig. 4.37(a). In addition, Fig. 4.37(b) yields the response of the system at $\tau = 9$ sec, where the result are very similar to the analysis given in one delayed case in Fig. 4.34(b).

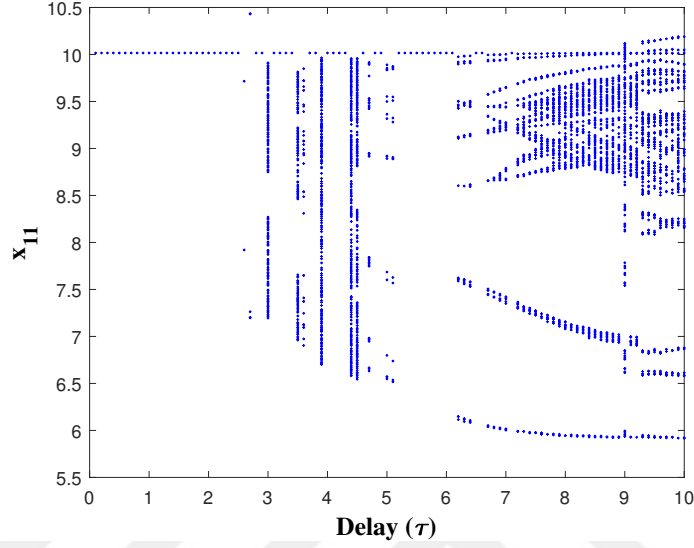


Figure 4.36: Bifurcation diagram of the variable x_{11} for a two-gene system with two equal delays, with respect to different values of τ .

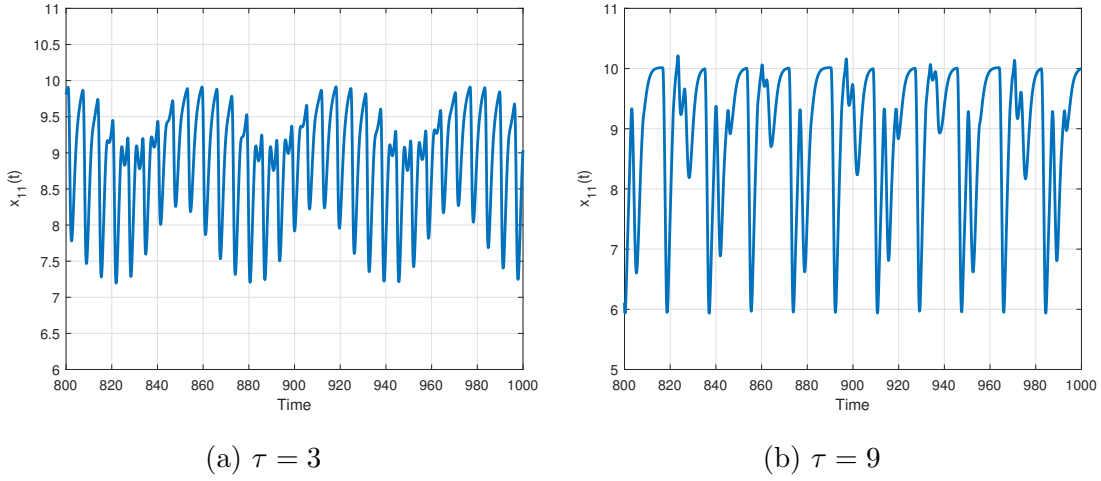


Figure 4.37: Time trajectory of $x_{11}(t)$ of the extended two equal delayed system after initial transients.

Based on our analysis, it is clear that the extended two-gene system's behavior is highly similar in the case of one delayed feedback and two coupled feedback loops having equal delays. Both system exhibit convergence to the equilibrium

and display aperiodic behavior at approximately the same delay values.

4.4.2.3 The model with two delayed feedback

In this section, we investigate the presence of chaotic behavior in the extended two-gene system including different delay parameters, considering it as a comprehensive model of the one delayed or two equal delayed cases. We have presented the extended two-gene model that incorporates various delay parameters in Fig. 4.4 (with a biological representation in Fig. 4.5) and conducted a thorough analysis of its equilibrium and stability properties, linearization and characteristic equation. In this system, it can be claimed that selecting one of the delay values as zero or choosing equal delays yields similar outcomes as previously discussed in the earlier parts.

We focus on studying the model by keeping the time delay τ_1 fixed at 5 sec while varying the time delay τ_2 , which allows us to observe the emergence of non-trivial dynamics in the system. The nonlinear functions $g_1(x)$ and $g_2(x)$ in Fig. 4.4 are selected as

$$g_1(x) = \frac{10}{1+x^4}, \quad g_2(x) = \frac{5}{1+x^4}. \quad (4.37)$$

For the chaos analysis, we have determined that the Hill coefficient, m , needs to be set to a minimum value of 4 in order to observe chaotic behavior. Note that each subsystem states include two variables in the extended structure, i.e. $\underline{x}_1 = [x_{11}, x_{12}]^\top$ and $\underline{x}_2 = [x_{21}, x_{22}]^\top$ in a two-gene extended model. In the analysis, we investigate the local maximum values of variable x_{11} by varying the second time delay τ_2 , relative to the first time delay $\tau_1 = 5$ sec. Fig. 4.38 shows the bifurcation diagram with local maxima over a wide range of τ_2 values. This indicates that the circuit exhibits predominantly non-periodic dynamics and displays steady-state dynamics within certain small intervals such as around τ_2 equals 1.01 sec, 1.8 sec, 5.5 sec and between 6.5 sec–7.5 sec. Fig. 4.39 presents zoomed versions of the bifurcation diagram, which provide a closer look at the system's dynamics. In these regions, the presence of non-periodic behavior is

evident. The bifurcation diagram captures the transitions and complexity of the system, allowing us to observe the chaotic dynamics.

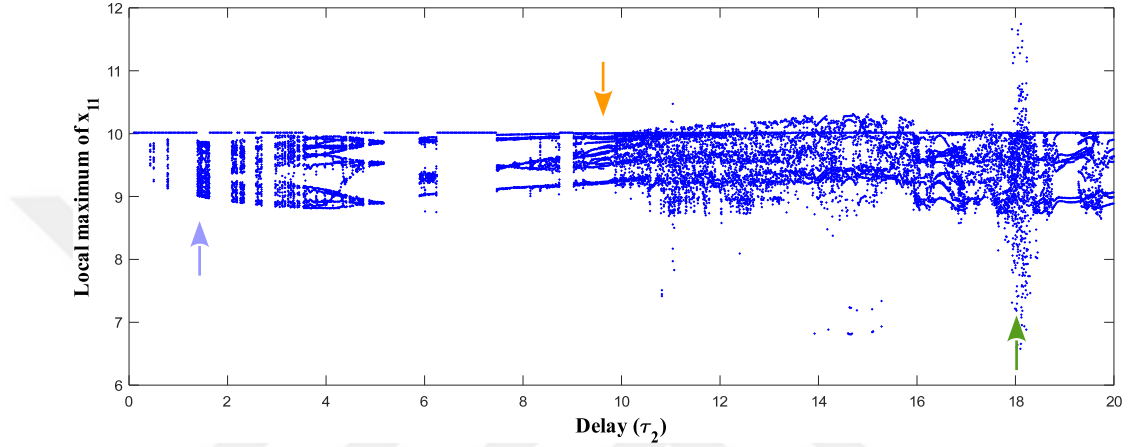


Figure 4.38: Bifurcation diagram with local maxima of x_{11} with respect to τ_2 , by setting $\tau_1 = 5$ sec.

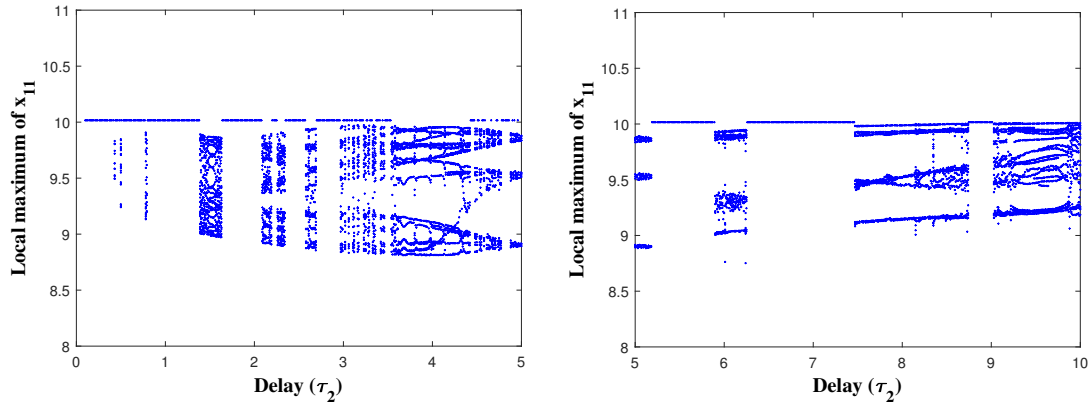


Figure 4.39: Bifurcation diagram with local maxima of x_{11} with respect to τ_2 , by setting $\tau_1 = 5$ sec.

We initially explore the dynamics for $\tau_2 = 1.5$ sec, indicated by the leftmost arrow in Fig. 4.38. For this case, the phase-space maps of $x_{11}(t)$ with its time

derivative $dx_{11}(t)/dt$ on the vertical axis and with $x_{11}(t - \tau_2)$ on the vertical axis are depicted in Fig. 4.40(a) and Fig. 4.40(b), respectively. Here, the phase map has a torus structure, indicating the presence of complex dynamics within the system. Time trajectory of $x_{11}(t)$ is demonstrated in Fig. 4.41(a) for $\tau_1 = 5$ sec and $\tau_2 = 1.5$ sec, where the time response clearly reveals the variable maximum values. Fig. 4.41(b) depicts the Fourier spectrum of $x_{11}(t)$, there are many peaks at some distinct frequencies.

We utilize the positive dominant Lyapunov exponent as numerical evidence to demonstrate that the dynamics of the extended model in this case is indeed chaotic. A dataset with 16384 samples taken every 0.05 seconds is analyzed by using an embedding dimension of 3 and a phase space reconstruction delay of 10 in the Wolf method [71]. The positive dominant Lyapunov exponent for the extended two-gene model with $\tau_1 = 5$ sec and $\tau_2 = 1.5$ sec is computed as 0.0435 bits/sec, which confirms the existence of chaos.

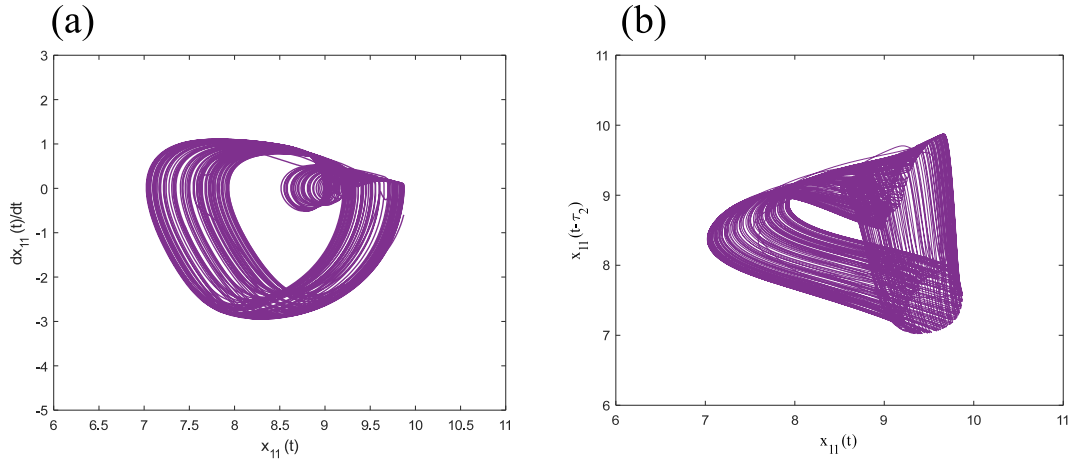


Figure 4.40: Chaotic dynamics for the two-gene extended model. (a) The phase-space map of $x_{11}(t)$ vs. its time derivative $dx_{11}(t)/dt$. (b) Trace of dynamics in phase space with $x_{11}(t - \tau_2)$ on the vertical axis, where $\tau_2 = 1.5$ sec.

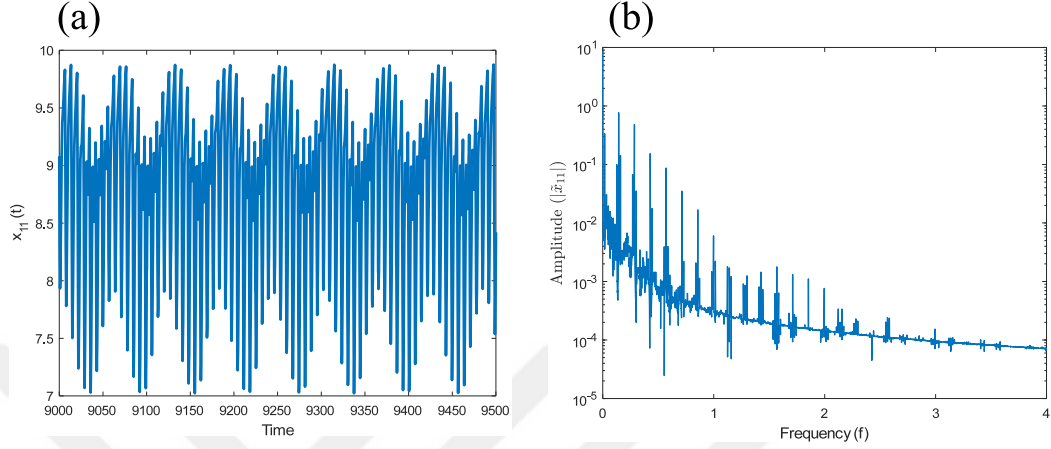


Figure 4.41: Chaotic dynamics for the two-gene extended model. (a) Time trajectory of concentration $x_{11}(t)$ after initial transients. (b) Power spectra of the concentration $x_{11}(t)$ for the case when $\tau_1 = 5$ sec and $\tau_2 = 1.5$ sec.

Secondly, we pick $\tau_2 = 9.6$ sec, indicated by the arrow in the middle in Fig. 4.38, to compare the behavior of the two-gene extended model with a different delay value. Likewise, the phase maps of the system with $\tau_1 = 5$ sec and $\tau_2 = 9.6$ sec are depicted in Fig. 4.42, where the maps cover the region somewhat regularly. We can clearly say that the results do not exhibit the characteristics of a simple oscillator as there is no presence of a periodic solution in the dynamics. Fig. 4.43(a) illustrates the time response of $x_{11}(t)$, which typically shows non-periodic behaviors. Also, the power spectrum of the whole time trajectory is given in Fig. 4.43(b), which has many spikes throughout the frequency range.

Similar to the previous scenario, we employ the positive dominant Lyapunov exponent to provide evidence for the chaotic nature of the dynamics in the extended model. The Wolf method [71] is applied to analyze a dataset consisting of 16384 samples, taken at a sampling interval of 0.05 seconds. In this analysis, an embedding dimension of 3 and a time delay reconstruction of 10 are used, and the dominant Lyapunov exponent is found as 0.1334 bits/sec. As the time delay τ_2 is increased, we noted a consistent rise in the positive Lyapunov estimate.

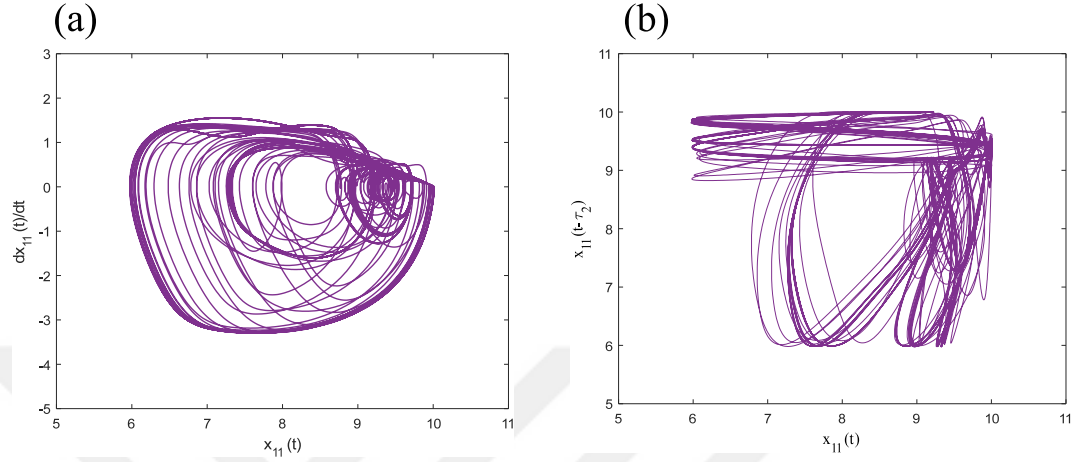


Figure 4.42: Chaotic dynamics for the two-gene extended model. (a) The phase-space map of $x_{11}(t)$ vs. its time derivative $dx_{11}(t)/dt$. (b) Trace of dynamics in phase space with $x_{11}(t - \tau_2)$ on the vertical axis, where $\tau_2 = 9.6$ sec.

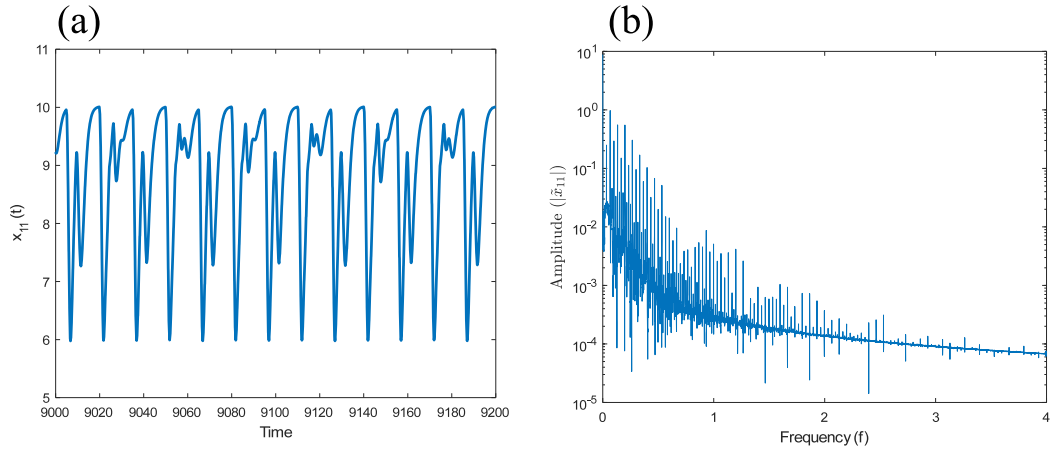


Figure 4.43: Chaotic dynamics for the two-gene extended model. (a) Time trajectory of concentration $x_{11}(t)$ after initial transients. (b) Power spectra of the concentration $x_{11}(t)$ for the case when $\tau_1 = 5$ sec and $\tau_2 = 9.6$ sec.

Finally, τ_2 is selected as 18, indicated by the rightmost arrow in Fig. 4.38, to conduct a more comprehensive and detailed analysis. Fig. 4.44 demonstrates

the phase maps of variable $x_{11}(t)$ with $dx_{11}(t)/dt$ and $x_{11}(t - \tau_2)$ on the vertical axis. In this case, the phase maps are more irregular compared to the analysis with $\tau_2 = 1.5$ sec and $\tau_2 = 9.6$ sec given in Fig. 4.40 and Fig. 4.42, respectively. The time trace of dynamics clearly shows the complex maxima in Fig. 4.45(a), which demonstrates the aperiodic behavior of the system. Additionally, Fourier transform of chaotic dynamics indicates many sharp peaks throughout the whole frequency range in Fig. 4.45(b).

The Wolf method [71] is similarly utilized to the dataset with 16384 samples taken every 0.05 seconds with an embedding dimension of 3 and reconstruction delay of 10, and the algorithm results in a positive dominant Lyapunov exponent of 0.6233 bits/sec. This value represents the highest Lyapunov exponent obtained throughout our analysis of both the original and extended models.

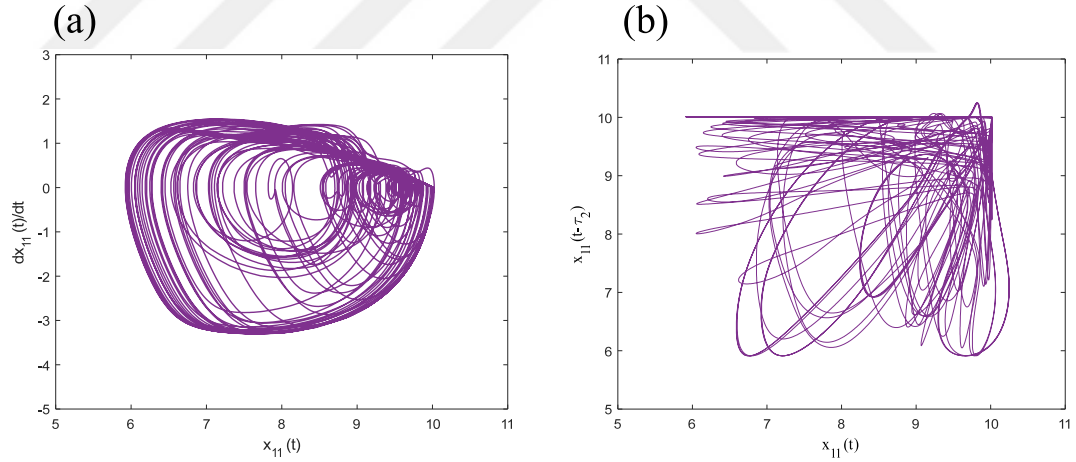


Figure 4.44: Chaotic dynamics for the two-gene extended model. (a) The phase-space map of $x_{11}(t)$ vs. its time derivative $dx_{11}(t)/dt$. (b) Trace of dynamics in phase space with $x_{11}(t - \tau_2)$ on the vertical axis, where $\tau_2 = 18$ sec.

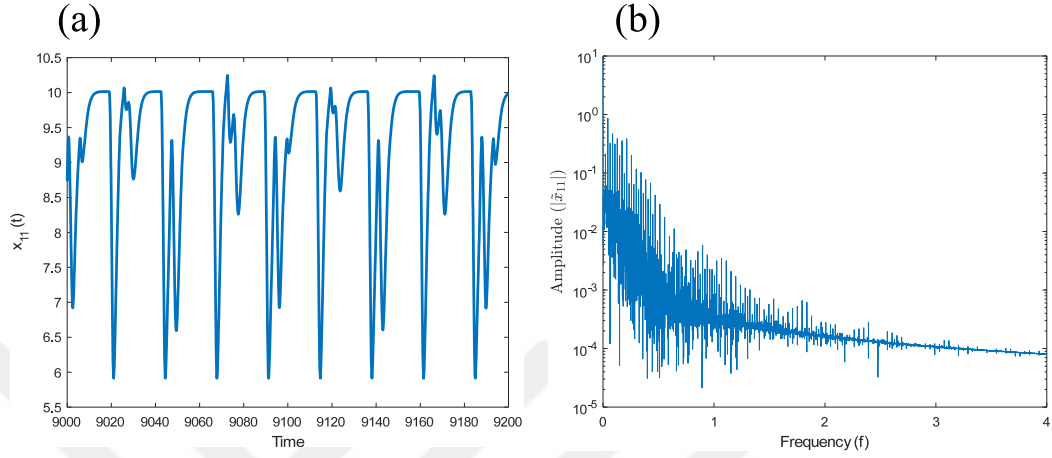


Figure 4.45: Chaotic dynamics for the two-gene extended model. (a) Time trajectory of concentration $x_{11}(t)$ after initial transients. (b) Power spectra of the concentration $x_{11}(t)$ for the case when $\tau_1 = 5$ sec and $\tau_2 = 18$ sec.

4.4.3 Summary of Chaotic Dynamics

In this section, we provide a summary of approach for the analysis of chaotic dynamics both in the original and extended gene network models. Two-gene model with one delayed and two delayed negative feedback loop exhibit a Hopf bifurcation from the steady-state to oscillatory dynamics in the original gene network. Hence, we have examined the modified original model including two-genes, with one of them exhibiting self-inhibition as given in Fig. 4.2 and Fig. 4.3. This configuration results in dual feedback within a single gene.

We have analyzed the extended model with a two-gene structure to investigate its potential for chaotic dynamics. It has been observed that the model, specifically the configuration involving one delayed feedback and two coupled feedback loops with equal delays, exhibits non-periodic behavior. Then, our attention shifts to the two-delayed extended model given in Fig. 4.4 and Fig. 4.5, where the delays differ. Remarkably, this model demonstrates the capability to exhibit more complex dynamics even without adding any self-inhibitory delay in one of

	Model Parameters	Positive Dominant Lyapunov Exponent	Dynamic Behavior
Modified Original Two-Gene Model	$\tau_1 = 15, \tau_2 = 5$ with $g_1(x) = \frac{10}{1+x^4}, g_2(x) = \frac{10}{1+x^4}$	6.0165e-04	Periodic Oscillations
	$\tau_1 = 15, \tau_2 = 3.9$ with $g_1(x) = \frac{10}{1+x^4}, g_2(x) = \frac{10}{1+x^4}$	0.0134	Chaotic
	$\tau_1 = 15, \tau_2 = 10$ with $g_1(x) = \frac{10}{1+x^4}, g_2(x) = \frac{5}{1+x^6}$	7.1395e-04	Periodic Oscillations
	$\tau_1 = 15, \tau_2 = 4.1$ with $g_1(x) = \frac{10}{1+x^4}, g_2(x) = \frac{5}{1+x^6}$	0.4701	Chaotic
	$\tau_1 = 15, \tau_2 = 6.8$ with $g_1(x) = \frac{10}{1+x^4}, g_2(x) = \frac{5}{1+x^6}$	0.5732	Chaotic
Extended Two-Gene Model	$\tau_1 = 5, \tau_2 = 1.5$ with $g_1(x) = \frac{10}{1+x^4}, g_2(x) = \frac{5}{1+x^4}$	0.0435	Chaotic
	$\tau_1 = 5, \tau_2 = 9.6$ with $g_1(x) = \frac{10}{1+x^4}, g_2(x) = \frac{5}{1+x^4}$	0.1334	Chaotic
	$\tau_1 = 5, \tau_2 = 18$ with $g_1(x) = \frac{10}{1+x^4}, g_2(x) = \frac{5}{1+x^4}$	0.6233	Chaotic

Table 4.1: Summary of all results provided in the chaos analysis for the modified original two-gene model and extended two-gene model.

the genes as in the modified original structure. It can be noted that our approach pertaining to the extension has effectively induced chaotic behavior in a simpler model.

The summary of all the results with model parameters including time delays and nonlinear functions, positive dominant Lyapunov exponents and dynamic behavior for the modified original and extended gene networks is provided in Table 4.1. Here, we have employed the Wolf method [71] to calculate the positive dominant Lyapunov exponent for various delay values in the system. When different nonlinear functions are used in the modified two-gene original model, we observe an increase in the Lyapunov exponent. The largest dominant Lyapunov exponent is obtained for the modified original model when the time delays are set to $\tau_1 = 15$ sec and $\tau_2 = 6.8$ sec.

We have conducted an analysis of the extended model using three distinct

delay values for τ_2 , while also considering the utilization of different nonlinear functions. In particular, the non-trivial dynamics are triggered by the increase in the value of τ_2 . The extended model exhibits the maximum Lyapunov exponent when the delay values are set to $\tau_1 = 5$ sec and $\tau_2 = 18$ sec.

4.5 Analysis with Larger Hill Coefficient m

This section covers the analysis of the modified original two-gene model described in Section 4.2.1 with larger Hill coefficient, m . The nonlinear functions used in this chapter are selected in the form of

$$g_i(x) = \frac{a_i}{1 + x^{m_i}}, \quad i = 1, 2. \quad (4.38)$$

As the parameter m increases, the nonlinearity function exhibits a behavior that resembles a switching function. This means that the function's output undergoes significant changes or switches rapidly as the input crosses certain thresholds or ranges, where the range is -1 and 1 in this case. The increasing nonlinearity of the function contributes to the system's complexity and can potentially lead to the chaos.

For larger m values, the function defined in (4.38) has the following form:

$$g_i(x) = \begin{cases} a_i & \text{if } |x| \leq 1 \\ 0 & \text{otherwise.} \end{cases} \quad (4.39)$$

Increasing the Hill coefficient m gives the nonlinear functions depicted in Fig. 4.46. It is worth noting that the constant a_i is assumed to be equal for each function, denoted as a . As the value of m increases, the functions become steeper in nature. The higher Hill coefficient emphasizes the threshold behavior of the functions, causing sharper transitions and potentially contributing to the emergence of chaotic behavior.

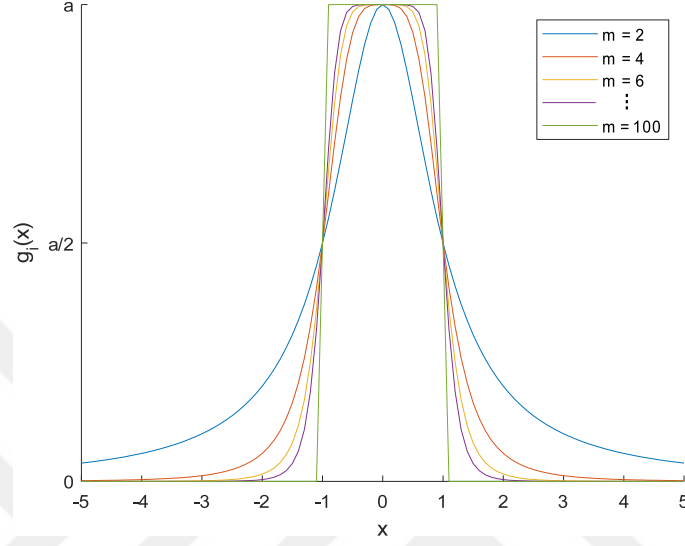


Figure 4.46: Nonlinear function $g_i(x)$ with different Hill coefficients.

In order to explore the non-trivial dynamics and compare the results given in Section 4.3 with $m = 2$ and in Section 4.4 with $m = 4$, the local maximum values of variable x_1 is examined with very large Hill coefficient m . In this analysis, the modified original two-gene model proposed in Fig. 4.2 is similarly used by selecting equal nonlinear functions $g_1(x)$ and $g_2(x)$.

Fig. 4.47 illustrates the local maximum of x_1 when time delay τ_1 is set to be 15 seconds while varying the time delay τ_2 . The system shows non-periodic dynamics when $3.25 \text{ sec} < \tau_2 < 4.6 \text{ sec}$. The behavior observed in Fig. 4.47 is indeed very similar to the analysis conducted for the two-gene modified original model with identical g_i 's, specifically for the case illustrated in Fig. 4.23 where the Hill coefficient m is set to 4. In both cases, the chaotic dynamics of the system are observed in very close regions. The similarity suggests that the choice of the Hill coefficient actually has not a significant impact on the dynamics of the system. However, as analyzed in Section 4.3, it was found that using a Hill coefficient of $m = 2$ in the modified original model leads to periodic dynamics, where the system exhibits regular oscillations. Therefore, to observe chaotic dynamics in the model, it is necessary to increase the value of the Hill coefficient to at least $m = 4$.

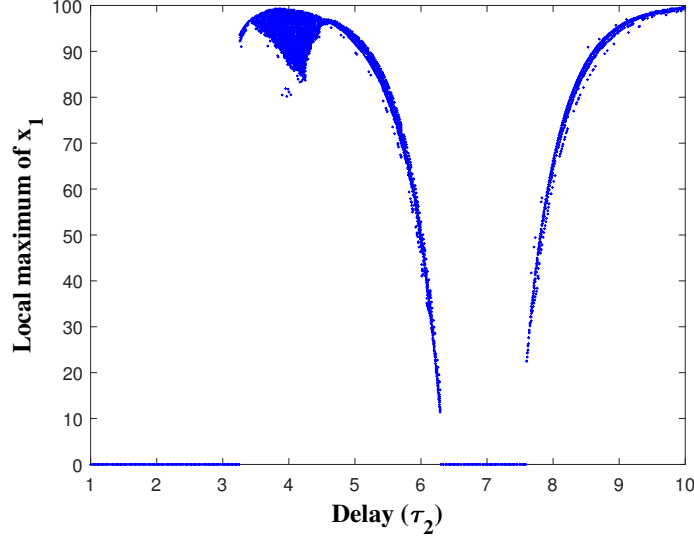


Figure 4.47: Bifurcation diagram with local maxima of x_1 with respect to τ_2 , by setting $\tau_1 = 15$ sec for the larger Hill coefficient. The non-periodic dynamics are shown when $3.25 \text{ sec} < \tau_2 < 4.6 \text{ sec}$.

This suggests that a higher degree of nonlinearity in the system, represented by a Hill coefficient greater than 2, is required to induce the complex and non-periodic behavior associated with chaos.

4.6 Conclusion

In this chapter, we have presented the analysis of nonlinear dynamics and chaos in gene regulatory network models. We first conducted the equilibrium and stability analysis of both the original and extended models, which were studied for their potential to exhibit chaotic behavior. We made modifications to the original gene network model based on insights from the literature in order to investigate chaos, and proposed the equilibrium and stability analysis of the modified original model. In the extended model, we performed the analysis without making any alterations or modifications.

Our objective was to investigate the behavior of the gene network by progressively increasing the Hill parameter. We thoroughly analyzed and provided a detailed explanation of the periodic behavior observed in the modified original two-gene model by choosing the Hill parameter $m = 2$. We conducted stability analysis of the model depending on the delay parameters in the system, and determined delay-dependent stability and instability regions. To validate the results, we also used stability-switching curves and bifurcation diagrams. These tools provided additional verification for our findings regarding the stability of the system at different delay values.

After conducting the stability analysis, we proceeded to analyze the presence of chaotic behavior in both the original and extended models. We aimed to investigate the emergence of non-periodic dynamics and assess the potential for chaotic behavior in these models. In the original model, which incorporates one delayed feedback and two coupled feedback loops, we observed a Hopf bifurcation phenomenon, consistent with our expectations. This transition occurs when the system undergoes a qualitative change from a steady-state regime to oscillatory dynamics. Then, we examined the modified original-two gene model to investigate its potential for chaotic behavior. We explored two scenarios: one where equal nonlinear functions were used, and another where different nonlinear functions were employed. Additionally, we varied the delay parameters to observe their impact on the system's dynamics. In the extended model, we found that introducing one delayed feedback and two equal delayed feedback loops resulted in non-periodic behavior. We then proceeded to explore the behavior of the model with different delay parameters, which gives more complex dynamics.

Motivated by the results of chaos analysis, we can claim that both the original and extended models demonstrate the potential for chaotic behavior to occur. The presence of chaos in the systems is not solely determined by the gene motif, but also by the specific values of the time delays in the feedback channel. Our numerical results indicate that increasing the value of delay parameter τ_2 in both the original and extended models enhances the possibility of observing chaos. The work presented in this chapter is significant in determining the presence of chaotic dynamics and understanding the underlying structure in the gene network

model being studied, which provides valuable insights into the complex behavior and potential mechanisms driving the system's dynamics.



Chapter 5

Conclusion and Future Works

Inspired by the functioning of biological cells, the study of gene expression levels is one of the prominent areas in mathematical biology, which aims to better understand and evaluate biological processes. By investigating gene expression patterns and mechanisms, we can discover about various aspects of biological processes involving specific mutations, disease progression and reactions to environmental stimuli. To this end, gene regulatory network models, which depict how genes and proteins interact with one another, play essential roles in performing and maintaining vital cellular functions.

The first part of the thesis covers an extension of a previously studied benchmark gene regulatory network model by considering the repressilator model as a transition to second order subsystems. We have employed the unmodeled dynamics and possibly ignored interactions including higher order dynamics in the system design as our tool, with the inspiration of synthetic gene networks. A thorough analysis of the extended gene network has been performed, and the characteristic equation of the linearized model has been derived. The stability characteristics of the extended model were examined using various design parameters and the results were compared with the benchmark original network. Our results show that how synthetic biological extensions can be utilized to improve the stability of GRNs.

In the second part, we have focused on an extension of a cyclic gene network model with multiplicative perturbations. Our aim was to introduce a novel framework that includes a type of multiplicative uncertainty with high-pass filter. In this extended model, an additional block has been introduced in each cascaded subsystem, and the effect of additional parameters on the stability robustness and delay margin of the system has been examined. We have showed that this extended framework can improve the stability characteristics under specific perturbations.

In the last part of the thesis, we have studied the nonlinear dynamics and chaos in the original and extended gene networks by utilizing two-gene models. We observed that the two-gene original model may show non-periodic dynamics if there exist a dual feedback in one gene. On the other hand, adding a self-inhibitory delay to one of the genes in the extended framework is not required to observe chaotic behavior. Two-gene extended model with one delayed feedback and two coupled feedback loops with equal and different delay parameters may exhibit chaotic behavior. Our focus has primarily been on the two delayed extended model where the delays differ, as this model has the potential to unveil non-periodic and chaotic dynamics across a wide range of delay parameters. We have seen that this novel framework pertaining to the extension leads to the existence of chaos in a simpler model. Consequently, nonlinear dynamics and chaos in gene regulatory networks depend not only on the specific gene motif but also on the values of time delays in the feedback channel.

As a future work, it may be possible to explore different delay structures, rather than relying on pointwise constant delays in gene network models. There are studies involving time varying delays, and analysis of the extended model and identifying the stability conditions in such cases require more involved techniques. This is a significant point worth investigating for the extended model under consideration. Studies on chaos demonstrate that introducing an extension to the gene network model by increasing the state space order and making modifications in each subsystem structure can result in the existence of non-periodic dynamics in a two-gene system. Our results now open doors worth investigating in the future. It is of interest to search the dynamics of the models depending on the

nonlinearity functions, to parametrically study the coefficients needed to observe chaotic behaviors, to analyze cases for the possibility of hyperchaos, and to further study on the networks with more than two genes. We anticipate that our study traces a different perspective for the analysis of gene regulatory network models and acts as a bridge between different disciplines such as engineering, applied mathematics and synthetic biology.



Bibliography

- [1] H. Kitano, “Systems biology: a brief overview,” *Science*, vol. 295, no. 5560, pp. 1662 – 1664, 2002.
- [2] C. V. Rao, D. M. Wolf, and A. P. Arkin, “Control, exploitation and tolerance of intracellular noise,” *Nature*, vol. 420, no. 6912, pp. 231 – 237, 2002.
- [3] D. Latchman, *Gene Regulation*, Taylor & Francis, 2007.
- [4] E. H. Davidson, *The Regulatory Genome: Gene Regulatory Networks in Development and Evolution*, Elsevier, 2010.
- [5] M. Levine, and E. H. Davidson, “Gene regulatory networks for development,” *Proceedings of the National Academy of Sciences*, vol. 102, no. 14, pp. 4936 – 4942, 2005.
- [6] J. Hasty, D. McMillen, and J. J. Collins, “Engineered gene circuits,” *Nature*, vol. 420, no. 6912, pp. 224 – 230, 2002.
- [7] S. Boccaletti, V. Latora, Y. Moreno, M. Chavez, and D-U Hwang, “Complex networks: Structure and dynamics,” *Physics Reports*, vol. 424, no. 4-5, pp. 175 – 308, 2006.
- [8] H. D. Jong, “Modeling and simulation of genetic regulatory systems: a literature review,” *Journal of Computational Biology*, vol. 9, no. 1, pp. 67 – 103, 2002.
- [9] S. S. Shen-Orr, R. Milo, S. Mangan, and U. Alon, “Network motifs in the transcriptional regulation network of *Escherichia coli*,” *Nature Genetics*, vol. 31, no. 1, pp. 64 – 68, 2002.

- [10] M. Madan Babu, and S. A. Teichmann, “Evolution of transcription factors and the gene regulatory network in *Escherichia coli*,” *Nucleic Acids Research*, vol. 31, no. 4, pp. 1234 – 1244, 2003.
- [11] S. Chandrasekaran, and N. D. Price, “Probabilistic integrative modeling of genome-scale metabolic and regulatory networks in *Escherichia coli* and *Mycobacterium tuberculosis*,” *Proceedings of the National Academy of Sciences*, vol. 107, no. 41, pp. 17845 – 17850, 2010.
- [12] H.-W. Ma, B. Kumar, U. Ditges, F. Gunzer, J. Buer and A.-P. Zeng, “An extended transcriptional regulatory network of *Escherichia coli* and analysis of its hierarchical structure and network motifs,” *Nucleic Acids Research*, vol. 32, no. 22, pp. 6643 – 6649, 2004.
- [13] D. Thieffry, A. M. Huerta, E. Pérez-Rueda, and J. Collado-Vides, “From specific gene regulation to genomic networks: a global analysis of transcriptional regulation in *Escherichia coli*,” *Bioessays*, vol. 20, no. 5, pp. 433 – 440, 1998.
- [14] X. Fang, A. Sastry, N. Mih, D. Kim, J. Tan, J. T. Yurkovich, C. J. Lloyd, Y. Gao, L. Yang, and B. O. Palsson, “Global transcriptional regulatory network for *Escherichia coli* robustly connects gene expression to transcription factor activities,” *Proceedings of the National Academy of Sciences*, vol. 114, no. 38, pp. 10286 – 10291, 2017.
- [15] M.B. Elowitz, and S. Leibler, “A synthetic oscillatory network of transcriptional regulators,” *Nature*, vol. 403, no. 6767, pp. 335 – 338, 2000.
- [16] O. Buse, R. Pérez, and A. Kuznetsov, “Dynamical properties of the repressilator model,” *Physical Review E*, vol. 81, no. 6, pp. 066206, 2010.
- [17] 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, pp. 905 – 937, 2006.
- [18] N. Strelkowa, and M. Barahona, “Transient dynamics around unstable periodic orbits in the generalized repressilator model,” *Chaos: An Interdisciplinary Journal of Nonlinear Science*, vol. 21, no. 2, 2011.

- [19] K.-Y. Kim, D. Lepzelter, and J. Wang, “Single molecule dynamics and statistical fluctuations of gene regulatory networks: A repressilator,” *The Journal of Chemical Physics*, vol. 126, no. 3, 2007.
- [20] G. Karlebach, and R. Shamir, “Modelling and analysis of gene regulatory networks,” *Nature Reviews Molecular Cell Biology*, vol. 9, no. 10, pp. 770 – 780, 2008.
- [21] L. Glass, and S. A. Kauffman, “The logical analysis of continuous, non-linear biochemical control networks,” *Journal of Theoretical Biology*, vol. 39, no. 1, pp. 103 – 129, 1973.
- [22] M. Andrecut, and S. A. Kauffman, “Chaos in a discrete model of a two-gene system,” *Physics Letters A*, vol. 367, no. 4-5, pp. 281 – 287, 2007.
- [23] 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.
- [24] A. Polynikis, S. J. 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.
- [25] 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.
- [26] F.M. Atay, J. Jost, and A. Wende, “Delays, connection topology, and synchronization of coupled chaotic maps,” *Physical Review Letters*, vol. 92, no. 14, pp. 144101, 2004.
- [27] F. Ren, and J. Cao, “Asymptotic and robust stability of genetic regulatory networks with time-varying delays,” *Neurocomputing*, vol. 71, no. 4-6, pp. 834 – 842, 2008.
- [28] Z. Li, D. Chen, Y. Liu, and Y. Zhao, “New delay-dependent stability criteria of genetic regulatory networks subject to time-varying delays,” *Neurocomputing*, vol. 207, pp. 763 – 771, 2016.

- [29] G. Ling, Z.-H. Guan, R.-Q. Liao, and X.-M. Cheng, “Stability and bifurcation analysis of cyclic genetic regulatory networks with mixed time delays,” *SIAM Journal on Applied Dynamical Systems*, vol. 14, no. 1, pp. 202 – 220, 2015.
- [30] T. Tian, K. Burrage, P. M. Burrage, and M. Carletti, “Stochastic delay differential equations for genetic regulatory networks,” *Journal of Computational and Applied Mathematics*, vol. 205, no. 2, pp. 696 – 707, 2007.
- [31] F.-X. Wu, “Stability and bifurcation of ring-structured genetic regulatory networks with time delays,” *IEEE Transactions on Circuits and Systems I: Regular Papers*, vol. 59, no. 6, pp. 1312 – 1320, 2011.
- [32] Y. Wang, Z. Wang, and J. Liang, “On robust stability of stochastic genetic regulatory networks with time delays: a delay fractioning approach,” *IEEE Transactions on Systems, Man, and Cybernetics*, vol. 40, no. 3, pp. 729 – 740, 2009.
- [33] M.E. Ahsen, H. Özbay, and S-I Niculescu, “Analysis of a gene regulatory network model with time delay using the secant condition,” *IEEE Life Sciences Letters*, vol. 2, no. 2, pp. 5 – 8, 2016.
- [34] E. Kaslik, and I. R. Rădulescu, “Stability and bifurcations in fractional-order gene regulatory networks,” *Applied Mathematics and Computation*, vol. 421, pp. 126916, 2022.
- [35] P. Li, Y. Li, R. Gao, C. Xu, and Y. Shang, “New exploration on bifurcation in fractional-order genetic regulatory networks incorporating both type delays,” *The European Physical Journal Plus*, vol. 137, no. 5, pp. 598, 2022.
- [36] D. Yue, Z.-H. Guan, J. Li, F. Liu, J.-W. Xiao, and G. Ling, “Stability and bifurcation of delay-coupled genetic regulatory networks with hub structure,” *Journal of the Franklin Institute*, vol. 356, no. 5, pp. 2847 – 2869, 2019.
- [37] D. Yue, Z.-H. Guan, J. Chen, G. Ling, and Y. Wu, “Bifurcations and chaos of a discrete-time model in genetic regulatory networks,” *Nonlinear Dynamics*, vol. 87, no. 1, pp. 567 – 586, 2017.

- [38] Z. Levnajić, and B. Tadić, “Stability and chaos in coupled two-dimensional maps on gene regulatory network of bacterium *E. coli*,” *Chaos: An Interdisciplinary Journal of Nonlinear Science*, vol. 20, no. 3, 2010.
- [39] V. Sevim, and P. A. Rikvold, “Chaotic gene regulatory networks can be robust against mutations and noise,” *Journal of Theoretical Biology*, vol. 253, no. 2, pp. 323 – 332, 2008.
- [40] V. A. Likhoshvai, S. I. Fadeev, V. V. Kogai, and T. M. Khlebodarova, “On the chaos in gene networks,” *Journal of Bioinformatics and Computational Biology*, vol. 11, no. 1, pp. 1340009, 2013.
- [41] C. Poignard, “Inducing chaos in a gene regulatory network by coupling an oscillating dynamics with a hysteresis-type one,” *Journal of Mathematical Biology*, vol. 69, no. 2, pp. 335 – 368, 2014.
- [42] Q. Lai, X.-W. Zhao, J.-N. Huang, V.-T. Pham, and K. Rajagopal, “Monostability, bistability, periodicity and chaos in gene regulatory network,” *The European Physical Journal Special Topics*, vol. 227, pp. 719 – 730, 2018.
- [43] M.W. Hirsch, S. Smale, and R. L. Devaney, *Differential equations, dynamical systems, and an introduction to chaos*, Academic press 2012.
- [44] F. M. Atay, “Delayed feedback control near Hopf bifurcation,” *Discrete and Continuous Dynamical Systems-S*, vol. 1, pp. 197 – 205, 2008.
- [45] L. P. Shayer, and S. A. Campbell, “Stability, bifurcation, and multistability in a system of two coupled neurons with multiple time delays,” *SIAM Journal on Applied Mathematics*, vol. 61, no. 2, pp. 673 – 700, 2000.
- [46] G. A. Enciso, “On the asymptotic behavior of a cyclic biochemical system with delay,” *Proceedings of the 45th IEEE Conference on Decision and Control*, pp. 2388 – 2393, 2006.
- [47] R. Wang, Z. Jing, and L. Chen, “Modelling periodic oscillation in gene regulatory networks by cyclic feedback systems,” *Bulletin of Mathematical Biology*, vol. 67, pp. 339 – 367, 2005.

- [48] I. Shmulevich, S. A. Kauffman, and M. Aldana, “Eukaryotic cells are dynamically ordered or critical but not chaotic,” *Proceedings of the National Academy of Sciences*, vol. 102, no. 38, pp. 13439 – 13444, 2005.
- [49] Z. Zhang, W. Ye, Y. Qian, Z. Zheng, X. Huang, and G. Hu, “Chaotic motifs in gene regulatory networks,” *Plos one*, vol. 7, no. 7, pp. e39355, 2012.
- [50] D. S. Glass, X. Jin, and I. H. Riedel-Kruse, “Nonlinear delay differential equations and their application to modeling biological network motifs,” *Nature Communications*, vol. 12, no. 1, pp. 1788, 2021.
- [51] Y. Suzuki, M. Lu, E. Ben-Jacob, and J. Onunhic, “Periodic, quasi-periodic and chaotic dynamics in simple gene elements with time delays,” *Scientific Reports*, vol. 6, no. 1, pp. 21037, 2016.
- [52] M.C. Mackey, and L. Glass, “Oscillation and chaos in physiological control systems,” *Science*, vol. 197, no. 4300, pp. 287 – 289, 1977.
- [53] M.E. 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, no. 11, pp. 1609 – 1627, 2014.
- [54] M.E. Ahsen, H. Özbay, and S-I Niculescu, *Analysis of Deterministic Cyclic Gene Regulatory Network Models with Delays*, Birkhauser, Cham, 2015.
- [55] M.E. Ahsen, H. Özbay, and S-I Niculescu, “A secant condition for cyclic systems with time delays and its application to gene regulatory networks,” *IFAC-PapersOnLine*, vol. 48, no. 12, pp. 171 – 176, 2015.
- [56] H. Özbay, *Introduction to Feedback Control Theory*, CRC Press, 1999.
- [57] D. Öztürk, and H. Özbay, “Analysis of an extended gene regulatory network model with time delay,” *Applied and Computational Mathematics*, vol. 22, no. 2, pp. 225 – 245, 2023.
- [58] D. Öztürk, H. Özbay, and F.M. Atay, “Robustness and delay margin analysis of a gene regulatory network model,” *IFAC-PapersOnLine*, vol. 55, no. 25, pp. 253 – 258, 2022.

- [59] D. Öztürk, and H. Özbay, “Extension of a gene regulatory network model and its stability analysis,” *Extended Abstracts of 7th International Conference on Control and Optimization with Industrial Applications*, vol. 1, pp. 314 – 317, 2020.
- [60] M. Heinemann, and S. Panke, “Synthetic biology—putting engineering into biology,” *Bioinformatics*, vol. 22, no. 22, pp. 2790 – 2799, 2006.
- [61] T.K. Lu, A.S. Khalil, and J. Collins, “Next-generation synthetic gene networks,” *Nature Biotechnology*, vol. 27, no. 12, pp. 1139 – 1150, 2009.
- [62] J. Ding, and A. Zhou, “Eigenvalues of rank-one updated matrices with some applications,” *Applied Mathematics Letters*, vol. 20, no. 12, pp. 1223 – 1226, 2007.
- [63] R.E. Thomas, A.J. Rosa, and G.J. Toussaint, *The Analysis and Design of Linear Circuits*, John Wiley & Sons, 2016.
- [64] T. Qi, J. Zhu, and J. Chen, “Fundamental Limits on Uncertain Delays: When Is a Delay System Stabilizable by LTI Controllers?,” *IEEE Transactions on Automatic Control*, vol. 62, no. 3, pp. 1314 – 1328, 2016.
- [65] H. Özbay, S. Gümüşsoy, K. Kashima, and Y. Yamamoto, *Frequency Domain Techniques for H_∞ Control of Distributed Parameter Systems*, SIAM, 2018.
- [66] M. K.H. Fan, A. L. Tits, and J. C. Doyle, “Robustness in the Presence of Joint Parametric Uncertainty and Unmodeled Dynamics,” *American Control Conference*, pp. 1195 – 1200, 1988.
- [67] A. Tözeren, and S. W. Byers, *New Biology for Engineers and Computer Scientists*, Prentice Hall, 2003.
- [68] R. Sipahi, S-I. Niculescu, C. T. Abdallah, W. Michiels, and K. Gu, “Stability and stabilization of systems with time delay,” *IEEE Control Systems Magazine*, vol. 31, no. 1, pp. 38 – 65, 2011.
- [69] K. Gu, S-I. Niculescu, and J. Chen, “On stability crossing curves for general systems with two delays,” *Journal of Mathematical Analysis and Applications*, vol. 311, no. 1, pp. 231 – 253, 2005.

- [70] D. Avanesoff, A. R. Fioravanti, and C. Bonnet, “YALTA: a Matlab toolbox for the $\mathcal{H}_\infty$ -stability analysis of classical and fractional systems with commensurate delays,” *IFAC Proceedings Volumes*, vol. 46, no. 2, pp. 839 – 844, 2013.
- [71] A. Wolf, J. B. Swift, H. L. Swinney, and J. A. Vastano, “Determining Lyapunov exponents from a time series,” *Physica D: Nonlinear Phenomena*, vol. 16, no. 3, pp. 285 – 317, 1985.
- [72] S. H. Strogatz, *Nonlinear Dynamics and Chaos: with Applications to Physics, Biology, Chemistry, and Engineering*, CRC Press, 2018.
- [73] E. N. Lorenz, “Deterministic Nonperiodic Flow,” *Journal of Atmospheric Sciences*, vol. 20, no. 2, pp. 130 – 141, 1963.
- [74] N. H. Packard, J. P. Crutchfield, J. D. Farmer, and R. S. Shaw, “Geometry from a time series,” *Physical Review Letters*, vol. 45, no. 9, pp. 712, 1980.
- [75] H. Poincaré, “Sur le problème des trois corps et les équations de la dynamique,” *Acta Mathematica*, vol. 13, pp. 1 – 271, 1890.
- [76] P. Grassberger, and I. Procaccia, “Characterization of strange attractors,” *Physical Review Letters*, vol. 50, no. 5, pp. 346, 1983.